Leaf Protein Concentrate from Paunch Grass and Green Waste:
A Technoeconomic Analysis and Lifecycle Assessment

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Abstract

The global supply of food and feed protein needs to double during the first half of the 21st century. At present, food and feed protein is produced by livestock and arable crop farming. Further expansion of this protein production model is unsustainable in many countries due to overallocated water resources, limited availability and accessibility of new farmlands, and the environmental impacts of widespread fertiliser and fossil fuel use in agriculture. Alternative sources of food and feed protein are therefore needed to meet growing demand. One potential source of protein is leaf protein concentrate (LPC) manufactured from negative-value paunch grass (PG) and leafy green waste (GW). The chemical compositions PG and GW have therefore been determined for the first time in New Zealand. Their protein contents (15.2 to 16.2 DM%) are comparable to those of alfalfa and ryegrass, making them suitable LPC feedstocks. The optimal manufacturing method uses two passes through a screw press with intermediary maceration to extract the PG and GW leaf proteins into a liquor; the liquor is then acidified with hydrochloric acid and the protein coagulated by steam injection (85°C, 30 seconds), to achieve 45.9 to 62.0 mass percent protein recovery. The industrial-scale manufacture of leaf protein concentrate from these waste materials is technologically and financially viable when it is co-sited with a meat processing facility or rendering plant, and the fibre fraction is treated as a valuable co-product instead of a waste stream. The LPC has a crude protein content (~37 DM%) and essential amino acid profile that is comparable to other plant protein products used for animal feed. Its Global Warming Potential (2094 kg CO_2-e / MT-LPC) is relatively high when compared with those of other animal feed protein sources; the Blue Water Footprint (39.3 m^3-H_2O / MT-LPC) is relatively low. Both impacts are within the range of protein sources already supplied to the animal feed industry. The fibre co-product has great potential; it could be used as a bulking agent for composting processes, a feedstock for anaerobic digestion, and a fuel for process heat. Further work in understanding the drying, extrusion, and palletisation properties of the LPC is needed for industrial-scale production and commercialisation.

Dedicated to my extraordinary wife, Diloromkhon Askarova,
and our beloved children.

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Contents

Abbreviations and Symbols

AA	Amino Acid
AWARE	Available Water Remaining
BL	Battery Limits
BWF	Blue Water Footprint
c	Concentration
C	Total Production Cost
c_{BM}	Bare Module Cost
C_{TBM}	Total Bare Module Cost (Installed Cost)
C_{TDC}	Total Depreciable Capital
C_{TPI}	Total Permanent Capital Investment
CE	Cumulative Extraction of Raw Material Protein
CEFIC	European Chemical Industry Council
CF	Cash Flow
CF-PV	Cash Flow Present Value
CL	Confidence Limit
CNR	Carbon to Nitrogen Ratio
COM	Cost of Manufacture
CP	Crude Protein
CPR	Cumulative Protein Recovery
CS	Carbon Steel
D	Depreciation
DM	Dry Matter
DM%	Dry Matter Percent
DW&B	Direct Wages & Benefits
DPL	Deproteinated liquor
EAA	Essential Amino Acids
ECTA	European Chemical Transport Association
f_{mx}	Cost Factor to Convert Between Material x and Carbon Steel Basis
f_{my}	Cost Factor for Delivered Cost in Material y
f_{er}	Cost Factor for Erection
f_p	Cost Factor for Piping, Ducting, and Chutes
f_i	Cost Factor for Instrumentation
f_{el}	Cost Factor for Electrical Work
f_c	Cost Factor for Civil Engineering Work
f_{sb}	Cost Factor for Surrounding Structures and Buildings
f_l	Cost Factor for Lagging
f_m	Functional Unit Weighting Factor
f_{CW}	Catchment Weighting Factor
f_{WC}	Water Consumption Factor
FC	Crude Fibre
FW	Food Waste
GHG	Greenhouse Gas
GW	Leafy Green Waste
GW-LPC	Leaf Protein Concentrate from Leafy Green Waste
GWP	Global Warming Potential
I	Interest Cost
I_a	Price Index Value for Year a
IRP	Integrated into Rendering Plant
IRR	Internal Rate of Return

LHV	Lower Heating Value
LP	Leaf Protein
LPC	Leaf Protein Concentrate
m	Mass
$\dot{m}_{in}$	Mass Flow In per Unit Time
$\dot{m}_{out}$	Mass Flow Out per Unit Time
$\dot{m}_{consumption}$	Mass Consumed per Unit Time
$\dot{m}_{generation}$	Mass Generated per Unit Time
$\dot{m}_{insoluble}$	Mass Flow of Insoluble Components per Unit Time
$\dot{m}_{soluble}$	Mass Flow of Soluble Components per Unit Time
$\dot{m}_{y}$	Mass Flow of Component y per Unit Time
M&O	Maintenance and Operations
MPIC	Main Plant Item Cost
MT	Metric Tonnes
MW&B	Maintenance Wages & Benefits
NPV	Net Present Value
NRC	National Research Council of the National Academies (USA)
PFD	Process Flow Diagram
PG	Paunch Grass
PG-LPC	Leaf Protein Concentrate from Paunch Grass
PLP	Press Liquor Protein
PMEI	Plant, Machinery, and Equipment Index
PRL	Protein Recovery from Liquor
q	Quantity of Energy, Fuel, or Material Used by a Process
R	Revenue from Sales, Gate Fees, and Internal Transfers
RCF	Relative Centrifugal Force (Multiple of Gravitational Force)
ROI	Return on Investment
SPBP	Simple Payback Period
SD	Standard Deviation
SLW	Soft Leafy Wastes (Paunch Grass and Leafy Green Waste)
SLW-LPC	Leaf Protein Concentrate from Soft Leafy Wastes
SS	Stainless Steel
SW&B	Salaries, Wages, and Benefits
TGA	Thermogravimetric Analysis
TW	Total Waste
U	Uncertainty Factor
VP	Venture Profit
VSD	Variable Speed Drive
WC	Water Consumption
WCF	Water Consumption Footprint
WF	Water Footprint
WHO	World Health Organisation
WSI	Water Stress Index
WTC	Well-to-Chimney
WTW	Well-to-Wheel
x_a	Mass Fraction of Component a
x_{water}	Mass Fraction of Water
$z_{i,soluble}$	Mass Fraction of Soluble Component i
μ	Specific Fuel Consumption
η	
η_a	Mass Ratio of Feedstock Crude Protein Extracted by a Single Maceration Step and a Single Pass Through the Screw Press

η_b	Mass Ratio of Feedstock Crude Protein Extracted by a Single Maceration Step and Dual Passes Through the Screw Press
η_c	Mass Ratio of Feedstock Crude Protein Extracted by Dual Maceration Steps and Dual Passes Through the Screw Press
η_{CP1}	Mass Ratio of Feedstock Crude Protein Extracted into Liquor
η_{CP2}	Mass Ratio of Feedstock Crude Protein Recovered from Liquor
η_M	Mass Ratio of Feedstock Crude Protein Extracted During the Maceration Step
η_{P1}	Mass Ratio of Feedstock Crude Protein Extracted During the First Pass Through the Screw Press
η_{P2}	Mass Ratio of Feedstock Crude Protein Extracted During the Second Pass Through the Screw Press
ρ	Price of a Process Input

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1 Introduction

1.1 Background

The world's population is projected to increase from 6 billion people in the year 2000 to 9.1 billion people in 2050. This population is also becoming more affluent, increasing the per capita demand for milk and meat, especially in the developing world [1; 2]. Globally, the average amount of meat consumed per capita is set to grow from 39 kg per person per year in 2005 to 49 kg per person per year in 2050; similarly, the amount of dairy is set to grow from 83 kg per person per year in 2005 to 99 kg per person per year in 2050. In developing countries, the growth is even more pronounced, with meat consumption growing from 28 kg per person per year to 42 kg per person per year; and milk consumption growing from 52 kg per person per year to 76 kg per person per year [2]. The overall demand for meat is thus predicted to increase from 228 million tonnes to 459 million tonnes per annum; and the demand for milk is predicted to increase from 475 to 883 million tonnes per annum [1].

A typical omnivorous diet in the USA consumes 994.9 kg of food per annum, including 124 kg of meat, 20.3 kg fish, and 256 kg of dairy protein, corresponding to approximately 61% of protein needs [3]. This requires 816 kg per annum of feed crops, most of which could be directly consumed by humans. 40% of the grain harvest and 75% of the soy harvest is used to feed livestock, but on average only 15% of the protein and energy in these crops is converted into edible animal products [1; 3]. By contrast, a typical lactoovovegetarian diet which uses dairy products to meet 33% of protein needs only requires 450 kg per annum of feed grains to produce dairy products [3]. The growth in both global population and per capita demand for milk and meat is therefore increasing pressure on global crop production: the per capita demand for crop protein is thus predicted to increase by approximately 110 percent between 2005 and 2050, largely to feed livestock [1].

While there is presently enough food in the world to adequately feed the global population, it is not equally distributed, resulting in widespread malnourishment in some parts of the world [2]. Similarly, although there are enough land and water resources to expand animal protein production to meet demand at a global level, the

land is often inaccessible and requires significant investment in infrastructure and disease eradication. The water resources also tend to be located away from accessible arable land or overallocated [1; 2]. Some countries do not have adequate land or water resources to meet growth in population and food demand, and as of 2006 there is no longer an abundant global supply of cereal crops [2]. Marine protein resources are already over-fished and aquaculture is closely tied to limited terrestrial feed production [4].

A comparison of studies by de Vries and de Boer [5] (Table 1.1), Noya *et al.* [6] (Table 1.2), and reveals that the lifecycle impacts of animal protein sources are typically several times greater than those of plant protein sources. Except for grass-fed pastoral systems, animal protein production systems use large quantities of plant protein crops as animal feed [1; 3; 7; 8]. Meanwhile, data from Mekonnen and Hoekstra [9] (Table 1.3 & Table 1.4) indicates that although plant proteins can be produced with less Blue Water - water from rivers, streams, lakes, and aquifers – than animal proteins, they require similar amounts of Green Water – rainwater falling directly onto the soil – and produce similar amounts of Grey Water – the amount of water needed to dilute pollutants to levels stipulated by environmental standards. However, on a product basis, the water footprints of animal proteins on a product basis are several times those of plant proteins [10; 11; 12].

Table 1.1: Lifecycle impacts of selected animal proteins.

Protein Source	Limit	Land Use *m^2 / kg protein*	Fossil Fuel Energy *MJ / kg protein*	Climate Change *kg CO_2-e / kg protein*	Acidification *kg SO_2-e / kg protein*	Freshwater Eutrophication Potential *kg PO_4-e / kg protein*
Pork	Upper	63.7	236.8	52.6	0.39	0.13
	Lower	46.8	94.7	20.5	0.23	0.02
Chicken	Upper	52.1	152.6	36.3	0.13	0.03
	Lower	42.6	78.9	19.5	0.08	0.01
Beef	Upper	257.9	273.7	168.4	0.29	0.11
	Lower	142.1	178.9	73.7	0.03	0.02
Milk	Upper	66.7	144.0	43.3	0.50	0.20
	Lower	36.7	37.0	28.0	0.17	0.07
Eggs	Upper	47.7	107.0	37.7	0.08	0.02
	Lower	34.6	87.0	30.0	0.02	0.01

Source: de Vries and de Boer [5]

Global supplies of surface water and rainwater for agriculture are already overallocated [4; 13; 14; 15; 16]; widespread deforestation is taking place to clear arable land for animal feed crops [1; 8]; and progressively greater quantities of nitrogen fertiliser are being applied to land to improve protein yields [1; 17]. Hence expanding current farming practices to meet the demand for animal protein will increase greenhouse gas emissions, water stress and pollution, deforestation, and lead to losses in biodiversity and ecosystem services; in some cases it may push utilisation of land and water resources beyond their sustainable limits [1; 13; 18; 19]. It is therefore necessary to explore alternative sources of protein for both human and animal nutrition that can help meet the unprecedented growth in global demand for protein, whilst also investigating ways to reduce demand [20].

Table 1.2: Lifecycle impacts of selected plant proteins.

Protein Source	Limit	Land Use *m^2 / kg protein*	Fossil Fuel Energy *MJ / kg protein*	Climate Change *kg CO_2-e / kg protein*	Acidification *kg SO_2-e / kg protein*	Freshwater Eutrophication Potential *kg PO_4-e / kg protein*
Barley	Upper	7.75	15.7	7.56	0.23	0.000322
	Lower	7.34	15.0	7.30	0.21	0.000312
Rye	Upper	7.80	11.2	6.95	0.23	0.000279
	Lower	4.06	11.0	5.71	0.19	0.000229
Sorghum	N/A	7.02	9.82	4.45	0.26	0.000246

Source: Noya, et al. [6]

Table 1.3: Global average water footprints of selected animal proteins.

Protein Source	Green Water Footprint *m^3 / kg protein*	Blue Water Footprint *m^3 / kg protein*	Grey Water Footprint *m^3 /kg protein*	Total Water Footprint *m^3 /kg protein*
Pork meat	25.8	2.4	3.3	31.5
Chicken meat	18.7	1.6	2.5	22.8
Beef	75.9	2.9	2.4	81.1
Sheep meat	51.6	2.7	0.4	54.8
Milk	28.8	2.9	2.4	34.0
Eggs	19.9	1.9	3.3	25.1

Adapted from Mekonnen and Hoekstra [9] with conversion factors from Lawrie and Ledward [21] & United States Department of Agriculture [22].

Table 1.4: Global average water footprints of selected plant proteins.

Protein Source	Green Water Footprint *m^3 / kg protein*	Blue Water Footprint *m^3 / kg protein*	Grey Water Footprint *m^3 /kg protein*	Total Water Footprint *m^3 /kg protein*
Barley	32.6	1.7	3.6	37.9
Rye	43.1	1.0	3.0	47.1
Sorghum	43.9	4.7	3.3	51.9

Adapted from Mekonnen and Hoekstra [9] with conversion factors from Noya, et al. [6].

An alternative source of protein is leaf protein concentrate. It is typically produced as per Figure 1.1: harvesting leaf crops such as alfalfa, ryegrass, and clovers [23; 24]; pulping them in a hammer mill; passing the pulp through a screw press to extract a protein-rich liquor; injecting steam into the liquor to rapidly heat it to approximately 80°C and coagulate the protein; then dewatering the protein curd in a decanting centrifuge and drying it in a drum dryer [25; 26; 27].

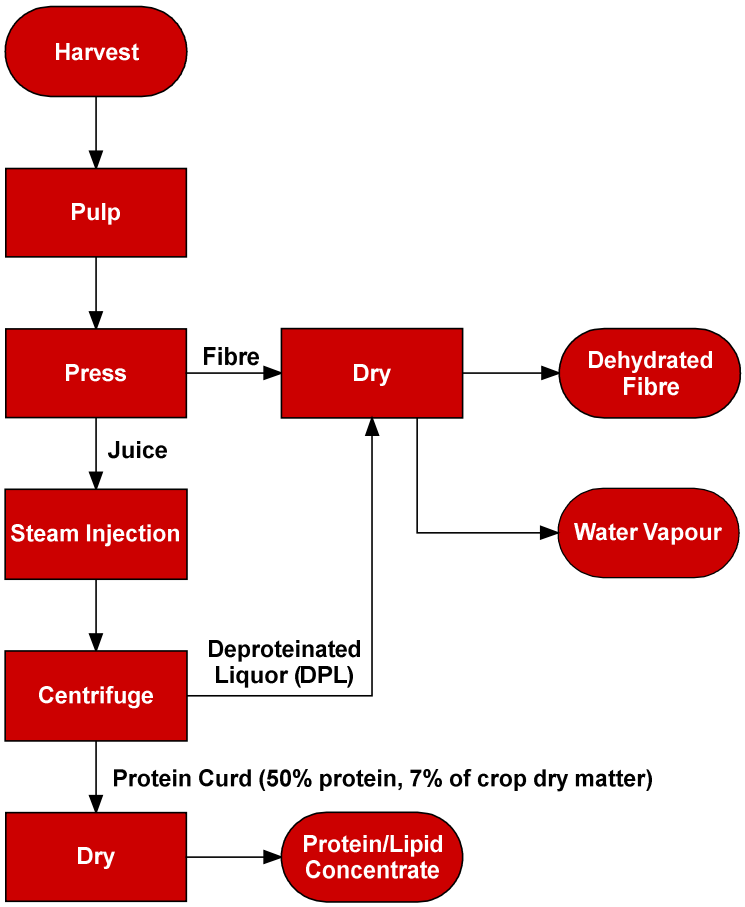

Figure 1.1: Established industrial process for extraction and recovery of leaf protein concentrate.

Source: McDonald [25]

Leaf protein concentrates (LPC) have been produced continuously since the 1950s [28]. They have been principally used in poultry feed applications but have also been considered for aquaculture feed and human nutrition. However, they have not been competitive with other animal feed ingredients to date [25]. For example, as of February 2020, alfalfa leaf protein concentrate cost approximately 1100 New Zealand dollars per tonne; whereas, soybean meal, a premium source of animal feed protein only cost NZD 550 per tonne [29; 30; 31]. This is because they were produced from crops grown solely for the purpose of producing LPC, which added

substantial production and harvesting costs to the processing costs. LPC production has also been the co-product of first-generation processes optimised to produce fibrous animal feed with a fixed protein content [25].

A second-generation process was optimised for LPC production was developed by New Zealand scientists and engineers in the 1980s (Figure 1.2), but it did not progress beyond the pilot plant stage [25; 32]. As per the main difference between the established industrial leaf protein process and the high-yield process was the introduction of a disc mill to re-pulp the fibre exiting the screw press; this second pulp was then passed through two more screw presses to maximise protein extraction [25; 32]. The bulk of research on LPC processing and properties was performed before 1991 [23; 26; 32; 33; 34; 35; 36; 37; 38; 39; 40; 41; 42; 43; 44; 45; 46], although there has been renewed interest since the turn of the 21st century [25; 47; 48; 49; 50; 51; 52; 53; 54; 55; 56; 57; 58; 59; 60; 61].

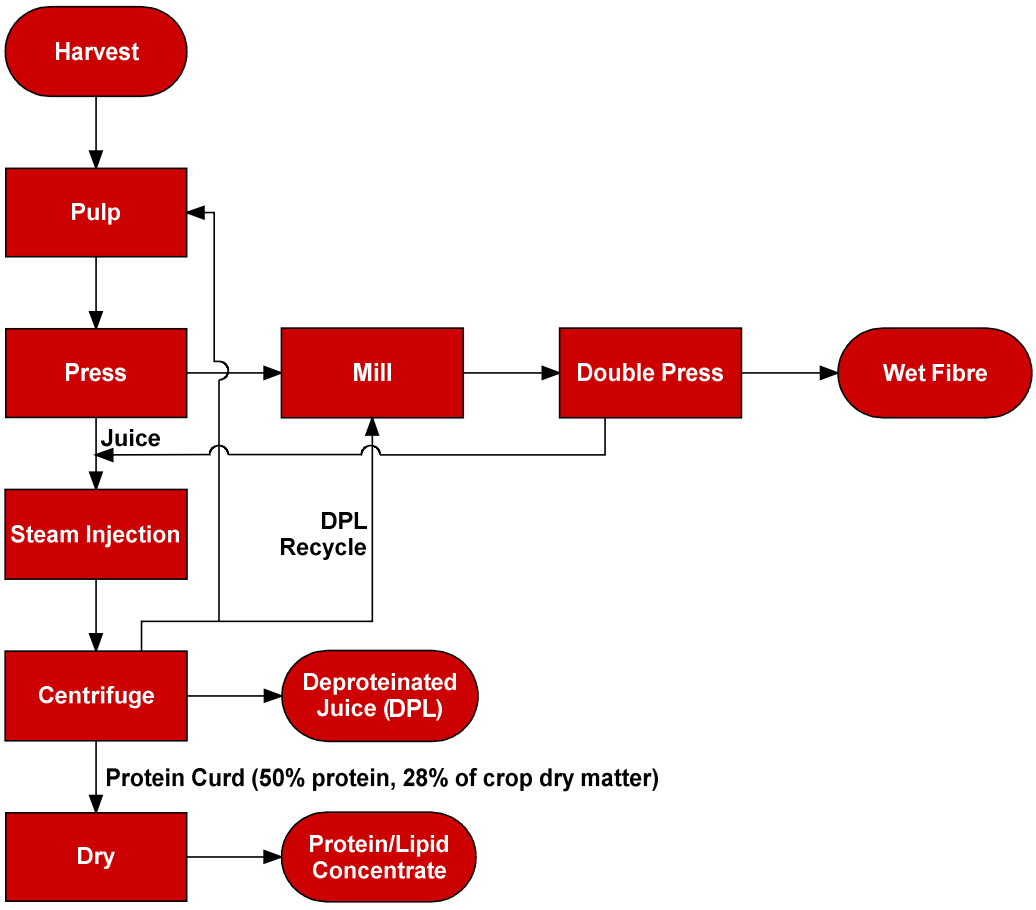

Figure 1.2: High-yield leaf protein concentrate manufacturing process.

An alternative source of LPC is negative-value materials such as leafy green waste from domestic and commercial gardening and horticulture, and paunch grass from animals from the meat processing industry. Producing LPCs from negative-value waste leafy green plant materials such as paunch grass and leafy green waste could reduce the difficulty and cost of their disposal. It will also create an innovative source of animal feed protein in response to the challenges presented by growing demands for protein in a world where further agricultural expansion is limited by environmental considerations.

Nationally, New Zealand produces 1,070,000 tonnes of paunch grass per year which has an average protein content of 15.2% on a dry basis, and has a Carbon to Nitrogen ratio (C:N) of 19:1. Additionally, NZ produces an estimated 169,000 tonnes of green waste per year, some of which is composted at home, some is composted commercially, and the remainder is landfilled. On average, this green waste contains 16.2% protein on a dry basis, and has a C:N of 47:1. Composting requires a C:N ratio of 25-30:1 meaning that green waste and paunch grass needs to be supplemented with carbon from wood chip or other high carbon sources. There is no published data available on the chemical composition of paunch grass and green waste in New Zealand. Globally, only limited information is available on the chemical composition of these paunch grass and green waste, and almost no attempt has been made to quantify the seasonal variation.

Some preliminary investigations were performed into using the established industrial leaf protein concentrate process on agricultural residues and the properties of the resulting leaf protein concentrates. However, this research either assumed obsolete economic scenarios or was only applicable to tropical climates [41; 42; 62; 63; 64]. Only one study by Bals and Dale [48] has reassessed producing alfalfa LPC in a temperate climate under contemporary economic conditions, but it did not attempt to determine the carbon or water footprint of the resulting LPC. Investigations into producing LPC using the second-generation process stopped at the pilot plant stage, and to date no investigations have been made into using this process to produce LPC from negative-value materials.

Hence the first aim of this investigation is to contribute towards filling the gaps in scientific knowledge of:

- The seasonal variation in the chemical composition of paunch grass and green waste in New Zealand;
- The technical viability of using the second-generation high-yield process to produce leaf protein concentrates from these materials;
- The basic chemical properties of those leaf protein concentrates; and
- The carbon and water footprint of those leaf protein concentrates.

The second aim of this investigation is to use the outcomes of the scientific investigations to predict the financial viability of industrial-scale production of leaf protein concentrate from paunch grass and leafy green waste.

1.1.1 Commercial Interest and Location of Processing Plant

There is commercial interest in this research from Wallace Corporation Ltd and Hamilton Garden Bags and Red Lid Bins Ltd, who are both interested in the potential to produce leaf protein from their waste streams. Wallace Corporation currently disposes of approximately 10,000 tonnes of paunch grass per annum at its Waitoa industrial estate, corresponding to approximately 12.5% of the paunch grass produced in the North Island each year; and Hamilton Garden Bags and Red Lid Bins collects approximately 2,300 m^3 of green waste per annum from its customers. Both organisations partnered with Callaghan Innovation to fund this research through a Capability Education Fellowship. As a condition of this funding, the model processing plant assessed by this research has been co-located with existing rendering facilities on the Waitoa industrial estate.

1.2 Research Aims and Questions

The overall aim of the research is to determine the technological, financial, and environmental viability of producing an animal feed leaf protein concentrate from paunch grass and leafy green waste. It may be further broken down into seven research questions.

- What is the seasonal production of paunch grass and green waste, and seasonal variation in composition?
- Do paunch grass and leafy green wastes contain enough protein to make extraction attempts worthwhile?

- What is the best method for extracting protein from the waste leafy green plant materials?
- Is the protein extracted from the waste green plant materials suitable for incorporation into animal feeds?
- What are the properties of the protein extraction co-products?
- Is the recovery of a leaf protein concentrate from waste leafy green plant materials financially viable under current economic conditions?
- Is the overall environmental impact of recovering leaf protein from waste leafy green plant materials better than that of disposing of these materials by present methods?

1.3 Structure of the Thesis

The thesis is divided into seven chapters:

- Chapter One presents a background to the research project, as well as an overview of the rationale for conducting the research, the aims of the research, and the structure of the thesis.
- Chapter Two presents a literature review background knowledge and relevant literature regarding food and feed proteins, present protein production practices, the challenges facing global protein production, alternative protein sources, leaf protein production and properties, current technoeconomic assessment practices, and current life-cycle assessment practices. It also identifies the gaps in leaf protein literature that are filled by this thesis.
- Chapter Three presents the experimental methodology involved with the multivariate characterisation of the seasonal variation in the biochemical compositions of paunch grass and leafy green wastes, and the experimental practices involved with the extraction, recovery, and characterisation of waste leaf proteins. It also presents the methodology used for the technoeconomic and life-cycle assessments of leaf protein production.
- Chapter Four presents the results of the seasonal multivariate characterisation of the seasonal variation in biochemical compositions of paunch grass and leafy green wastes and discusses their impacts on the leaf protein production potential of these materials.

- Chapter Five presents the results of the process to optimise leaf protein extraction and recovery methods for paunch grass and leafy green waste, as well as the biochemical and thermal characterisation of leaf proteins recovered by the optimised method.
- Chapter Six presents the outcomes of the technoeconomic assessment performed to determine the technological and financial viabilities of producing leaf protein concentrate from paunch grass and leafy green wastes on an industrial scale.
- Chapter Seven presents the outcomes of the lifecycle assessment performed to determine the carbon and water footprints of leaf protein concentrate and leafy green waste and compare them to those of other animal feed protein products.
- Chapter Eight presents the key outcomes of the research, the overall feasibility of leaf protein production from paunch grass and leafy green waste, and recommendations for supplementary future research.

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2 Literature Review

This research had three objectives: to determine the potential to recover leaf proteins from paunch grass and garden waste; to perform a technoeconomic analysis of using these materials for industrial-scale leaf protein concentrate manufacturing; and to perform a lifecycle assessment to determine the environmental impacts of the industrial leaf protein concentrate production. This literature review therefore begins with a summary of proteins in general, as the research aims to provide an additional protein source for animal nutrition. Global protein supply and barriers to increasing animal protein production are explored next, as they provide an environmental and economic context for the research. Meat processing is then reviewed as the sole source of paunch grass and one of the protein resources investigated by this research. This naturally leads to investigation of the characteristics and supplies of paunch grass available in the Upper North Island of New Zealand. The characteristics and supplies of garden waste are reviewed next, as it is the second protein resource investigated by this research; feedstock quantities are again estimated. The review of potential leaf protein resources is followed by a summary of alternative protein sources and exploration of the literature on leaf proteins. The latter emphasises existing leaf protein resources, LPC production technology, and by-products of the LPC production process. This informs a discussion of fish protein and how LPC can enable sustainable expansion of aquaculture. The review ends with a discussion of lifecycle assessment and its relevance to sustainable production of food and feed, before concluding with an analysis of gaps in the literature and the novelty of this research.

2.1 Proteins

Proteins are polymers of amino acids, and consist of many amino acids joined by peptide bonds [1]. They are key agents of biological function, and play an essential role in human nutrition, with adults requiring an average of 0.83 g of protein per kilogram of body weight per day [2]. The main sources of food protein are plants and animal products. The former can be subdivided into grains, pulses, oilseeds, nuts, starchy roots, vegetables, and fruits. The latter can be subdivided into meat, dairy products, eggs and fish [3].

Despite the diverse range of proteins found in nature, the almost all proteins are formed from just 20 amino acids. The amino acids are classified according to their polarity, charge and acid/base properties [1]. The structures of the 20 common amino acids are depicted in Figure 2.1.

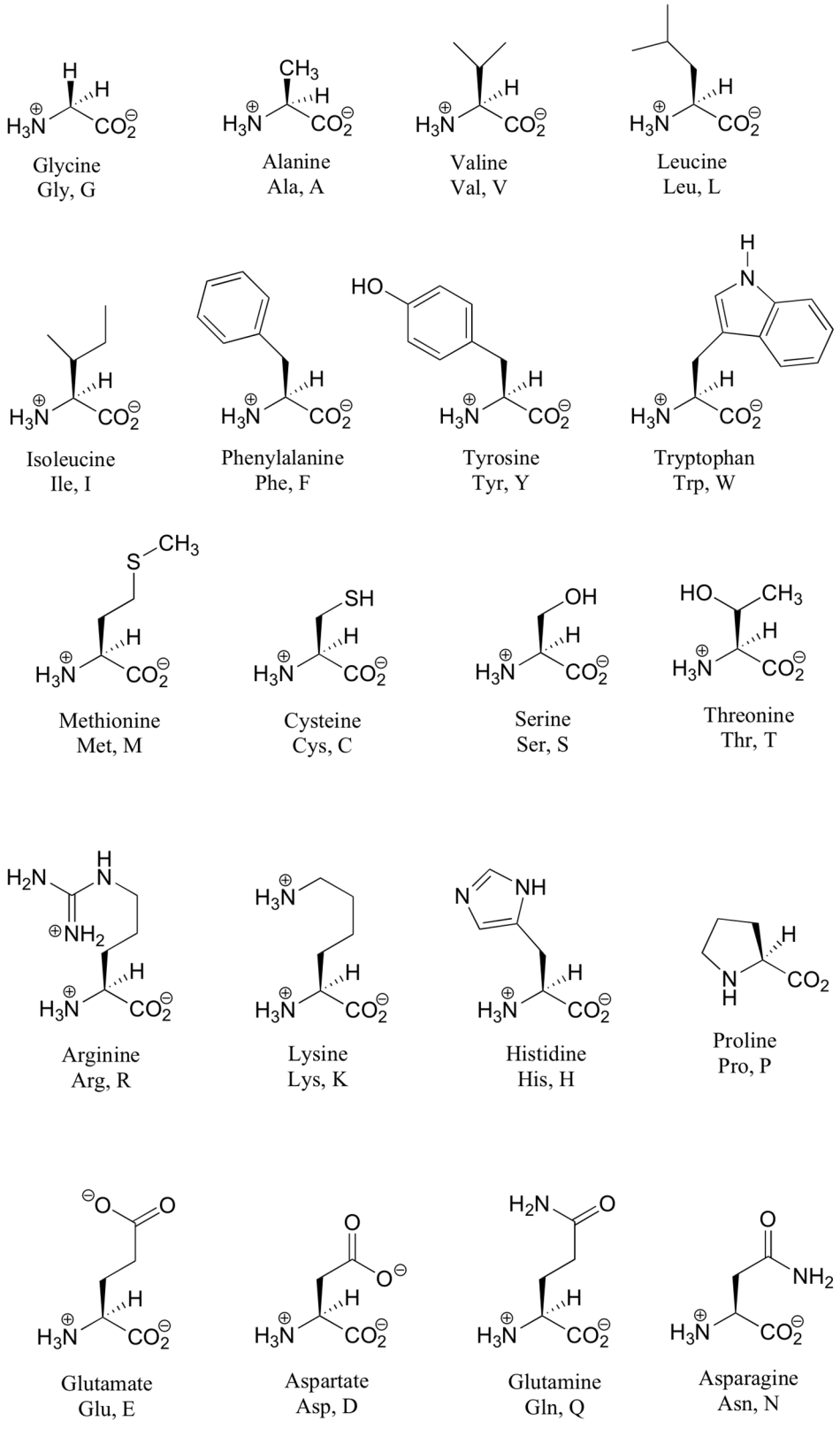

Figure 2.1: The principal 20 common amino acids found in protein.

Source: Soderberg [4]

There are three main types of protein [1]:

- Fibrous proteins, which usually have simple, linear structures.
- Globular proteins, which are spherical in shape and are folded so that hydrophobic amino acid side chains face into the globule, and hydrophilic amino acid side chains are on the outside of the globule.
- Membrane proteins, which are an essential component of cell membrane systems. These proteins have the hydrophilic amino acid side chains facing outwards.

Furthermore, proteins typically have four levels of structure [1]:

- Primary structure: the amino acid sequence
- Secondary structure: amino acid chains often arrange themselves into helical (α-helix) or pleated segments (β-strand).
- Tertiary structure: The three-dimensional folding of the protein's polypeptide chains into a more complex shape.
- Quaternary structure: Interactions between two or more polypeptide chains of characteristic tertiary structure.

2.2 Global Protein Supply

There has been a global shortage of protein for some time. According to Abbott [3], global protein production needed to be increased by 45% between 1960 and 1975. Simultaneous intensification and expansion of agriculture since 1961 has helped to close this gap [5; 6]. Approximately 20% more protein is available in typical diets today compared with 20 years ago, although South Asia and Africa have not benefited from these increases – diets there remain of a poor quality, and heavily reliant on grains, roots and tubers [7]. Approximately one in eight people worldwide remain undernourished [7].

The world will need to approximately double its food supply by 2050 to accommodate a projected population increase from 6.0 billion people in the year 2000 to 9.1 billion people in the year 2050 [8]; this population is also becoming more affluent, and is thus increasing both the per capita and absolute demand for animal products [6; 9; 10; 11]. Hence global meat production is projected to increase from 229 billion kg in 2000 to 465 billion kg in 2050 to meet increased

demand (Table 2.1) [8]. This corresponds to livestock numbers increasing from 60 billion in 2010 to 120 billion in 2050 [12]. Similarly, Tilman*, et al.* [10] have calculated that the demand for crop protein is likely to increase by approximately 110 percent between 2005 and 2050, largely to feed livestock [13].

Table 2.1: Meat and dairy demand in 2000, and predicted demand in 2050 [11].

Description	Year 2000	Year 2050
Global Population	6 billion	9 billion
Average per capita annual global demand – meat (tonnes)	0.0374	0.052
Average per capita annual global demand – milk (tonnes)	0.0783	0.115
Total annual demand – meat (million tonnes)	228	459
Total annual demand – milk (million tonnes)	475	883

Unfortunately, limitations of environment and ecology will likely prevent expansion of the global food supply to such a great extent. These limitations are discussed later in this review. Meat production is one of the most resource-intensive and inefficient forms of food production, and consumes vast quantities of food that could otherwise be used for human consumption [14]. For example, 94 per cent of soya production is currently used to feed cattle, pigs and poultry, and almost 40 per cent of cereals are used for livestock production [12]. More specifically, *"cattle, pigs, and poultry now consume half the world's wheat (the principal staple), 80 per cent of the world's maize, virtually all of the barley that is not used for brewing and distilling, and well over 90 per cent of the world's soya [13]."* Hence it will be essential to increase the relative amount of plant protein in the global diet as the global population increases [14].

2.3 Barriers to Increasing Animal Protein Production

During the latter half of the 20th century, food production kept pace with population growth by expanding and intensifying agriculture [6; 14]. However, this strategy is not sustainable in the long term, as many barriers exist to continued intensification and expansion of agriculture in the 21st century. The barriers include limited land resources, limited water resources, and environmental damage caused by agricultural intensification and expansion.

2.3.1 Land Restrictions

Overall, 38 per cent of the terrestrial surface of the planet is presently used for agriculture, and 30 per cent of the terrestrial surface of the Earth is presently used for livestock production. 26 per cent of the ice-free terrestrial area is used for grazing, whilst 33 per cent of all arable land is used for feed crop production [5; 14]. Furthermore, most of the land suitable for agriculture is already in use, limiting the potential for further expansion [5]. Most recent expansions have come at the expense of deforestation in tropical areas, but did little to increase global food supplies as the cleared land was used to grow biofuel crops or animal feed [5].

As producing animal products is a relatively inefficient use of land, the relative amount of animal products in the global diet must decrease to minimise the amount of land required to expand the world's food supply. For example, 4 million km^2 is currently used to provide feed crops for intensive livestock farming. The feed from this land can produce 29 million tonnes of animal protein. In contrast, only 0.25 million km^2 of land would be required to produce the same amount of plant protein. Such a conversion would free up 3.75 million km^2 of land for additional plant protein production or ecological restoration [14].

2.3.2 Water Restrictions

Approximately 2800 km^3 of fresh water from lakes, rivers and ground water is used to irrigate croplands each year, and approximately 80 per cent of humanity's water use is to sustain irrigated agriculture [5; 15]. Approximately 24 per cent of croplands are irrigated and maintain approximately 34 per cent of all agricultural production. Without irrigation, global cereal production would fall by 20 per cent [5]. However, the demand for fresh water resources is increasing at a time when the amount of fresh water available is decreasing [15]. Half a billion people already live within water scarce or water stressed countries, and as much as two-thirds of the world's population could be water-stressed by 2025. Many of the water shortages will occur in the world's most densely populated regions (e.g. Mediterranean, Middle East, China, India and Pakistan) Furthermore, it is estimated that 20 per cent more water than what is currently available will be needed to feed the additional 3 billion people on the planet in 2025 [15]. Humanity will clearly

need to use less water in the 21st century and reducing the use of irrigation will be essential to reducing overall water use.

Reducing meat consumption is one way to achieve reductions in the use of irrigated agriculture. Approximately 85 per cent of humanity's water consumption is related to the production of agricultural products, and a large proportion of agricultural products are used to feed animals to produce meat [16]. Raising animals on feed crops is a particularly inefficient use of water resources, as a large amount of water must be used to produce the feed crop. Depending on the feed crop and irrigation intensity, producing 1 kg of grain fed beef can use between 5 and 1000 times as much water as producing 1 kg of wheat [14]. Table 2.2 demonstrates the difference in water footprint between animal and plant products, whilst Table 2.3 demonstrates the difference in water footprint between vegetarian diets, and diets which include meat. Hence the global trade in animal products leads to large net imports and exports of virtual water between countries. The total virtual water flows associated with the international trade in crops and crop products add up to 979 billion m^3 per year [16].

Table 2.2: The global average water footprint of animal products versus crops [17].

Animal Product	Water Footprint (L/kg)	Crop	Water Footprint (L/kg)
Bovine leather	16600	Rice	3400
Beef	15600	Groundnuts (in shell)	3100
Sheep meat	6100	Wheat	1399
Cheese	5000	Maize	900
Pork	4800	Apple or pear	700
Milk powder	4600	Orange	460
Goat meat	4000	Potato	250
Chicken	3900	Cabbage	200
Eggs	3300	Tomato	180
Milk	1000	Lettuce	130

Note that water footprint of a product does not just water directly used to produce a product – it also includes water polluted and evaporated during the production of a product [16]. The overall water footprint can be subdivided into three component footprints: the Blue Water footprint, which is the water evaporated from surface and groundwater (i.e. irrigation); the Green Water footprint, which is the water evaporated from soil (i.e. rainwater that would otherwise have remained in the soil);

and the Grey Water footprint, which is the amount of water required to dilute pollutants (e.g. fertilizers and pesticides) below the maximum tolerances set by water quality standards [16]. The water inputs required to produce an animal product are summarised in Figure 2.2

Table 2.3: The daily water footprints of omnivorous and vegetarian diets for industrialized and developing countries [17].

	Omnivorous Diet	**kcal**	**L/kcal**	**L**	**Vegetarian Diet**	**kcal**	**L/kcal**	**L**
Industrialized countries	Animal protein	950	2.5	2375	Animal protein	300	2.5	750
	Vegetable protein	2450	0.5	1225	Vegetable protein	3100	0.5	1550
	Total	**3499**		**3600**	**Total**	**3400**		**2300**
Developing countries	Animal protein	350	2.5	875	Animal protein	200	2.5	500
	Vegetable protein	2350	0.5	1175	Vegetable protein	2500	0.5	1250
	Total	**2700**		**2050**	**Total**	**2700**		**1750**

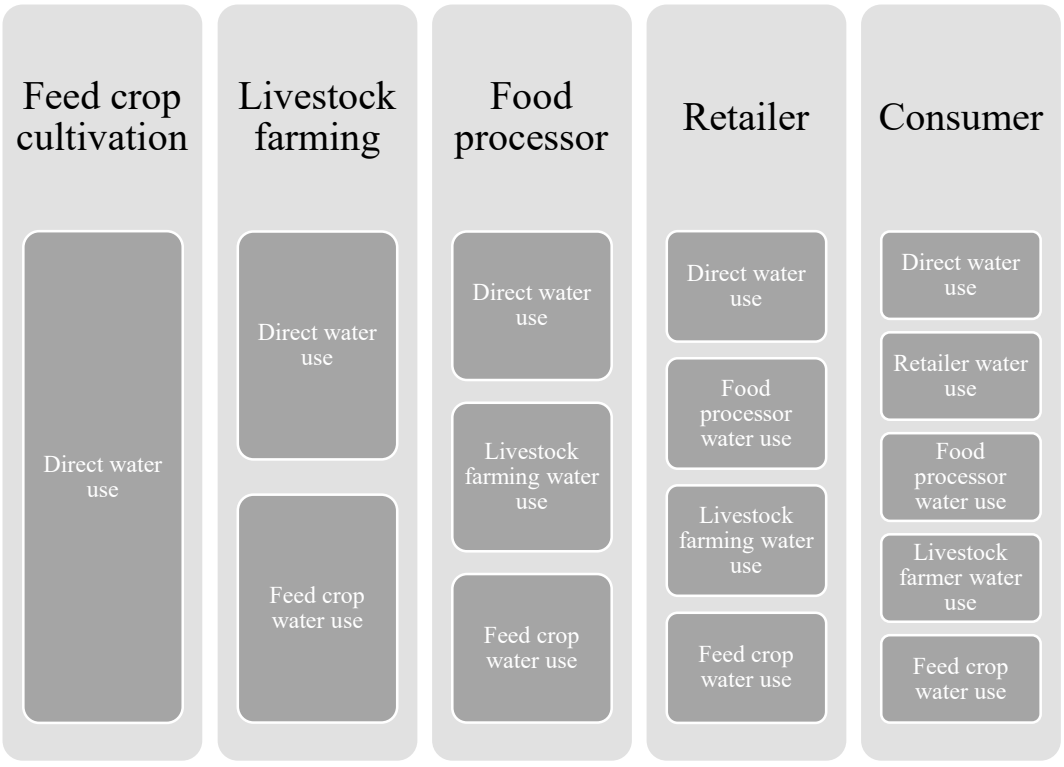

Figure 2.2: Total water footprint at each stage of animal product production [17].

Table 2.4: Virtual water imports and exports related to the animal product trade [17].

Country	Import	Export	Net Change
Argentina	811	4180	-3370
Australia	745	26400	-25600
Bangladesh	86	652	-566
Brazil	1910	11900	-10000
Canada	4950	17400	-12500
China	15200	5640	9610
Egypt	1470	221	1250
France	11800	13200	-1390
Germany	16100	17400	-1370
India	343	221	-3060
Indonesia	1670	371	1300
Italy	28300	14900	13400
Japan	20300	955	19400
Jordan	462	165	297
South Korea	6100	3930	2170
Mexico	13400	5760	7660
Netherlands	7850	15100	-7300
Pakistan	98	612	-514
Russia	12200	2500	9740
South Africa	1020	1310	-293
Spain	5970	8540	-2570
Thailand	1760	2860	-1100
United Kingdom	10200	3790	6380
United States of America	32900	35500	-2560

2.3.3 Environmental Concerns

The expansion and intensification of agriculture has already caused much environmental damage, and further expansion will continue to do so. For example, livestock ranching is directly responsible for 85 per cent of the topsoil loss in the USA, whilst 80 per cent of new croplands are currently obtained by clearing tropical rainforests, which causes substantial greenhouse gas emissions and a loss of ecosystem services and biodiversity [5; 8; 14]. On a worldwide basis, 70 per cent of grasslands, 50 per cent of the savannah, 45 per cent of the temperate deciduous forests, and 27 per cent of the tropical forests have already been cleared or converted for agricultural purposes [5]. Further expansion of agriculture will diminish the ability of the natural environment to provide ecosystem services, many

of which are essential for human life (e.g. soil regeneration, aquifer recharge, and water purification) [18].

Furthermore, widespread fertiliser and pesticide use has caused nutrient overload and eutrophication in many aquatic ecosystems. In some areas (e.g. northern Gulf of Mexico, Chesapeake Bay, and the Kattegat), dissolved oxygen levels have decreased to hypoxic levels, creating dead zones which cannot be inhabited by marine life and endanger fisheries [18; 19]. Nutrients deposited on land allow weedy species to flourish, causing a loss in biodiversity [18]. Increased used of nitrogen fertilisers have also led to increased emissions of nitrogen oxides, which can increase emissions of tropospheric ozone, a component of photochemical smog. It has also lead to increased emissions of nitrous oxide, which is a greenhouse gas [18].

In addition, agricultural systems are becoming less nutrient-efficient, and hence further additions of fertiliser will lead to diminishing returns with respect to yields (e.g. the nitrogen efficiency of cereal crops was approximately 85 per cent in 1960, but is approximately 25 per cent today), but will greatly exacerbate environmental problems [18]. Further expansion of agriculture will exacerbate these problems. Therefore any expansion of protein supplies should be plant-based rather than animal-based, as the former requires far less agricultural output than the latter [14]. Producing animal products also releases substantial greenhouse gas emissions. Livestock are estimated to cause 18 per cent of global greenhouse gas emissions worldwide [8].

The carbon footprints of various animal products are presented in Table 2.5. Fish are generally less carbon-intensive than terrestrial animals, but there is a large variation between species and harvesting methods [11]. Some forms of aquaculture are more sustainable than others. Carnivorous fish, such as salmon, trout, and tuna, as well as omnivores like shrimps, are popular, but often fed wild fish from stressed or depleted fisheries. On the other hand, aquatic plant (e.g. seaweed) and mollusc cultivation, and most forms of freshwater fish farming require few marine inputs, and are more sustainable [11].

Table 2.5: Greenhouse gas intensity of livestock types in the United Kingdom [11; 20].

Livestock Type	Tonnes CO_2-equivalent per Tonne of Product
Beef	16.0
Pig (pork and bacon)	6.4
Poultry	4.6
Sheep	17.0
Eggs	5.5
Milk and products	10.6

2.4 Meat Processing

A typical New Zealand meat processing facility processes the entire animal on one site [21]. Every NZ meat processing facility is therefore expected to produce paunch grass as a by-product. Further processing of most meat processing by-products is often co-sited with meat processing facilities [21]. The exception is paunch grass (PG), which is generally disposed of by composting. This is typically performed on-site at rural processing facilities, which generally have sufficient land available to operate a composting process. However, processors based in urban areas typically transfer their PG waste to an off-site composting facility for disposal [22]. Wallace Corporation, a sponsor of this research, operates a PG composting at Waitoa in the Waikato use of New Zealand. They are therefore interested in finding a higher-value application for PG.

2.4.1 Animal Products

Although meat is the principal product of a slaughtered animal, many other by-products are also created. A breakdown of the products from a slaughtered animal can be found in Figure 2.3.

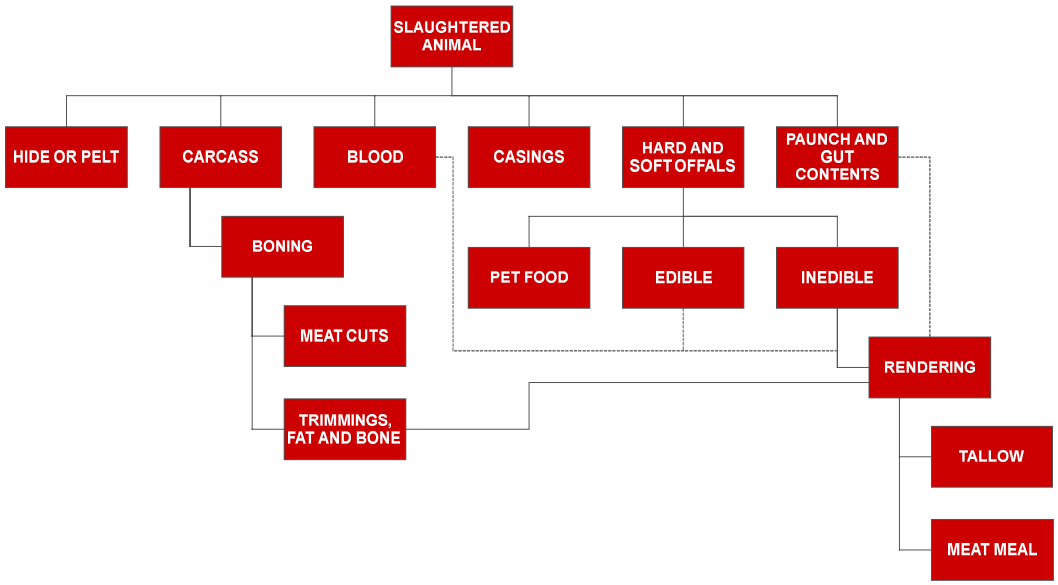

Figure 2.3: Flow diagram of material from a slaughtered animal. ***Source: Swan [23]***

Edible Products

The NZ meat industry has several three main meat products: lamb, mutton and beef. They can be exported either in bulk or in consumer packages. The industry also exports ovine and bovine variety meats (i.e. edible offals), such as tongues, sweetbreads, hearts, livers, brains, kidneys, bovine tripe and bovine tails. Variety meats are almost always exported in consumer packages. Other valuable by-products are lamb, sheep, and cattle intestines (i.e. casings), which are processed into sausage skins [21].

Inedible Products

There are many inedible by-products from slaughtered animals. These include hides, foetal blood, cattle-tail hair, glue pieces and inedible offal. Some of the inedible offal (e.g. lungs, trachea and spleens) can be used for pet food. Fine chemicals and pharmaceuticals can also be extracted from the endocrine and pancreas glands [21; 23]. Many of the inedible parts of the animal can be rendered into valuable blood meal, meat meal, bone meal and fats [23].

2.4.2 Abattoir Processes

The slaughtering of animals is a complex process. The abattoir processes used to handle cattle and sheep are summarised in Figure 2.4. They can be split into three main categories: holding and inspection; dressing; and edibles processing.

Holding and Inspection

The animals are initially transported from the farm or sales yard to the abattoir, where they are placed in holding areas. The livestock are then inspected to identify sick animals and animals unfit for human consumption. These animals are removed from the rest of the livestock and are slaughtered separately. The remaining animals are then weighed prior to stunning [24].

Dressing

- In New Zealand, nearly all animals are rendered unconscious by electrical stunning (i.e. passing an electrical current across the brain of the animal) prior to slaughter [25; 26]. The alternative method is to shoot the animal in the head with a captive bolt or free-bullet firearm [26; 27]. Captive bolt firearms may or may not penetrate the skull but must cause sufficient brain damage to render the animal unconscious. Free bullet firearms always penetrate the skull and cause catastrophic damage to brain tissue [27].
- The stunned animals are then shackled, hoisted by their hind quarters, and suspended from the ceiling. They are then exsanguinated (sticked) by severing the carotid artery and jugular vein. The blood is collected through a floor drain or in specialised containers (receivers) and is sent to a rendering facility for further processing [24].
- The carcasses are then electrically stimulated to induce rigor before chilling. This prevents cold shortening from occurring when the muscle temperature decreases to between 15°C and 16°C. It also controls pH to ensure that the meat is neither pale, soft and exudative (PSE) or dry, firm and dark (DFD) [24].
- The carcasses are then dressed (i.e. cleaned). Initially, the oesophagus is separated from the trachea. This can also be done after the opening of the chest cavity, to aid evisceration. A knot is then made in the oesophagus, or the oesophagus sealed with a band. This prevents the contents of the rumen from spilling and contaminating the carcass [24].

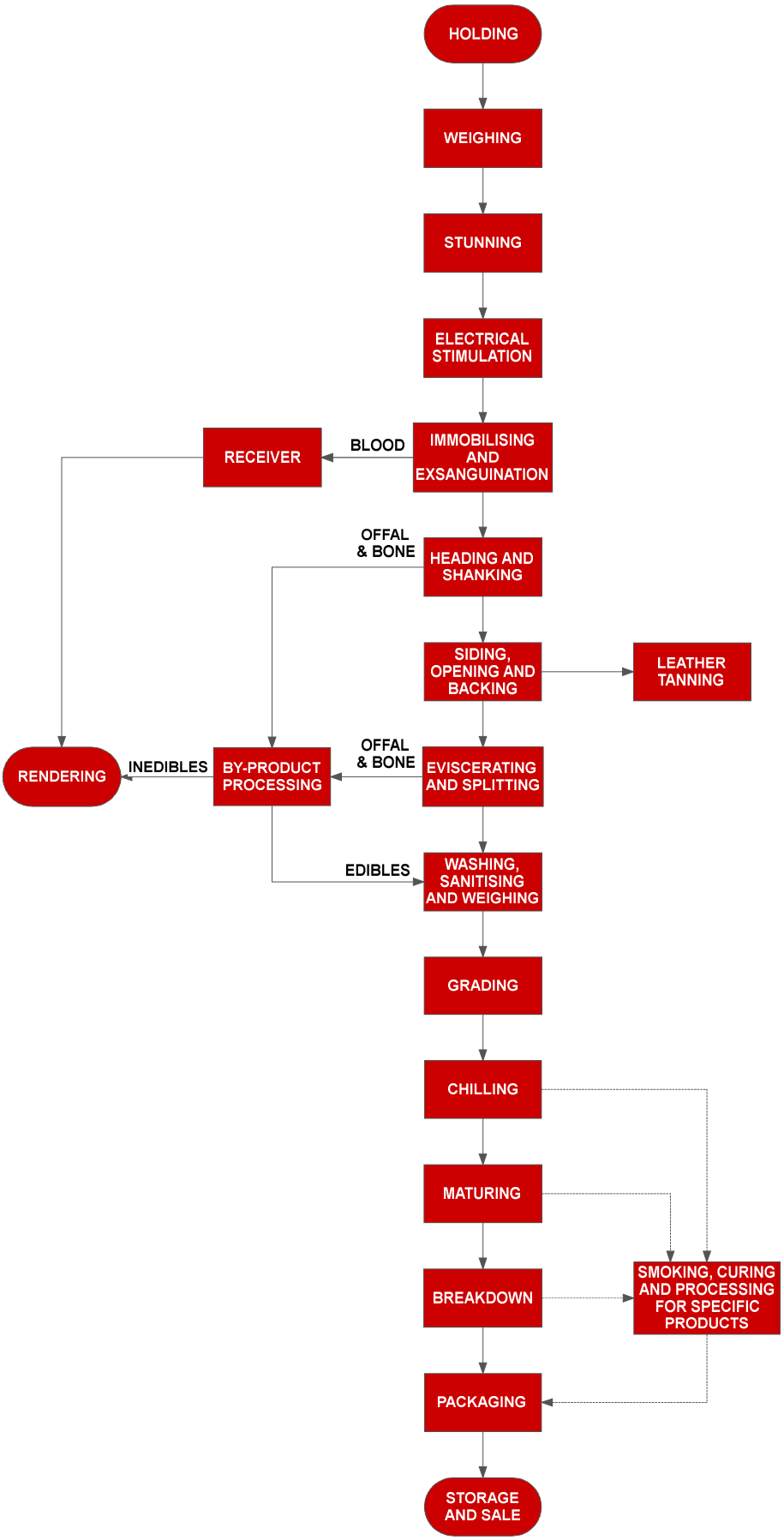

Figure 2.4: Abattoir processes.

Adapted from Environmental Protection Agency [24].

- The skin is then removed from the animal's head, and the animal is decapitated. This is achieved by cutting through the animal's Adam's apple and the Atlas joint. This process is known as heading [24; 26].
- The fore and hind feet are then removed, as this prevents the carcass from being contaminated by manure and soil dropped from the hooves (shanking). The legs are then skinned [24].
- The carcass is then cut along its ventral length to open the hide, which is then pulled away over the sides of the animal. This process is known as siding. The siding and skinning processes are often aided by electric or pneumatic rotary skinning knives [24]. The removal of cattle hides is often entirely automated (i.e. done by "hide-pulling" machines), whilst sheep pelts are normally removed by hand (i.e. "fisting"). Note that hides and pelts are usually salted to preserve them for leather tanning [26].
- The carcass is then eviscerated. This involves using a knife to slit the abdomen open from top to bottom, loosening the membranes and fat which hold the internal organs in place, and cutting the urethras which connect the bladder to the kidneys. The previously loosened oesophagus is pulled up through the diaphragm, which allows the abdominal organs to fall into an inspection cart. The liver is removed for inspection and the viscera (i.e. liver, stomach, bladder, intestines and reproductive organs), pluck (i.e. thoracic organs, including the heart and lungs), and kidneys are chilled then sent for by-product processing [24; 26]. This process separates the paunch grass from the viscera and transfers it to by-product processing.
- The carcass is then halved (split) by cutting through the exact centre of the backbone with a handsaw or electric saw. The inedible portions of the carcass are removed and sent to a rendering facility for further processing [24]. This concludes the dressing process.

Edibles Processing

- Post-dressing processing includes washing and sanitising the split carcass, followed by weighing, chilling and maturation. This is followed by breakdown into wholesale cuts and vacuum packaging prior to storage and sale [24].

- The washing process involves spraying the split carcass with either high pressure steam or hot water. This may be sufficient to sanitise the carcass, but typically, an additional chemical sanitising agent such as acetic acid or lactic acid is also used [24].
- Split carcasses are then chilled at 0°C for 24 hours to prevent spoilage and are usually kept in the cooler for at least one week to allow them to mature. In some cases, carcasses are separated from the main process flow to enable additional processing (e.g. smoking and curing) into specific products. The intact carcass halves (sides) can then be packaged and shipped to a butcher. Alternatively, the split carcass may be broken down into wholesale meat cuts, vacuum packed and sent to a butcher [24].

2.4.3 Rendering

Rendering can convert 30 per cent to 60 per cent of the weight of a slaughtered animal into valuable products. Rendering processes typically separate by-products, such as "fatty tissue, trimmings, bones, hooves and soft offal" into water, fat and protein components, which are commercially valuable. Rendering products include tallow, inedible fats, bone meal, and meat meal [23]. Dried blood (i.e. blood meal) is also produced using rendering equipment [23; 28]. Paunches (i.e. guts) are included in the raw material sent for rendering. However, they are typically emptied and washed at the meat processor before they are transferred to a rendering facility as the paunch grass inside them can reduce the quality of the rendered product [23]. The emptied paunches and other soft offal is then size-reduced and transported to the rendering plant [23]. A generic flow diagram of the rendering process is presented in Figure 2.5.

Rendering processes can subdivided into three main systems: wet rendering, dry rendering, and low temperature rendering. Although these systems have different operating principles, they all require steam utilities, decanting centrifuges, screw presses, and thermal driers [23]. Wallace Corporation, a sponsor of this research owns and operates a rendering process in Waitoa, New Zealand [29], which is co-sited with a paunch grass composting facility. There is thus scope to integrate an LPC manufacturing process with the rendering process already established on this site.

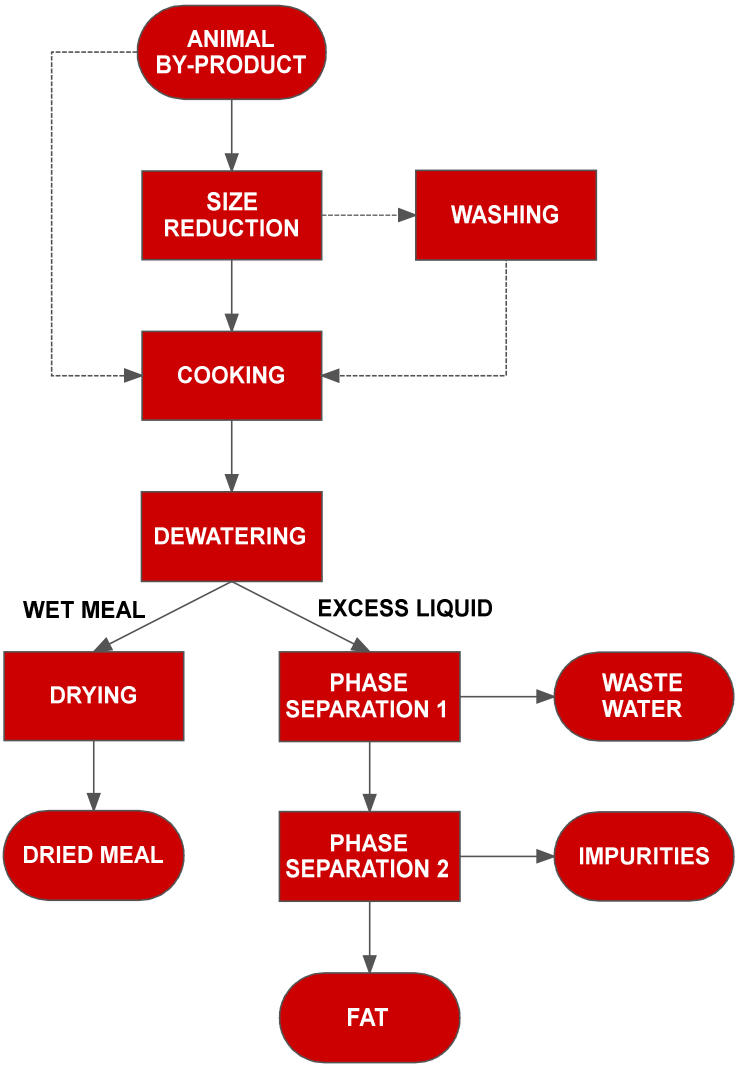

Figure 2.5: Generic rendering process.

2.5 Paunch Grass

Paunch grass (PG) (also referred to as paunch manure) is the partially digested feed removed the stomachs of slaughtered cattle and sheep during the final evisceration step of the dressing process (Figure 2.4) [22; 23; 30]. There is only limited information available about the chemical composition of PG: it is generally considered to be rich in crude fibre; rich in organic content; and moist (75-90 w/w%) despite mechanical dewatering before discharge from the meat processing facility [30; 31; 32]. The most comprehensive profile was published by Tritt and Schuchardt [31] and based on data collected in West Germany in the late 1980s and early 1990s (Table 2.6). Moisture content is controlled both by the type of feed eaten by the slaughtered animals and the amount of water added during processing [30; 32; 33], although the latter is largely removed by mechanical dewatering [31]. McPhail [32] hence indicated that PG from US slaughterhouses (where the animals

are fed feed crops) has a moisture content of approximately 85%, whereas Australian grass-fed paunch has a moisture content of approximately 92%. It is important to note variation in moisture content can be caused by the different ways in which samples are collected. Some studies use the paunch contents directly sampled from the animal, whereas the more common approach is to sample dewatered PG from the disposal skip. To the best of the author's knowledge, there have been no comprehensive chemical profiles of PG measured in New Zealand; nor have there been any studies of seasonal variation in PG chemical profiles measured anywhere in the world.

Table 2.6: Chemical profile of German paunch grass.

Paunch Grass Characteristic	Lower Limit	Upper Limit
Dry matter (w/w%)	25%	30%
Carbon to Nitrogen ratio	11	20
Organic content (DM%)	80%	90%
Minerals (DM%)	5.3%	10.3%
Fat (DM%)	2.5%	8.3%
Protein (DM%)	7.1%	11.1%
Crude fibre (DM%)	75.7%	82.5%

PG has long been a waste management issue for the meat processing industry due to its high moisture content, high ratio of suspended solids, high organic matter content, strong and objectionable odour, and being a media for growth of pathogens and vermin [30]. Hence sterility is an important consideration for products manufactured from PG Historically, there were several options for disposing of PG: dumping (land-spreading), landfilling, direct use as animal feed, and composting [22; 23; 30; 32]. By the early 1990s all but composting had become illegal in many developed countries; hence composting became the disposal method of choice [31; 32]. However, The high moisture content and low carbon to nitrogen ratio of PG makes it difficult to compost [32; 34]. There has thus been substantial research into optimising PG composting processes [30; 35; 36; 37]. More recent studies have investigated the potential to use PG as a fertiliser [38]; a fuel for process heat [39]; a resource for nitrogen and phosphorous recovery [40]; and as a feedstock for methane production [40]. To the best of the author's knowledge, no investigations have been made into PG as a protein resource.

The quantities of PG available in the Upper North Island of New Zealand have not been measured in the scientific literature. The best estimates therefore come from multiplying livestock slaughter data from Statistics New Zealand [41] by various estimates of PG per head of animal slaughtered (Table 2.7). This indicates there is approximately 31,000 to 80,000 tonnes per annum of PG generated in the Upper North Island of New Zealand. This PG is generated by just 11 meat processing facilities distributed throughout the Upper North Island [42]. The roundtrip driving distances between these facilities and Wallace Corporation's Waitoa rendering and PG composting facilities are presented in Table 2.8.

Table 2.7: Estimates of annual quantities paunch grass available in the Upper North Island of New Zealand.

Cattle paunch mass data source	*Tritt and Schuchardt [31]*	*Tritt and Kang [36]*	*Wilson [30]*	*Pagan et al. [43]*
Cattle slaughtered (head)	970322	970322	970322	970322
Sheep slaughtered (head)	3956984	3956984	3956984	3956984
Paunch grass per head of cattle (kg)	62	60	24.9	11.28
Paunch grass per head of sheep (kg)	5	5	5	5
Cattle PG (MT)	60160	58219	24203	10945
Sheep PG (MT)	19785	19785	19785	19785
Total PG (MT)	**79945**	**78004**	**43988**	**30730**

Livestock slaughter data from Statistics New Zealand [41], average of years 2014-2019 | Sheep paunch mass data from Filstrup [44]

Table 2.8: Roundtrip driving distances between Waitoa LPC Manufacturing Plant and Meat Processing Facilities [42; 45].

Meat Processing Facility	**Roundtrip Driving Distance from Waitoa Manufacturing Plant (km)**
Greenlea Morrinsville	26
Silver Fern Farms Te Aroha	28
Greenlea Hamilton	94
AFFCO Horotiu	102
Universal Beef Packers	226
AFFCO Rangiuru	226
Te Kuiti Meat Processors	228
Auckland Meat Processors	252
Crusader Meats	296
Silver Fern Farms Dargaville	620
AFFCO Moerewa	704

2.6 Alternative Protein Sources

Humanity gets approximately 70 per cent of its protein from plant sources, and the remaining 30 per cent from animal products [3]. If the world is to feed the 9.1 billion people living on it by 2050, then a greater emphasis will need to be placed on obtaining protein from plant sources. Many protein sources are currently underutilised, and this lead to an extensive investigation of alternative protein sources as part of the International Biological Programme (IBP), which ran between 1964 and 1974 and identified many potential sources of protein [46]. Sources which were edible after minimal processing included seeds, vegetables and algae. Other protein sources included concentrates made by mechanical extraction, and concentrates made by biological conversion. The former category included concentrates from coconuts, soybeans, rapeseeds, sunflowers, safflowers, sesames, castors, groundnuts, broad beans, cereals, and leaves. The latter category included terrestrial animals, terrestrial animal by-products, fish, fungi and yeasts. Leaf protein and fish are of particular interest.

2.6.1 Leaf Proteins

Leaf proteins can be broadly defined as protein extracted from plant material which contains leaves. At present, humans consume leaf proteins indirectly by eating animal products from ruminants which eat the leaves. However, this only allows between 10 per cent and 30 per cent of the protein in forage to be recovered for consumption Pirie [47]. According to Pirie [47], direct extraction of protein from forage allows for between 40 per cent and 60 per cent recovery of protein. Improved extraction methods could allow for up to 80 per cent recovery of protein [48].

Sources

Many different crops have been used for leaf protein extraction. Crops grown solely for leaf protein extraction are typically grasses and legumes, such as ryegrass, alfalfa (lucerne), white clover, and red clover [49; 50; 51]. Protein concentrates have also been made from the green leaves of food crops, such as carrots, cucumbers, mangolds, tomatoes, peas, potatoes, kumara, bitter leaf, green tete, fluted pumpkins, winter wheat, mustard beans, jute and sugar beet [47; 52; 53]. Leaf proteins can also be obtained from aquatic weeds, fodder radish, and dandelions [47; 54; 55; 56]. More recently, investigations have been made into producing leaf

protein as a by-product of biofuel production [57; 58]. The chemical compositions of ryegrass and alfalfa are thus summarized in Table 2.9 to Table 2.12; the protein recoveries from selected sources are summarized in Table 2.13.

Table 2.9: Seasonal variation in the chemical composition of ryegrass (wet basis).

Mass Fraction	Summer	Autumn	Winter	Spring	Drought
Water	0.800	0.780	0.770	0.800	0.560
Protein	0.029	0.033	0.041	0.033	0.066
Lipids	0.009	0.010	0.013	0.010	0.020
Sugars	0.031	0.036	0.030	0.026	0.069
Fibre	0.115	0.131	0.110	0.096	0.251
Minerals	0.002	0.002	0.003	0.002	0.005
Other	0.014	0.008	0.033	0.032	0.030

Source: Stockdale et al. [59]

Table 2.10: Seasonal variation in the chemical composition of ryegrass (dry basis).

Mass Fraction	Summer	Autumn	Winter	Spring	Drought
Protein	0.147	0.152	0.180	0.167	0.150
Lipids	0.045	0.047	0.055	0.051	0.046
Sugars	0.157	0.162	0.130	0.131	0.156
Fibre	0.573	0.593	0.477	0.480	0.570
Minerals	0.010	0.010	0.012	0.012	0.010
Other	0.068	0.036	0.145	0.159	0.068

Source: Stockdale, et al. [59]

Table 2.11: Seasonal variation in the chemical composition of alfalfa.

Mass Fraction	Summer	Autumn	Winter	Spring	Drought
Water	0.801	0.782	0.770	0.797	0.572
Protein	0.038	0.039	0.041	0.036	0.072
Lipids	0.012	0.012	0.013	0.011	0.022
Sugars	0.049	0.056	0.058	0.057	0.083
Fibre	0.079	0.085	0.073	0.074	0.184
Minerals	0.016	0.017	0.018	0.015	0.031
Other	0.004	0.009	0.029	0.010	0.037

Source: Homolka et al. [60]

Table 2.12: Seasonal variation in the chemical composition of alfalfa (dry basis).

Mass Fraction	Summer	Autumn	Winter	Spring	Drought
Protein	0.191	0.179	0.177	0.177	0.168
Lipids	0.059	0.055	0.055	0.054	0.052
Sugars	0.246	0.258	0.251	0.280	0.193
Fibre	0.399	0.389	0.315	0.362	0.430
Minerals	0.083	0.077	0.077	0.076	0.072
Other	0.022	0.041	0.125	0.051	0.086

Source: Homolka, et al. [60]

Table 2.13: Recovery of leaf protein concentrates from various sources.

Protein Source	Crude Protein in Concentrate (% DM)	Protein Extracted (t/ha)	Reference
Alfalfa (high-yield)	50	3.15	McDonald [48]
Alfalfa (PRO-XAN®)	50	1.575	
Ryegrass	50-54	-	Sinclair [61]
Ryegrass and white clover	45-47	-	
Red clover	47.4	-	
White clover	56.2	-	
Carrot tops	22.5	0.045	Carlsson and Hanczakowski [52]
Cucumber leaves	48.2	0.600	
Mangold leaves	31.1	0.178	
Pea vines	44.3	0.083	
Potato haulm	50.0	0.600	
Tomato leaves	32.7	0.600	
Bitter leaf	52.2	-	Aletor, *et al.* [53]
Green tete	35.1	-	
Fluted pumpkins	54.9	-	
Solanum africanus	46.1	-	
Aquatic weed *(Elocharis dulcis)*	56.6	2.16	Pandey and Srivastava [55]
Semi-aquatic weed *(Monochoria hastata)*	71.93	1.700	Pandey and Srivastava [54]

Extraction Technology

The most common leaf protein extraction technology is the PRO-XAN® process [48; 62]. This process well-established, but has become uneconomic to operate, due to its relatively low yield and high energy costs. The sole remaining plant is located in France, and processes alfalfa [48]. A high-yield process was developed during the 1980s, but was never commercialised [48; 63]. The PRO-XAN® process has two products: leaf protein concentrate (LPC), and dehydrated fibre. The high-extraction process produces LPC, wet fibre and deproteinated liquor (DPL). The former process is summarised in Figure 2.6, whilst the latter process is summarised in Figure 2.7.

The green leaves are harvested from the field and transported to the processing plant. The leaves are then pulped using a hammer mill. This ruptures the cytoplasm of the cell and liberates the protein [49; 63]. In the PRO-XAN® process, the pulp is then passed through a press. This causes the liquor to be expressed and taken to a holding

tank. The press also ejects a wet fibre stream, which is combined with concentrated DPL and dried [48]. In the high-yield process, the pulp is passed through a screw press, causing liquor to be expressed and fibre ejected. The fibre is washed with recycled process liquor (DPL) and re-milled in a disc mill before it is subjected to a second pressing. The fibre from the second press is re-washed with DPL, and pressed a third and final time to produce a wet fibre stream [63].

Liquor from the press or presses accumulates in a central holding tank. The pH of the accumulated liquor is adjusted to between 8.0 and 8.5 with ammonia or sodium hydroxide, and the protein is coagulated by direct steam injection, which typically heats the liquor to 85°C [49; 62]. Note that it is possible to coagulate the green 'chloroplastic' and white 'cytoplasmic' proteins separately. This is achieved by heating to between 50°C and 60°C, centrifuging to remove the coagulated 'chloroplastic' protein, and then heating to 70°C [47; 49]. Rapidly heating the liquor to 100°C ensures an almost sterile product, and prevents undesirable enzymatic degradation of the LPC [47].

The coagulated protein slurry is then separated using a decanting centrifuge. The solid product is a wet protein curd, which is then dried to produce LPC. The wet product is DPL. PRO-XAN® DPL is evaporated to approximately 50 per cent solids and combined with the fibre stream prior to drying. High-yield process DPL has usually been discharged to waste or sprayed on the land as fertiliser, but has numerous other applications [61]. A normalised mass balance comparing the PRO-XAN® and high-yield processes can be found in Table 2.14.

Table 2.14: Normalised mass balance comparison between PRO-XAN® and high-yield processes.

Streams	Wet Tonnes per Hour		Dry Tonnes per Hour	
	Low Yield	High Yield	Low Yield	High Yield
Lucerne feed (IN)	100	100	20	20
Fibre outstream (OUT)	50	30	13.35	11.1
Leaf protein concentrate (OUT)	3.5	5.4	3.15	5.2
Molasses product if DPL evaporated (OUT)	7	7.5	3.5	3.8
Protein extraction %	-	-	49	71
Fibre protein content % of DM	-	-	19	13
Fibre DM%	-	-	27	37

Source: Sinclair [61]

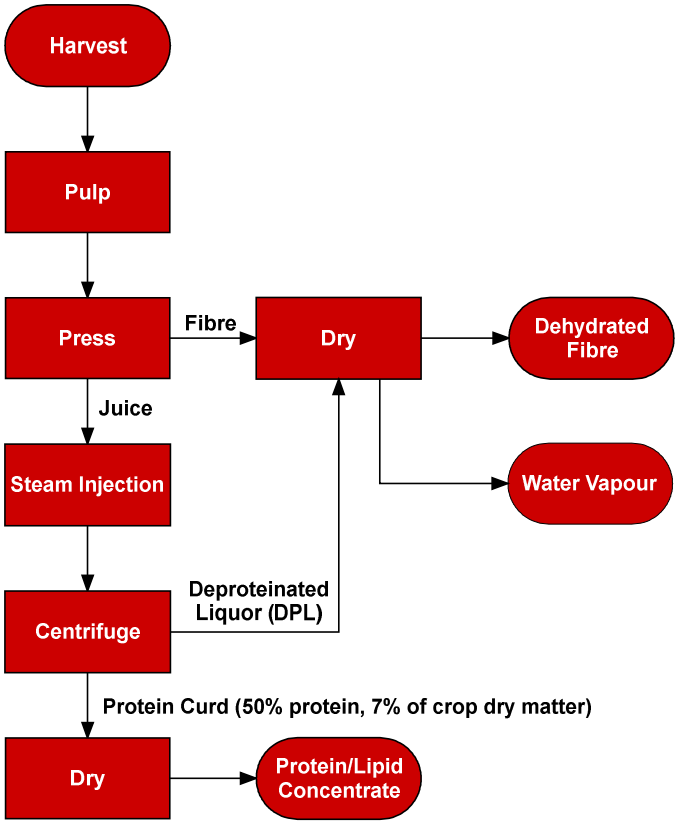

Figure 2.6: PRO-XAN® leaf protein extraction process.

Source McDonald [48].

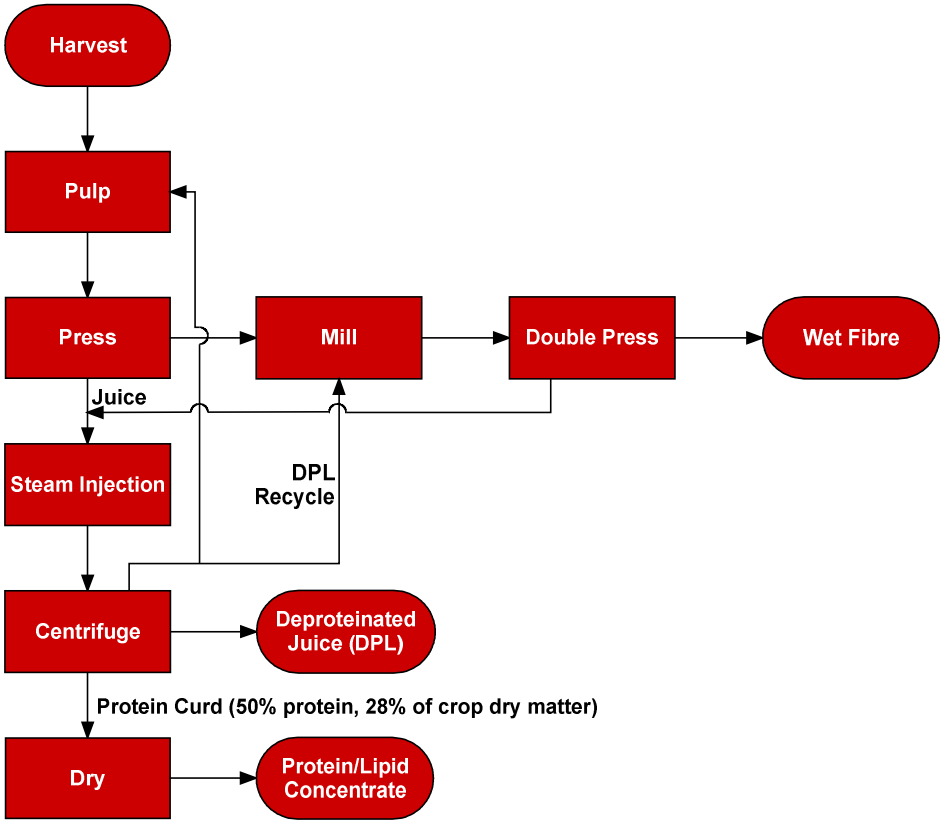

Figure 2.7: High-yield extraction process.

Source: McDonald [48]

Leaf Protein Concentrate

The leaf protein concentrate (LPC) is the main product of the extraction process and is usually dried. It was a secondary product of the PRO-XAN® process, but the second-generation process was optimised to produce LPC as it much more valuable than fibre [48]. The dried curd typically contains more than 90 per cent dry matter by weight, and 45 per cent to 55 per cent crude protein (as a percentage of dry matter). Lipid content usually varies between 15 per cent and 25 per cent of dry matter, whilst ash and fibre content are typically less than 10 per cent and 1 per cent of dry matter, respectively (Table 2.15) [61]. PRO-XAN® technology can typically extract up to 50 per cent of the protein in the forage, whilst high-extraction technology can recover up to 85 per cent of the protein in the forage, with a typical recovery of 70 per cent (Table 2.14). The typical yield of LPC varies between 22 per cent and 30 per cent of the crop dry matter [61].

Table 2.15: Chemical composition of alfalfa and ryegrass LPCs.

Leaf Protein Concentrate Source	Alfalfa	Ryegrass
Dry matter (wt%)	90.0	94.8
Crude protein (%DM)	48-52	50.5
Total lipids (%DM)	20-22	17.1
Crude fibre (%DM)	1.0	0.9
Crude ash (%DM)	10.0	8.1

Source: Sinclair [61]

Alfalfa protein concentrate has a similar amino acid profile to fish meal, and hence it has been trialled as a fishmeal substitute [61; 64]. It has also been used as an animal feed ingredient, principally for monogastric animals (pigs and poultry), but also for cattle and pet food [61; 65]. The full amino acid profile for New Zealand alfalfa LPC can be found in Table 2.16.

Table 2.16: Amino acid profile of New Zealand alfalfa leaf protein concentrate.

Amino Acid	Grams of Amino Acid / 18 grams of Nitrogen
Alanine	5.4
Arginine	6.1
Aspartic acid	9.2
Cystine	1.0
Glutamic acid	9.7
Glycine	4.8
Histidine	2.6
Isoleucine	4.6
Leucine	8.4
Lysine	6.1
Methionine	2.0
Phenylalanine	5.8
Proline	3.8
Serine	4.9
Threonine	5.0
Tryptophan	2.5
Tyrosine	4.7
Valine	5.4

Source: Sinclair [61]

Fibre

PRO-XAN® fibre typically has a crude protein content of between 15 per cent and 25 per cent of dry matter, and a dry matter content of approximately 25 per cent. Dehydrated fibre is the main product of the PRO-XAN® process, but is less valuable than LPC. It must also have a relatively high crude protein content to comply with animal feed specifications [48]. High extraction fibre has a crude protein content of approximately 7 per cent, but this fibre cannot be used for animal feed [61]. Adding value to the fibre stream is essential for making a leaf protein extraction process economic. Suggested uses include energy recovery, or alternatively hydrolysis and fermentation to long-chain omega-3 fatty acids [48]. The fibre could also be composted, however key composting parameters such as the carbon to nitrogen ratio, and presence of heavy metals and trace elements have not yet been published in the scientific literature.

Deproteinated Liquor

Deproteinated Liquor (DPL) is the supernatant remaining after the protein has been removed from the liquor expressed from the presses. The DPL contains between 50 per cent and 70 per cent of the original liquor dry matter. The DPL itself typically has a dry matter content of less than 5 per cent. The dry matter in the DPL typically contains 2 per cent to 4 per cent nitrogen, 30 per cent to 35 per cent sugars, and 15 per cent to 30 per cent crude ash [61].

The DPL has a high biochemical oxygen demand (typically 15,000 mg O_2/L to 20,000 mg O_2/L), and this makes disposing of the DPL difficult. It was originally applied to the land, but this approach can be infeasible due to environmental concerns [61]. Alternatively, it can be concentrated by evaporation to approximately 50 per cent solids. It is then sold as a molasses-like concentrate or incorporated to the fibre stream before dehydration [61]. However, these approaches are very energy-intensive, and often results in poor process economics [48]. McDonald [48] has suggested that the DPL could be used as a fermentation feedstock to produce long-chain omega-3 fatty acids, but there have been no investigations into the fermentation potential of the DPL.

Recent Research

Most leaf proteins research activities wound up in the late 1980s and early 1990s, but since 2005 there has been a renewed interest in using leaf protein extraction for human nutrition. The 2000s saw food technologists investigate enzymatic solubilisation, gelation, and hydrolysis of alfalfa leaf proteins to improve their functional properties and produce functional peptides [66; 67; 68; 69; 70]. There have also been ongoing efforts since 2003 to improve the fractionation and recovery of the white and green leaf protein fractions using chromatographic, membrane, and solvent extraction processes [71; 72; 73; 74; 75; 76; 77; 78].

There has also been renewed interest in producing leaf proteins for animal feed from crop by-product leaves as well as dedicated leaf crops. To this end, Tangka [79] investigated using the mature industrial first-generation mechanical extraction process developed by Bickoff*, et al.* [62] on crop residues. Urribarrí *et al.* [80] investigated ammonia solvent extraction of elephant grass proteins.

More extensive investigations at the University of Michigan explored producing leaf protein concentrate for animal feed from alfalfa or switchgrass, then using the fibre co-product as a substrate for biofuel and bio-based chemical production [57; 81; 82]. In addition to the established industrial mechanical extraction process, the University of Michigan study also investigated using ammonia solvent extraction and ultrafiltration for leaf protein extraction and recovery [57; 81; 82]; parallel research in France investigated using a twin-screw extrusion equipment to improve protein extraction into the liquid draining from the extruder [83; 84]. This renewed interest coincided with renewed advocacy for high-yield extraction and recovery of leaf protein for human and animal nutrition by McDonald [48].

2.6.2 Green Waste

One potential untapped source of leaf protein is green waste, defined as the soft leafy component of the plant waste generated during domestic and municipal garden maintenance. As per the definition of Boldrin and Christensen [85], this includes flowers, grass clippings, hedge cuttings, and soil; but excludes branches and other woody material. To the best of the author's knowledge, data on the chemical composition of GW is limited to five studies that sought to determine its value as a compost substrate [85; 86; 87; 88; 89]. These studies thus focused on the elemental compositions rather than the macromolecular (or biochemical) composition, and results are therefore limited to measurements of moisture, organic carbon, nitrogen, and various metallic elements. At the time of writing, no studies have published the chemical composition of GW at a macromolecular level (e.g. protein, lipids, total carbohydrates, and crude fibre).

There are presently no published studies or centralised records about the volumes of GW generated annually in New Zealand. There is currently no centralised record of GW collections across New Zealand. Although some individual providers have publicly reported organic material tonnages collected for composting [90; 91; 92], they usually do not distinguish between food waste and green waste. The best estimate of GW generated in New Zealand thus comes from refuse transfer station data published by Purchas and Reeve [93] and Ministry for the Environment [94]. This data indicates that GW comprised 6.02% of the 2.461 million metric tonnes of

waste sent to landfills in New Zealand in 2007 [95]. 148,100 metric tonnes of GW were therefore generated nationwide in 2007.

As of 2006, approximately 70% of territorial authorities (i.e. city and district councils) provided green waste composting facilities [96]; as of 2017, there are 39 composting facilities in New Zealand; virtually all of them accept GW [97]. New Zealand composting facilities are predominantly windrow-based facilities – a 2017 study found that 67% of facilities suitable for composting biodegradable packaging used the windrow composting method; the next most prevalent methods were in-vessel composting (15%) and vermicomposting (7%) [97]. Windrow composting entails stacking the organic material in large outdoor piles which are then broken down by microorganisms [98; 99]; in-vessel processes do this using microbes inside a closed reactor [99]; whilst vermicomposting processes do this using earthworms [99]. The piles in windrow systems are usually turned periodically to sufficient aeration for the microbes [34], whilst in-vessel systems are designed to keep the organic matter sufficiently aerated [100]. An overview of composting methods is provided by Schaub and Leonard [99]; detailed discussions can be found in EPA [100] and Diaz *et al.* [101].

GW should be composted in an efficient aerobic, composting process with a carbon to nitrogen ratio (C:N) of the feedstock is between 25:1 and 35:1 [100]. The feedstock material must also permit sufficient airflow through the compost pile to maintain aerobic composting conditions. When these conditions are not met, the process will become anaerobic and break down the feedstock more slowly than an aerobic process. Anaerobic processes also emit nitrous oxides and ammonia which cause odour problems, and methane which is a greenhouse gas [100]. Detailed discussions of composting microbiology and process parameters can be found in de Bertoldi *et al.* [102], Diaz and Savage [34], and Insam and de Bertoldi [98].

2.6.3 Aquaculture

Approximately 3 billion people around the world get at least 15 per cent of their animal protein supply from fish. 80 million tonnes of fish was harvested from marine fisheries in 2008, and as of 2009 the marine fisheries provided direct employment to 34 million people [103]. Pirie [46] identified fish as one of the foods

that could be used to increase global protein supplies. However, there is very limited potential to increase the catch of wild fish. As of 2009, 57.4 per cent of global fish stocks were estimated to be fully exploited and 29.9 percent were estimated to be over-exploited. Only 12.7 per cent were estimated to be non-fully exploited, but these usually do not have a high production potential [103].

At first glance, aquaculture appears to be one method of increasing fish supplies without placing any further pressure on fish stocks. Unfortunately, popular farmed carnivorous fish (e.g. salmon, trout, tuna, sea bream, and yellowtail) and farmed shrimp are presently net fish consumers, due to the inclusion of fish oil and fish meal in their diets to meet protein and essential amino acid requirements [11; 14; 104]. As of 2006, carnivorous and omnivorous fish already accounted for 45 per cent and 21 per cent of aquaculture fish meal use respectively, and salmon feed manufacturing was responsible for 45 per cent of all aquaculture fish oil use [104]. Global fish oil and fish meal supplies are unlikely to increase, making the sustainable expansion of the aquaculture industry impossible. As of 2008 already used 65 per cent of the global fish meal supply, an order of magnitude increase over the 10 per cent it used in the early 1980s. Similarly, the aquaculture feed industry uses 85 per cent of the global fish oil supply, a drastic increase from 15 per cent it used in the early 1980s. This increase occurred due to the enormous growth of the aquaculture industry, and in spite of a 25 per cent to 50 per cent reduction in the use of fish meal in aquaculture diets over the same period [104]. Aquaculture feed production was therefore a cornerstone of the renewed advocacy by McDonald [48] to expand and improve the manufacture of leaf protein concentrates from alfalfa and other fresh leaf crops.

Fish Meal Substitutes

Substantial efforts have been made to reduce the amount of fish meal in aquaculture diets, and it is relatively easy to substitute up to half of the fish meal in an aquaculture diet with plant proteins [104]. So far, the substitution of fish meal with plant protein has been driven by economics. Historically, fish meal was by far the cheapest protein source for aquaculture feeds, and cost between USD 400 and USD 900 per metric tonne. However, in 2006 the price increased to over USD 1500 per metric tonne, and has remained above USD 1100 per metric since then [104]. In

There are sustainability concerns about the amount of fish meal used for aquaculture diets. Hence there are both economic and sustainability concerns driving the minimisation of fish meal inclusion in aquaculture feed products.

Most of the plant protein used in aquaculture feed comes from either corn or soybean sources, although distiller's dried grains are also being used for some omnivorous species [104]. However these too have rapidly increased in price. Between 2007 and 2008, the price of corn gluten meal jumped from USD 257 per metric tonne to USD 575 per metric tonne. Soybean meal experienced a similar jump from USD 179 per metric tonne to USD 335 per metric tonne. This was due to an increased demand for raw materials to produce biofuel and terrestrial animal feed [104]. Indicative prices of common protein meals are presented in Table 2.17.

Note that neither corn gluten meal nor soy meal are particularly good substitutes for fish meal. Corn gluten meal is easily digested by fish, but is deficient in lysine. It also contains a relatively high amount of insoluble carbohydrates, which are poorly digested by fish. Soy meal contains relatively little protein (48 per cent crude protein), and cannot comprise more than 30 per cent of a feed formulation of salmonids and some other fish – at higher concentrations it causes intestinal enteritis. Soy meal protein concentrate (75 per cent crude protein) and wheat gluten meal (75 per cent to 80 per cent crude protein) do not have these limitations and can be used to replace up to 75 per cent of the fish meal in salmonid aquaculture feed formulations. However, they cost at least USD 1100 per tonne, which makes them less economically competitive [105].

Table 2.17: Indicative prices of protein meals

Protein Source	Price (USD per metric tonne)	Source
Corn gluten meal	652	[106]
Soy meal	638	[106]
Fish meal	1991	[107]
Fish oil	2150	[107]

Leaf proteins have a similar amino acid profile to fishmeal, which makes them an ideal fishmeal substitute [61]. However, alfalfa leaf protein concentrate sells for a similar price to fishmeal, which makes it too expensive to use in many aquaculture feed formulations [48]. Rendered products, such as blood meal, feather meal, and poultry meal are also used as fish meal substitutes [108].

There are several additional barriers to replacing fish meal with plant protein. Much remains unknown about the nutritional limitations of plant proteins in aquaculture feed. Furthermore, many sources of plant protein contain anti-nutritional compounds which must be removed before they are used in aquaculture diets. Many plant-based fishmeal substitutes also contain compounds that are poorly digested by fish, such as non-soluble carbohydrates and fibre. These are excreted and can contribute to the pollution of aquatic environments. Aquaculture nutrition strategies will need to be developed to overcome and control these problems [104].

Fish Oil Substitutes

Substituting fish oil is more difficult, as there are no plant sources of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), although some animals can synthesise them from α-linolenic acid [109]. These polyunsaturated fatty acids (PUFA) are essential components of aquaculture feed and are easily obtained from fish oil, which is 20 per cent to 25 per cent EPA (by mass) and 8 per cent to 20 per cent DHA (by mass) [109; 110]. Fish cannot synthesise EPA and DHA themselves – they obtain it by eating marine algae, which can produce de novo EPA and DHA [15].

Numerous attempts have been made to produce EPA and DHA by commercial fermentation. The algal fermentation methods of Cellana [111] (culturing *Haematococcus pluvialis* to produce EPA and DHA) and Photonz Corporation [112] (culturing *Nitzschia laevis* to produce EPA and DHA) have been patented and commercialised [113; 114; 115]. Cellana have also conducted aquaculture feed trials on their product [116]. DuPont has used genetically modified yeast (*Yarrowia lipolytica*) to produce EPA [117].

Recent research attempts have used *Schizochytrium limacinum* to produce DHA from glycerol [118; 119]. Earlier attempts used: *Crypthecodinium cohnii* to produce DHA from acetic acid, ethanol and glucose; *Thraustochytrium* strain G13 to produce DHA from glucose; and *Schizochytrium* sp./ *Schizochytrium* sp. SP21 to produce DHA from glucose [120].

2.7 Lifecycle Assessment

Lifecycle Assessment (LCA) is based on the ISO 14040 family of standards and seeks to determine the impact of an activity, process, or product on the environment throughout its life [121; 122; 123; 124; 125]. The first step in an LCA is to set the goal and scope as per ISO 141040 4 [121; 122; 125]. This is followed by an inventory analysis, which seeks to identify and quantify the material and energy flows required for the activity, product, or process [121; 122; 125]; then by Lifecycle Impact Assessment (LCIA), which seeks to quantify the environmental impacts of those material and energy flows and allocate them to the different inputs and/or outputs of the system under study [126]. There are several impact categories which can be selected for a study: abiotic resource use; acidification; climate change; ecotoxicity; eutrophication; human toxicity; land use; particulate matter formation; photochemical ozone formation; stratospheric ozone depletion; and water use [126]. These impacts are then normalised and weighted to enable comparison and aggregation across impact categories based on value choices [126]. A more detailed overview of LCIA can be found in Hauschild and Huijbregts [126]. Water Footprint Assessment as per Hoekstra *et al.* [127] complements the traditional ISO 14040 Lifecycle Assessment as it gives more detailed information about impacts on water resources in an easy to understand and comparable format.

A small number of LCA and Water Footprint Assessments have been performed on protein sources in the New Zealand context – all have focused on milk or milk products [128; 129; 130; 131; 132]. However, there have been numerous international studies performed on food protein sources, animal feed production, alternative proteins, and composting, including:

- Impacts of red meat produced in Australia and the USA [133; 134; 135; 136];
- Impacts of dairy production [137; 138; 139; 140; 141; 142; 143];
- Impacts of animal feed production and ingredients in the USA and Europe [144; 145; 146; 147];
- Impacts of manufacturing plant-based meat substitutes [148];
- Impacts of composting in Australia, Denmark, South Korea, and the USA [149; 150; 151; 152; 153; 154; 155; 156; 157; 158; 159; 160]; and
- Water Footprint Assessment of meat, non-meat animal products, crops, and meat analogs [161; 162; 163; 164; 165].

Despite commercial production of alfalfa leaf protein concentrate started in the 1960s, no Lifecycle Impact or Water Footprint Assessments could be found for leaf protein concentrates produced by the processes described by Bickoff*, et al.* [62], Donnelly*, et al.* [63], and Bals and Dale [57]. There is thus a gap in the literature for the lifecycle impacts of leaf proteins extracted by mechanical attrition then recovered by steam coagulation. Once these impacts are known, they can be compared to those of other animal feed proteins; or the impact of alternative PG and GW disposal methods such as composting.

2.8 Conclusion

The global protein supply will need approximately double between 2000 and 2050. This need cannot be met by expanding the current protein production model due to constraints on water resources and arable croplands available to grow animal feed crops, and a need to contain or reverse the environmental damage caused by current agricultural practices. There is hence a need to find alternative sources of protein for animal feed as well as human nutrition. This principally entails manufacturing protein concentrates from plants, extracting protein from industrially grown microbes, and better utilisation of animal by-products.

Leaf protein concentrates for animal and human nutrition have been manufactured from alfalfa since the 1960s. However, the technology has seen very limited use with other leaf crops and has not been used on waste materials, improved process technologies have never been implemented at an industrial scale, and the environmental impacts have never been assessed. The soft leafy green component of domestic and municipal garden wastes is thus an untapped source of leaf protein for animal feed; similarly, the paunch grass recovered from the stomachs of animals slaughtered during meat processing is another potential source of animal feed protein. The latter is a particularly challenging waste management issue for the meat processing industry due to its high moisture content, high ratio of suspended solids, high organic matter content, strong and objectionable odour, and being a media for growth of pathogens and vermin. Both materials are presently disposed of through composting; using them as leaf protein feedstocks for a manufacturing process based in the Upper North Island of New Zealand could result in a higher-value end-use that helps to meet the increasing demand for animal feed protein.

There are several knowledge gaps that must be filled to assess the economic and environmental value of this proposal: the volumes and chemical composition of the materials; the technological and economic viability of producing a leaf protein concentrate from these materials; and the environmental impact must be assessed. This thesis fills these gaps by performing year-round sampling and characterisation of the feedstocks available in the Upper North Island of New Zealand; experimentally verifying their suitability for use in an improved leaf protein concentrate production process; performing a technoeconomic analysis to establish the economic viability of processing them; and performing a lifecycle assessment to determine their environmental impacts.

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3 Experimental Methodology

The experimental investigation was comprised of two main steps: a multivariate characterisation of the raw paunch grass and leafy green waste; followed by the optimisation of the protein recovery process and characterisation of its products.

3.1 Materials and Equipment

The materials used in experimental work are listed in Table 3.1. Laboratory equipment items are listed in Table 3.2. The details of analytical instruments are specified in their respective methods.

Table 3.1: List of experimental materials

Material	Grade	Supplier
Petroleum benzine / petroleum spirits (Boiling range 40-60°C)	Analytical	Merck, Darmstadt, Germany UniVar, Sydney, Australia
Methanol	Analytical	Merck, Darmstadt, Germany
N-trimethylsilylimizadole	Analytical	Sigma Aldrich, St. Louis, MO, USA
N-heptane	Analytical	Merck, Darmstadt, Germany
Starch	Food	Sigma Aldrich, St. Louis, MO, USA
Glucose	Analytical	Sigma Aldrich, St. Louis, MO, USA
Sodium benzoate	Analytical	BDH, London, England
Sodium hydroxide	Analytical	Merck, Darmstadt, Germany
Hydrochloric acid	Analytical	Merck, Darmstadt, Germany
Benzoic acid	Analytical	UniVar, Sydney, Australia
Phenol	Analytical	UniVar, Sydney, Australia
Sulfuric acid	Analytical	Merck, Darmstadt, Germany
Boiling chips	Analytical	UniVar, Sydney, Australia
Antifoam A	Analytical	Sigma Aldrich, St. Louis, MO, USA
Isopropanol	Analytical	Merck, Darmstadt, Germany
Calcium bentonite clay	Industrial	Transform Minerals, Coalgate, New Zealand
Calcium hydroxide	Analytical	UniVar, Sydney, Australia
Magnesium hydroxide	Analytical	UniVar, Sydney, Australia

Table 3.2: Laboratory equipment items

Equipment Item	Supplier
RHFP750 Food Processor	Russell Hobbs, Failsworth, England
Oscar DA-1000 Screw Juicer	Tasman Trading, Whanganui, New Zealand
Contherm 245M Laboratory Oven	Contherm Scientific, Lower Hutt, New Zealand
Sigma 4-5L unrefrigerated laboratory centrifuge	Sigma, Osterod am Harz, Germany
MF 10 hammer mill	IKA-Werke, Staufen im Breisgau, Germany
Autopipettes (100 µL, 1000 µL, 5000 µL)	Eppendorf, Hamburg, Germany
Laboratory balances (100 g, 300 g)	Mettler Toledo, Columbus, OH, USA
Laboratory balance (5 kg)	A&D Company Ltd, Tokyo, Japan

3.2 Multivariate Characterisation of Waste Leafy Plant Materials

It was anticipated that there would be substantial seasonal variation in the biochemical composition of the paunch grass and leafy green waste raw material streams, and that this seasonal variation could have a significant impact upon the outcomes of the technoeconomic and lifecycle assessments. To mitigate this uncertainty, fortnightly samples of paunch grass and leafy garden were collected between June 2014 and May 2015. The samples were sourced from an abattoir in the Waikato region of New Zealand and Hamilton Garden Bags and Red Lid Bins Ltd of Hamilton New Zealand, respectively. The samples were then analysed in the laboratory to determine their absolute and relative levels of moisture, crude protein, crude lipids, total carbohydrates, crude fibre and ash, as well as their carbon to nitrogen ratio. This data was then collated and statistically analysed to produce seasonal biochemical profiles for paunch grass and leafy green waste.

3.2.1 Sampling Protocol

Samples of bovine paunch grass were collected fortnightly from an abattoir in the Waikato region of New Zealand. These samples were collected at approximately 11:00 am by suspending a 10 L bucket underneath the screw conveyor which discharged paunch grass from the processing hall into a skip bin for disposal. The abattoir process was to spill paunch contents from each animal onto the processing hall floor before washing them into a receptacle which was subsequently emptied by a screw conveyor and dewatered before discharged into the skip via a second screw conveyor. Each sample therefore contained paunch grass from multiple animals and was thus considered heterogeneous and representative. Three 10 L

buckets of paunch grass were collected during each visit. These samples were then taken transported to Red Lid Bins depot for storage where they were kept at approximately -20°C until needed for analysis.

Samples of leafy green waste (i.e. lawn clippings and soft leafy weeds) were collected fortnightly by Hamilton Garden Bags and Red Lid Bins Ltd of Hamilton New Zealand, respectively. Samples were collected by Red Lid Bins staff from a random truck within the Red Lids fleet. The collection machinery on the back of the truck mixed and compressed the collected leafy green waste as it was collected, thus ensuring that the truck contents were heterogeneous. At the dump site, the mixed leafy green waste inside the truck was spread into a linear windrow, and samples were scooped into a 10 L bucket at three points along the windrow: the beginning, the middle, and the end. These samples were then taken back to Red Lid Bins depot and stored at approximately -20°C until needed for analysis.

3.2.2 Characterisation of Samples

Samples were removed from frozen storage as required and determinations carried out for: moisture content; physical composition; crude protein; carbon to nitrogen ratio (CNR); organic nitrogen content; crude lipids; total carbohydrates; crude fibre; and ash as per the workflow Figure 3.1.

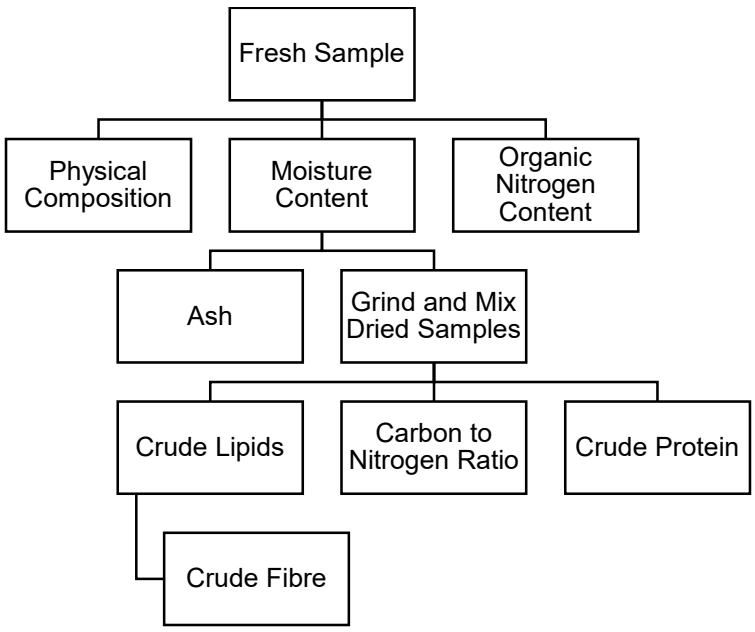

Figure 3.1: Sample characterisation laboratory workflow.

Moisture by Oven Drying

The samples were defrosted and weighed into disposable aluminium oven trays and dried to constant mass in a Contherm laboratory oven at 100°C. The sample moisture content was then calculated from the change in mass as per Equation 3.1. The dried samples were then stored in airtight containers for further analysis.

Equation 3.1: Calculation of sample moisture content.

$$x_{moisture} = \frac{m_{wet\ sample} - m_{dry\ sample}}{m_{wet\ sample}}$$

Physical Composition of Leafy Green Waste Samples

After the leafy green waste samples had been dried, they were visually inspected and categorised as one of three sample types: grass and weeds, tree leaves, or other.

Crude Protein and Carbon to Nitrogen Ratio

Dried samples were ground in an IKA®-Werke MF 10 hammer mill fitted with a 250 µm screen. The resulting powder was then analysed on a PDZ Europa 20-20 isotope ratio mass spectrometer to determine the total nitrogen and total carbon contents of each sample. The crude protein content and carbon to nitrogen ratio were then calculated (Equation 3.2 and Equation 3.3)

Equation 3.2: Calculation of sample crude protein content.

$$x_{CP} = 6.25 x_{total\ nitrogen}$$

Equation 3.3: Calculation of sample carbon to nitrogen ratio.

$$CNR = \frac{x_{total\ carbon}}{x_{total\ nitrogen}}$$

Organic Nitrogen

50 g samples were mixed with 100 ml of water and blended using a Russell Hobbs RHFP750 food processor for 30 seconds. The slurry was then fed into an Oscar DA-1000 screw juicer, which separated the slurry into a liquor fraction which was weighed and collected; and a fibre fraction, which was weighed and discarded. The liquor was then analysed on a Lachat QuikChem 8500 flow injection analyser fitted with a cadmium column and autosampler. The liquor samples were analysed by QuikChem® Methods to determine their total nitrogen content [1]; nitrate plus nitrite content [2]; nitrite content [3]; and ammonia content [4]. A 5 ml sample liquor sample was also pipetted into an aluminium cup and dried in a laboratory oven at 100°C to determine its moisture content as per Equation 3.1. The solids content per unit volume was also determined. These results were then used to

calculate the mass fraction of organic nitrogen, ammonia, nitrites, and nitrates as a proportion of total nitrogen (Equation 3.4 through Equation 3.7).

Equation 3.4: Calculation of relative ammonium level in sample liquor.	**Equation 3.5: Calculation of relative nitrite level in sample liquor.**	**Equation 3.6: Calculation of relative nitrate level in sample liquor.**
$x_{NH_4^+} = \frac{c_{NH_4^+}}{c_{total\ nitrogen}}$	$x_{NO_2^-} = \frac{c_{NO_2^-}}{c_{total\ nitrogen}}$	$x_{NO_3^-} = \frac{c_{NO_3^-}}{c_{total\ nitrogen}}$

Equation 3.7: Calculation of relative organic nitrogen level in sample liquor.

$$x_{organic\ N} = \frac{c_{total\ nitrogen} - \left(c_{NO_3^-} + c_{NO_2^-} + c_{NH_4^+}\right)}{c_{total\ nitrogen}}$$

Crude Lipids

Samples for crude lipid analysis were weighed into cellulose extraction thimbles and capped with glass wool. Samples were then extracted overnight in a Soxhlet extraction apparatus, using petroleum spirits (boiling range 40°C to 60°C) as the solvent. The samples were then left under a fume hood to allow excess solvent to evaporate. The thimbles were emptied into disposable aluminium oven trays and dried to constant mass at 100°C. Lipid content was calculated from the change in mass (Equation 3.8). The extracted sample was then set aside for crude fibre and total carbohydrate analysis.

Equation 3.8: Calculation of sample crude lipid content.

$$x_{lipids} = \frac{m_{pre-extraction} - m_{post-extraction}}{m_{pre-extraction}}$$

Preliminary GC-MS Detection of Sugars

The samples for sugar analysis were ground in an IKA-Werke MF 10 hammer mill fitted with a 250 µm screen, placed in a centrifuge tube, and shaken in 10 ml of methanol overnight in a rotary end-over-end shaker. 5 ml of methanol was then autopipetted into a glass vial and blown down to dryness using nitrogen gas. 1 mg of sugar residue was then scraped into a silylated glass vial and silylated using 200 µl of N-trimethylsilylimizadole. The sample was then transferred into a gas chromatography (GC) vial, diluted with 0.5 ml of n-heptane, and run on a Hewlett-Packard gas chromatography – mass spectroscopy (GC-MS) system to qualitatively detect sugars (Table 3.3).

Table 3.3: Components of the multiplexed Hewlett Packard GC-MS system

System Component	Description
Gas Chromatography Unit	Hewlett Packard 6890
Mass Spectrometer	Hewlett Packard 5973 quadrupole
Column	Phenomenex Zebron ZB5 capillary column *5 % polysiloxane* *Nominal length: 30.0 m* *Nominal diameter: 250.00 um* *Nominal film thickness: 0.25 um*

A constant helium gas flow (45.0 mL per minute) and column temperature (20°C) were maintained whilst 1.00 µL of sample was injected through the programmable temperature vaporizing (PTV) inlet. The column was then heated to 200°C and the mass spectroscopy detector activated and set to detect all species with molecular masses between 10 and 550 Da at a 20 Hz sampling rate. The column was then heated from 200°C to 310°C minutes at a ramp of 20°C per minute. The total run time was 19 minutes.

Total Carbohydrates

Samples were assayed for sugars by a procedure developed from the methods of DuBois *et al.* [5] and Brummer and Cui [6].

Preliminary digestion

14 mg of dry defatted residues from lipid analysis were weighed into autoclavable centrifuge tubes. Two quality control checks (14 mg starch; 14 mg glucose) were prepared after every tenth sample. 3 mL of a mixture containing 0.25 M sodium hydroxide and 1.7% (w/v) sodium benzoate was added into each centrifuge tube, which was then capped. Each tube was vortexed for 10 seconds before all the tubes were placed in a water bath set to 100°C for 4 minutes. The tubes were then removed from the water bath and cooled to ambient temperature. 5 mL of 4.25 M hydrochloric acid was added to the tubes, which were then capped, vortexed for 10 seconds and digested for 3 hours in an oven set to 85°C. The tubes were then removed from the oven and allowed to cool to ambient temperature. 5 mL of 4 M sodium hydroxide and 1 mL of MilliQ water were added to each tube before it was vortexed for 10 seconds. The tubes were then centrifuged for 10 minutes at 2000 relative centrifugal force.

Assay

400 µL of supernatant from each centrifuge tube was pipetted into a test tube and diluted to 1 mL total volume with 0.1% (w/v) benzoic acid. 1 mL of 5.0% (w/v) phenol solution was added the test tubes, which were then placed in an ice bath. 5 mL of concentrated (>96%) sulfuric acid was added to each tube in a rapid stream. Each test tube was vortexed for 10 seconds immediately after acidification. The test tubes were then in a 25°C water bath for 30 minutes before absorbance measurements commenced.

Measurement

A spectrophotometer (ThermoFisher Jenway) was zeroed using the blank prepared during the assay. A 100 µg/mL glucose standard (preserved with 0.1% w/v benzoic acid) was then diluted with 0.1% benzoic acid to prepare triplicates of six different glucose concentrations (0, 20, 40, 60, 80, and 100 µg/mL). The absorbance of each replicate was measured twice at 490 nm. These absorbance measurements were then used to prepare a calibration curve. The absorbance of the samples and QCs were measured twice at 490 nm and their concentrations determined from the calibration curve. Each sample was left in the water bath until immediately before measurement.

Fibre

Crude fibre determinations were performed on the residues produced during crude lipid analysis by a procedure developed from the method of AOAC International [7]. These residues were ground in an IKA-Werke MF 10 hammer mill fitted with a 250 µm screen. Approximately 150 mg of each sample was weighed into a digestion tube. 20 ml of 0.128 M sulfuric acid, a few boiling chips, and a drop of Antifoam A (CAS: 8050-81-5) diluted in petroleum spirits (boiling range 40°C-60°C) were then added to the tube. The tubes were capped, wrapped in aluminium foil, and placed in a digestion block set to 140°C for 40 minutes. The tube were then removed from the block and allowed to cool to room temperature.

The tubes were inverted three times to mix its contents, which were then vacuum filtered through California Buchner funnels modified with a 200 mesh stainless steel screens (Figure 3.2). The filtered samples were then washed with 6 ml of boiling distilled water, and 3x4 ml of cold distilled water, and allowed to suck dry.

They were then transferred into clean digestion tubes. 20 ml of 0.128 M sodium hydroxide and a few boiling chips were then added to each digestion tube. The tubes were capped, wrapped in aluminium foil, and placed in a digestion block set to 140°C for 40 minutes. The tube was then removed from the block and allowed to cool to room temperature.

The tube was inverted three times to mix its contents, which were then vacuum filtered through a 200 mesh stainless steel screen. It was then washed with 3 mL of boiling 0.128 M sulfuric acid, 3x4 ml of cold distilled water, 3 ml of isopropanol, and allowed to suck dry. It was then transferred to a crucible and dried in an oven set to 130°C for 2 hours. The crucible was then weighed and placed in an electric furnace and held at 600°C for 60 minutes to ignite the digested residue. The crucible was cooled and re-weighed, and the crude fibre content determined from the change in mass (Equation 3.9).

Equation 3.9: Calculation of sample crude fibre content.

$$x_{crude\ fibre} = \frac{m_{dry\ digested\ sample} - m_{ash} - \Delta m_{control}}{m_{undigested\ sample}}$$

Figure 3.2: Vacuum manifold with modified California Buchner funnels attached.

Ash

The samples for ash analysis were weighed into crucibles (either porcelain or stainless steel) and ignited at 600°C for two hours in an electric furnace. They were then left in the furnace to cool to room temperature and re-weighed. Ash content was calculated from the change in mass (Equation 3.10).

Equation 3.10: Calculation of sample ash content.

$$x_{ash} = \frac{m_{ash}}{m_{sample}}$$

3.2.3 Statistical Analysis of Characterisation Data

A one-way analysis of variance (ANOVA) was performed on each variable for each sample type to determine whether statistically significant differences between each were present for in any of the variables. A Tukey-Kramer analysis was then performed to determine the significant differences between seasonal means. Student's t-tests were performed to determine whether there were statistically significant differences between sample types for the mean of each variable. The calculation methods for ANOVA and Student's t-test are described in detail by Ross [8]. The Tukey-Kramer calculation is described by Zaiontz [9].

The significance level for the ANOVA and Student's t-tests was 10 per cent. This significance level was chosen because of the small size of the sample set relative to the population. The null hypothesis for the Student's t-tests was the mean of population F1 (the larger of the two means) was less than or equal to F2 (the smaller of the two means). Pearson's correlation coefficients were used to determine the strength of the correlation between some variables.

3.2.4 Estimation of Waste Leaf Proteins Available in New Zealand

The data from the characterisation experiments was then combined with publicly available datasets to calculate the total mass of waste leaf proteins produced each year in New Zealand.

Estimate of Leafy Green Waste Protein Biomass

The monthly mass of leafy green waste was calculated from data published by Ministry for the Environment [10] and Ministry for the Environment [11]. The latter was collected in 2011 indicated that the annual mass of waste sent to landfill in New Zealand was stable at the time of publication. Hence it was assumed that the annual mass of waste sent to landfill had not changed substantially since 2011. Ministry for the Environment [10] measured the mass of all the different waste components collected at four indicator landfill sites across New Zealand, including leafy green waste. Measurements were taken over a week in each season from Winter 2007 until Autumn 2008 and were assumed to be representative of the entire season. This data was used to calculate the amount of leafy green waste collected each season as a mass fraction of the total mass of waste sent to landfill at during that season (Equation 3.11). The annual mass of leafy green waste produced in New Zealand was calculated by multiplying the annual mass of solid waste sent to landfill in 2011 [11] by the mass fraction of leafy green waste present in the overall waste stream (Equation 3.12). The total mass of solid waste sent to landfill was determined from data published by Ministry for the Environment [11]. The mass fraction of leafy green waste in landfill waste (6.02%) was a weighted average based on the leafy green waste collected at four indicator sites from across New Zealand [10]. The annual mass of green waste was then multiplied by the mean annual leafy green waste dry matter, crude protein, and organic nitrogen contents determined by this study to yield the total annual mass of leafy green waste protein (Equation 3.13).

Equation 3.11: Calculation of leafy green waste as a mass fraction of total waste sent to landfill in 2007.

$$x_{GW} = m_{TW(2007)} \sum_{s=1}^{4} \frac{m_{GW(i)}}{m_{TW(i)}}$$

Where s = 1 (Winter 2007); 2 (Spring 2007); 3 (Summer 2008); 4 (Autumn 2008).

Equation 3.12: Calculation of total GW biomass produced in New Zealand in 2011.

$$m_{GW(2011)} = x_{GW} m_{TW}$$

Equation 3.13: Calculation of extractable GW protein produced in New Zealand in 2011.

$$m_{protein\,(GW)} = m_{GW(2011)} x_{organic\,N} \left(1 - x_{moisture\,(LGW)}\right)$$

Estimate of Paunch Grass Protein Biomass

The annual mass of paunch grass produced in New Zealand was calculated by multiplying the monthly head of cattle slaughtered by average mass of paunch grass recovered per animal and the volumetric solids content of paunch grass [12; 13]. These particular averages were used as they were based on dewatered paunch grass, which is consistent with the samples taken. The monthly paunch grass masses were then multiplied by the mean monthly solids, crude protein, and organic nitrogen contents determined by this study to yield to monthly masses of paunch grass protein (Equation 3.14). The monthly masses of paunch grass protein were then added to yield the annual total of paunch grass protein (Equation 3.15).

Equation 3.14: Calculation of total paunch grass biomass in New Zealand.

$$\left[m_{protein\,(PG)}\right]_m = \dot{m}_{PG} v_{S\,(PG)} \left[n_{cattle} x_{CP} x_{organic\,N} \left(1 - x_{moisture\,(PG)}\right)\right]_m$$

Where m corresponds to the Gregorian calendar month.

Equation 3.15: Calculation of extractable paunch grass protein in New Zealand.

$$m_{protein\,(PG)} = \sum_{m=1}^{12} \left[m_{protein\,(PG)}\right]_m$$

Where m corresponds to the Gregorian calendar month.

The mass of recoverable protein in each waste stream was calculated multiplying the annual mass of protein from each source by a protein recovery factor of 80%, based on the findings of Donnelly *et al.* [14] and McDonald [15]. It was assumed that the recovered protein could be used in a product equivalent to soybean meal, which has a crude protein content of at least 48% by mass [16]. Therefore the financial value of the protein product was determined by multiplying the annual product mass by the commodity price of soybean meal, which was NZD 457.59 per tonne as of June 2017 [16].

3.3 Optimisation of Leaf Protein Recovery

There are two steps in the recovery of leaf protein from soft leafy wastes: the extraction of protein into liquor and the recovery of this protein from the liquor. Both steps need to be optimised independently to maximise overall protein recovery.

3.3.1 Optimisation of Leaf Protein Extraction into Press Liquor

The first step in the leaf protein recovery process is the extraction of leafy protein into press liquor. It was optimised by independently controlling:

i. The amount of water added to samples prior to maceration;
ii. The number of times a sample was passed through the screw press; and
iii. Whether or not a sample was pre-treated with alkali solution.

Control Extractions

The control extractions were based on the method developed to express liquor from raw waste leafy green plant materials for organic nitrogen content determinations. 50 g samples were mixed with 100 ml of water and blended using a Russell Hobbs RHFP750 food processor for 30 seconds. The slurry was then fed into an Oscar DA-1000 screw press, which separated the slurry into liquor and fibre fractions. These fractions were weighed before the moisture content of the raw material, 5.000 mL of liquor (transferred by autopipette), and ca. 10 g of fibre was determined by the oven drying method described in Section 3.2.2. The crude protein contents of the raw waste leafy green plant material and fibre were determined by isotope ratio mass spectroscopy (see Section 3.2.2). A nitrogen balance was then carried out to determine the extent of protein extraction into the liquor (Equation 3.16).

Equation 3.16: Calculation of crude protein extracted from raw feed material into screw press liquor.

$$\eta_{CP1} = \frac{x_{CP(feed)} m_{feed} - x_{CP(fibre)} m_{fibre}}{x_{CP(feed)} m_{CP(feed)}}$$

Addition of Water to Soft Leafy Wastes

The control method adds water to soft leafy wastes at a 2:1 wet mass ratio prior to maceration in the food processor. This ratio worked well during initial trials, but three further experiments were required to determine whether it was the optimal method:

i. Determination of the water holding (i.e. maximum water absorption and adsorption) capacity of soft leafy wastes;
ii. Determination of the optimal amount of time between water addition and maceration (i.e. soaking time).
iii. Determination of protein extraction from samples at half water holding capacity.

Determination of water holding capacity

The maximum amount of water that can be absorbed by a sample was determined by mixing pre-weighed wet samples (ca. 5 g) with varying masses of water (Table 3.4) and allowing the mixture to soak for 30 minutes. Excess water was removed from the samples by vacuum filtration through California Buchner funnels fitted with 200 mesh stainless steel screens (Figure 3.2). Samples were placed inside the modified California Buchner funnels and watch glasses were added to seal the system. Vacuum was applied for five minutes to remove the excess water. Moisture content was then determined by the method described in Section 3.2.2.

Table 3.4: Addition of water to soft leafy waste samples to determine their holding capacity.

Water Mass (g)	5.000	10.000	15.000	20.000	25.000	30.000	35.000	40.000
Sample Mass (g)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

Determination of optimal soaking time

In each trial, enough water to achieve complete soaking was added to approximately 100 g of sample. The samples were then left to soak for 20, 30, or 40 minutes and processed according to the control method. The crude protein contents of the fibre fractions, and the moisture contents of the liquor and fibre fractions were determined according the methods described in Section 3.2.2. Protein extractions were calculated using Equation 3.16.

Determination of protein extraction at half water holding capacity

To determine whether adsorbed water affects protein extraction, sufficient water to reach 50 percent of the soaking capacity was added to approximately 100 g of sample. The sample was left to soak in water for 30 minutes, before it was processed according to the control extraction method. The crude protein content of the fibre fraction, and the moisture contents of the liquor and fibre fractions were determined according the methods described in Section 3.2.2. Protein extraction was calculated using Equation 3.16.

Multiple Pressing

Samples were initially processed according to the control method. Water was added to the fibre fractions at a mass ratio of 2:1. The resulting fibre slurries were then re-macerated in the food processor and re-extracted in the screw press; or just re-extracted in the screw press (Table 3.5).

Table 3.5: Investigation of how multiple passes through the screw press and fibre re-maceration effect protein extraction from samples of leafy green waste and paunch grass.

Passes through screw press	ONE				TWO				THREE			
Fibre maceration	YES		NO		YES		NO		YES		NO	
Sample type	GW	PG	GW	PG	GW	PG	GW	PG	GW	PG	GW	PG

This step was then repeated prior to a third pass through the screw press. The crude protein contents of the fibre fractions, and the moisture contents of the liquor and fibre fractions were determined according the methods described in Section 1.1.2. Protein extraction was calculated using Equation 1.12.

Alkali Pre-Treatment

Samples (ca. 50 g) were processed in duplicate under the conditions described in Table 3.6. The mixtures of sample and sodium hydroxide solution were incubated for 30 minutes inside beakers placed in a serological water bath. Samples were then extracted according to the control method. The crude protein content of the fibre fractions, and the moisture contents of the liquor and fibre fractions were determined according to the methods described in Section 1.1.2. Protein extraction was calculated using Equation 1.12.

Table 3.6: Investigation of how alkali pre-treatments affect protein extraction from samples of leafy green waste and paunch grass.

NaOH concentration ($molL^{-1}$)	NaOH addition (mL)	Incubation temperature (°C)	Sample type
0.125	2.000	40	Green waste
0.125	2.000	40	Paunch grass
0.625	2.000	40	Green waste
0.625	2.000	40	Paunch grass
0.125	2.000	50	Green waste
0.125	2.000	50	Paunch grass
0.625	2.000	50	Green waste
0.625	2.000	50	Paunch grass
0.125	2.000	60	Green waste
0.125	2.000	60	Paunch grass
0.625	2.000	60	Green waste
0.625	2.000	60	Paunch grass

3.3.2 Optimisation of Protein Recovery from Press Liquor

The second step in the leaf protein recovery process is the recovery of leaf protein from the press liquor by steam coagulation and centrifugation. It was optimised by independently controlling:

i. The Zeta potential of the protein liquor prior to steam coagulation; and
ii. The length of time that the protein liquor was held at coagulation temperature.

Zeta Potential Adjustment

Minimising the Zeta potential (i.e. particle charge) of the proteins suspended in the liquor prior to coagulation facilitates aggregation and precipitation of proteins, thereby increasing the amount of protein that can be coagulated from the solution.

Identification of Precipitation Agent

Samples of leafy green waste and paunch grass liquor were produced by a preparing liquor according to the control method and passing the resulting slurry through the screw press twice without macerating the fibre between passes (see Section 3.3.1). Samples of liquor (10.000 mL) were pipetted into the holding cell of a Mütek PCD 03 particle charge detector, and various precipitation agents (Table) were added in 100 µL increments until the Zeta potential stabilised. The Zeta potential was plotted against the amount of precipitation agent added to identify the most effective precipitation agent.

Table 3.7: Precipitation agents used to adjust Zeta potential of screw press liquors

Precipitation Agent	Concentration (gL^{-1})
Calcium Bentonite Clay	40.00
Calcium Hydroxide	50.00
Hydrochloric Acid	36.46
Magnesium Hydroxide	40.00
Sodium Hydroxide	40.00

Precipitation of Liquor without Steam Injection

Samples of leafy green waste and paunch grass liquor were produced according to a modified version of the control method which passed a fibre slurry through the screw press twice without macerating the fibre between passes (see Section 1.2.1). 1 M hydrochloric acid (500 µL) was added to each 10.000 mL sample of press liquor. The acidified liquor samples were then weighed and centrifuged at 2000 RCF for 15 minutes. The supernatant was decanted off into a pottle, weighed, and

frozen; the settled solids were weighed, freeze-dried, and analysed for crude protein by isotope ratio mass spectroscopy (see Section 3.2.2). Protein recovery was calculated using Equation 3.17.

Equation 3.17: Calculation of protein recovery from screw press liquor

$$\eta_{CP2} = \frac{x_{CP(ss)} m_{ss}}{x_{CP(feed)} m_{CP(feed)} - x_{CP(fibre)} m_{CP(fibre)}}$$

Precipitation of Liquor with Steam Injection

Samples of leafy green waste and paunch grass liquor were produced according to a modified version of the control method which passed a fibre slurry through the screw press twice without macerating the fibre between passes (see Section 1.2.1). Approximately 25 mL of press liquor was treated with a precipitation agent listed in Table 3.8, then heated to 85°C with 3 bar saturated steam using a Bellman CX-25S stovetop steam injector. The coagulated liquor samples were then weighed and centrifuged at 2000 RCF for 15 minutes. The supernatant was decanted off into a pottle, weighed, and frozen; the settled solids were weighed, freeze-dried, and analysed for crude protein by isotope ratio mass spectroscopy (see Section 1.1.2). Protein recovery was calculated using Equation 1.13.

Table 3.8: Precipitation agents used to mimimise Zeta potential of screw press liquors prior to steam coagulation.

Precipitation Agent	Concentration (gL^{-1})	Volume Added (µL)
None (Control)	0.00	0
Calcium Hydroxide	50.00	2640
Hydrochloric Acid	36.46	1440

Variation of Steam Coagulation Residence Time

Samples of leafy green waste and paunch grass liquor were produced according to a modified version of the control method which passed a fibre slurry through the screw press twice without macerating the fibre between passes (see Section 1.2.1). Approximately 25 mL of press liquor was treated with 1440 µL of 1 36.46 gL^{-1} hydrochloric acid, then heated to 85°C with 3 bar saturated steam using a Bellman CX-25S stovetop steam injector. The coagulated liquor samples were then placed in a water bath for a fixed amount of time: 13 seconds, 30 seconds, 60 seconds, 270 seconds, or 480 seconds. Control samples were not placed in the water bath and left to cool at ambient temperature.

The samples were then weighed and centrifuged at 2000 RCF for 15 minutes. The supernatant was decanted off into a pottle, weighed, and frozen; the settled solids were weighed, freeze-dried, and analysed for crude protein by isotope ratio mass spectroscopy (see Section 1.1.2). Protein recovery was calculated using Equation 1.13.

3.4 Characterisation of Recovered Leaf Protein and its Coproducts

The lyophilised leaf protein concentrate, press fibre, and waste liquor were characterised according to various methods:

i. The leaf protein concentrate was characterised by its amino acid profile, molecular weight distribution, crude protein content and carbon to nitrogen ratio, thermal properties, and ash content;
ii. The fibre was characterised by its moisture content, crude protein content and carbon to nitrogen ratio; crude lipid content, crude fibre content, total carbohydrate content, thermal properties, and ash content; and
iii. The waste liquor was characterised by its solids content as per Section 3.2.2.

3.4.1 Characterisation of Leaf Protein Concentrate

Samples of leaf protein concentrate were lyophilised and ground into a fine powder using a mortar and pestle. They were then characterised by a commercial amino acid assay, differential scanning calorimetry, and thermogravimetric analysis. Isotope ratio mass spectroscopy (IRMS) was also used for determination of crude protein content and carbon to nitrogen ratio.

Amino Acid Assay

Samples of leaf protein concentrate recovered from paunch grass and green waste were submitted to the Nutrition Laboratory at the School of Food and Advanced Technology, Massey University (Palmerston North, New Zealand). The laboratory analysed the samples for the: standard acid stable amino acid profile; cysteine and methionine; and tryptophan as per Table 3.9.

Table 3.9: Amino acids analysed and corresponding test methods.

Amino Acids Analysed	Test Method
Aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, taurine (i.e. acid-stable AA)	HCl hydrolysis followed by RP HPLC using AccQ Tag derivatisation as per AOAC 994.12
Cysteine and methionine	Performic acid oxidation as per AOAC 994.12
Tryptophan	Alkaline hydrolysis

Thermal Analysis

Two thermal analysis methods were applied to the ground and lyophilised leaf protein concentrates: differential scanning calorimetry (DSC) was used to determine the glass transition points of the protein concentrates; and thermogravimetric analysis (TGA) was used to determine their thermal degradation properties, and residual moisture contents.

Differential Scanning Calorimetry

Samples were analysed in a PerkinElmer DSC8500 under 20 mL per minute of nitrogen gas. Each sample was weighed into an aluminium DSC pan, which was then sealed by crimping an aluminium lid onto the pan. Samples were rapidly cooled to -50°C, then heated to 140°C at a rate of 20°C per minute. The DSC8500 recorded instantaneous heat flow against time; traces were analysed using PerkinElmer Pyris software.

Thermogravimetric Analysis

Samples were analysed in a PerkinElmer STA8000 under 20 mL per minute of air. The STA8000 was purged with 30 mL per minute of argon prior to each run. Each sample was weighed into a specialised crucible supplied with the STA8000, placed inside the instrument furnace, and its mass compared with an identical empty crucible. The sample was then heated from 35°C to 700°C at a rate of 10°C per minute.

3.4.2 Characterisation of Press Fibre

Samples of press fibre were lyophilised, and their moisture content calculated with Equation 3.1. Their crude protein contents and carbon to nitrogen ratios, crude lipid contents, crude fibre contents, total carbohydrate contents, and ash contents were

determined by the methods described in Section 3.2.2. The residual moisture contents and thermal stabilities of the fibre samples were determined by the TGA method described in Section 3.4.2.

3.4.3 Characterisation of Press Liquor

5.000 mL of press liquor was auto-pipetted into disposable aluminium pie dishes and dried to constant mass in a Contherm laboratory oven at 100°C. The sample moisture content was then calculated from the change in mass as per Equation 3.1.

3.5 References

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4 Characterisation of Soft Leafy Wastes

Materials such as paunch grass (PG) and leafy green waste (GW) are negative value materials that are presently composted prior to disposal. However, they are not well-suited to composting, and this creates environmental issues such as odour, leachate, and methane emissions. PG and GW could be a potential source of leaf protein (LP), but at this stage the chemical and biochemical compositions of New Zealand PG and GW are unknown.

To inform the technoeconomic analysis and life cycle assessment of LP production from PG and GW, the chemical and biochemical parameters of these materials were measured fortnightly over a 13-month period (Table 4.1): moisture, crude protein, crude lipids, total carbohydrates, crude fibre, ash, and carbon to nitrogen ratio.

Table 4.1: Annual mean levels of PG and GW biochemical parameters.

Parameter	PG Annual Mean	GW Annual Mean
Moisture (w/w%)	81.1 %	64.7 %
Crude protein (DM %)	15.2 %	16.2 %
Crude lipids (DM %)	10.4 %	11.9 %
Total carbohydrates (DM %)	20.4 %	17.1 %
Crude fibre (DM %)	23.1 %	15.6 %
Ash (DM %)	13.1 %	16.1 %
C:N ratio (DM %)	19.0	17.2

Liquors expressed from the fortnightly samples were also tested for relative concentrations of organic nitrogen, nitrites, nitrates, and ammonia (Table 4.2).

Table 4.2: Relative annual mean concentrations of nitrogen species in PG and GW.

Parameter	PG Annual Mean	GW Annual Mean
Organic nitrogen (Total N %)	15.9 %	74.5 %
Ammonia (Total N %)	19.7 %	17.9 %
Nitrite (Total N %)	0.5 %	0.7 %
Nitrate (Total N %)	1.3 %	2.0 %

This was the first study in New Zealand to measure most of these parameters in PG and GW on a regular basis, and one of only a handful worldwide. The differences in overall parameter means between sample types were statistically significant for all parameters except crude protein, with significance levels ranging from 1 to 10 per cent. Statistically significant differences between parameter the seasonal means

within a sample type were also present. On an overall wet basis, leafy green wastes and paunch grass contain 43.8 and 45.3 g of protein per kg of fresh material. This is comparable to fresh leaf crops which have been grown for leaf protein extraction, including: alfalfa, ryegrass, white clover, and red clover. Hence PG and GW may be a feasible source of leaf protein.

4.1 Summary of Seasonal Variations

The proximate analysis of leafy green waste and paunch grass is summarised on a seasonal basis in Figure 4.1 and Figure 4.2, respectively. The moisture contents of leafy green waste followed a different trend to that of the paunch grass. The typical moisture content of paunch grass was also noticeably higher than that of the leafy green waste. The crude protein and lipid components of the leafy green waste and paunch grass also follow different trends. The leafy green waste and paunch grass displayed similar trends for the remaining dry matter components (i.e. total carbohydrates, crude fibre, and ash). The components do not add to 100 percent. One explanation is that crude fibre was destroyed during the digestion step of the analysis. Nevertheless, it is unusual for a biochemical profile of a plant to add to 100 percent – the fresh crop profiles reviewed during this study had either missing or overlapping biochemical components [1; 2; 3; 4; 5; 6; 7; 8; 9].

The protein and moisture contents of leafy green waste was highest during Spring 2014 and steadily decreased with each successive season until it reached its lowest level in Winter 2015. A similarly low level was observed in Winter 2014. The lipid content of the leafy green waste is lowest during Autumn 2015 and consistent during the rest of the year. The total carbohydrate contents of leafy green waste were highest during summer and consistent during the remainder of the year. The crude fibre and ash contents of leafy green waste were lowest during Spring 2014 and Autumn 2015. They were at consistent levels in the remaining seasons.

The crude protein content of paunch grass remains consistent from Winter 2014 until Summer 2014/2015, then drops by approximately five per cent. Hence the crude protein contents of paunch grass during Autumn and Winter 2015 are considerably lower than during the earlier seasons. The lipid contents of paunch grass are highest during Spring 2014 and Autumn 2015, and consistent during the

other seasons. The total carbohydrate contents of paunch grass are at their minimum during Winter and Spring 2014, and at their maximum thereafter. Conversely, the crude fibre and ash contents of paunch grass are at their highest from Winter 2014 until Summer 2014/2015, then drop to a lower level during Autumn and Winter 2015.

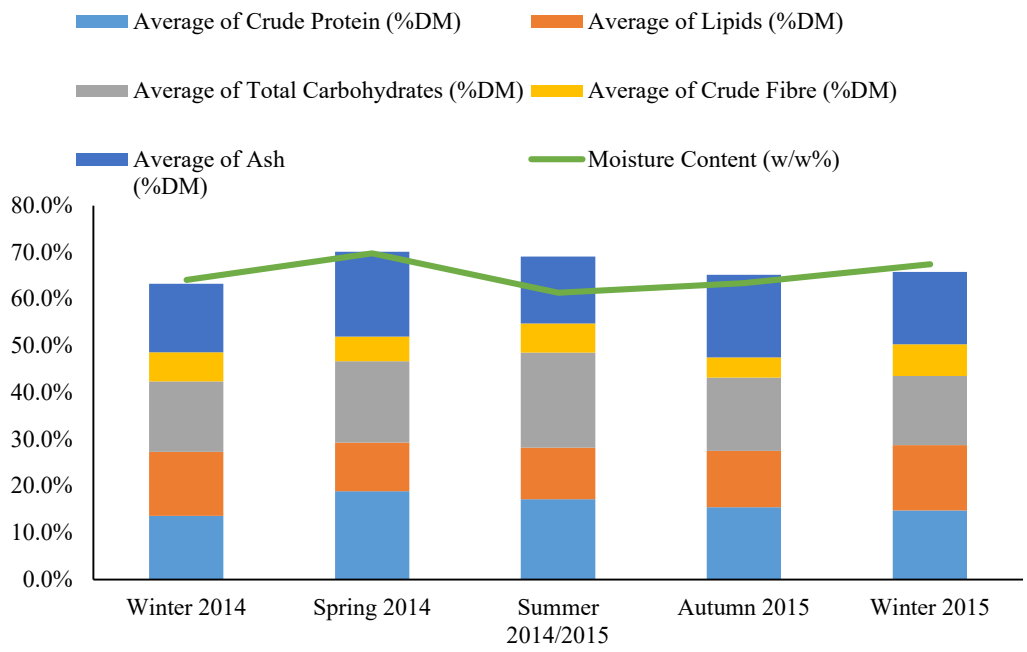

Figure 4.1: Seasonal summary of leafy green waste biochemical analysis.

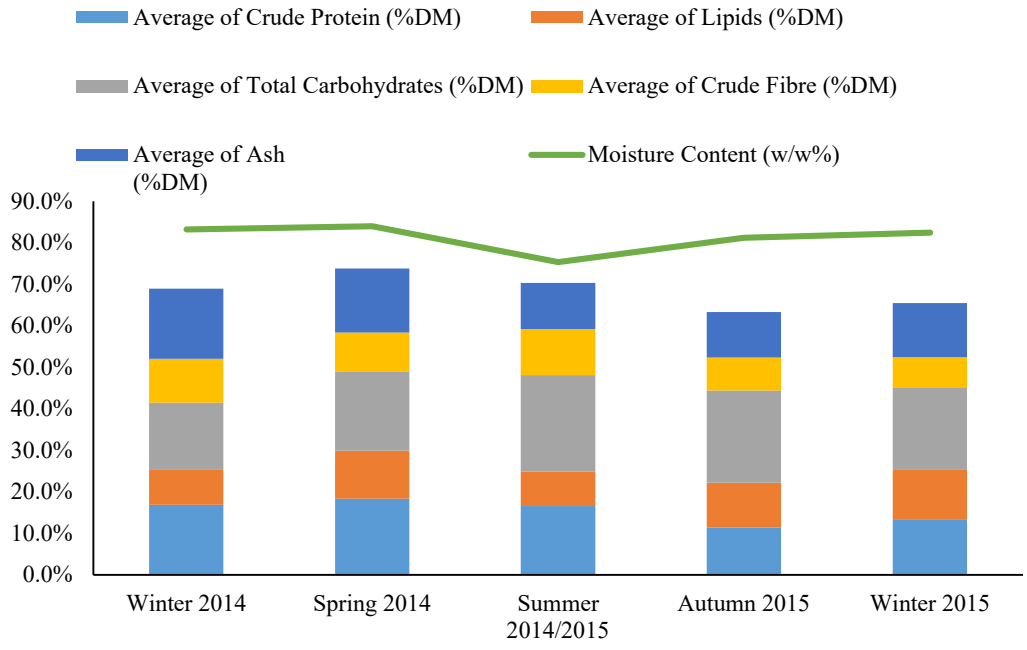

Figure 4.2: Seasonal summary of paunch grass biochemical analysis.

4.1.1 Statistical Analysis

The Student's t-tests summarised in Table 4.3 indicate statistically significant seasonal differences between sample types for all mean overall parameter levels except crude protein. In addition, all parameters had statistically significant differences between sample types in at least one season. Many of these differences were significant at the 1 per cent level. Detailed Student's t-tests for each parameter can be found in Appendix A.

Table 4.3: Summary of Student's t-tests for statistical significance of differences between parameter means of leafy green waste and paunch grass. Significance levels: ***, P<=0.01 | **, P<=0.05 | *, P<=0.1 | -, P>0.1

Variable	Overall	Winter 2014	Spring 2014	Summer 2014/2015	Autumn 2015	Winter 2015
Moisture	***	***	***	***	***	***
Crude protein	-	*	-	-	***	-
Lipids	**	**	-	***	-	-
Total CHOs	***	-	-	-	***	***
Crude fibre	***	*	***	***	***	-
Ash	***	*	-	***	***	*
C:N	*	-	-	-	***	-
Organic N	***	***	-	-	*	**
Ammonia	**	-	-	-	-	**
Nitrite	*	*	-	-	*	**
Nitrate	***	***	***	-	-	**

The ANOVA tests summarised in Table 4.4 indicate statistically significant differences between at least one pair of seasons for some GW parameters and most paunch grass parameters. Most of these differences were significant at the 1 per cent level, and all except one were significant at the 5 per cent level. Detailed ANOVA and Tukey Kramer tests for each parameter can be found in Appendix A.

Table 4.4: Summary of ANOVA tests for statistically significant differences between seasonal mean parameter levels within GW and PG data sets. Significance levels: ***, P<=0.01; **, P<=0.05; *, P<=0.1; -, P>0.1

Variable	Significance of Differences Between Seasonal GW Means	Significance of Differences Between Seasonal PG Means
Moisture	-	***
Crude protein	-	***
Lipids	-	-
Total CHOs	**	***
Crude fibre	-	-
Ash	-	***
C:N	-	***
Organic N	**	-
Ammonia	*	-
Nitrite	-	**
Nitrate	***	***

4.2 Physical Composition of Leafy Green Waste

Grass and weeds (i.e. soft green material) are clearly the dominant fraction in all seasons and comprise at least 94 per cent of leafy green waste collected in Summer 2014/2015 (Figure 4.3). A review of the literature determined that the physical composition of garden waste varies between locations and is difficult to predict [10; 11; 12]. The results in Figure 4.3 are consistent with the observations of Boldrin and Christensen [10] from Denmark, which indicated that grass was most dominant in spring and summer, whilst leaves and other materials were more dominant in winter. It is important to note that Denmark has a harsher winter than the Waikato region of New Zealand, and this could affect the composition of garden waste collected during this season.

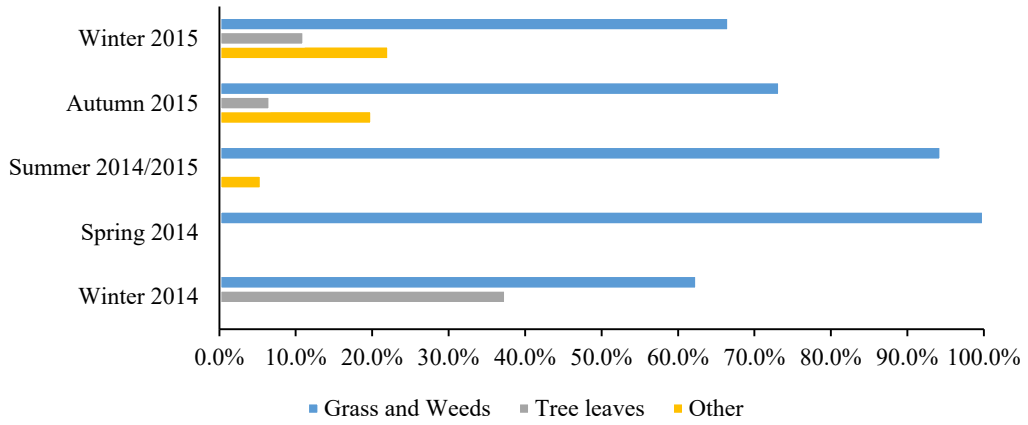

Figure 4.3: Physical composition of leafy green waste.

4.3 Chemical Compositions of Leafy Green Wastes

The laboratory methods used to characterise the seasonal variation in PG and GW are summarised in Table 4.5 Student's t-tests were used to identify statistically significant differences between PG and GW means for each season; ANOVA tests were performed to identify statistically significant differences The protein production potential across New Zealand was determined by combining publicly available statistics [13; 14; 15; 16] with experimental results to estimate the mass of protein available for processing. Further details are available in Section 3.2.4.

Table 4.5: Characterisation methods for PG and GW biochemical parameters.

Variable	Characterisation Method
Moisture content	Oven drying at 100°C
Crude protein Carbon to nitrogen ratio	Isotope ratio mass spectroscopy
Liquor nitrogen composition	Flow injection analysis
Crude lipids	Soxhlet extraction in petroleum benzine (B.R. 40°C to 60°C)
Total carbohydrates	GC-MS (for preliminary screening) Phenol-sulfuric acid assay
Crude fibre	Gravimetric combustion of oven dried residue from dry samples digested with 0.128 M HCl and 0.128 NaOH
Ash	Gravimetric combustion of dried samples

4.3.1 Moisture Content

The moisture content of the samples is summarised in Table 4.6. The overall mean moisture contents were 64.7 per cent and 81.1 per cent for leafy green waste and paunch grass, respectively. The Student's t-tests in Table 4.3 confirm there is a statistically significant difference between the means on both an overall and seasonal basis. The overall standard deviation is approximately 24 percent of the mean. Similarly, the seasonal standard deviations vary from 18 percent to 28 percent of the mean. This indicated a large amount of variability between samples. The ANOVA tests summarised in Table 4.4 indicate no statistically significant seasonal differences between the seasonal means for leafy green waste. However, the subsequent Tukey-Kramer test identified a single statistically significant difference (Table 4.7). These tests also indicate there are statistically significant seasonal differences between a single pair of seasonal means for paunch grass (Table 4.8). Krogmann [17] and Hanc *et al.* [18] noted that green waste is often left in a collection container for several days or weeks prior to collection. This is enough

time for moisture to be lost to the atmosphere through transpiration. As expected, the mean moisture content is lowest in summer. Statistical correlation tests indicated that the moisture content of the green waste was moderately correlated to the number of rainy days in a month: the Pearson's Correlation Coefficient was 0.426 when outliers were included, and -0.471 when outliers were excluded. The plot of leafy green waste moisture content against the number of rainy days in a month (Figure 4.4) indicates that extreme data points significantly affect the correlation and should be included in the analysis. A negative correlation implies the moisture content of leafy green waste should be highest under drought conditions, which is unrealistic.

Table 4.6: Moisture content of leafy green waste and paunch grass samples.

	Leafy green waste		**Paunch Grass**	
Time Period	**Mean (w/w%)**	**Standard Deviation (w/w%)**	**Mean (w/w%)**	**Standard Deviation (w/w%)**
Winter 2014	**64.1%**	**18.0%**	**83.2%**	**2.4%**
Jul	75.0%	2.5%	83.7%	1.3%
Aug	58.6%	19.9%	82.9%	2.7%
Spring 2014	**69.8%**	**9.9%**	**84.0%**	**2.0%**
Sep	67.5%	13.9%	85.2%	1.4%
Oct	70.9%	5.4%	84.0%	2.5%
Nov	69.7%	11.7%	83.4%	0.8%
Summer 2014/2015	**61.3%**	**16.9%**	**75.3%**	**16.7%**
Jan	66.3%	12.6%	82.8%	0.9%
Feb	46.0%	17.7%	83.5%	1.8%
Dec	71.7%	4.0%	44.3%	13.1%
Autumn 2015	**63.4%**	**16.9%**	**81.2%**	**1.9%**
Mar	58.6%	20.6%	79.7%	0.7%
Apr	62.9%	3.7%	81.5%	1.9%
May	68.4%	15.4%	82.2%	1.6%
Winter 2015	**67.4%**	**12.3%**	**82.4%**	**1.5%**
Jun	69.5%	11.4%	82.4%	1.7%
Jul	63.3%	13.0%	82.5%	1.1%
Overall	**64.7%**	**15.7%**	**81.1%**	**8.2%**

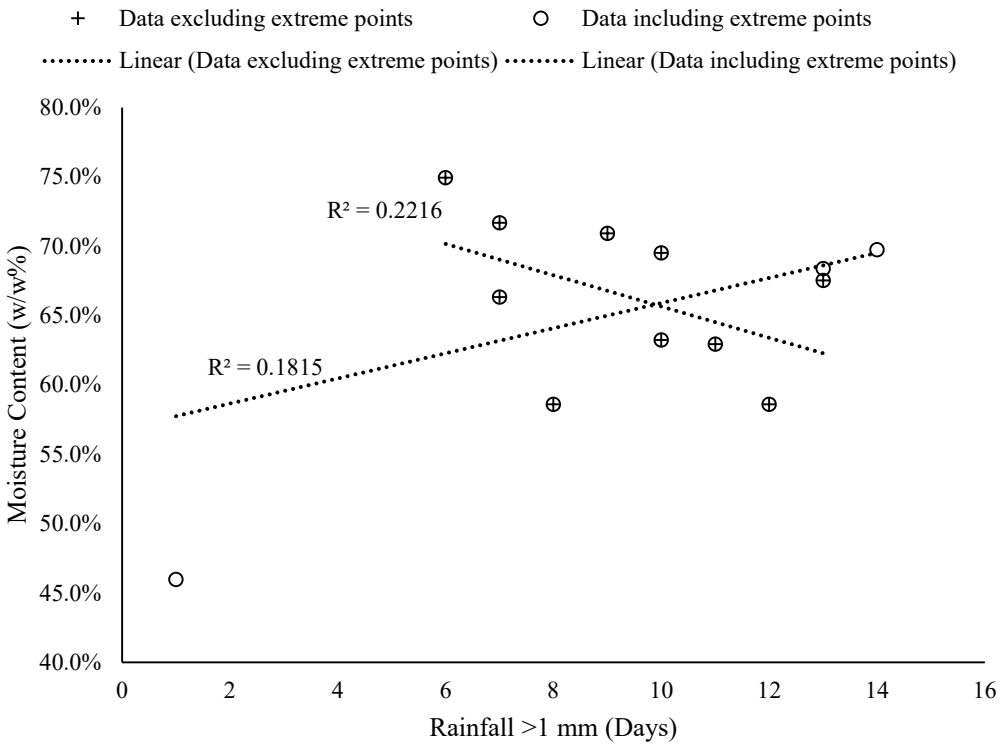

Figure 4.4: Correlation between monthly mean moisture content of leafy green waste and rainy days in a month.

Apart from Summer 2014/2015, the seasonal average moisture contents for paunch grass were within three percent of the overall average. This is less than one standard deviation. The December 2014 moisture contents were unusually low and affected the seasonal mean for Summer 2014/2015. A likely explanation is that the samples collected in December 2014 were removed from an open skip that had been left in the hot sun outside the slaughterhouse. This allowed water to evaporate from the sample prior to collection. All other samples were collected directly from the paunch grass discharge outlet inside the slaughterhouse, so measurements were not subject to evaporative effects.

Table 4.7: Statistically significant difference between seasonal mean moisture contents of leafy green waste.

q-Crit	3.505						
Group 1	**Group 2**	**Mean Δ**	**Std Err**	**q-Stat**	**Lower Δ**	**Upper Δ**	**p-Value**
Spring 2014	Summer 2014/2015	0.08439	0.02372	3.558	0.00125	0.16753	0.0915

The paunch grass moisture content can be assumed to be within a constant range for the rest of the year, which seems counter-intuitive given the season variation in grass moisture content reported by [citations needed]. However, it is logical upon consideration of rumen and slaughterhouse processes: the rumen regulates the water

content of ruminal material at 95 percent [19]; and the liquid and solid fractions of the rumen are usually separated in a screw press which expels solid material with a consistent moisture content [16]. The overall and Summer 2014/2015 standard deviations for the paunch grass are much larger than most of the monthly standard deviations. This is because the monthly means are spread from 44.3 per cent to 83.7 per cent, and the data points for each month are tightly clustered around their respective means.

Table 4.8: Statistically significant differences between seasonal mean moisture contents of paunch grass.

q-Crit	3.501						
Group 1	**Group 2**	**Mean Δ**	**Std Err**	**q-Stat**	**Lower Δ**	**Upper Δ**	**p-Value**
Spring 2014	Summer 2014/2015	0.0865	0.0110	7.839	0.0479	0.1252	8.41E-07
Winter 2014	Summer 2014/2015	0.0784	0.0133	5.888	0.0318	0.1250	4.29E-04
Summer 2014/2015	Winter 2015	0.0706	0.0122	5.773	0.0278	0.1134	5.93E-04
Summer 2014/2015	Autumn 2015	0.0585	0.0107	5.483	0.0212	0.0959	1.31E-03

4.3.2 Crude Protein

The crude protein (CP) content of the samples is summarised in Table 4.9. The mean CP content of leafy green waste was between 13 to 19 percent of dry matter for the across the entire sampling period. The seasonal means were within one standard deviation of the overall mean. The overall standard deviation was high, at 33 percent of the mean. Similarly, the seasonal standard deviation ranged from 27 to 51 percent of the seasonal means. This indicates a high degree of short-term variability in the leafy green waste.

There is no significant difference in the overall CP content of the two sample types on dry basis, and most of the overall and seasonal means for each sample type are all within one standard deviation of each other. This is consistent with the rumen converting feed protein to microbial protein rather than absorbing it [19]. However, the t-tests summarised in Table 4.3 indicate that there was a significant difference between the seasonal means of crude protein during Winter 2014 and Autumn 2015. This may have been due to the use of supplementary feed. There is also a significant difference in moisture content between the two sample types due to rumen and slaughterhouse processes which give paunch grass a consistently higher moisture

content than leafy green waste. There will be a significant difference in CP content on a wet basis.

Table 4.9: Crude protein content of leafy green waste and paunch grass samples.

	Leafy green waste		Paunch Grass	
Time Period	**Mean (%DM)**	**Standard Deviation (%DM)**	**Mean (%DM)**	**Standard Deviation (%DM)**
Winter 2014	**13.6%**	**4.1%**	**16.9%**	**4.7%**
Jul	13.5%	4.0%	11.9%	0.3%
Aug	13.6%	4.1%	19.4%	3.8%
Spring 2014	**18.9%**	**4.4%**	**18.3%**	**3.4%**
Sep	16.4%	3.3%	15.2%	3.7%
Oct	20.0%	5.4%	19.4%	3.6%
Nov	19.1%	0.8%	18.3%	1.6%
Summer 2014/2015	**17.1%**	**4.7%**	**16.6%**	**3.1%**
Jan	19.0%	2.3%	16.3%	3.5%
Feb	13.2%	3.6%	14.9%	2.1%
Dec	21.3%	2.7%	18.3%	2.1%
Autumn 2015	**15.4%**	**5.1%**	**11.4%**	**1.3%**
Mar	14.1%	3.9%	11.5%	0.5%
Apr	15.9%	4.2%	10.9%	1.3%
May	16.3%	6.5%	11.9%	1.6%
Winter 2015	**14.7%**	**7.5%**	**13.4%**	**3.7%**
Jun	14.2%	6.5%	11.2%	2.0%
Jul	15.7%	9.1%	15.6%	3.6%
Overall	**16.2%**	**5.4%**	**15.2%**	**4.3%**

The ANOVA tests summarised in Table 4.4 indicate the seasonal differences in the mean CP contents of leafy green waste are not statistically significant. This was surprising given the seasonal variation in the crude protein contents of grass crops reported by Fulkerson*, et al.* [5], Tremblay *et al.* [20], and Wilman [6]. However, these authors also reported that grass cut at the same phenological stage had similar crude protein contents. The observations in Table 4.9 are consistent with lawns being consistently cut at a similar length.

The mean CP of the paunch grass was 15.2 percent. There were two distinct ranges of mean CP: 16 to 18 percent from Winter 2014 until the end of Summer 2014/2015; and 11 to 15 percent during Autumn 2015 and Winter 2015. This is reflected in the

relatively high overall standard deviation, which sits at 28 percent of the overall mean.

The ANOVA tests summarised in Table 4.4 indicate there are statistically significant differences between the means of at least two of the groups. The statistically significant differences are presented in Table 4.12. These differences could be explained by supplementary feed use during the winter season. The solid paunch contents were almost entirely grassy material during the former period. During the latter period, supplementary feed residues could be observed with the naked eye. The demand for non-pasture silage supplementary feed has increased markedly from 397000 tonnes in 1990/1991 to 4451000 tonnes in 2014/2015 [21]. A breakdown of non-pasture silage supplementary feed demand is presented in Table 4.10. These figures are for the dairy herd only, and do not include additional feed fed to dry stock.

Supplementary feed costs money, and therefore farmers do not feed more supplement than what is required to meet a cow's nutritional needs [22]. The protein contents of common supplements are presented in Table 4.11. They contain less protein than the pasture crops in Table 4.10. This explains the lower mean crude protein content during the supplementary feeding period. Short-term variability was also high, with standard deviations ranging from 11 to 28 percent of the mean. This can be explained by slaughterhouse and farm operations.

The slaughterhouse sources its cattle from many different farms, which use heterogeneous feed sources and hold cattle for different amounts of time before sending them away for slaughter. It cannot be assumed that the rumen from each slaughtered animal contained the same type of feed for the same amount of time. It is likely that the grass ingested by the cattle on different farms will be at various stages of maturity. There is a well-established correlation between the protein content and age of grass [6]. As the samples tested contained mixed paunch contents from several animals, the standard deviation of the crude protein content was high.

Table 4.10: Supplementary feed use in the New Zealand dairy industry during the 2014/2015 production season. Dairy NZ [21].

Supplementary Feed	Mass Fed to Cattle (Tonnes DM)	Fraction of Supplementary Feed Supply
Palm kernel expeller	1452787	32.6%
Maize silage	1269500	28.5%
Fodder beet	698250	15.7%
Other supplements	487235	10.9%
Barley	144505	3.2%
Kale	121080	2.7%
Wheat	55310	1.2%
Maize grain	53746	1.2%
Swedes	45830	1.0%
Cereal whole crop silage	40320	0.9%
Turnips	20400	0.5%
Soyabean meal	18746	0.4%
Cottonseed	13199	0.3%
Other brassicas	12960	0.3%
Rape	10920	0.2%
Brewe"s grain	4502	0.1%
Oats	2539	0.1%
Tapioca	2307	0.1%
TOTAL	**4454136**	**100.0%**

Table 4.11: Protein contents of common supplements. Source: Dairy NZ [22].

Supplement	Crude Protein Content (%DM)
Pasture silage (high quality)	17.0%
Pasture silage (low quality)	14.0% (Upper) 12.0% (Lower)
Maize silage (high grain)	8.0%
Maize silage (low grain)	8.0%
Palm kernel expeller	14.0%

Table 4.12: Statistically significant differences between seasonal mean crude protein contents of paunch grass.

q-Crit	3.558						
Group 1	**Group 2**	**Mean Δ**	**Std Err**	**q-Stat**	**Lower Δ**	**Upper Δ**	**p-Value**
Spring 2014	Autumn 2015	0.0691	0.00793	8.706	0.0408	0.0973	5.66E-07
Summer 2014/2015	Autumn 2015	0.0515	0.00911	5.656	0.0191	0.0839	1.54E-03
Winter 2014	Autumn 2015	0.0544	0.00971	5.600	0.0198	0.0890	1.76E-03
Spring 2014	Winter 2015	0.0494	0.00887	5.573	0.0179	0.0810	1.87E-03

4.3.3 Relative Organic Nitrogen

The organic nitrogen content of the samples is summarised in Table 4.13. Significant variability was present in the green waste samples. Although the seasonal mean organic nitrogen contents were within one standard deviation of the overall mean organic nitrogen content, the standard deviation was 21.3 percent of the mean, which is relatively high. A maximum mean green waste organic nitrogen content of approximately 80 per cent was reached during Spring 2014 and Summer 2014/2015. The relative standard deviation was also at its minimum during these seasons, ranging from 9 to 15 percent of the mean. The mean organic nitrogen content of green waste varied from 62 to 75 percent during the rest of the year, and the standard deviation ranged from 15 to 45 percent of the mean.

The Student's t-tests summarised in Table 4.3 indicate there are statistically significant differences between the mean organic nitrogen contents of leafy green waste and paunch grass on an overall basis, as well as during Winter 2014, Autumn 2015, and Winter 2015. The seasonal differences all occur during the cooler seasons. During these seasons, leafy green waste is produced more slowly and is generally left in the collection bin for longer. This gives more time for the degradative processes described by Insam and de Bertoldi [23] to occur. Such a drastic increase in residence time is not expected in the rumen [19]. Advanced degradation of organic nitrogen by microbes could explain why the organic nitrogen content of the leafy green waste is significantly lower than that of paunch grass during the cooler months.

The ANOVA tests summarised in Table 4.4 indicate there were statistically significant differences between the mean organic nitrogen contents of leafy green waste. These statistically significant differences are presented in Table 4.14. This in turn indicates seasonal differences in the extent of nitrogen mineralisation.

This was unsurprising. Leafy green waste can be left in a collection bin which is not emptied for several weeks [17; 18]. This allows sufficient time for heterotrophic bacteria to convert organic nitrogen to ammonia, nitrate, and nitrogen gas. The extent of mineralisation depends on time, temperature, and aeration [23]. A truck load of leafy green waste is comprised of waste from multiple sources, and each source is subject to different treatments and conditions.

Table 4.13: Organic nitrogen content of leafy green waste and paunch grass samples.

	Leafy green waste		Paunch Grass	
Time Period	**Mean (%DM)**	**Standard Deviation (%DM)**	**Mean (%DM)**	**Standard Deviation (%DM)**
Winter 2014	**68.5%**	**10.5%**	**81.3%**	**3.8%**
Jul	69.2%	2.7%	83.2%	1.2%
Aug	68.2%	12.7%	80.7%	4.0%
Spring 2014	**79.5%**	**11.9%**	**82.8%**	**8.8%**
Sep	85.7%	2.5%	89.5%	6.7%
Oct	78.8%	13.8%	85.4%	4.2%
Nov	74.9%	10.8%	75.4%	9.9%
Summer 2014/2015	**79.9%**	**7.7%**	**79.1%**	**11.8%**
Jan	78.7%	6.0%	82.2%	10.9%
Feb	76.7%	9.7%	75.5%	13.6%
Dec	84.2%	4.2%	80.2%	7.0%
Autumn 2015	**75.2%**	**13.5%**	**82.3%**	**13.1%**
Mar	66.9%	16.5%	87.0%	5.2%
Apr	77.5%	4.0%	76.6%	17.5%
May	81.3%	9.0%	85.9%	5.3%
Winter 2015	**62.1%**	**27.7%**	**85.0%**	**8.3%**
Jun	61.7%	30.9%	80.8%	10.0%
Jul	62.9%	20.0%	89.2%	1.8%
Overall	***74.5%***	***15.9%***	***82.1%***	***10.5%***

Table 4.14: Statistically significant differences between seasonal mean organic nitrogen contents of leafy green waste.

q-Crit	3.563						
Group 1	**Group 2**	**Mean Δ**	**Std Err**	**q-Stat**	**Lower Δ**	**Upper Δ**	**p-Value**
Summer 2014/2015	Winter 2015	0.178	0.0438	4.0537	0.02149	0.334	0.0438
Spring 2014	Winter 2015	0.175	0.0474	3.6865	0.00583	0.343	0.0821

The higher means and lower standard deviations observed in sample collected during Spring 2014 and Summer 2014/2015 were not unexpected. Boldrin and Christensen [10] and Krogmann [17] reported that the volume of garden waste peaked during spring and summer. Their observations are consistent with the experiences of the leafy green waste supplier for this study. Higher rates of garden waste generation imply a higher collection frequency, and therefore less opportunity for nitrogen mineralisation to occur.

The ANOVA tests summarised in Table 4.4 indicate the differences between the seasonal mean organic nitrogen contents of paunch grass were not statistically significant. However, the relative standard deviations were considerably large: the overall relative standard deviation was 12.8 percent of the overall mean, and the seasonal standard deviations varied from 4 to 16 percent of the seasonal means. Hence the mean organic nitrogen contents of paunch grass did not vary with the seasons but were subject to considerable short-term variability. As the samples tested contained the mixed paunch contents of several animals, this was likely due to subsamples originating from different farms and ingestion of the plant material occurring at different times.

These results are not surprising, as the feed material is only retained in the rumen until it is reduced to pieces shorter than 1 millimetre [19]. The microbial flora inside the rumen constantly convert feed protein to ammonia, which is then converted to microbial protein [19]. Hence virgin protein from fresh feed is broken down in a controlled environment. This implies a relatively constant organic nitrogen concentration, which is consistent with the results in Table 4.13. Hence the organic nitrogen content of paunch grass is much less variable than that of leafy green waste: the former is produced in a relatively controlled environment, where degradation processes are constant and predictable; the latter is produced in a largely uncontrolled environment, where degradation processes are very difficult to predict quantitatively.

4.3.4 Inorganic Nitrogen

The inorganic nitrogen in the samples is present due to the microbial degradation of protein. Microbes metabolise protein to ammonia. This ammonia is used for microbial protein synthesis but can also be metabolised further to nitrites and then nitrates. The latter two steps are known as nitrification, and are usually limited by availability of ammonia [24].

Ammonia

The relative concentrations of ammonia are summarised in Table 4.15. The Student's t-tests summarised in Table 4.3 indicate there was a statistically

significant difference in the overall mean ammonia contents of the two liquors. On a seasonal basis, a statistically significant difference was only observed in Winter 2015. This insignificant difference between the two sample types was likely due to ammonia synthesis being the rate-limiting step of the nitrification process [24]. The relative concentration of ammonia will vary little when available protein exceeds the metabolic requirements of the microbes.

Table 4.15: Relative concentrations of ammonia present in GW and PG samples.

	Leafy green waste		Paunch Grass	
Time Period	**Mean (%DM)**	**Standard Deviation (%DM)**	**Mean (%DM)**	**Standard Deviation (%DM)**
Winter 2014	**13.8%**	**8.6%**	**12.2%**	**5.4%**
Jul	13.4%	1.7%	7.0%	0.5%
Aug	14.0%	10.4%	13.7%	5.3%
Spring 2014	**16.6%**	**16.7%**	**15.5%**	**13.2%**
Sep	10.6%	3.6%	7.5%	6.9%
Oct	11.3%	8.6%	16.1%	14.2%
Nov	33.3%	24.3%	18.8%	12.4%
Summer 2014/2015	**12.4%**	**11.0%**	**13.5%**	**11.8%**
Jan	11.6%	6.1%	9.2%	6.3%
Feb	16.8%	16.9%	17.9%	16.2%
Dec	8.9%	2.6%	13.2%	3.4%
Autumn 2015	**18.1%**	**12.8%**	**13.4%**	**20.8%**
Mar	26.4%	14.5%	7.1%	5.6%
Apr	19.0%	8.7%	23.3%	28.5%
May	10.5%	6.5%	4.7%	0.8%
Winter 2015	**34.5%**	**37.7%**	**5.3%**	**2.1%**
Jun	35.7%	42.0%	5.4%	1.2%
Jul	32.2%	27.2%	5.1%	2.8%
Overall	**17.9%**	**19.7%**	**12.5%**	**14.4%**

Table 4.16: Statistically significant difference between seasonal mean ammonia contents of GW liquors.

q-Crit	3.563						
Group 1	**Group 2**	**Mean Δ**	**Std Err**	**q-Stat**	**Lower Δ**	**Upper Δ**	**p-Value**
Summer 2014/2015	Winter 2015	0.221	0.0552	3.9988	0.0240	0.418	0.0483

The ANOVA tests summarised in Table 4.4 indicate there was statistically significant seasonal variation in the mean ammonia content of the leafy green waste liquor. These tests also indicate there was no statistically significant variation in the

mean ammonia content of paunch grass liquor. The statistically significant difference is presented in Table 4.16.

The overall and seasonal relative standard deviations were very high. The overall seasonal standard deviations for green waste and paunch grass were 109.9 percent and 115.2 percent, respectively. Similarly, the seasonal standard deviations ranged between 40.6 percent and 155.9 percent. There was very noticeable day-to-day variability in the relative concentrations of ammonia present in both sample types. This could be explained variable treatments prior to sampling. The leafy green waste is placed in collection bins for variable amounts of time prior to collection [17; 18]. Similarly, the length of time feed material is contained within the rumen prior to slaughter can be expected to vary from animal to animal. This led to a varied extent of protein degradation within a sample set, and hence a high standard deviation for that sample set.

Nitrites

The relative nitrite concentrations of the samples are summarised in Table 4.17. The Student's t-tests summarised in Table 4.3 indicate the differences between the mean nitrite contents of the liquors were statistically significant on an overall basis. On a seasonal basis, statistically significant differences were detected during the autumn and winter sampling periods. These differences may be due to different microorganisms converting the ammonia to nitrite in each sample type, or differences in microbial metabolism [24]. The ANOVA tests summarised in Table 4.4 confirm there is no statistically significant seasonal variation in the mean nitrite contents of the leafy green waste liquor. These tests also indicate there are statistically significant differences in the seasonal mean nitrite contents of the paunch grass liquors.

The overall standard deviations for green waste and paunch grass are 78.8 and 50.0 percent of their respective overall means, which is very high. Hence there was very noticeable day to day variability in the nitrite content of both sample sets. There is also seasonal variability present, as the mean Winter 2014 nitrite content of green waste is significantly different to the means observed in Spring 2014 and Autumn 2015.

Table 4.17: Relative concentrations of nitrites present in GW and PG samples.

	Leafy green waste		Paunch Grass	
Time Period	**Mean (%DM)**	**Standard Deviation (%DM)**	**Mean (%DM)**	**Standard Deviation (%DM)**
Winter 2014	**0.5%**	**0.1%**	**0.6%**	**0.1%**
Jul	0.4%	0.1%	0.5%	0.0%
Aug	0.5%	0.2%	0.6%	0.1%
Spring 2014	**0.7%**	**0.3%**	**0.6%**	**0.3%**
Sep	0.9%	0.3%	0.5%	0.0%
Oct	0.6%	0.1%	0.8%	0.3%
Nov	0.9%	0.3%	0.5%	0.0%
Summer 2014/2015	**0.5%**	**0.2%**	**0.7%**	**0.4%**
Jan	0.5%	0.2%	0.5%	0.3%
Feb	0.5%	0.1%	0.9%	0.5%
Dec	0.6%	0.2%	0.6%	0.1%
Autumn 2015	**0.9%**	**0.9%**	**0.5%**	**0.2%**
Mar	1.6%	1.1%	0.5%	0.2%
Apr	0.4%	0.1%	0.6%	0.2%
May	0.4%	0.2%	0.3%	0.1%
Winter 2015	**0.8%**	**0.5%**	**0.4%**	**0.1%**
Jun	0.5%	0.3%	0.4%	0.1%
Jul	1.3%	0.5%	0.4%	0.1%
Overall	***0.7%***	***0.5%***	***0.6%***	***0.3%***

Table 4.18: Statistically significant differences between mean nitrite contents of PG liquors.

q-Crit	3.550						
Group 1	**Group 2**	**Mean Δ**	**Std Err**	**q-Stat**	**Lower Δ**	**Upper Δ**	**p-Value**
Summer 2014/2015	Winter 2015	0.00292	0.000736	3.9627	0.000304	0.00553	0.0498

However, these results are not particularly relevant, as nitrites are a non-rate limiting intermediate in nitrate production from ammonia [24]. The Pearson's correlation coefficients between the relative nitrite and ammonia contents were 0.499 and 0.639 for leafy green waste liquor and paunch grass liquor, respectively. This is demonstrated graphically in Figure 4.5 The correlation explains the relatively small, but highly variable nitrite concentrations.

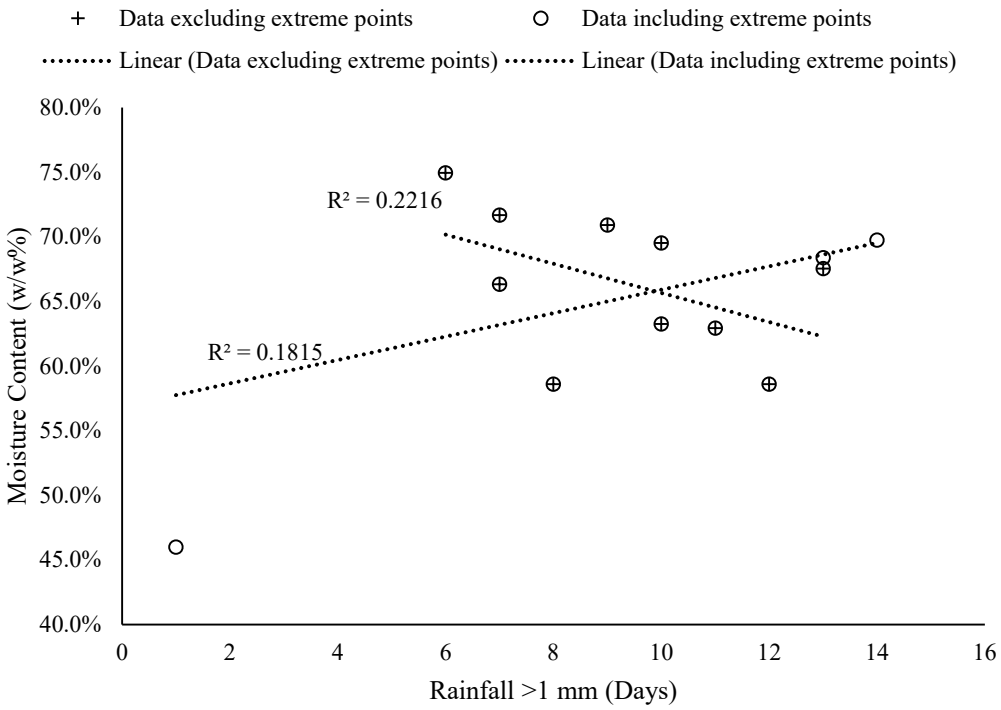

Figure 4.5: Correlation between relative nitrite and ammonia contents of liquors expressed from leafy green waste and paunch grass

Nitrates

The relative concentrations of nitrates in the samples are summarised in Table 4.19. The Student's t-tests summarised in Table 4.3 indicate there are statistically significant differences between the mean nitrate contents of the two liquor types. This occurs both on an overall basis, and during most sampling periods. This is not surprising. Microbes convert the feed in both sample types to ammonia, which are in turn converted to nitrite, and then nitrate [19; 24]. In cattle, excess nitrogen is removed across the rumen wall and is excreted [19]. This does not occur in a garden waste collection bin.

The ANOVA summarised in Table 4.4 indicates there were statistically significant seasonal differences in the mean seasonal nitrate contents of leafy green waste and paunch grass liquors. These statistically significant differences are presented in Table 4.20 and Table 4.21. The samples taken in Winter 2015 had particularly low relative concentrations of nitrates in comparison to the samples taken in the other seasons. This could be explained by the relatively low crude protein levels observed in both leafy green waste and paunch grass during Winter 2015. Excess proteins in paunch grass and leafy green waste are converted to excess ammonia by microbial

processes [19; 24]. Microbes then produce nitrates from the excess ammonia [24]. If there is less protein in the fresh materials, there will be less excess nitrogen for microbial metabolism, and therefore less nitrates produced.

Table 4.19: Relative concentrations of nitrates present in GW and PG liquors.

	Leafy green waste		Paunch Grass	
Time Period	**Mean (%DM)**	**Standard Deviation (%DM)**	**Mean (%DM)**	**Standard Deviation (%DM)**
Winter 2014	**3.1%**	**0.1%**	**2.1%**	**0.0%**
Jul	3.1%	0.1%	2.1%	0.0%
Aug	3.1%	0.1%	2.1%	0.0%
Spring 2014	**3.0%**	**0.1%**	**2.1%**	**0.1%**
Sep	2.9%	0.2%	2.1%	0.0%
Oct	3.1%	0.1%	2.0%	0.1%
Nov	3.0%	0.1%	2.1%	0.1%
Summer 2014/2015	**2.5%**	**0.8%**	**2.1%**	**1.1%**
Jan	2.4%	1.0%	1.9%	0.1%
Feb	2.3%	1.0%	2.5%	1.6%
Dec	2.8%	0.1%	1.9%	0.0%
Autumn 2015	**1.2%**	**1.3%**	**0.8%**	**0.9%**
Mar	0.3%	0.3%	0.9%	0.9%
Apr	2.8%	0.0%	1.2%	0.9%
May	1.2%	1.4%	0.0%	0.0%
Winter 2015	**0.1%**	**0.1%**	**0.0%**	**0.0%**
Jun	0.0%	0.0%	0.0%	0.0%
Jul	0.1%	0.1%	0.0%	0.0%
Overall	**2.0%**	**1.3%**	**1.4%**	**1.1%**

The relative standard deviations for the Winter 2014 and Spring 2014 samples do not exceed 6 percent of the mean, but the relative standard deviations for the remaining seasons are at least 33.7 percent of the mean, and often exceed 100 percent of the mean. This indicates a high level of variability in the relative concentrations of nitrates on a day-to-day basis for both sample types. This can variability can be explained by the heterogeneity of the materials.

The paunch grass samples were taken from cattle sourced from numerous farms. These cattle will have ingested grass at varying stages of maturity and times before slaughter. Leafy green waste may have originated from a freshly filled bin, or from a bin that has been left for several weeks. This variability leads to high standard

deviations in summer and autumn. The standard deviations for Winter 2014 and Spring 2014 were thus lower than during other seasons.

Table 4.20: Statistically significant differences between mean seasonal nitrate contents of GW liquors.

q-Crit	3.563						
Group 1	**Group 2**	**Mean Δ**	**Std Err**	**q-Stat**	**Lower Δ**	**Upper Δ**	**p-Value**
Spring 2014	Winter 2015	0.0295	0.00256	11.5257	0.0203	0.0386	3.04E-10
Winter 2014	Winter 2015	0.0307	0.00273	11.2335	0.0210	0.0404	6.79E-10
Summer 2014/2015	Winter 2015	0.0244	0.00237	10.3089	0.0160	0.0328	8.68E-09
Spring 2014	Autumn 2015	0.0182	0.00221	8.2313	0.0103	0.0261	2.51E-06
Winter 2014	Autumn 2015	0.0195	0.00241	8.0557	0.0108	0.0281	4.01E-06
Summer 2014/2015	Autumn 2015	0.0132	0.00199	6.6055	0.0061	0.0203	1.69E-04
Autumn 2015	Winter 2015	0.0112	0.00241	4.6535	0.0026	0.0198	1.41E-02

Table 4.21: Statistically significant differences between seasonal mean nitrate contents of paunch grass liquors

q-Crit	3.550						
Group 1	**Group 2**	**Mean Δ**	**Std Err**	**q-Stat**	**Lower Δ**	**Upper Δ**	**p-Value**
Summer 2014/2015	Winter 2015	0.0214	0.00190	11.2369	0.01462	0.0281	2.29E-10
Spring 2014	Winter 2015	0.0205	0.00183	11.2078	0.01401	0.0270	2.50E-10
Winter 2014	Winter 2015	0.0208	0.00217	9.6067	0.01311	0.0285	2.95E-08
Summer 2014/2015	Autumn 2015	0.0136	0.00166	8.1690	0.00767	0.0195	1.90E-06
Spring 2014	Autumn 2015	0.0127	0.00158	8.0524	0.00710	0.0183	2.64E-06
Winter 2014	Autumn 2015	0.0130	0.00196	6.6408	0.00605	0.0199	1.23E-04
Autumn 2015	Winter 2015	0.0078	0.00178	4.3949	0.00150	0.0141	2.21E-02

During winter, animals are fed supplements, which stabilise the level of protein and nitrogen in their diets, albeit at a lower level when the animals are eating fresh forage [21]. Nitrates are metabolites of ammonia, which is in turn a metabolite of protein [25]. This reduces the variability in the relative concentration of nitrates in paunch grass samples. Similarly, green waste bins are emptied infrequently in

winter. This gives time for degradative processes to run their course, thereby reducing the variability in the relative concentrations of nitrates.

During spring, most grass crops follow similar protein content trends [6; 9]. Hence degradation of grass-based feed in the rumen varies less between animals. This makes the relative concentration of nitrates in the paunch grass less variable. Similarly, a large amount of young grass is placed into green waste bins during spring. These bins are emptied frequently. These circumstances combine to make protein degradation, and hence the relative concentration of nitrates in leafy green waste less variable.

4.3.5 Crude Lipids

The crude lipid contents of the samples are summarised in Table 4.22. The overall mean lipid contents of leafy green waste and paunch grass were 11.9 and 10.4 percent of dry matter content, respectively. The Student's t-tests summarised in Table 4.3 indicate there is a statistically significant difference between the two sample types on an overall basis, and during the Winter 2014 and Summer 2014/2015 sampling periods. When there was a significant difference in the mean lipid contents of the two sample types, the paunch grass samples had consistently lower lipid contents than the paunch grass samples. This could be due to conversion of plant lipids to volatile fatty acids (VFAs) by ruminal microbes [25]. It is likely some VFAs would be lost during the Soxhlet extraction process used to measure the crude lipid content of the samples. The ANOVA tests summarised in Table 4.4 indicate there were no statistically significant differences between the seasonal means for leafy green waste and paunch grass.

The relative standard deviations were very high. The overall relative standard deviations were 58.5 percent of the overall mean for green waste, and 79.4 percent of the overall mean for paunch grass. Similarly, the seasonal relative standard deviations ranged from 33 to 85 percent of the seasonal means for green waste, and from 24 to 98 percent of the seasonal means for paunch grass. This indicated the lipid content of the samples varied noticeably in the short-term, but the long-term mean was stable. The variation of in lipid content can be explained by the dependence of lipid synthesis on environmental factors (e.g. soil salt and moisture

content) and the maturity of plants [26]. The lipid content of a given plant species converges upon a specific value as plants matures [26]. Plants require a minimum amount of lipid for surface waxes, biological membranes, and energy storage [27].

Table 4.22: Lipid content of GW and PG samples.

	Leafy green waste		Paunch Grass	
Time Period	**Mean (%DM)**	**Standard Deviation (%DM)**	**Mean (%DM)**	**Standard Deviation (%DM)**
Winter 2014	**13.7%**	**11.5%**	**8.6%**	**4.9%**
Jul	22.9%	8.1%	11.7%	3.3%
Aug	7.6%	9.2%	6.4%	4.7%
Spring 2014	**10.4%**	**6.0%**	**11.5%**	**10.8%**
Sep	13.2%	5.6%	7.5%	0.6%
Oct	9.3%	4.9%	11.6%	7.6%
Nov	9.9%	7.3%	11.9%	14.7%
Summer 2014/2015	**11.1%**	**5.0%**	**8.3%**	**5.2%**
Jan	10.1%	3.2%	10.6%	2.7%
Feb	13.8%	6.6%	4.2%	1.9%
Dec	9.2%	2.8%	12.4%	7.7%
Autumn 2015	**12.1%**	**4.1%**	**10.8%**	**10.5%**
Mar	13.1%	3.7%	7.6%	4.1%
Apr	7.6%	1.7%	10.6%	2.3%
May	13.5%	3.6%	13.8%	17.1%
Winter 2015	**14.0%**	**9.6%**	**12.0%**	**2.9%**
Jun	16.3%	10.7%	10.7%	2.2%
Jul	9.2%	3.7%	13.4%	3.0%
Overall	**11.9%**	**7.0%**	**10.4%**	**8.2%**

The paunch grass samples consistently produced a yellow lipid extract, whilst the colour of the leafy green waste lipid extracts varied from green to yellow. This variation in colour is caused by chlorophyll degradation. When plant cells die, the chlorophyll protective mechanisms are overcome by free radicals generated during wilting [28]. This causes lipids to be oxidised to malondialdehyde [28]. Linolenic acid makes up 90 per cent of the fatty acids in chloroplasts, and is particularly susceptible to attack by lipoxygenase [26]. The lipids in dead plant tissues also polymerise to form lignin-like compounds in Milliard reactions [25]. The change in colour from green is indicative of linolenic acid degradation [26]. Ruminal microbes usually rupture the porphyrin rings in the chlorophyll to scavenge the

magnesium ion [25]. This explains why the paunch grass lipid extracts are consistently yellow.

4.3.6 Total Carbohydrates

The total carbohydrate contents of the GW and PG samples are summarised in Table 4.23. The overall mean carbohydrate contents of green waste and paunch grass were 17.1 and 20.4 percent of dry matter, respectively. The paunch grass overall mean is within the range of 250 g to 190 g of water soluble carbohydrates per kilogram of dry matter reported by Wilman [6]. The leafy green waste mean is just below it. This indicates that some carbohydrate degradation has already taken place. This is consistent with the literature [23].

No simple sugars were detected in either sample set. Hence all carbohydrates present were either storage or structural carbohydrates. Composting and ruminal microbes always digest simple sugars first [23; 25]. This result indicates that some microbial degradation of the carbohydrate had already taken place at the time of sampling.

The Student's t-tests summarised in Table 4.3 indicate there is a statistically significant difference between the mean total carbohydrate contents of the leafy green waste and paunch grass. This also applies to the differences between the seasonal means in Autumn 2015 and Winter 2015. Paunch grass had a consistently higher total carbohydrate content. This is not surprising given the protein component of the feed is readily broken down by ruminal bacteria, increasing the relative abundance of the other components [19].

The ANOVA tests summarised in Table 4.4 indicate there are statistically significant differences between the seasonal means for leafy green waste. These statistically significant differences are presented in Table 4.24. They are not surprising, as the total carbohydrate content of fresh grass varies with the seasons [5], although weather conditions can also have a large effect [6]. The higher total carbohydrate contents for leafy green waste are observed during the warmer growth seasons (Table 4.23). This is consistent with the trends described by Fulkerson*, et al.* [5] and Wilman [6]. Storage carbohydrates are generally produced during spring

and summer and consumed during autumn and winter [5; 6; 25]. As all carbohydrates detected were either storage or structural carbohydrates, the leafy green waste trends observed in Table 4.23 are sensible.

Table 4.23: Total carbohydrate content of leafy green waste and paunch grass samples

	Leafy green waste		Paunch Grass	
Time Period	**Mean (%DM)**	**Standard Deviation (%DM)**	**Mean (%DM)**	**Standard Deviation (%DM)**
Winter 2014	**15.1%**	**7.3%**	**16.0%**	**6.6%**
Jul	19.5%	9.2%	21.7%	4.6%
Aug	12.8%	4.7%	13.1%	5.5%
Spring 2014	**17.4%**	**3.6%**	**19.0%**	**2.8%**
Sep	13.9%	2.9%	18.9%	1.6%
Oct	18.7%	2.4%	17.3%	1.9%
Nov	18.4%	3.9%	21.5%	2.4%
Summer 2014/2015	**20.3%**	**4.6%**	**23.2%**	**7.9%**
Jan	21.6%	7.3%	24.1%	10.9%
Feb	19.7%	1.9%	20.8%	3.4%
Dec	19.7%	2.0%	26.2%	5.3%
Autumn 2015	**15.7%**	**5.2%**	**22.1%**	**2.8%**
Mar	19.6%	4.1%	22.5%	1.5%
Apr	9.3%	1.0%	20.9%	3.5%
May	14.9%	3.5%	23.3%	2.2%
Winter 2015	**14.8%**	**2.8%**	**19.7%**	**3.5%**
Jun	14.8%	3.0%	20.9%	3.3%
Jul	14.8%	2.3%	18.6%	3.4%
Overall	**17.1%**	**5.3%**	**20.4%**	**5.4%**

Table 4.24: Statistically significant differences between seasonal mean total carbohydrate contents of GW.

q-Crit	3.565						
Group 1	**Group 2**	**Mean Δ**	**Std Err**	**q-Stat**	**Lower Δ**	**Upper Δ**	**p-Value**
Summer 2014/2015	Winter 2015	0.0554	0.0146	3.7960	0.00337	0.107	6.87E-02
Summer 2014/2015	Autumn 2015	0.0469	0.0125	3.7500	0.00231	0.091	7.42E-02
Winter 2014	Summer 2014/2015	0.0529	0.0146	3.6224	0.00084	0.105	9.13E-02

The overall relative standard deviation for leafy green waste was 31.1 percent of the mean. The seasonal relative standard deviations varied from 18.9 to 48.4 percent of the mean for leafy green waste. This indicates a high degree of variation about the mean. The variation for leafy green waste can be explained by the inherent

heterogeneity of the material. There is also variation in the amount of time the leafy green waste is left in the collection container before it is picked up [17]. This affects the extent of microbial carbohydrate degradation prior to sample collection [23].

The ANOVA tests summarised in Table 4.4 indicate there were statistically significant differences between the seasonal mean total carbohydrate contents of paunch grass. These statistically significant differences are presented in Table 4.25. The total carbohydrate contents of paunch grass also followed the seasonal trends described by Fulkerson*, et al.* [5] and Wilman [6], but the seasonal differences between the means are smaller. This is likely due to the use of supplementary feed to ensure sufficient animal nutrition [21; 22]. The overall paunch grass relative standard deviation was 26.6 percent of the mean. The seasonal relative standard deviations varied from 12.5 to 41.3 percent of the mean. This also indicates a high degree of variation about the mean. This variation could be explained by the heterogeneity of the raw material. The cattle slaughtered at the abattoir will have ingested a range of feed materials at varying lengths of times prior to slaughter. These materials will vary both in their total carbohydrate contents and the type of carbohydrates present. This will lead to varying degrees of degradation [25], and hence variation in the levels of total carbohydrates measured.

Table 4.25: Statistically significant differences between seasonal mean total carbohydrate contents of PG.

q-Crit	3.565						
Group 1	**Group 2**	**Mean Δ**	**Std Err**	**q-Stat**	**Lower Δ**	**Upper Δ**	**p-Value**
Winter 2014	Summer 2014/2015	0.0722	0.0151	4.7677	0.01841	0.126	1.05E-02
Winter 2014	Autumn 2015	0.0616	0.0145	4.2371	0.00996	0.113	3.02E-02
Spring 2014	Summer 2014/2015	0.0426	0.0126	3.3895	-0.00204	0.087	1.29E-01

4.3.7 Crude Fibre

The crude fibre results are presented in Table 4.26. The mean overall crude fibre contents for green waste and paunch grass were 5.7 and 9.1 percent of dry matter, respectively. These are much lower than the neutral detergent fibre results for ryegrass reported by Wilman [6] (40.0 to 65.0 per cent of dry matter), and the crude fibre results reported by Tritt and Schuchardt [16] (75.7 to 82.5 per cent of dry matter). There are several possible explanations for the low result. A substantial fraction of the crude fibre may have already been partially or completely digested

by the microbial processes as described by Insam and de Bertoldi [23] and Van Soest [25]. The crude fibre method involves two harsh digestions, and partially digested material may have not survived them. This would lead to an unusually low result, with most of the material detected being lignin, which is more resistant to digestion [27]. The digestion procedure was based on the methods of AOAC International [29], but used much smaller amounts of sample. This could have introduced substantial error to the crude fibre determination.

Table 4.26: Crude fibre content of leafy green waste and paunch grass samples

	Leafy green waste		Paunch Grass	
Time Period	**Mean (%DM)**	**Standard Deviation (%DM)**	**Mean (%DM)**	**Standard Deviation (%DM)**
Winter 2014	**6.3%**	**2.7%**	**10.6%**	**4.9%**
Jul	8.2%	1.8%	15.1%	2.2%
Aug	4.3%	1.9%	6.1%	1.6%
Spring 2014	**5.3%**	**1.9%**	**9.5%**	**5.6%**
Sep	4.6%	0.5%	27.6%	0.0%
Oct	5.3%	2.0%	8.1%	3.6%
Nov	5.8%	2.1%	8.6%	2.5%
Summer 2014/2015	**6.2%**	**2.7%**	**11.1%**	**3.6%**
Jan	6.0%	2.6%	11.6%	2.5%
Feb	4.1%	1.3%	11.1%	4.3%
Dec	8.6%	2.0%	10.2%	3.3%
Autumn 2015	**4.3%**	**3.0%**	**8.0%**	**3.9%**
Mar	5.7%	2.0%	10.6%	3.3%
Apr	1.2%	0.6%	6.0%	2.9%
May	4.6%	3.3%	7.7%	4.0%
Winter 2015	**6.8%**	**5.4%**	**7.2%**	**4.1%**
Jun	7.1%	4.7%	7.0%	4.7%
Jul	6.1%	6.4%	7.4%	3.3%
Overall	**5.7%**	**3.3%**	**9.1%**	**4.7%**

It is well known that structural carbohydrates are slowly degraded by composting microbes in the environment, as well as by bacteria present in the rumen [23; 25]. Krogmann [17] reported that garden waste can be left in a collection bin for up to two weeks prior to being transported to the composting site. The suppliers of the leafy green waste for this study have customers that put their garden waste out for collection even less often. This would give ample opportunity for composting microbes to reduce the crude fibre content. The crude fibre levels determined by

this study are similar to the lignin levels (5 to 16 per cent of dry matter) in non-woody yard wastes determined by Bary*, et al.* [11]. This suggests that substantial microbial degradation of the original crude fibre has taken place.

The Student's t-tests summarised in Table 4.3 indicate that the differences between the means of the two sample types are not statistically significant, except during Winter 2014 and Autumn 2015. The overall relative standard deviations were very high: 58.8 and 51.2 percent of the mean for green waste, and paunch grass respectively. The seasonal relative standard deviations varied from 35.1 to 78.9 percent of the seasonal means for green waste, and from 32.3 to 59.0 percent of the seasonal means for paunch grass. This indicates that there was substantial variation between individual samples. This was likely caused by heterogeneity in the raw material and the degree of microbial degradation.

The ANOVA presented in Table 4.4 indicates the seasonal variation in the mean crude fibre contents of leafy green waste and paunch grass was not statistically significant. They can therefore be assumed to be constant throughout the year.
The decrease in the crude fibre content of paunch grass observed during the winter months was unexpected. Wilman [6] reported that the neutral detergent fibre (NDF) (i.e. structural carbohydrate) content of grasses increases rapidly during spring growth, and then does not change. Mature grasses which would normally be ingested during winter have the highest NDF content [6]. This drop can be attributed to the use of supplementary feed, which is typically added to cattle diets when pasture is of insufficient nutritional value. The most common supplement is pasture silage [30]. Other common supplements include palm kernel expeller, maize silage, and fodder beet [22]. The NDF contents of these feeds are presented in (Table 4.27). Supplementary feed residues could be observed with the naked eye during the cooler seasons. Most supplements have a lower NDF content than the 66.0 dry matter percent for very mature grasses reported by Wilman [6].

The mean crude fibre content of paunch grass in Winter 2015 is 3.2 dry matter per cent lower than during Winter 2014. This can be explained by the difference in total rainfall in Hamilton during that period. Both winters contained exactly 32 wet days [31]. However, 382.8 mm of rain fell during Winter 2014, compared with just 286.2 mm during Winter 2015 [31]. This was 48.0 mm above and 48.6 mm below the

average rainfall from 1981 to 2010, respectively [31]. Hence the rainfall deficit during Winter 2015 may have limited the growth of grass and therefore its crude fibre content.

Table 4.27: Neutral detergent fibre contents of supplementary cattle feeds. Source: Dairy NZ [22]

Supplement	Neutral Detergent Fibre Content (%DM)
Pasture silage (high quality)	45.0% to 50.0%
Pasture silage (low quality)	53.0% to 57.0%
Maize silage (high grain)	42.0% to 45.0%
Maize silage (low grain)	45.0% to 50.0%
Palm kernel expeller	70.0%

4.3.8 Ash

The ash results are summarised in Table 4.28. The mean ash contents for leafy green waste and paunch grass are 16.1 and 13.1 per cent of dry matter, respectively. The results differ from values given in the literature. The ash content of the leafy green waste samples in this study is well below the 40 to 80 per cent range reported by Boldrin and Christensen [10]. It also within a much narrower range. The climate of the Waikato region in New Zealand climate is much milder than that of Denmark, the location of the Boldrin and Christensen [10] study. Boldrin and Christensen [10] correlated ash content with seasonal weather changes. It therefore follows that milder weather changes will lead to less variation in the ash content. Additionally, the samples taken by Boldrin and Christensen [10] were heavily contaminated with soil, which also explains their higher ash results.

The ash content of the paunch grass samples in this study is slightly higher than the 5.3 to 10.3 per cent range reported by Tritt and Schuchardt [16]. Ash content can depend on multiple factors, including growing conditions and the amount of soil in the sample [10]. Soil is known to be present in both leafy green waste and paunch grass [10; 19], and could have been ingested by animals eating supplementary feed spread on the ground. The deviations from previously published ash contents are therefore unsurprising.

The Student's t-tests summarised in Table 4.3 indicate there were statistically significant differences between the mean ash contents of leafy green waste and paunch grass on both and overall and seasonal basis. The ash content of leafy green

waste is consistently higher than that of paunch grass (Table 4.28). This is not surprising, as minerals can be removed from paunch grass by digestive processes [19].

Table 4.28: Ash content of leafy green waste and paunch grass samples

	Leafy green waste		Paunch Grass	
Time Period	**Mean (%DM)**	**Standard Deviation (%DM)**	**Mean (%DM)**	**Standard Deviation (%DM)**
Winter 2014	**14.7%**	**4.6%**	**16.8%**	**6.1%**
Jul	13.0%	4.6%	11.3%	2.9%
Aug	15.4%	4.4%	19.6%	5.3%
Spring 2014	**18.2%**	**14.1%**	**15.4%**	**5.9%**
Sep	21.2%	11.7%	23.3%	10.3%
Oct	17.6%	17.5%	13.4%	2.7%
Nov	16.0%	5.0%	14.3%	1.2%
Summer 2014/2015	**14.3%**	**4.7%**	**11.1%**	**1.1%**
Jan	14.6%	5.3%	11.1%	0.9%
Feb	16.2%	5.2%	11.0%	1.3%
Dec	12.1%	1.7%	11.6%	0.6%
Autumn 2015	**17.7%**	**10.3%**	**10.9%**	**4.0%**
Mar	12.7%	4.0%	10.7%	0.5%
Apr	35.0%	10.6%	11.0%	6.0%
May	13.9%	2.7%	11.0%	1.6%
Winter 2015	**15.5%**	**7.9%**	**13.0%**	**2.2%**
Jun	17.1%	8.0%	13.2%	1.9%
Jul	12.0%	6.2%	12.8%	2.4%
Overall	**16.1%**	**9.1%**	**13.1%**	**4.8%**

The ANOVA tests summarised in Table 4.4 indicate the seasonal differences in the mean ash contents of the leafy green waste samples was not statistically significant. Hence the mean ash content of leafy green waste can be assumed to be constant throughout the year. They also indicate there were statistically significant differences between the seasonal mean ash contents of paunch grass samples. These statistically significant differences are presented in Table 4.29. The highest means occur in Winter 2014, Spring 2014, and Winter 2015. Most of the mean ash contents are close to the range reported by Cosgrove*, et al.* [4] (9.2 to 11.0 per cent). There are also minerals in the ruminal fluid which could contribute to the ash content [19]. The highest mean ash contents occurred the winter months, when ingestion of soil and supplements is more likely.

Table 4.29: Statistically significant differences between seasonal mean ash contents of paunch grass

q-Crit	3.501						
Group 1	**Group 2**	**Mean Δ**	**Std Err**	**q-Stat**	**Lower Δ**	**Upper Δ**	**p-Value**
Winter 2014	Autumn 2015	0.0593	0.00704	8.4199	0.0346	0.0839	1.03E-07
Spring 2014	Autumn 2015	0.0445	0.00567	7.8502	0.0247	0.0644	8.12E-07
Winter 2014	Summer 2014/2015	0.0571	0.00745	7.6691	0.0310	0.0832	1.53E-06
Spring 2014	Summer 2014/2015	0.0424	0.00618	6.8665	0.0208	0.0640	2.24E-05
Winter 2014	Winter 2015	0.0380	0.00784	4.8456	0.0105	0.0654	6.50E-03
Spring 2014	Winter 2015	0.0233	0.00664	3.5032	0.0000	0.0465	9.96E-02

4.4 Carbon to Nitrogen Ratio

The GW and PG carbon to nitrogen ratios are summarised in Table 4.30. The overall mean carbon to nitrogen ratios for leafy green waste and paunch grass were 17.2 and 19.0, respectively. These are consistent with carbon to nitrogen ratios published in the literature. Bary*, et al.* [11] reported carbon to nitrogen ratios of 12 for grass-rich material, and 15-20 for mixed leafy green waste. Tritt and Schuchardt [16] reported a carbon to nitrogen ratio of 11-20 for paunch grass.

The overall relative standard deviations for leafy green waste was 47.0 percent of the overall mean. Similarly, the seasonal standard deviations varied from 40.3 to 53.3 percent of the seasonal means. This indicates that a high degree of variability in the individual populations. Leafy green waste can be left in a collection container for several weeks before it is transported to the composting facility [17]. This allows ample time for microbes to consume nitrogenous compounds, thereby increasing the carbon to nitrogen ratio [23].

The overall standard deviation was 26.5 percent of the overall mean for paunch grass. The seasonal standard deviations varied from 11.1 to 30.2 percent of the seasonal means. This is likely due to inherent heterogeneity in the paunch contents. The cattle slaughtered at the abattoir have come from multiple farms which use multiple feed sources. The contents of their rumens will have also been ingested at varying times and stages of maturity prior to slaughter. This means that ruminal

processes have progressed to varying degrees at the time of slaughter. These two factors lead to inevitable variation in the composition of the paunch contents between animals.

Table 4.30: Carbon to nitrogen ratios of leafy green waste and paunch grass samples

	Leafy green waste		Paunch Grass	
Time Period	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**
Winter 2014	**20.6**	**8.3**	**16.4**	**4.9**
Jul	21.4	8.2	22.3	1.5
Aug	20.2	8.3	13.4	3.0
Spring 2014	**14.4**	**7.3**	**15.3**	**2.4**
Sep	13.5	3.5	16.2	3.0
Oct	15.8	9.8	15.0	2.5
Nov	12.6	0.7	15.1	1.6
Summer 2014/2015	**15.3**	**5.6**	**17.3**	**3.1**
Jan	12.9	2.2	17.4	3.5
Feb	18.6	6.8	19.2	2.3
Dec	12.2	1.2	15.8	1.8
Autumn 2015	**17.6**	**7.5**	**23.7**	**2.6**
Mar	19.2	5.6	23.3	1.0
Apr	14.0	1.5	25.3	2.5
May	19.6	10.7	22.6	3.1
Winter 2015	**21.1**	**11.3**	**21.1**	**5.5**
Jun	21.3	9.3	24.3	4.9
Jul	20.8	14.4	17.9	4.1
Overall	**17.2**	**8.1**	**19.0**	**5.0**

The ANOVA tests summarised in Table 4.4 indicate there were no statistically significant differences between the seasonal mean carbon to nitrogen ratios. At a superficial level, this result seems inconsistent with the literature. Boldrin and Christensen [10] reported the highest carbon to nitrogen ratios in winter, and the lowest carbon to nitrogen ratios in spring. This variation was due to an increased proportion of woody material in the garden waste during winter. The leafy green waste excludes woody material which automatically excludes the main source of variation in carbon to nitrogen ratio. It is hence unsurprising that the differences between the seasonal mean carbon to nitrogen ratios were not statistically significant.

The ANOVA tests summarised in Table 4.4 indicate there were statistically significant differences between the seasonal mean carbon to nitrogen ratios of paunch grass. These statistically significant differences are presented in Table 4.31. They most likely occur due to variation in the protein content of the cattle feed ingested by the cattle.

Table 4.31: Statistically significant differences between seasonal mean carbon to nitrogen ratios of paunch grass

q-Crit	3.558						
Group 1	**Group 2**	**Mean Δ**	**Std Err**	**q-Stat**	**Lower Δ**	**Upper Δ**	**p-Value**
Spring 2014	Autumn 2015	8.50	0.90	9.4479	5.30	11.69	7.10E-08
Winter 2014	Autumn 2015	7.40	1.10	6.7169	3.48	11.31	1.16E-04
Summer 2014/2015	Autumn 2015	6.44	1.03	6.2413	2.77	10.12	3.81E-04
Spring 2014	Winter 2015	5.85	1.01	5.8227	2.28	9.43	1.04E-03
Winter 2014	Winter 2015	4.76	1.19	3.9977	0.52	8.99	4.77E-02

The protein content of grasses is relatively high during its rapid growth stages spring and summer, and relatively low when it is in a vegetative state during autumn and winter [6]. This explains much of the seasonal variations in the means. Supplementary feed use may also be a factor. Supplementary feeds are typically used in the cooler months to provide additional protein and energy to cattle when the volume or nutritive value of the primary feed (e.g. grass) is insufficient. Supplementary feed incurs a cost to the farmer. Hence it is unlikely that farmers will feed more supplement than what is required to meet nutritional needs. This protein restriction leads to a lower carbon to nitrogen ratio limit during autumn and winter.

4.5 New Zealand's Protein Production Potential

The leaf protein concentrate production potential of paunch grass and leafy green waste is summarised in Table 4.14. Assuming the leaf protein concentrate produced is comparable to soybean meal, 21,679 tonnes of leaf protein concentrate containing 48% protein could have been produced from paunch grass and green waste between

the 1st of August 2014 and the 31st of July 2015. 65.1% of the protein concentrate would have been produced from green waste, and the remaining 34.9% produced from paunch grass. Assuming the protein concentrate can be sold at the same price as soybean meal (NZD 457.59 per tonne) [32], sales of the concentrate would be worth approximately NZD 9.92 million.

Table 4.32: Leaf protein concentrate production potential of leafy green wastes

Description	Leafy green waste	Paunch Grass	Total of Combined Waste Streams
Total waste stream mass (MT)	2,461,000	106,399	2,567,399
Soft leafy waste (MT)	148,114	106,399	254,513
Soft leafy waste as proportion of total waste stream	6.0%	100.0%	9.9%
Protein in soft leafy waste (MT)	8,470	4,537	13,007
Protein as proportion of total soft leafy waste	5.7%	4.3%	5.1%
Recoverable protein in waste stream (MT)	6,776	3,630	10,406
Leaf protein concentrate production potential (MT)	14,117	7,562	21,679
Leaf protein concentrate as proportion of combined stream production potential	65.1%	34.9%	100.0%
Potential revenue from leaf protein concentrate sales (NZD)	$6.46 million	$3.46 million	$9.92 million

4.6 Comparison to Fresh Leaf Protein Crops

Table 4.33 summarises the recoverable protein present in alfalfa, white clover, red clover, ryegrass, green waste, and paunch grass. It also presents a wet-basis estimate of the yield of protein that could be recovered from those materials. This estimate assumes that the leaf protein production process will recover 80 percent of the recoverable protein in the leaf crops and soft leafy wastes, which is consistent with the performance of the high-yield extraction process developed by Donnelly *et al.* [35]. Surprisingly, the estimated protein recoveries from paunch grass and green waste were slightly greater than the estimated recoveries from all leaf protein crops. This was because the soft leafy wastes contain almost double the dry matter of leaf protein crops. This compensates for their lower protein contents, which are approximately 50 to 60 percent of the protein contents of most leaf protein crops.

Table 4.33: Protein content of leaf crops, paunch grass, and leafy green waste with estimated protein yields

Crop	Dry Matter (w/w%)	Protein Content (g/kg DM)	Recoverable Protein (g/kg fresh material)	Estimated Protein Yield (g/kg fresh material)
Alfalfa [33]	19.9%	206	41.0	32.8
White Clover [33]	16.8%	249	41.8	33.5
Red Clover [33]	19.0%	197	37.4	29.9
Ryegrass [4; 34]	15.0%	236	35.4	28.3
Paunch grass	35.6%	123	43.8	35.1
Green waste	36.2%	125	45.3	36.3

4.7 Implications for Process Design

A summary of results relevant to process design is presented in Table 4.34. Any protein extraction process will need to cope with a significant seasonal variation in moisture content of each material, as well as short-term variation in all other components. The six-sigma operating limits for a leafy green waste and paunch grass protein extraction process are thus summarised in Table 4.35. Note that limits that were greater than unity or less than zero were truncated at that those levels. It is also unlikely that a single sample would present multiple extremes at once. These limits indicate the extraction process will need to cope with significant differences between feedstocks. Green waste and paunch grass have significantly different moisture contents. This difference is crucial, as they will lead to different yields of protein per tonne of delivered material for each material type, even when the crude protein content of the samples is equal on a dry basis. There are also significant differences in the mean amount of crude protein, total carbohydrates, and crude fibre present in each sample.

Any process designed to handle both sample types must be able to cope with these differences. These differences will also lead to different dry basis yields for crude protein, total carbohydrates, and crude fibre. Some of this variation can be attributed to the small sample size relative to the product stream. Although the fortnightly frequency gives good coverage of the variation over time, three samples of approximately 300 grams is unlikely to be fully representative of the 3 to 22 tonnes of material that can be contained in a single delivery of paunch grass or green waste. A larger number of samples would be more representative of the bulk mass and

would thus allow smaller and more accurate standard deviations to be estimated for the chemical composition of the paunch grass and green waste. At a laboratory scale, leaf protein concentrate for analysis can be standardised by combining samples produced from material collected from across the bulk mass and on different days.

Table 4.34: Statistically significant differences relevant to process design

	Leafy green waste		Paunch Grass		GW-PG Comparison	
Component	**Seasonal Variation**	**Short-Term Variation**	**Seasonal Variation**	**Short-Term Variation**	**Annual Means**	**Seasonal Means**
Moisture	✓	✓	×	×	✓	✓
Crude Protein	×	✓	✓	✓	×	×
Organic Nitrogen	×	✓	×	✓	×	×
Lipids	×	✓	×	✓	×	×
Total Carbohydrates	×	✓	×	✓	×	×
Crude Fibre	×	✓	×	✓	×	✓
Ash	×	✓	×	✓	×	×
Carbon to Nitrogen Ratio	×	✓	×	✓	×	×
Nitrite	✓	✓	✓	✓	×	×
Nitrate	✓	✓	✓	✓	×	✓
Ammonia	×	✓	×	✓	×	×

Table 4.35: Mean values for chemical components of leafy green waste and operating limits based on six sigma principles. Asterisk (*) denotes limits calculated from seasonal extremes.

Component	Leafy Green Waste Mean	Paunch Grass Mean	Upper Process Limit	Lower Process Limit
Moisture (percent total mass)	64.7%	81.1%	*89.9%**	*12.6%**
Crude Protein (percent dry matter)	16.2%	15.2%	*32.5%*	*0.0%*
Organic Nitrogen (percent Total N)	74.5%	82.1%	*100.0%*	*26.9%*
Lipids (percent dry matter)	11.9%	10.4%	*35.1%*	*0.0%*
Total Carbohydrates (percent dry matter)	17.1%	20.4%	*36.7%*	*0.1%**
Crude Fibre (percent dry matter)	15.6%	23.1%	*100%*	*26.9%*
Ash (percent dry matter)	16.1%	13.1%	*43%*	*0.0%*
Carbon to Nitrogen Ratio	17.2	19.0	*41.5*	*0.0*

4.8 Leaf Protein Yields and Markets

2.461 million tonnes of solid waste are sent to New Zealand municipal landfills each year. 6.02% of this material is green waste [13]. If this green waste was put through a protein recovery process, 6,676 tonnes of protein could be recovered from leafy green waste, and 3,860 tonnes of protein could be recovered from paunch grass. This assumes that that the resulting LPC is sufficiently sterile and free from toxins.

These yields assume all leafy green waste and paunch grass from across New Zealand is processed through a protein recovery process. As both leafy green waste and paunch grass are not considered human food sources, the protein recovered from them would most likely be used for animal feed. The recovered protein could be used to produce 21,679 tonnes of animal feed containing 48% protein by wet mass (i.e. the same protein content as soybean meal) [32]. If the feed product can be sold for the same price as soybean meal, sales of the feed product would be worth 9.92 million New Zealand dollars per year.

The recovered leaf protein could be used to make an animal feed ingredient similar to Vitalfa, a leaf protein concentrate produced from alfalfa [36]. It could also be pelletised and used as a stand-alone animal feed.

4.9 Composting

An aerobic, efficient composting process can only be achieved when the carbon to nitrogen ratio (C:N) of the feedstock is between 25:1 and 35:1. When the C:N of the feedstock is below 25:1, the feedstock must be mixed with a carbon source to shift the C:N into the optimal range [37]. The feedstock material must also permit sufficient airflow through the compost pile to maintain aerobic composting conditions [37]. If there is too much nitrogen in the raw material, or insufficient airflow through the compost pile, the composting process will become anaerobic. An anaerobic composting process breaks down the feedstock more slowly than an aerobic process. Anaerobic processes also emit nitrous oxides and ammonia which cause odour problems, and methane which is a greenhouse gas [37].

Leafy green wastes typically have a C:N below the optimal range and do not permit sufficient airflow [37]. Wood chips are mixed with these feedstocks as a bulking agent to ensure sufficient airflow and increase the C:N to approximately 30:1 [37]. The mean C:N were 17.2 for leafy green waste and 19.0 for paunch grass, respectively. A large majority of the nitrogen in the samples is organic. The organic nitrogen contents of the leafy green wastes and paunch grass were 75.4 and 82.1 percent of total nitrogen, respectively. Protein is the main form of organic nitrogen [38]. Hence there is potential to shift the C:N of leafy green wastes into the optimal range by removing organic nitrogen in the form of protein.

4.10 Conclusion

Leafy green waste and paunch grass are scientifically feasible feedstocks for leaf protein extraction. On a dry basis, they contain less protein than fresh leaf crops, but also have a higher dry matter content. Hence the amount of protein present in the waste materials on a wet basis (43.5 to 48.3 g recovered protein per kg of fresh material) is comparable to that of fresh crops (35.4 to 41.8 g recovered protein per kg fresh material). The protein present in New Zealand paunch grass could be recovered to produce 21,679 tonnes of protein concentrate, which would contain 48 per cent protein on a wet mass basis. Assuming the protein is similar to soy protein, this protein concentrate could be sold for NZD 9.92 million at July 2017 prices.

The differences between the overall mean moisture, lipid, total carbohydrate, crude fibre, ash, organic nitrogen, nitrite, nitrate, and ammonia contents of leafy green waste and paunch grass are statistically significant at the 10 per cent level, and most are significant at the 1 per cent level. There are statistically differences between the leafy green waste seasonal means for total carbohydrate, organic nitrogen, ammonia, and nitrate levels. These differences are significant at the 10 per cent level, and most are significant at the 5 per cent level. There are also statistically significant differences between the paunch grass seasonal means for moisture, crude protein, total carbohydrate, ash, nitrite, and nitrate levels, as well as for carbon to nitrogen ratios. These differences are significant at the 5 per cent level, and most are significant at the 1 per cent level.

This study has confirmed that leafy green waste and paunch grass should not be composted without a supplementary carbon source and bulking agent. The overall mean carbon to nitrogen ratios for leafy green waste and paunch grass are 17.2 and 19.0, respectively. This is below the 20.0 to 25.0 range recommended for composting. Hence a supplementary carbon source is required to avoid issues with odour, leachate, and ammonia volatilisation. The overall mean moisture contents for these materials are 64.7 and 81.1 per cent by mass, respectively. This is above the 60 per cent upper limit recommended for composting, and hence a bulking agent is required to ensure adequate aeration.

Further work is required to optimise the extraction and recovery of leaf protein from these waste materials. Subsequent research should then investigate the quality of the recovered protein, valorisation potential of the co-products, and financial viability of leaf protein recovery from these materials.

4.11 References

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5 Process Optimisation and Product Characterisation

Three steps were required to maximise the value of leaf protein concentrates (LPCs) recovered from soft leafy wastes such as paunch grass (PG) and leafy green waste (GW):

i. Optimisation of protein extraction from PG and GW into press liquor.
ii. Optimisation of protein concentrate recovery from the press liquor.
iii. Characterisation of the resulting LPCs and their press fibre (PF) and post-recovery liquor (PRL) co-products.

The optimal yield could be achieved by sequential double-extraction (without re-pulping) of PG and GW, acidification treatment of the press liquor, and coagulation by steam injection between 80°C and 85°C for 13 seconds (Table 3.4).

Table 5.1: Protein yields for optimised process steps and overall LPC production process.

Process Step	PG	GW
Raw Material Crude Protein	17.9 % DM	23.6 % DM
Maceration and First Pass through Screw Press	75.2 % CE	37.1 % CE
Second Pass through Screw Press	90.7 % CE	89.7 % CE
Acidification and Steam Injection	64.6 % PRL	50.6 % PRL
Overall LPC Production Process	62.0 % CPR	45.9% CPR

CE = Cumulative Extraction of Raw Material Protein
PRL = Protein Recovery from Liquor
CPR = Cumulative Protein Recovery

The resulting SLW-LPCs contain comparable levels of crude protein and ash to commercial alfalfa LPC (Table 5.2).

Table 5.2: Biochemical profiles of PG-LPC, GW-LPC, and commercial alfalfa LPC.

Component	PG-LPC	GW-LPC	Alfalfa LPC
Crude Protein (DM%)	37.7 %	24.3 %	51.69 %
Ash (DM%)	10.2 %	27.1 %	14.1 %

Their amino acid profiles are also similar (Section 5.3.1). This should allow them to be sold at a protein-content adjusted price based on that of alfalfa leaf protein concentrate (NZD 1670 per metric tonne [1]). This suggests that production of leaf protein concentrates from leafy green wastes may be financially viable.

5.1 Leaf Protein Extraction Optimisation

Initial protein extractions were performed according to a control method that essentially mixed water and the raw material at a 2:1 mass ratio in an open container, transferred and macerated the resulting mixture in a food processor, and passed the pulp through the screw press once. The control extractions (Table 5.3 and Table 5.4) indicate baseline demonstrate that on average, the control method extracts 76.2 percent of the crude protein in paunch grass, and 63.2 percent of the crude protein in leafy green waste.

Table 5.3: Control paunch grass protein extractions.

Quantity	Run 1	Run 2	Run 3	Run 4	Run 5	Mean
Paunch Grass Wet Mass (g)	100.26	100.78	204.75	187.32	201.07	158.84
Paunch Grass DM (g)	25.08	25.21	33.21	30.38	32.61	29.30
Paunch Grass CP (g)	4.50	4.52	5.96	5.45	5.85	5.26
Press Fibre Wet Mass (g)	7.74	5.41	38.41	43.76	38.63	26.79
Press Fibre MC (w/w%)	63.5%	72.1%	51.0%	57.9%	48.6%	58.6%
Press Fibre DM (g)	2.83	1.51	18.81	18.41	19.85	12.28
Press Fibre CP (g)	2.48	2.06	1.40	1.43	1.32	1.74
Press Liquor Wet Mass (g)	244.94	226.08	506.37	465.57	494.30	*387.45*
Press Liquor CP (g)	2.02	2.46	4.56	4.02	4.53	3.52
Protein Extraction Ratio (g PLP/100 g PG protein)	69.1%	84.0%	76.5%	73.8%	77.5%	76.2%
Protein Yield (g PLP/100 g dry PG)	12.4%	15.1%	13.7%	13.2%	13.9%	13.7%

Table 5.4: Control leafy green waste protein extractions.

Quantity	Run 1	Run 2	Run 3	Run 4	Run 5	Mean
Leafy Green Waste Wet Mass (g)	99.30	99.56	100.08	100.22	81.22	96.08
Leafy Green Waste DM (g)	24.84	24.91	25.04	25.07	27.31	25.44
Leafy Green Waste CP (g)	5.87	5.88	5.92	5.92	6.45	6.01
Press Fibre Wet Mass (g)	76.76	60.86	47.35	43.89	25.22	50.82
Press Fibre MC (w/w%)	55.7%	55.6%	53.7%	56.4%	56.7%	56.0%
Press Fibre DM (g)	34.04	27.01	21.94	19.12	10.92	22.61
Press Liquor Wet Mass (g)	180.88	193.24	212.16	202.53	207.52	*199.27*
Press Fibre CP (g)	1.04	0.93	4.46	2.86	1.60	2.18
Press Liquor CP (g)	4.83	4.95	1.46	3.07	4.86	3.83
Protein Extraction Ratio (g PLP/100 g PG protein)	82.3%	84.2%	23.9%	50.2%	75.2%	63.2%
Protein Yield (g PLP/100 g dry PG)	19.4%	19.9%	5.7%	11.9%	17.8%	14.9%

Several approaches were trialled to improve this method, including:

iv. Varying the amount of water added to samples prior to maceration;

v. Varying the number passes through the screw press, both with and without re-maceration between passes; and

vi. Pre-treating samples of raw material with alkali solutions.

Detailed methodology can be found in Chapter 3 (§3.3.2).

5.1.1 Water Addition Ratios

Initial experiments determined the amount of water that could be absorbed and adsorbed by the leafy green wastes, followed by the time required to achieve maximal binding of water to the leafy green wastes. Extractions were then performed on samples that had been soaked in enough water for enough time to absorb and adsorb half of the maximum amount of water that can be bound to the wet mass of each sample type. The water addition ratio used in the control method turned out the most sensible. Only marginal improvements in protein extraction could be obtained by adding additional water, and the costs of doing so on an industrial scale were likely to outweigh the benefits.

Paunch Grass

The effect of water addition on the moisture contents of paunch grass is presented in Figure 5.1. It appears that paunch grass can take up water weighing approximately 1.5 times its mass. As the control method already mixes water with paunch grass at a 2:1 wet mass ratio, adding further water is unlikely to improve protein extraction.

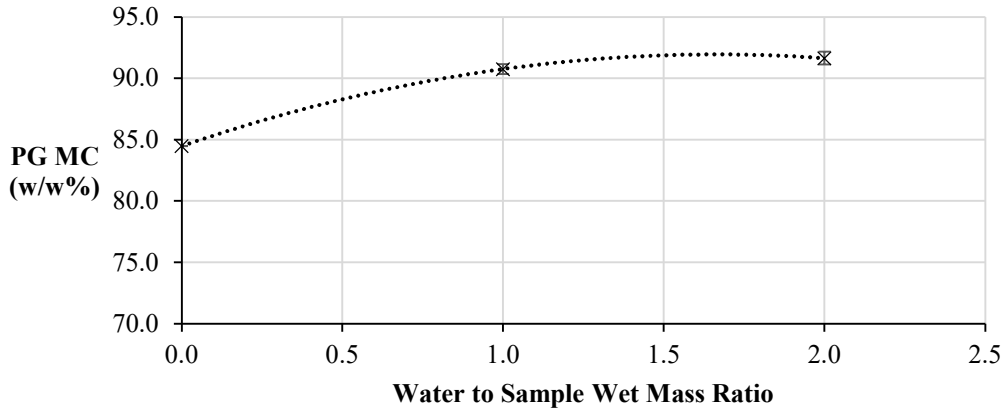

Figure 5.1: Water adsorption and adsorption by paunch grass (wet mass basis).

The outcomes of the soaking time experiments are presented in Table 5.5. It appears that the maximum protein recovery ratio and yield are achieved after a 20-minute soak. The lower yields achieved thereafter are likely due to enzymatic and microbial action degrading protein and volatilising nitrogen in the sample. This is unsurprising given the high levels of microbes present in paunch grass. Therefore, industrial scale processing should keep the time between the initial wetting of the paunch grass and coagulation of protein to under 20 minutes. Whilst the 20-minute protein extraction ratio is higher than the control extractions (Table 5.3), the protein yield only slightly higher. Given that longer residence times require larger equipment that incurs greater capital costs, deliberately increasing the residence time beyond the normal time required to pass through processing equipment cannot be justified. Soak times under 20 minutes could not be tested due to limitations of the experimental equipment and setup; however it would be interesting to investigate this in a future study.

Table 5.5: Protein extraction from soaked paunch grass samples.

Soaking Time (min)	**20**	**30**	**40**
Average Press Liquor Crude Protein (g)	2.52	2.22	2.36
Average Protein Recovery Ratio (g PLP/100 g paunch grass protein)	85.74	77.57	80.32
Average Protein Yield (g PLP/100 g dry paunch grass)	15.38	13.91	14.41

Leafy green waste

The effect of water addition on the moisture contents of paunch grass is presented in Figure 5.2. It appears that leafy green waste can take up water weighing approximately 3.0 times its wet mass, although there is only a small change in moisture content after adding water equal to 2.0 times the sample wet mass. As the control method already mixes water with paunch grass at a 2:1 wet mass ratio, adding further water is unlikely to improve protein extraction.

The outcomes of the soaking time experiments are presented in Table 5.6. It appears that the maximum protein recovery ratio and yield are achieved after a 20-minute soak, and that further soaking time has a negligible effect on the protein extraction ratio. However, a 20-minute soak results in a substantially higher protein extraction ratio and protein yield than in the control experiments (Table 5.4). The water may

have softened the plant cell walls, enabling more effective mechanical lysis of the cells to extract cytoplasmic protein into the press liquor.

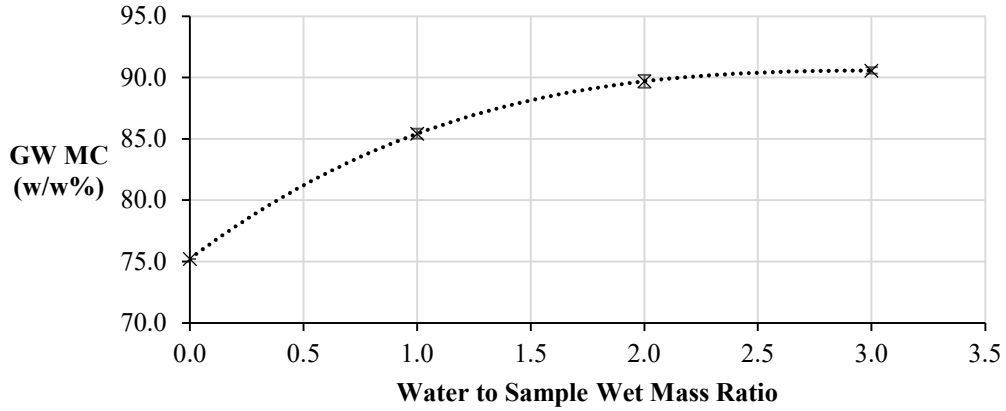

Figure 5.2: Water adsorption and adsorption by leafy green waste.

Industrial scale processes should therefore be designed to keep the time between initial wetting of the paunch grass and coagulation of protein to approximately 20 minutes. Longer residence times require larger equipment that incurs greater capital costs, and the improvements in protein extraction ratio and protein yield are both marginal and inconclusive, so a residence time greater than 20 minutes cannot be justified. Soak times under 20 minutes could not be tested due to limitations of the experimental equipment and setup; however it would be interesting to investigate this in a future study.

Table 5.6: Protein extraction from soaked leafy green waste samples.

Soaking Time (min)	**20**	**30**	**40**
Average Press Liquor Protein (g)	4.32	4.16	3.56
Average Protein Extraction Ratio (g protein/100 g leafy green waste protein)	72.52	69.47	72.97
Average Protein Yield (g protein/100 g dry leafy green waste)	17.13	16.41	17.24

Leafy green waste samples were also soaked in half the mass of water they could take up and extracted according to the control method. The protein extraction ratios and yields from these experiments (Table 5.7) are similar to those achieved during the control experiments (Table 5.4). This justifies adding enough water to fully-soak leafy green waste and allowing sufficient residence time for it to soften.

Table 5.7: Protein extraction from half-soaked leafy green waste samples.

Quantity	1	2	Mean
Wet Leafy green waste (g)	49.93	50.74	50.34
Leafy Green Waste Moisture Content (w/w%)	0.749816	0.749816	0.75
Leafy Green Waste DM (g)	12.49169	12.69433	12.59
Water Added (g)	34.01	34.02	34.02
Press Liquor Protein (g)	1.63	1.81	1.72
Protein Extraction Ratio (g protein/100 g leafy green waste protein)	55.29	60.26	57.78
Protein Yield (g protein/100 g dry leafy green waste)	13.06	14.24	13.65

5.1.2 Number of Passes Through Screw Press

Initial experiments soaked the press fibre in water before passing it through the screw press. Subsequent experiments macerated the soaked fibre before it was passed through the screw press again and attempted to make three passes through the screw press without re-macerating the soaked press fibres. The initial trial that soaked the press fibre before passing it through the screw press was the most effective. Re-maceration of soaked fibres yielded moderate improvements in protein extraction efficiency for paunch grass and major improvements in protein extraction efficiency from leafy green waste. Passing the plant material through the screw press thrice proved to be non-viable. The third pass through the screw press produced a paunch grass protein extraction efficiency similar to that obtained from two passes with intermediary maceration. A third pass through the screw press extracted no further protein from leafy green waste fibre, and often led to complete disintegration of the fibre fraction. This leads to all the fibrous content being entrained into the process liquor, thus causing the extraction to fail.

Paunch Grass

Both dual-pass screw press extraction methods (Table 5.8 and Table 5.9) result in a substantial (6.1-27.7%) improvement in protein extraction ratio and protein yield over the controls in Table 5.3. Intermediary maceration may be justified, as its protein extraction ratio and protein yield are substantially higher than when intermediary maceration are not used. However, industrial-scale intermediary maceration will require additional conveyors and a disc mill to be placed between the two screw presses. This will increase the capital and operating costs for the protein extraction process and will need to be justified in the process design.

Table 5.8: Dual-pass paunch grass protein extractions (without intermediary maceration).

Quantity	Run 1	Run 2	Run 3	Mean
Paunch Grass Mass (g)	82.09	70.54	182.96	*111.86*
Paunch Grass MC (w/w%)	83.78%	83.78%	83.78%	*83.78%*
Paunch Grass CP (g)	2.39	2.05	5.32	*3.25*
Press Fibre Mass (g)	15.40	16.48	35.15	22.34
Press Fibre MC (w/w%)	51.0%	57.9%	48.6%	52.5%
Press Fibre CP (g)	0.56	0.54	1.20	0.76
Double-Pressed Fibre Wet Mass (g)	10.95	14.59	50.91	25.48
Double-Pressed Fibre MC (w/w%)	59.4%	64.3%	65.0%	62.9%
Double-Pressed Fibre CP (g)	0.30	0.30	0.89	0.50
Press Liquor CP (g)	0.26	0.23	0.30	0.27
Double-Pass Protein Extraction Ratio [g JP/100 g PG protein]	80.94	78.13	82.73	80.60
Single-Pass Protein Extraction Ratio [g JP/100 g PG protein]	76.52	73.83	77.52	75.96
Protein Extraction Ratio Improvement (cf. single pass)	5.8%	5.8%	6.7%	6.1%
Double-Pass Protein Yield (paunch grass basis) [g JP/100 g dry PG]	14.52	14.01	14.84	14.46
Single-Pass Protein Yield (paunch grass basis) [g JP/100 g dry PG]	13.73	13.24	13.90	13.62
Yield Improvement	5.8%	5.8%	6.7%	6.1%

Table 5.9: Dual-pass paunch grass protein extractions (with intermediary maceration).

Quantity	Run 1	Run 2	Mean
Paunch Grass Mass (g)	63.75	70.72	*67.24*
Paunch Grass MC (w/w%)	83.78%	83.78%	*83.78%*
Paunch Grass CP (g)	1.85	2.06	*1.96*
Press Fibre Mass (g)	11.96	16.52	14.24
Press Fibre MC (w/w%)	51.0%	57.9%	54.5%
Press Fibre CP (g)	0.44	0.54	0.49
Double-Pressed Fibre Wet Mass (g)	11.96	16.52	14.24
Double-Pressed Fibre MC (w/w%)	70.7%	58.1%	64.4%
Double-Pressed Fibre CP (g)	0.23	0.39	0.31
Press Liquor CP (g)	0.20	0.14	0.17
Double-Pass Protein Extraction Ratio [g JP/100 g PG protein]	96.98	94.99	95.98
Single-Pass Protein Extraction Ratio [g JP/100 g PG protein]	76.52	73.83	75.17
Protein Extraction Ratio Improvement (cf. single pass)	26.7%	28.7%	27.7%
Double-Pass Protein Yield (paunch grass basis) [g JP/100 g dry GW]	17.40	17.04	17.22
Single-Pass Protein Yield (paunch grass basis) [g JP/100 g dry GW]	13.73	13.24	13.48
Yield Improvement	26.7%	28.7%	27.7%

The triple-pass extraction results in Table 5.10 indicate that the protein extraction ratio and protein yield from washing the fibre-washing and passing it through the screw-press for a third time are similar to that achieved by two passes through the screw press with intermediary maceration. On an industrial scale, a third screw-press stage and the associated larger liquor recycle system or water use will incur unjustifiable additional capital and operating costs. This demonstrates that a dual-pass screw-press extraction (with intermediary maceration) is sufficient to achieve maximal paunch grass protein extraction.

Table 5.10: Triple-pass paunch grass protein extractions (without intermediary maceration).

Quantity	Value
Double-Pressed Fibre Mass (g)	16.22
Double-Pressed Fibre MC (w/w%)	65.0%
Double-Pressed Fibre CP (g)	0.28
Triple-Pressed Fibre Wet Mass (g)	15.15
Triple-Pressed Fibre MC (w/w%)	62.6%
Triple-Pressed Fibre CP (g)	0.24
Triple-Pressed Liquor CP (g)	0.05
Triple-Pass Protein Extraction Ratio [g JP/100 g PG protein]	95.43
Single-Pass Protein Extraction Ratio [g JP/100 g PG protein]	77.52
Protein Extraction Ratio Improvement (cf. single pass)	23.1%
Triple-Pass Protein Yield (paunch grass basis) [g JP/100 g dry PG]	17.12
Single-Pass Protein Yield (paunch grass basis) [g JP/100 g dry PG]	13.90
Yield Improvement	23.1%

Leafy green waste

Both dual-pass screw press extraction methods (Table 5.11 and Table 5.12) result in very substantial improvements (92-176%) in protein extraction ratio and yield over the controls in Table 5.4. The Run 1 in both Table 5.11 and Table 5.12 showed an especially large improvement. The variation in improvement could be explained by heterogeneity of the GW samples. The Run 1 samples seem to contain plant material which is more susceptible to mechanical disruption (i.e., softer) than those used in subsequent runs. The additional processing stesps therefore liberate protein to extract into the press liquor. Unlike with paunch grass, intermediary maceration can likely be justified, as it substantially increases the protein extraction ratio and

protein yield; it also leads to more consistent results. However, it will require a disc mill and additional conveyors, which will incur greater capital and operating costs.

Table 5.11: Dual-pass leafy green waste protein extractions (without intermediary maceration).

Quantity	Run 1	Run 2	Run 3	Mean
Leafy Green Waste Mass (g)	38.26	38.54	62.28	*46.36*
Leafy Green Waste MC (w/w%)	74.23%	74.23%	66.37%	*71.61%*
Leafy Green Waste CP (g)	2.33	2.35	4.95	*3.21*
Press Fibre Mass (g)	18.10	16.88	19.34	18.11
Press Fibre MC (w/w%)	53.7%	56.4%	56.7%	56.0%
Press Fibre CP (g)	1.77	1.17	1.23	1.39
Double-Pressed Fibre Wet Mass (g)	9.86	8.69	10.99	9.85
Double-Pressed Fibre MC (w/w%)	60.7%	60.0%	60.8%	60.5%
Double-Pressed Fibre CP (g)	0.76	0.50	0.57	0.61
Press Liquor CP (g)	1.01	0.67	0.66	0.78
Double-Pass Protein Extraction Ratio [g JP/100 g GW protein]	67.33	78.87	103.75	83.32
Single-Pass Protein Extraction Ratio [g JP/100 g GW protein]	23.95	50.25	75.24	49.81
Protein Extraction Ratio Improvement (cf. single pass)	181.2%	57.0%	37.9%	92.0%
Double-Pass Protein Yield (paunch grass basis) [g JP/100 g dry GW]	15.91	18.63	24.51	19.68
Single-Pass Protein Yield (paunch grass basis) [g JP/100 g dry GW]	5.66	11.87	17.78	11.77
Yield Improvement	181.2%	57.0%	37.9%	92.0%

The first triple-pass extraction led to complete disintegration of the fibre, which was then entrained into the liquor. A second triple-pass extraction carried out with a larger fibre sample (Table 5.13) indicated that no further improvement in protein extraction ratio and protein yield is achieved by a second washing of the fibre and pass through the screw-press. A dual-pass screw-press extraction (without intermediary maceration) is therefore the best approach to achieve maximal extraction of leafy green waste protein into the press liquor. This approach achieved large improvements in protein extraction ratio and yield over the the control

extractions but is simpler and therefore less costly than including intermediary maceration step between presses.

Table 5.12: Dual-pass leafy green waste protein extractions (with intermediary maceration).

Quantity	Run 1	Run 2	Mean
Leafy Green Waste Mass (g)	38.38	38.41	*38.40*
Leafy Green Waste MC (w/w%)	74.23%	74.23%	*74.23%*
Leafy Green Waste CP (g)	2.34	2.34	*2.34*
Press Fibre Mass (g)	18.16	16.82	17.49
Press Fibre MC (w/w%)	53.7%	56.4%	55.0%
Press Fibre CP (g)	1.78	1.16	1.47
Double-Pressed Fibre Wet Mass (g)	10.65	8.73	9.69
Double-Pressed Fibre MC (w/w%)	64.5%	70.1%	67.3%
Double-Pressed Fibre CP (g)	0.77	0.36	0.56
Press Liquor CP (g)	1.01	0.80	0.91
Double-Pass Protein Extraction Ratio [g JP/100 g GW protein]	87.43	94.07	90.75
Single-Pass Protein Extraction Ratio [g JP/100 g GW protein]	23.95	50.25	37.10
Protein Extraction Ratio Improvement (cf. single pass)	265.1%	87.2%	176.2%
Double-Pass Protein Yield (paunch grass basis) [g JP/100 g dry GW]	20.66	22.22	21.44
Single-Pass Protein Yield (paunch grass basis) [g JP/100 g dry GW]	5.66	11.87	8.76
Yield Improvement	265.1%	87.2%	176.2%

Table 5.13: Triple-pass leafy green waste protein extractions (without intermediary maceration).

Quantity	Value
Double-Pressed Fibre Mass (g)	45.43
Double-Pressed Fibre MC (w/w%)	60.8%
Double-Pressed Fibre CP (g)	2.35
Triple-Pressed Fibre Wet Mass (g)	31.25
Triple-Pressed Fibre MC (w/w%)	57.0%
Triple-Pressed Fibre CP (g)	2.35
Triple-Pressed Liquor CP (g)	0.00
Third-Pass Protein Extraction Ratio [g JP/100 g Double-Press Fibre Protein]	0.00

Biological Explanation of Mechanical Disruption Efficacy

These outcomes demonstrate that two maceration and two pressing steps will fully disrupt the plant cells to maximise the cytoplasmic protein completely available for screw press extraction. This is approach is consistent with the designs patented by Donnelly *et al.* [2] and proposed by McDonald [3], which macerated the plant

material prior to its first pass through the screw press, pass it through a disc mill, and then through a second screw press.

The disc mill and second pass through the screw press ensure consistent extraction of cytoplasmic protein into the liquor. This is likely due to the plant material being more mature and containing a greater lignin content, which makes mechanical disruption of the cell walls more difficult. The second maceration step also leads to greater improvements in the leafy green waste protein extraction ratio than it does for the paunch grass ratio. This is because plant material is churned and mechanically disrupted inside the rumen, which starts to break down the plant cell walls and partially accomplishes the purpose of the second maceration step.

The double-press extractions without intermediary maceration (Table 5.8 and Table 5.11) approximate the design of Donnelly*, et al.* [2]; the triple-press extractions (Table 5.10 and Table 5.13) approximate the approach proposed by McDonald [3]; and the double-press extractions with intermediary-maceration (Table 5.9 and Table 5.12) approximate a novel approach to screw-press extraction of leaf proteins. The latter approach achieved maximal protein extraction with only two passes through the screw press, rather than the three passes proposed by McDonald [3]. It is postulated that the third pass through the screw press acts primarily as a maceration step and therefore redundant when intermediary maceration is used. As the second maceration step uses cutting and pulsing actions, it can further disrupt plant cell walls without causing the fibre to disintegrate; an additional pass through the screw press primarily utilises a rubbing motion that causes the plant cell walls to tear and separate from each other, leading to fibre disintegration. This should be verified by future research.

5.1.3 Alkali Pre-Treatments

Samples of paunch grass and leafy green waste were treated with weak sodium hydroxide solutions (0.125 M and 0.625 M) at three temperature levels (40°C, 50°C, and 60°C) according to a full-factorial design prior to screw-press protein extraction by the control method. Detailed methodology can be found in Section 3.3.1.

The resulting protein extraction yields (Table 5.14 and Table 5.15) were similar to those achieved by double-screw press extraction and intermediary maceration of paunch grass and leafy green waste (Table 5.9 and Table 5.12). This demonstrates that the weakening cell-walls by hydrolysis leads to outcomes comparable to what can be achieved by extensive mechanical disruption. The cost of providing chemicals, process heat, and wastewater treatment for alkali pre-treatment is likely to be more expensive than a using extensive mechanical treatments of waste green plant materials. This should be confirmed by a future technoeconomic analysis.

Table 5.14: Protein extractions from alkali-treated paunch grass samples using control screw-press method.

		Protein Extraction Ratio (g PLP/100 g GW)		Protein Yield (g PLP/100 g dry GW)	
NaOH Concentration ($molL^{-1}$)	Temperature (°C)	Replicate A	Replicate B	Replicate A	Replicate B
0.125	40	90.9	91.6	16.3	16.4
0.125	50	92.1	92.7	16.5	16.6
0.125	60	89.2	91.2	16.0	16.4
0.625	40	93.6	94.2	16.8	16.9
0.625	50	91.3	89.7	16.4	16.1
0.625	60	91.3	92.1	16.4	16.5

Table 5.15: Protein extractions from alkali-treated leafy green waste using control screw-press method.

		Protein Extraction Ratio (g PLP/100 g GW)		Protein Yield (g PLP/100 g dry GW)	
NaOH Concentration ($molL^{-1}$)	Temperature (°C)	Replicate A	Replicate B	Replicate A	Replicate B
0.125	40	32.0	46.0	7.5	10.9
0.125	50	33.4	18.2	7.9	4.3
0.125	60	71.9	70.5	17.0	16.7
0.625	40	52.8	60.5	12.5	14.3
0.625	50	51.2	42.9	12.1	10.1
0.625	60	47.7	57.0	11.3	13.5

Additionally, although the acid and base treatments weaken the plant cell walls, they will also alter the pH of the press liquor if the treated material is not neutralised or washed prior to passing through the screw press. A strongly acid or alkali liquor may or may not be beneficial. An acidic or alkali liquor pH could inhibit enzymatic and microbial degradation of the protein; yet it could also degrade the protein which has been extracted into the liquor. The effect of liquor pH on the preservation and

degradation of extracted proteins could be another interesting topic to investigate during future work.

5.2 Leaf Protein Recovery Optimisation

Preliminary protein recoveries were performed by injecting press liquors with steam to heat them to approximately 85°C. Two main approaches were trialled to improve this method:

i. Minimising the Zeta potential of the press liquor prior to coagulation; and
ii. Varying the amount of time that the press liquor was held at 85°C.

The most effective method treated the press liquor with hydrochloric acid prior to coagulation prior by steam injection to 85°C for 13 seconds. Detailed methodology can be found in Section 3.3.2.

5.2.1 Zeta Potential Minimisation

Hydrochloric acid proved to be the only treatment that increased the Zeta potential of both paunch grass and leafy green waste press liquors to zero and was the most effective flocculating agent for both press liquor types (Figure 5.3 and Figure 5.4) on a mass basis.

On a molar basis, similar amounts of hydrochloric acid and calcium hydroxide were required to minimise the Zeta potential of the paunch grass press liquor (Figure 5.5). The Zeta potential of the leafy green waste press liquor can be minimised with a smaller amount of hydrochloric acid than calcium hydroxide (Figure 5.6). Calcium hydroxide and sodium hydroxide substantially increased the Zeta potentials of both liquor types without them approaching zero (Figure 5.3 and Figure 5.4).

Although magnesium hydroxide caused some improvement in the Zeta potential of the paunch grass press liquor, it was even less effective than calcium hydroxide. When used to treat leafy green waste liquor, it caused the Zeta potential to diverge from zero, making the flocculation even more difficult. The poor performance of the calcium hydroxide and magnesium hydroxide treatments are surprising and are inconsistent with the principles underpinning the industrial-scale recovery method patented by De Jong *et al.* [4] that proscribe the use of divalent cations as a preferred treatment to assist protein precipitation.

One explanation for the principles of De Jong*, et al.* [4] being inconsistent with the outcomes of these experiments is the low total solids concentration in the press liquor. The De Jong*, et al.* [4] method calls for the total solids press liquor to be concentrated by ultrafiltration to produce a "soluble plant protein concentrate, preferably comprising 25 to 50 mass percent protein, wherein said soluble plant protein concentrate is essentially free of salts and phenolic compounds." The total solids contents of paunch grass (2.13 mass percent) and leafy green waste (3.38 mass percent) are an order of magnitude below the minimum concentration specified by De Jong*, et al.* [4]. The total solids were not entirely protein (Table 5.2), so the protein content of the press liquors will be even lower again. Flocculation is generally more effective at higher concentrations of total solids [5], so it is possible the liquor contained insufficient total solids for the electrochemical flocculation interactions to take effect.

Unlike in De Jong*, et al.* [4], the salts and phenolic compounds were not removed from the press liquor. They may have therefore inhibited the biochemical and electrochemical interactions between divalent cations and proteins that lead to floc formation. Similarly, adding calcium bentonite clay also proved to be an ineffective protein flocculation treatment (Figure 5.3 and Figure 5.4) despite its success in other studies [6]. Characterising potential inhibitory effects of salts and phenolic compounds on floc formation by is outside the scope of this investigation but could be an interesting topic for future research.

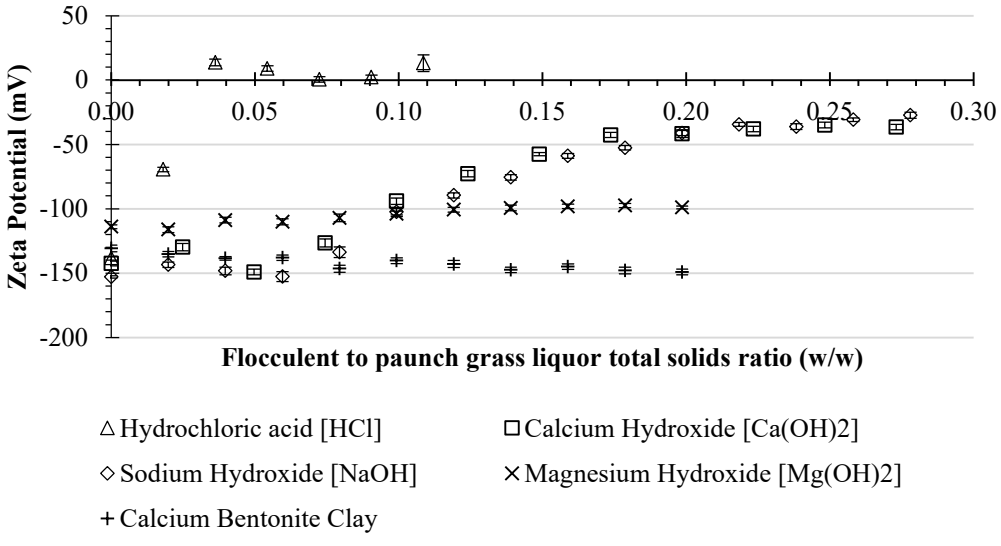

Figure 5.3: Cumulative effect of flocculent mass on paunch grass liquor zeta potential. Error bars represent instrument measurement uncertainties.

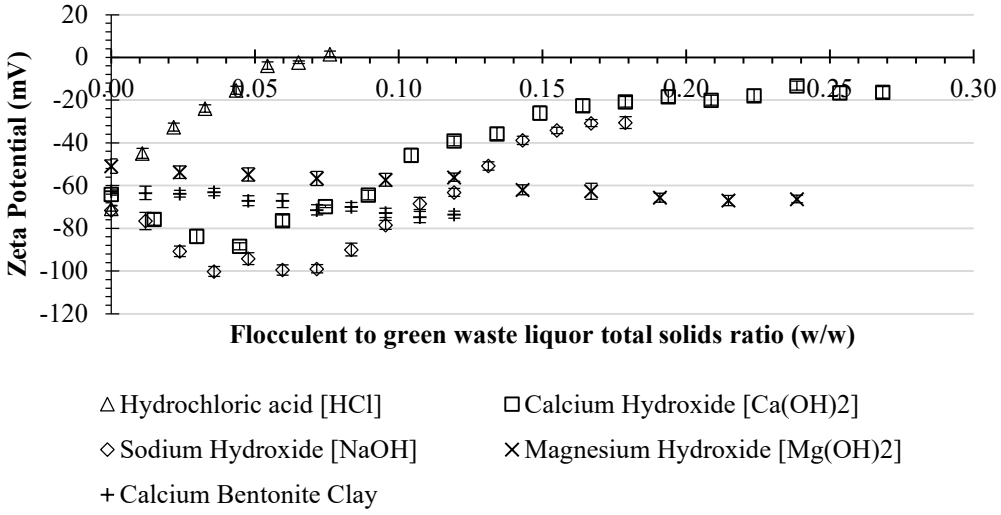

Figure 5.4: Cumulative effect of flocculent mass on leafy green waste liquor zeta potential. Error bars represent instrument measurement uncertainties.

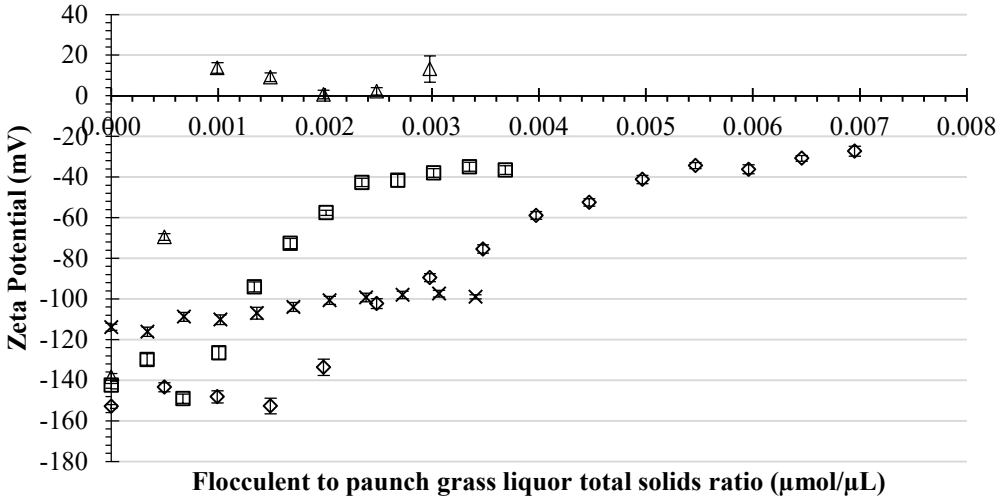

Figure 5.5: Cumulative effect of flocculent amount on paunch grass liquor zeta potential. Error bars represent instrument measurement uncertainties.

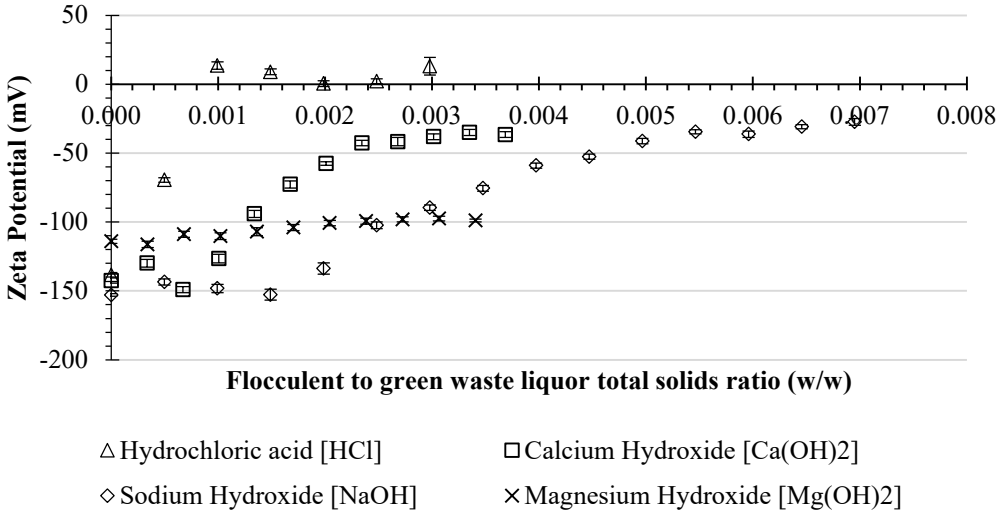

Figure 5.6: Cumulative effect of flocculent amount on leafy green waste liquor zeta potential. Error bars represent instrument measurement uncertainties.

5.2.2 Variation of Holding Time

The mean cumulative protein recoveries for the various SLW liquors extracted by a dual-pressing process are presented in Table 5.16. Analysis of Variance (ANOVA) was performed on the dataset for each liquor type; the differences in mean cumulative protein recovery (CPR) corresponding to the various holding times proved to be insignificant ($P>0.05$). This allows the mean CPR for each liquor dataset to be used in mass balance calculations. It also allows the steam injection unit to be optimised for product sterility rather than maximum protein recovery. The partial sterilisation achieved during the steam injection process is a key topic for further investigation; pending experimental data it is best assume a conservative 30 second residence time for the steam injection unit. This residence time is based on the upper time limit for the heat treatment used for partial sterilisation (i.e., pasteurisation) of milk, which is for a lower operating temperature than the steam injection unit (72°C cf. 85°C) [7]. An acceptable degree of sterility should therefor be achieved by this time and temperature [7; 8].

Table 5.16: Cumulative recovery of protein from SLW liquors by steam coagulation and centrifugation

Holding Time (s)	Acidified PG Liquor	Acidified GW Liquor	PG Liquor	GW Liquor
0	62.94 %	47.62 %	48.33 %	36.75 %
13	61.98 %	46.80 %	67.54 %	31.84 %
16	63.42 %	45.61 %	45.80 %	34.02 %
60	63.95 %	46.65 %	46.57 %	35.84 %
270	60.32 %	43.49 %	38.03 %	36.89 %
480	59.40 %	44.47 %	51.93 %	37.38 %
Mean	62.00 %	45.93 %	45.77 %	35.45 %

5.3 Characterisation of Leaf Protein Concentrates and Process Co-Products

The leaf protein concentrates recovered from the press liquor, press fibre, and post-recovery liquor were characterised to determine their processing properties as well as their nutritive and financial values (see [Section] for methodology):

i. The leaf protein concentrate samples were characterised for their moisture contents, crude protein contents, amino acid profiles, thermal properties, and ash contents.

ii. The press fibre samples were characterised for their moisture, crude protein, crude lipid, carbon to nitrogen ratios, ash contents, and thermal properties.

5.3.1 Leaf Protein Concentrates

The crude protein and ash contents of the PG and GW leaf protein concentrates are compared with those of alfalfa LPC in Table 5.17. The average crude protein contents of the PG LPCs and GW-LPCs are substantially less (ca. 15-26 % DM) than the crude protein content of the alfalfa leaf protein concentrate. The average ash contents of the PG LPCs are slightly lower than alfalfa LPC; those of the GW LPCs are considerably higher. Elevated ash levels may be problematic, as they could indicate unacceptable levels of heavy metals in the LPCs.

Table 5.17: Comparison of LPC crude protein and ash contents

Component	PG-LPC (Acid)	PG-LPC	GW-LPC (Acid)	GW-LPC	Alfalfa LPC
Crude Protein (DM%)	37.7 ± 0.6 %	33.9 ± 0.9 %	24.3 ± 0.5 %	21.5 ± 0.3 %	51.7 %
Ash (DM%)	10.2 ± 0.4 %	11.0 %	27.1 ± 0.3 %	33.1 %	14.10 ± 0.04%

ICP-MS analyses of ash produced from LPC samples (Table 5.18) indicated unacceptable levels of arsenic, cadmium, and lead in the LPCs. High levels of heavy metals in the LPC could limit its inclusion in animal feed formulations as per Table 5.18., or make it entirely unsuitable for animal feed. Boldrin and Christensen [9] suggest this heavy metal contamination may have occurred due to soil contamination of garden waste. Similarly, soil ingested by cattle could lead to elevated levels of heavy metals in the ruminal contents. Relatively small feedstock samples (ca. 50 g) were used to produce leaf protein concentrate samples for analysis, so soil contamination of feedstock materials could have a particularly large effect on the heavy metal profiles of the LPC materials. There is potential for the LPC heavy metal levels to decrease when larger sample sizes and equipment are used. This is the most straightforward approach to reducing the heavy metal content of the LPC. This is a key area of future research, as meeting heavy metal limits is a critical requirement for using the LPC in animal feed.

Table 5.18: Heavy metal contents of leaf protein concentrates cf. EU standard animal feed specifications [10]

Material	Arsenic (75, 75-91)	Cadmium (110, 111, 114)	Lead (206-208)	Maximum LPC Content of Whole Feed (DM %)
Steam Coagulated Leafy Green Waste Protein	23.2	1.92	123.8	19.6%
Acid-Steam Coagulated Leafy Green Waste Protein	33.0	1.82	146.7	13.8%
Steam Coagulated Paunch Grass Protein	3.77	6.12	22.6	18.6%
Acid Steam Coagulated Paunch Grass Protein	2.99	12.1	42.0	9.4%
Maximum Level in EU Standard Feed (mg/kg)	**4**	**1**	**10**	**-**

Alternatively, the LPC heavy metal levels could be reduced by additional process steps. This would likely involve adjusting the protein slurry pH prior to centrifugation to dissolve metal cations in the process effluent [5]. Another approach would be to add a selective coagulant to the screw press liquor to remove heavy metals prior to the recovery of protein [5]. Heavy metals could also be removed by an ion-exchange system, but this is unlikely to be cost-effective [11].

Amino Acid Profiles

The amino acid profiles of alfalfa LPC [12], PG-LPC, and GW-LPC are compared in Figure 5.7 and agree with the general conclusion of Pirie [13]: all three LPC amino acid profiles are consistent with the general pattern of AA profiles published for other leaf proteins [12; 13; 14; 15].

The essential amino acid (EEA) profiles of PG-LPC and GW-LPC recovered from acidified press liquor as well as commercially produced alfalfa LPC are presented and compared with the standard EAA profiles specified by the World Health Organization [16] in Figure 5.8. The PG-LPC exceeds all EAA specifications; whilst the GW-LPC is deficient in histidine, lysine, and methionine. It is therefore an inadequate source of these EAAs when used as the sole dietary source of protein.

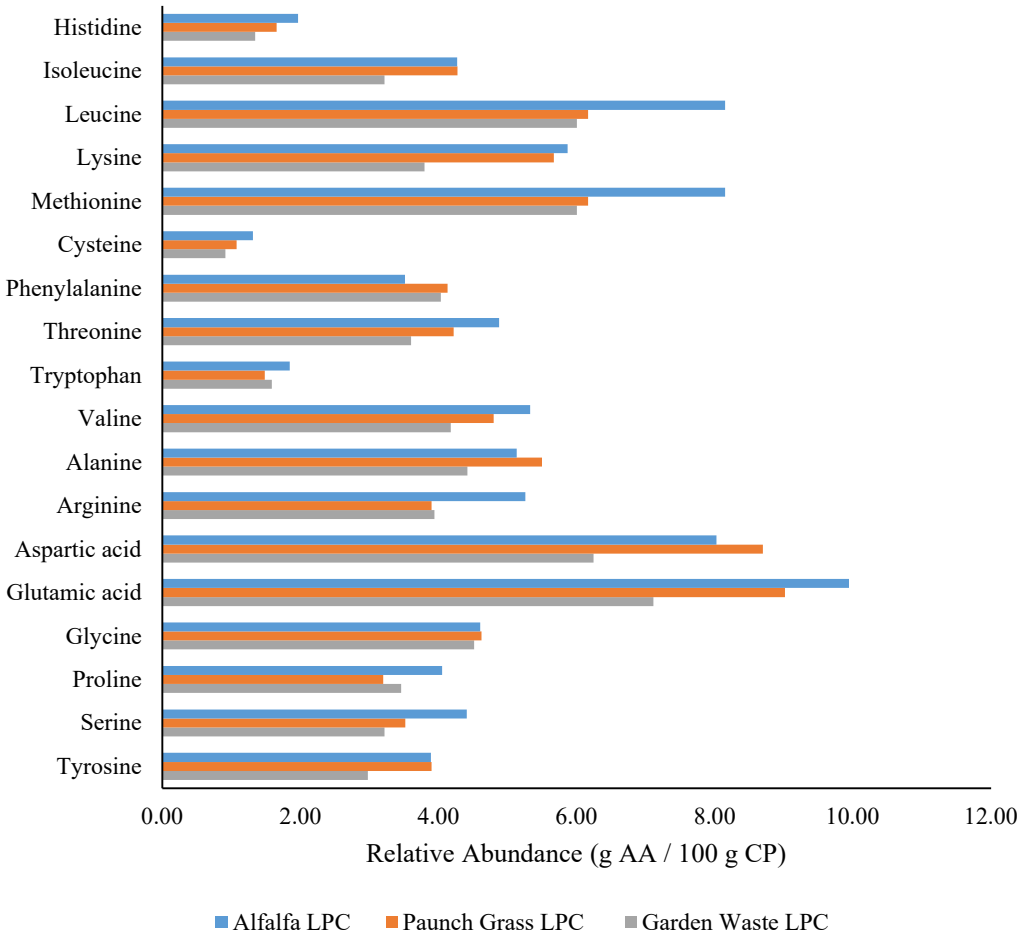

Figure 5.7: Amino acid profiles of alfalfa LPC, PG-LPC, and GW-LPC

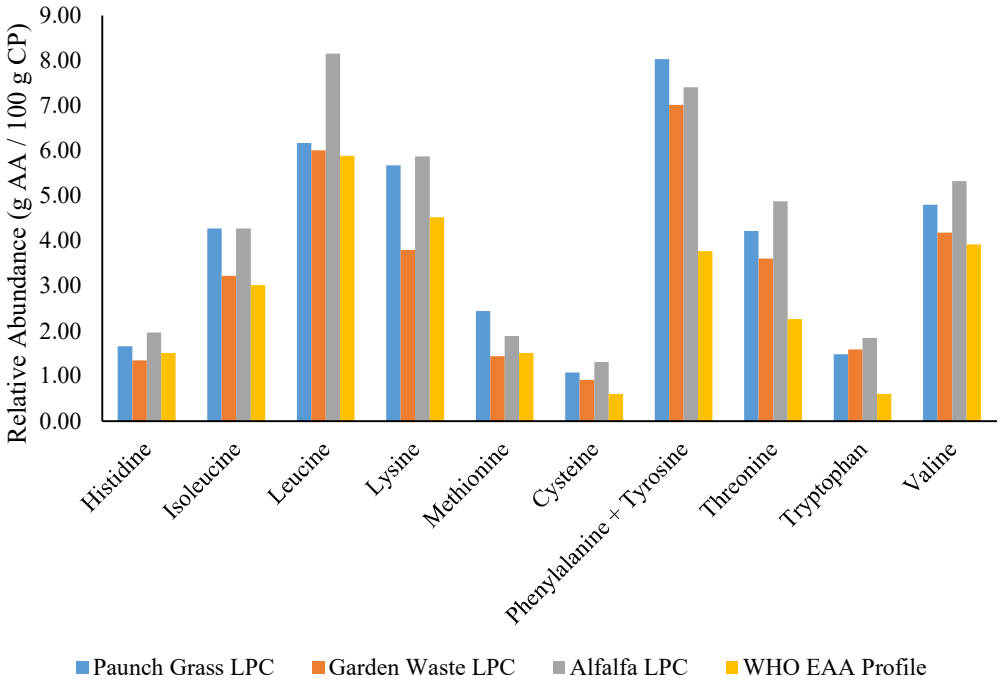

Figure 5.8: Essential amino acid profiles of PG-LPC, GW-LPC, and alfalfa LPC compared with the WHO EAA profile

The EAA requirements of most production animals (e.g. cattle, pigs, sheep) are poorly understood so the WHO adult human EEA profile is used as a reference point in these applications [17]. The exception is poultry diets, where the National Research Council [18] specify EAA profiles for various poultry feed applications.

EAA profiles for commercially produced alfalfa LPC, PG-LPC, GW-LPC, are compared with the recommended EAA profile for chickens producing brown eggs in Figure 5.9. In this application, both the PG-LPC and GW-LPC are deficient in and limited by their arginine contents; GW-LPC is also slightly deficient and limited by its valine content. These limitations can be overcome by blending LPC with canola and soy proteins (Figure 5.10 and Figure 5.11) The PG-LPC is slightly deficient in phenylalanine but its excess tyrosine content should prevent phenylalanine-limitation of the LPC [16].

The alfalfa LPC exceeds all the levels in the WHO EAA profile (Figure 5.8), but is deficient in histidine and leucine when compared with the NRC brown egg layers' EAA profile (Figure 5.9). The PG-LPC and GW-LPC both exceed the histidine and leucine specifications in this EAA profile; the alfalfa LPC exceeds the arginine and valine specification. The three LPCs could therefore be mixed into an LPC blend that meets all EAA specifications for brown egg laying hens.

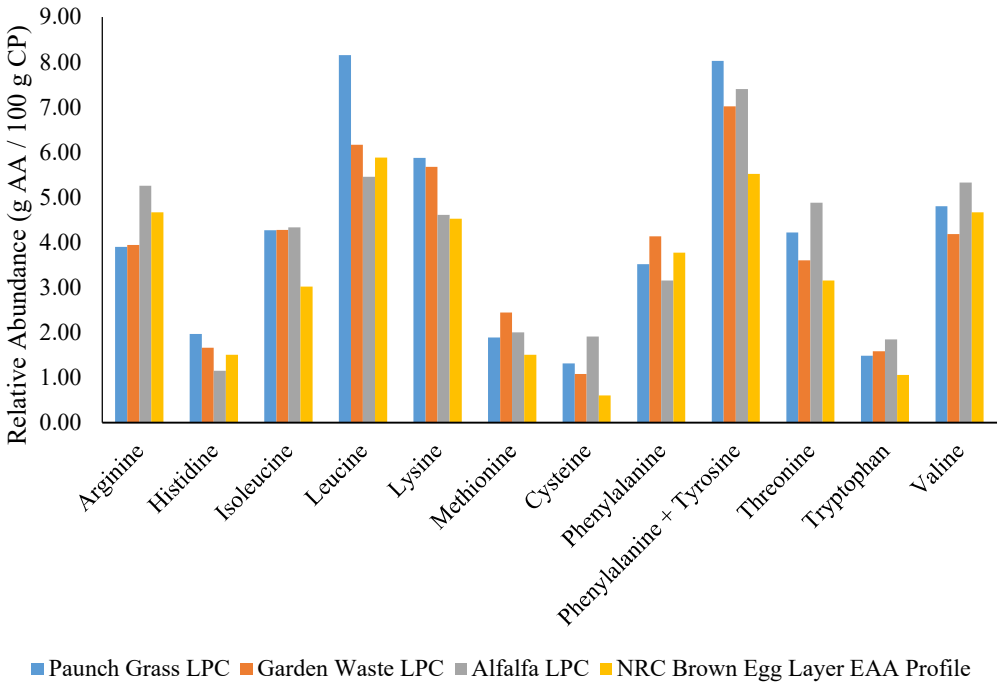

Figure 5.9: Essential amino acid profiles of PG-LPC, GW-LPC, and alfalfa LPC compared with the NRC brown egg layer EAA profile

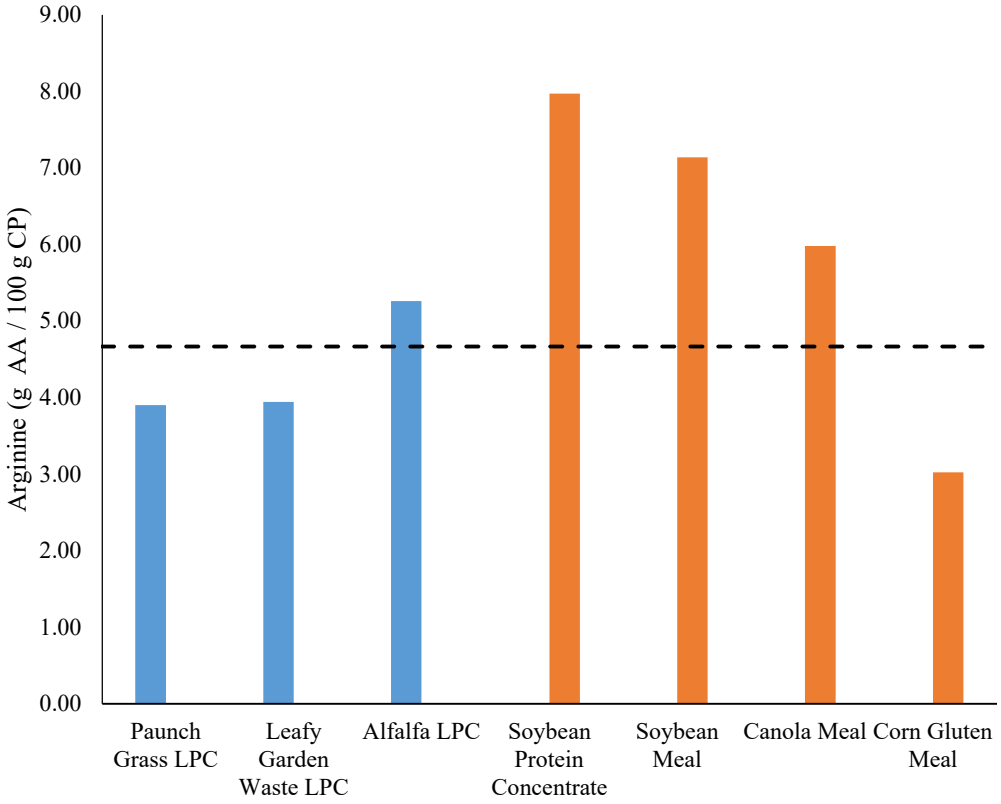

Figure 5.10: Arginine levels of alfalfa LPC, PG-LPC, GW-LPC, and plant protein meals compared with the NRC brown egg layer and WHO EAA profile requirements

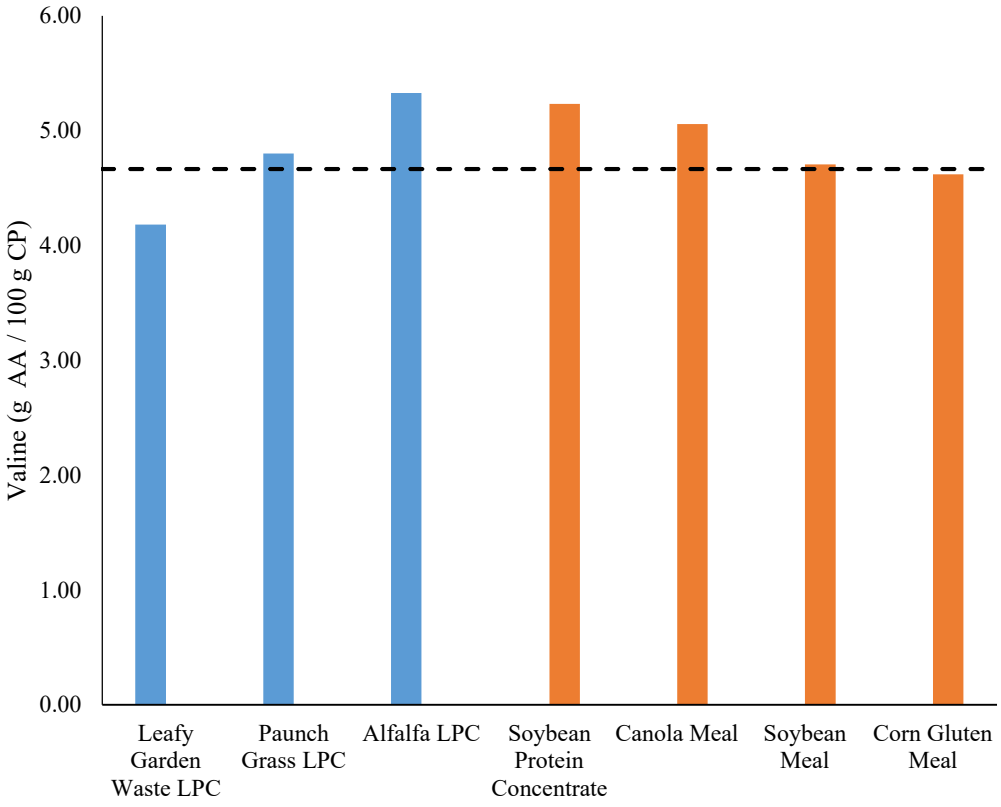

Figure 5.11: Valine levels of alfalfa LPC, PG-LPC, GW-LPC, and plant protein meals compared with the NRC brown egg layer and WHO EAA profile requirements

A comparison of the LPC EAA profiles with those of various protein crops, animal proteins, and protein meals indicates the LPCs can also be used to compensate for deficiencies in the EAA profiles of these feedstuffs. Plant proteins are generally deficient in isoleucine, lysine, methionine, cysteine, threonine and valine; blending these proteins with LPCs could compensate for these AA deficiencies and allow them to meet the AA levels specified in the WHO and NRC brown egg layer EAA profiles (Figure 5.12 to Figure 5.16).

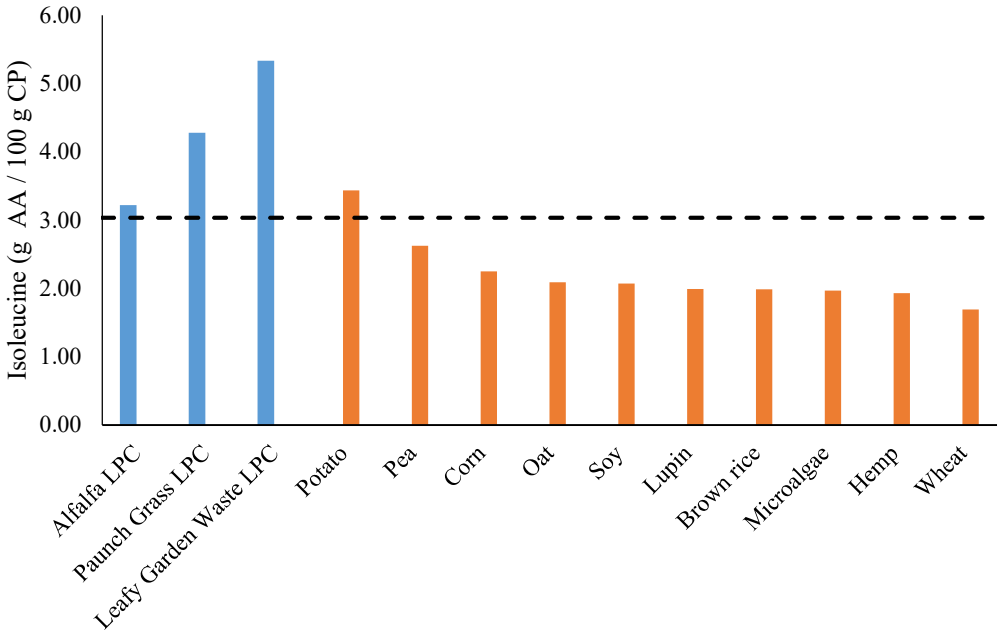

Figure 5.12: Isoleucine levels of alfalfa LPC, PG-LPC, GW-LPC, and protein crops compared with the NRC brown egg layer and WHO EAA profile requirement.

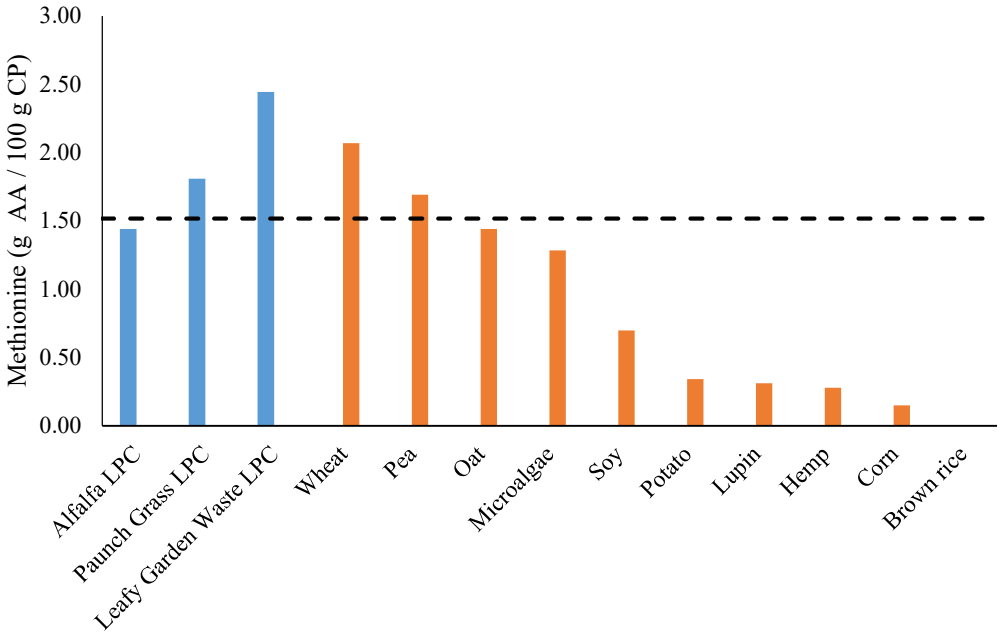

Figure 5.13: Methionine levels of alfalfa LPC, PG-LPC, GW-LPC, and protein crops compared with the NRC brown egg layer and WHO EAA profile requirement.

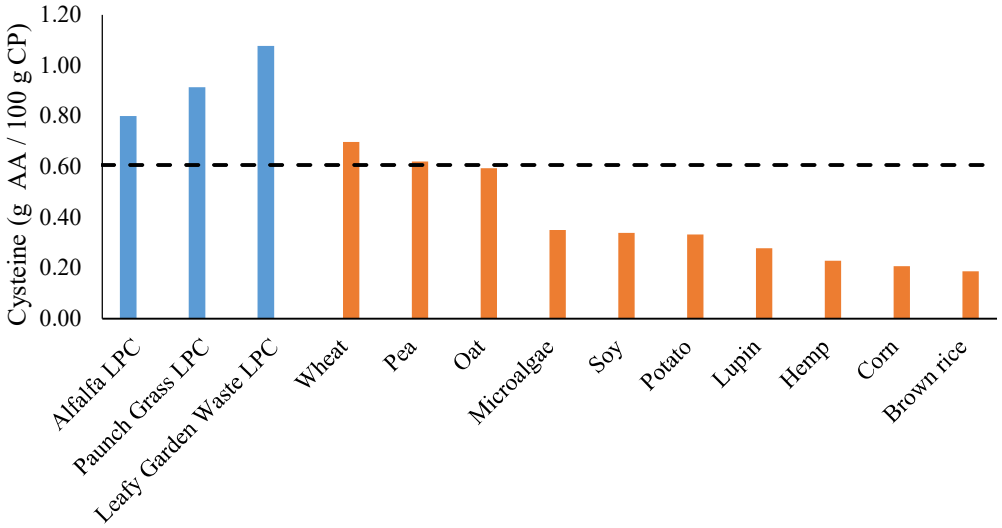

Figure 5.14: Cysteine levels of alfalfa LPC, PG-LPC, GW-LPC, and protein crops compared with the NRC brown egg layer and WHO EAA profile requirement

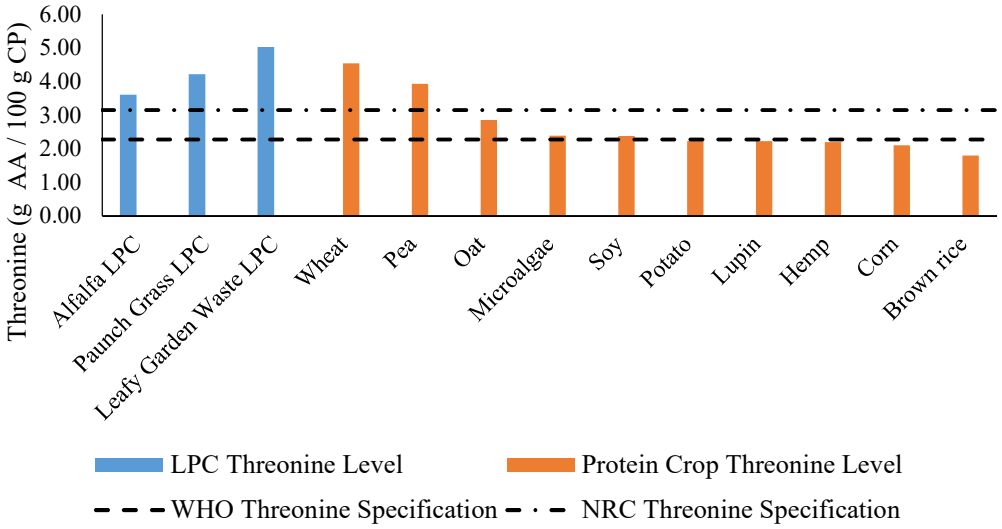

Figure 5.15: Threonine levels of alfalfa LPC, PG-LPC, GW-LPC, and protein crops compared with the NRC brown egg layer and WHO EAA profile requirements

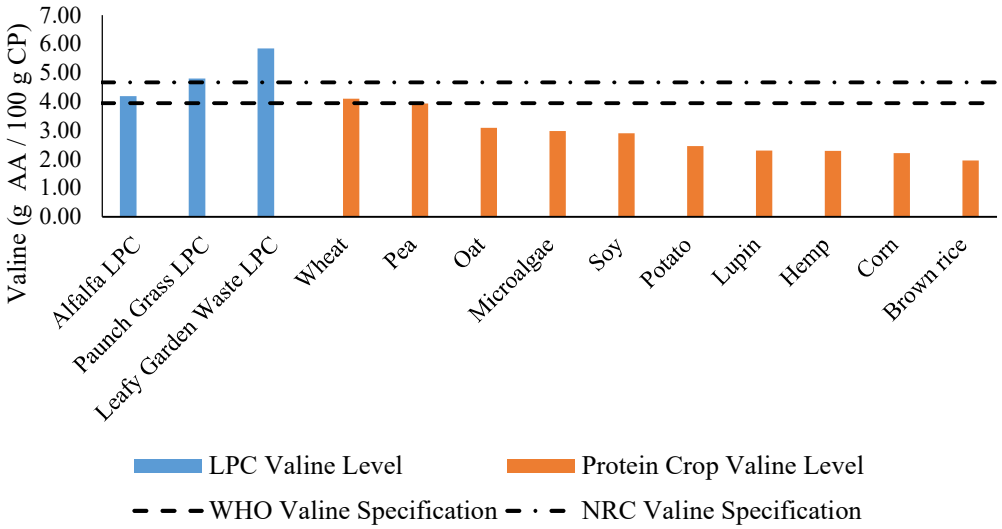

Figure 5.16: Valine levels of alfalfa LPC, PG-LPC, GW-LPC, and protein crops compared with the NRC brown egg layer and WHO EAA profile requirements

Similarly, LPCs could be used to compensate for lysine and tryptophan deficiencies in corn gluten meal (Figure 5.17 and Figure 5.18); methionine deficiencies in soybean meal and soy protein concentrates (Figure 5.19); as well as methionine, phenylalanine, tyrosine, and tryptophan deficiencies in several animal-based protein meals (Figure 5.20 to Figure 5.22).

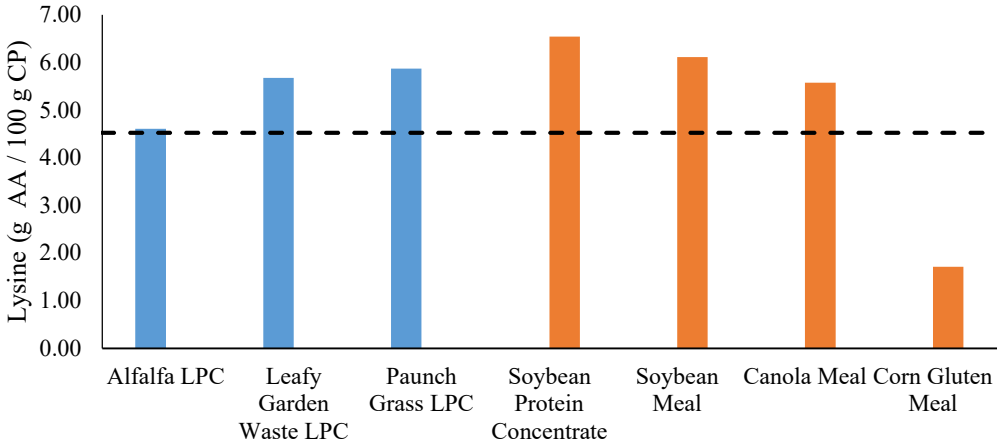

Figure 5.17: Lysine levels of alfalfa LPC, PG-LPC, GW-LPC, and plant protein meals compared with the NRC brown egg layer and WHO EAA profile requirements

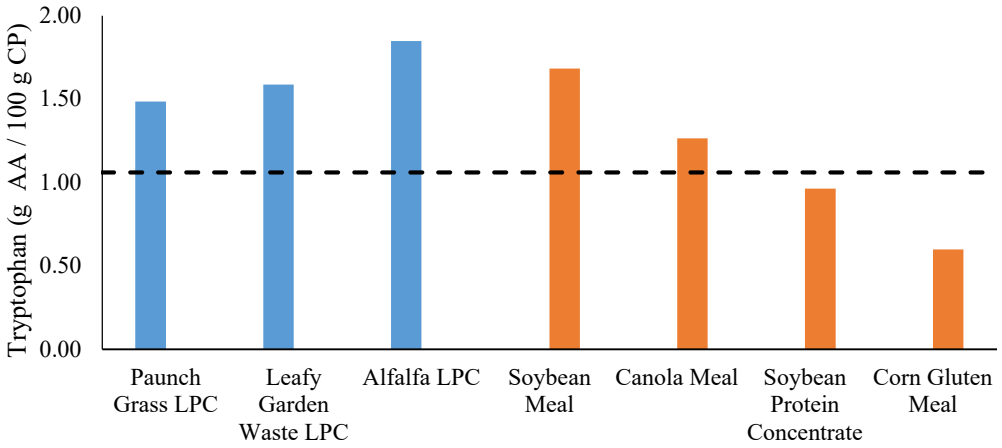

Figure 5.18: Tryptophan levels of alfalfa LPC, PG-LPC, GW-LPC, and plant protein meals compared with the NRC brown egg layer and WHO EAA profile requirements

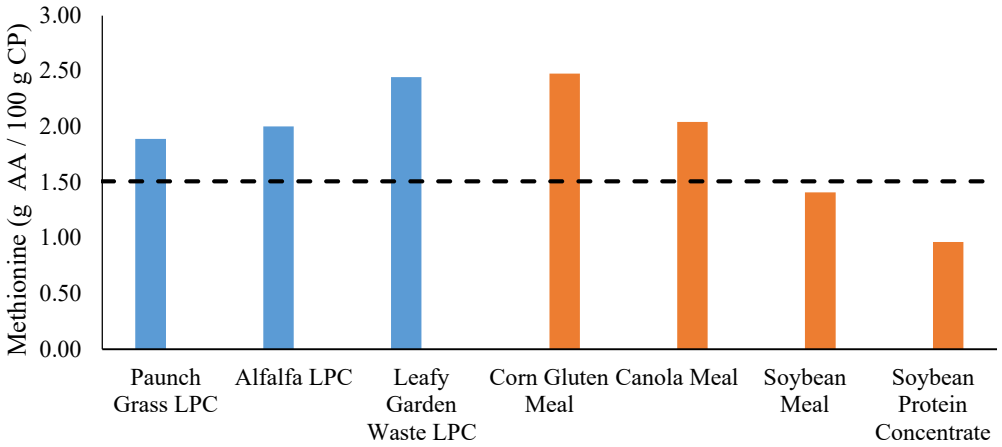

Figure 5.19: Methionine levels of alfalfa LPC, PG-LPC, GW-LPC, and plant protein meals compared with the NRC brown egg layer and WHO EAA profile requirements

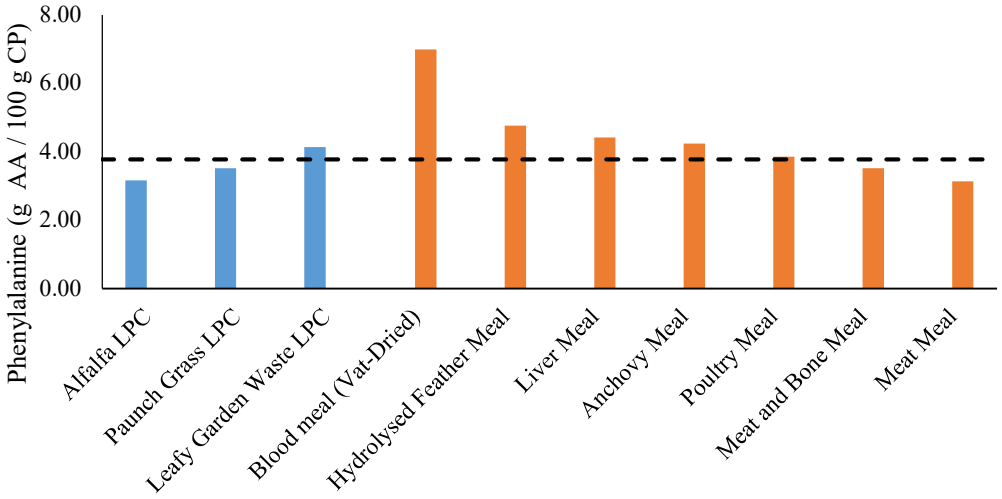

Figure 5.20: Phenylalanine levels of alfalfa LPC, PG-LPC, GW-LPC, and animal protein meals compared with the NRC brown egg layer and WHO EAA profile requirements

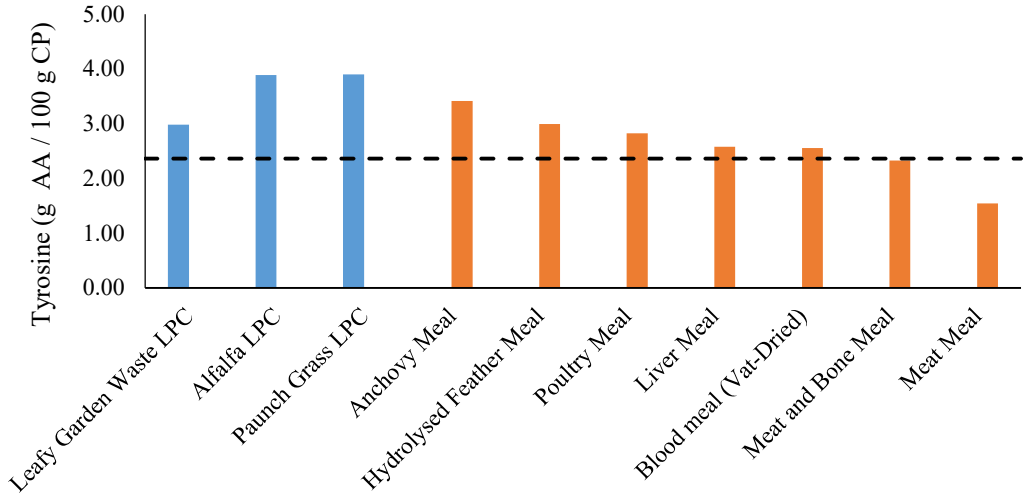

Figure 5.21: Tyrosine levels of alfalfa LPC, PG-LPC, GW-LPC, and animal protein meals compared with the NRC brown egg layer and WHO EAA profile requirements

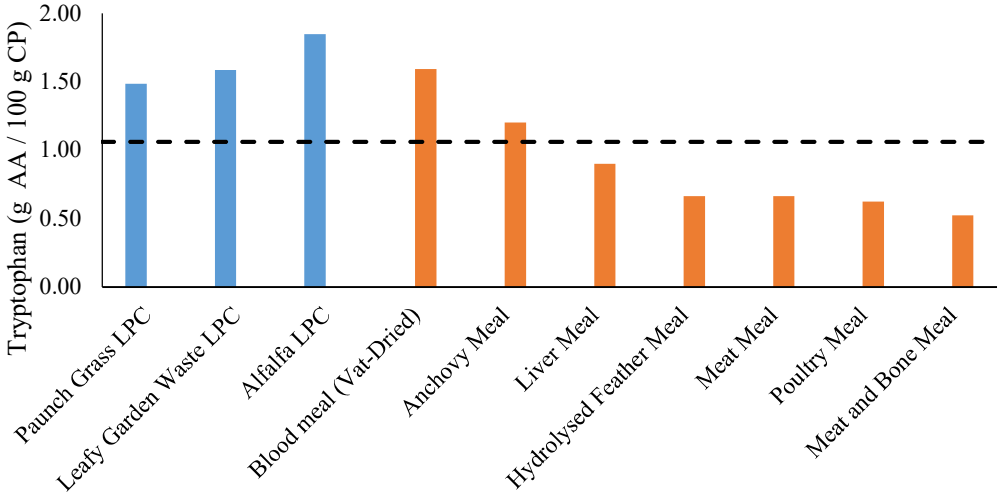

Figure 5.22: Tryptophan levels of alfalfa LPC, PG-LPC, GW-LPC, and animal protein meals compared with the NRC brown egg layer and WHO EAA profile requirements

Chickens raised for meat have a substantially higher EAA requirements than chickens raised to produce eggs [18]. This becomes evident when the EAA profiles for alfalfa LPC, PG-LPC, and GW-LPC are compared with the National Research Council [18] EAA profiles for broiler hens (Figure 5.23). Whilst the PG-LPC and GW-LPCs only had two limiting amino acids when compared against the brown egg laying hens' EAA profile, they have four limiting EAA when compared against the broiler chicken EAA profiles: arginine, leucine, lysine, and valine.

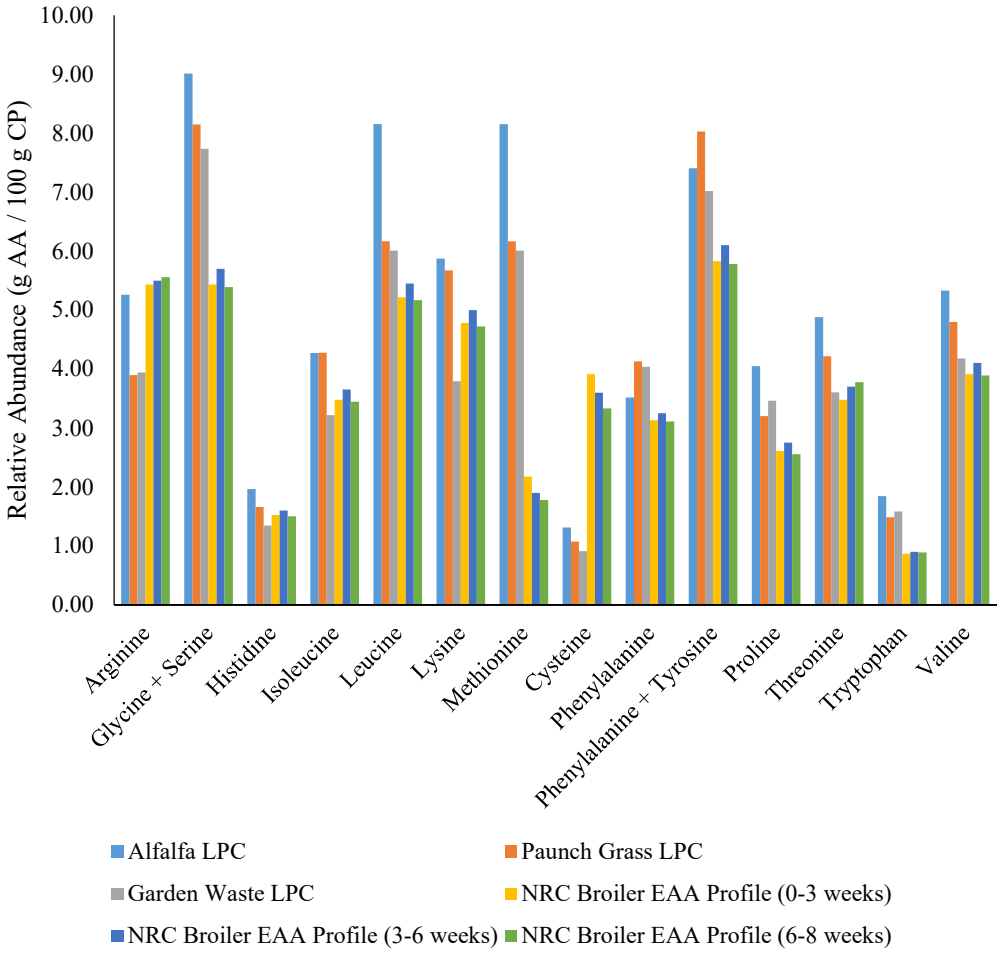

Figure 5.23: Essential amino acid profiles of PG, GW, and alfalfa LPC compared with NRC broiler EAA profiles

No mixture of LPCs can solely meet the EAA requirements for broiler hens as all LPCs are contain limiting quantities of arginine and cysteine. However an LPC mixture can meet arginine specifications by blending with soy and canola proteins (Figure 5.24) or most animal-based protein feed meals (Figure 5.24 and Figure 5.25). There are no common plant-based sources of feed protein that meet the cysteine specification in the broiler hen EAA profile; it can only be met by blending LPC with dairy protein or hydrolysed feather meal (Figure 5.26).

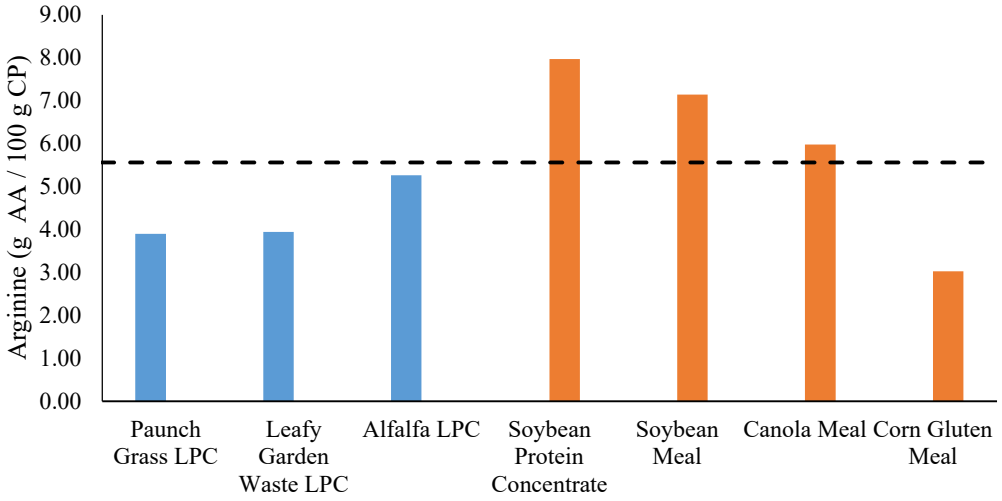

Figure 5.24: Arginine levels of PG-LPC, GW-LPC, alfalfa LPC, and plant protein meals compared with the maximum NRC broiler hen EAA requirement

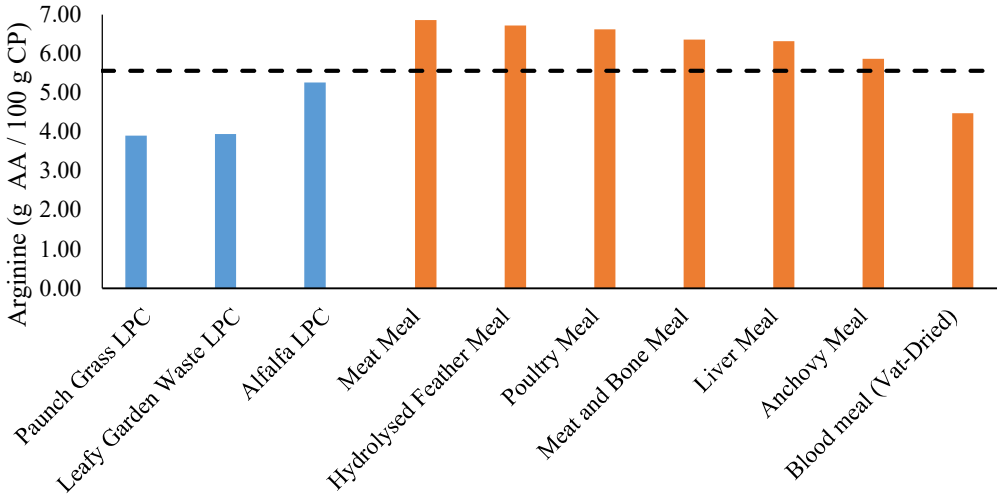

Figure 5.25: Arginine levels of PG-LPC, GW-LPC, alfalfa LPC, and animal protein meals compared with the maximum NRC broiler hen EAA profile requirement

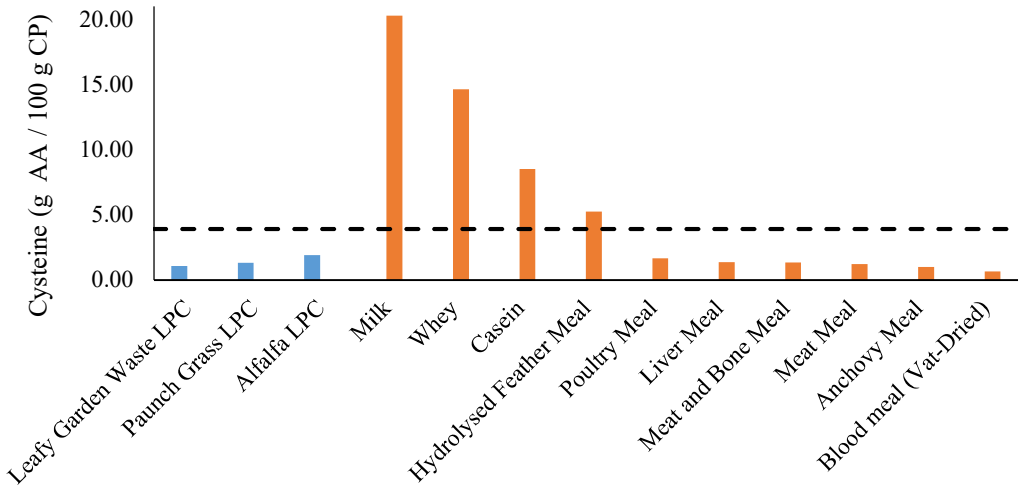

Figure 5.26: Cysteine levels of PG-LPC, GW-LPC, alfalfa LPC, dairy proteins, and animal protein meals compared with the maximum NRC broiler hen EAA profile requirement

Thermal Analysis

Thermogravimetric analyses for PG-LPC and GW-LPC are compared with an analysis for alfalfa LPC in Figure 5.27 and Figure 5.28, respectively. The protein concentrates coagulated from acidified press liquor follow a similar profile to alfalfa LPC; whilst the protein concentrates recovered from untreated liquor follow a slightly different profile and maintain a constant rate of mass loss between 300°C and 490°C. These LPCs have relatively high ash contents and therefore less organic material; this reduces the proportion of the sample that is combusted between 300°C and 490°C and hence allows for a more uniform the rate of change in sample mass.

The most interesting part of the thermogravimetric analyses lies between 35°C and 125°C. The mass change in this region corresponds to residual moisture evaporating off and the steepest part of the trace corresponds to the greatest rate of evaporation. This occurs from 65°C to 85°C for PG-LPC and 65°C to 80°C for GW-LPC; this is similar to the temperature range for alfalfa LPC (70°C to 95°C) and indicates the ideal drying temperature for the PG-LPC and GW-LPC is approximately 70°C. This temperature is consistent with the range specified by Mani and Sokhansanj [19] for drying delicate products such as proteins in a direct heat rotary drum dryer.

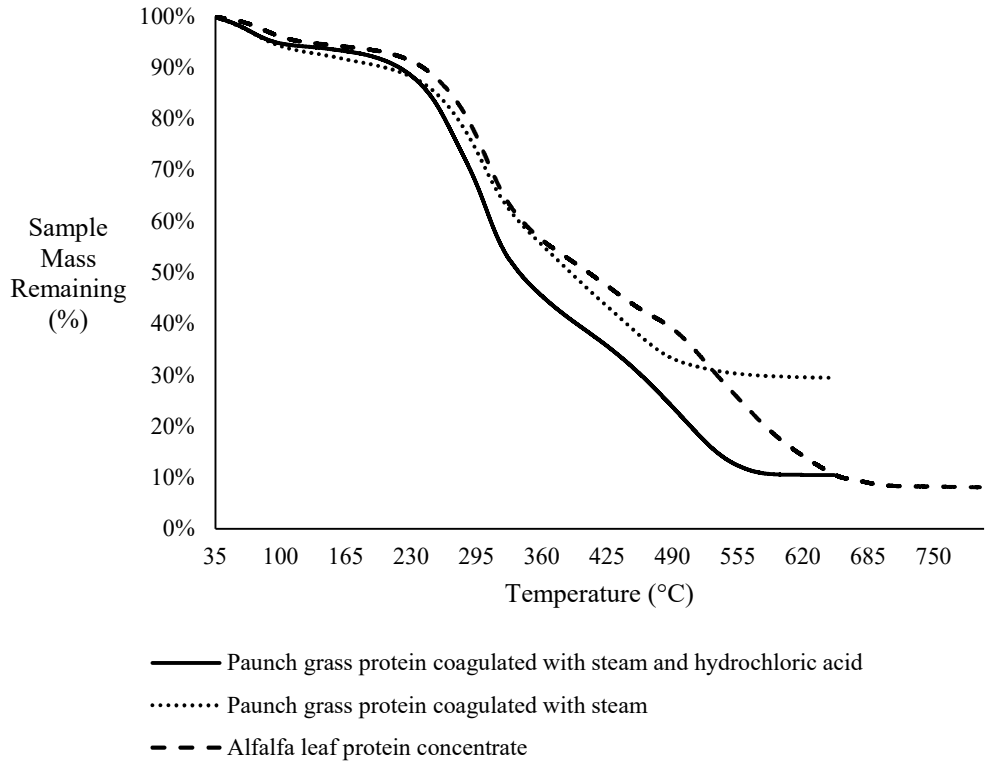

Figure 5.27: TGA traces for PG-LPCs and alfalfa LPC

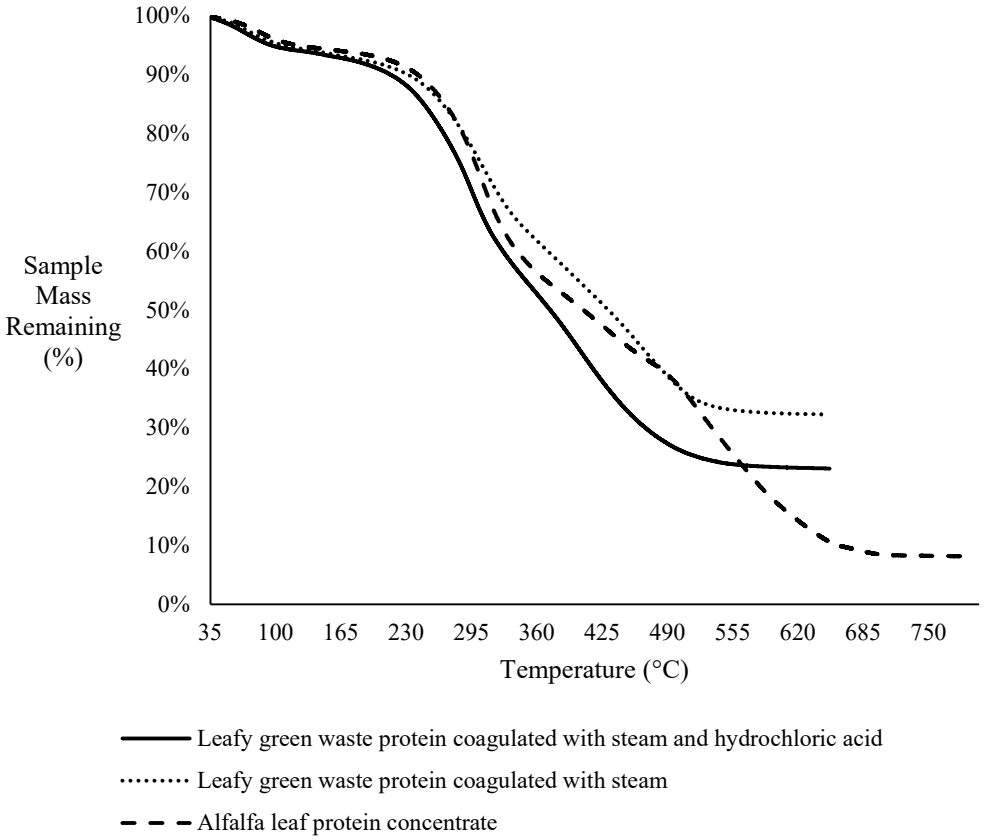

Figure 5.28: TGA traces for GW-LPCs and alfalfa LPC

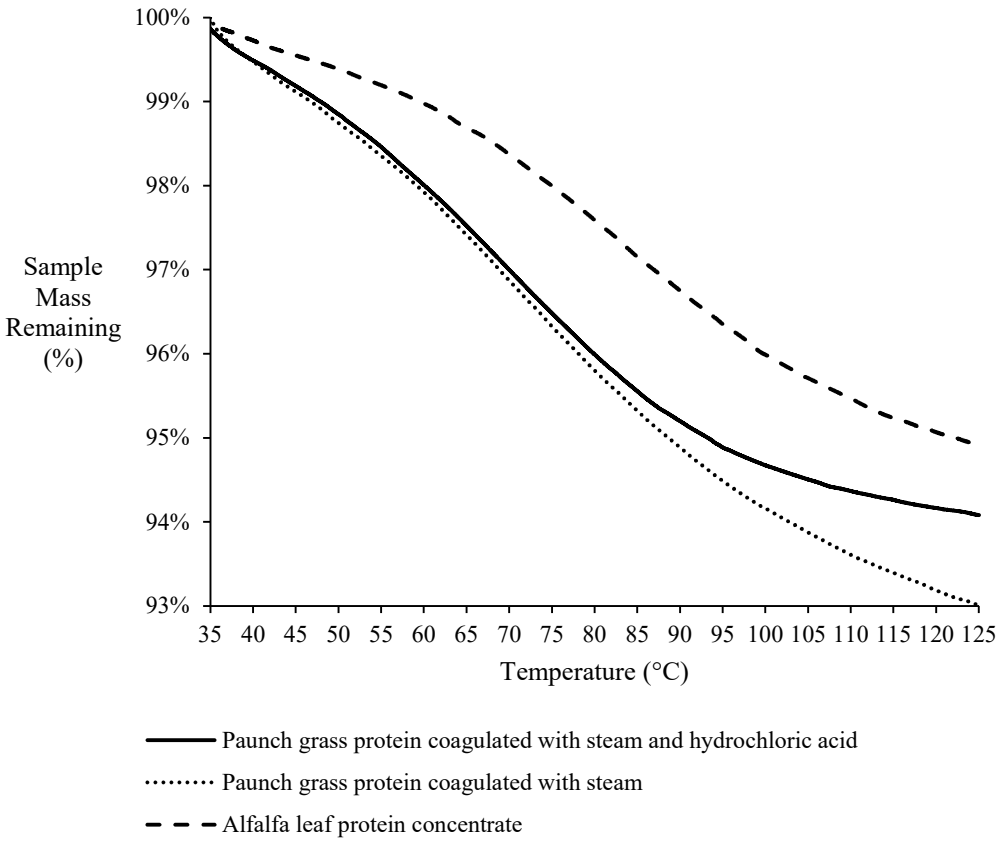

Figure 5.29: Low temperature TGA traces for PG-LPCs and alfalfa LPC

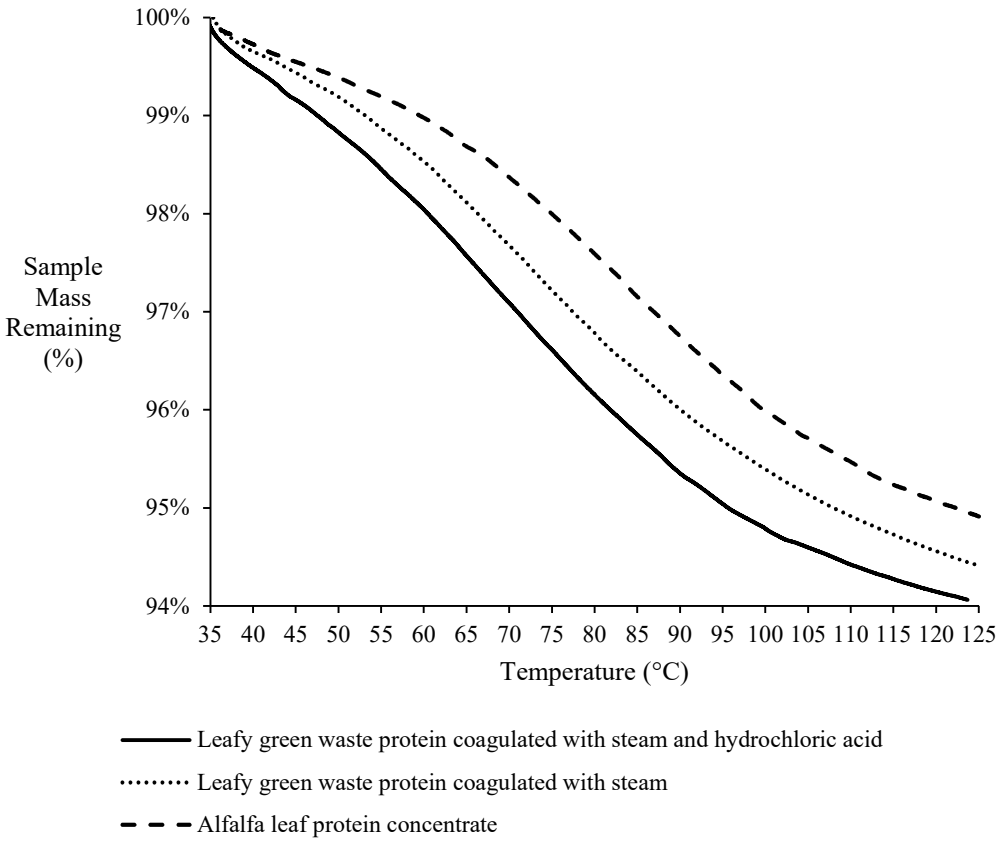

Figure 5.30: Low temperature TGA traces for GW-LPCs and alfalfa LPC

The relevant sections of the differential scanning calorimeter traces for PG and GW LPCs, alfalfa LPC, as well as PG and GW press liquor are presented in Figure 5.31 and Figure 5.32. The endothermic liquor peaks correspond to the temperature range required to coagulate the leaf protein in the liquor [20]; they indicate that most of the leaf proteins in the liquor can be recovered by heating it to ca. 85°C.

The endothermic peaks for the LPCs indicate their glass transition temperatures (T_g) are between 40°C and 55°C [21; 22; 23], allowing them to be extruded and pelletised well below the onset of thermal decomposition observed in Figure 5.27 and Figure 5.28 [24]. These temperatures are substantially lower than those characteristic of pure lyophilised amorphous proteins (120°C to 210°C) [25], so it is likely that the non-protein components of the LPC (e.g. lipids and carbohydrates) have plasticised the leaf protein [22; 23]. This is further supported by the fact that the alfalfa LPC has both a higher T_g (ca. 70°C) and crude protein content (Table 5.17) than PG-LPC or GW-LPC. These outcomes indicate that PG-LPC and GW-LPC are easily processible in their original forms and do not require modification or blending with plasticisers prior to extrusion into pellets. Successful extrusion of

PG-LPC and GW-LPC into protein pellets will be a key area of research that should be undertaken before industrial-scale production commences. Potential topics could include blending with binders and other protein meals to produce an easier to handle or more nutritionally valuable product, respectively.

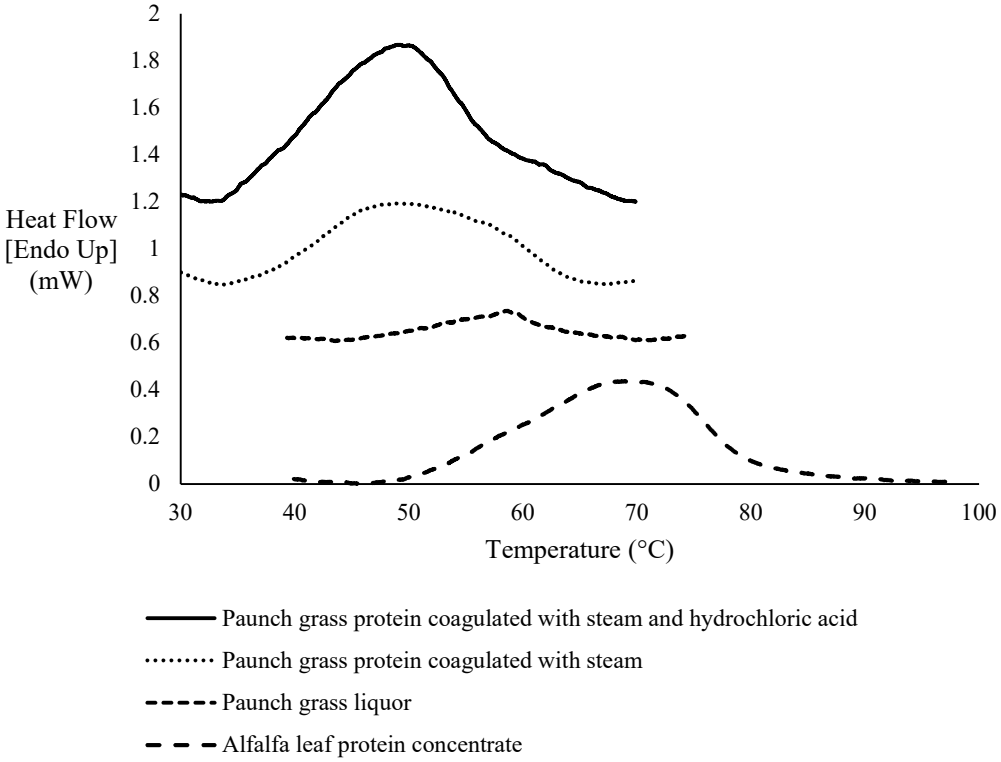

Figure 5.31: DSC traces for PG-LPCs, lyophilised PG liquor, and alfalfa LPC

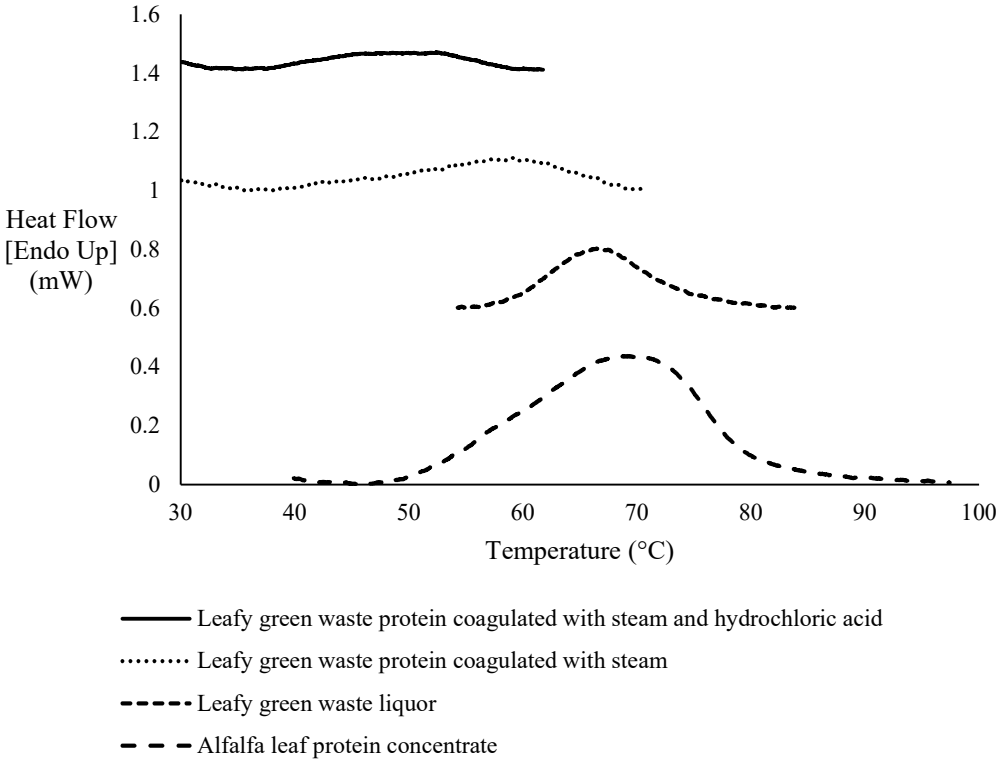

Figure 5.32: DSC traces for PG-LPCs, lyophilised PG liquor, and alfalfa LPC

5.3.2 Fibre

The press fibre fraction compositions are presented in Table 5.19. The moisture contents of both fibre types are below the optimal starting moisture content for a composting process (ca. 60 %) [26]. Similarly, both the PG and GW fibres have carbon to nitrogen ratios outside the recommended initial ratio for composting (25-30) [26]. However, the high carbon to nitrogen ratio suggests that the press fibre is rich in organic material and well-suited to anaerobic digestion [27]. The protein extraction process also mechanically disrupts the press fibre by hammer milling, disc milling, and screw pressing – similar mechanical treatments are used to prepare other plant materials for anaerobic digestion [27]. The fibre thus both chemically suitable for anaerobic digestion and has already been mechanically pre-treated. The biogas resulting from the anaerobic digestion process could be used to provide heat and power for the leaf protein concentrate manufacturing process; this potential should be investigated during future work.

Both paunch grass and green waste fibre contain substantial amounts of crude protein and lipids. These findings are consistent with those of Smith [12] who concluded that as portion of leaf proteins and lipids are integrated into plant cell walls and therefore cannot be extracted into solution [28; 29]. The ash levels are consistent with the lower end of the range determined for garden wastes by Boldrin and Christensen [9] and are therefore unlikely to be problematic in a composting process.

Table 5.19: Composition of press fibre fractions from double-pass protein extraction of leaf protein

Component	Paunch Grass Fibre	Garden Waste Fibre
Moisture (w/w%)	45.0 ± 0.5 %	44.9 ± 0.5%
Crude Protein (DM%)	6.2 ± 0.3%	16.5 ± 2.1 %
Lipids (DM%)	4 ± 2 %	7.7 ± 0.8 %
Ash (DM%)	14 ± 8 %	12 ± 8 %
Carbon to Nitrogen Ratio	45.2 ± 2.4 %	16.5 ± 2.1 %

Furthermore, ICP-MS analysis of ash from both fibre fractions (Table 5.20) indicates that the levels of heavy metals in the fibres are below the concentration limits specified for Contaminant Grade Biosolids in New Zealand [30]. This should allow compost from these fibres to be applied to land in accordance with regulatory guidelines [30]. Blending the fibres with other materials prior to composting could

sufficiently reduce concentrations of copper and zinc to allow the resultant compost to comply with the concentration limits specified for Remediation Grade biosolids, which allows them to be safely handled by the public and applied to land without restriction [30]. The full ICP-MS profile of the fibres is presented in Table 5.21**.** These results suggest that the combined fibre fractions could be sold as a bulking agent and carbon source for commercial composting operations. They also indicate that the fibre contains substantial levels of calcium, phosphorus, potassium, and sulfur, which could give the fibre or its compost value as a fertiliser as suggested for leafy green waste by Boldrin and Christensen [9].

Table 5.20: Heavy metal contents of PG and GW fibre

Heavy Metal	Paunch Grass Fibre Concentration (mg/kg DM)	Leafy Green Waste Fibre Concentration (mg/kg DM)	Remediation Grade Biosolids Concentration Limit [Grade a] (mg/kg DM)	Contaminant Grade Biosolids Concentration Limit [Grade b] (mg/kg DM)
Arsenic	1.05	14.63	20	30
Cadmium	3.25	0.93	1	10
Chromium	71.3	233.7	600	1500
Copper	127	139	100	1250
Lead	14.4	45.4	300	300
Mercury	0.11	0.02	1	7.5
Nickel	18.7	43.2	60	135
Zinc	1111	601	300	1500

Table 5.21: ICP-MS profile of PG and GW fibre

Element	Level Present in PG Fibre (mg/kg DM)	Level Present in GW Fibre (mg/kg DM)
11 B corrected	74.36	57.43
23 Na corrected	20674	5246
24 Mg corrected	6041	8116
27 Al	9684	21557
31 P	20827	8981
34 S	13399	5827
39 K	12428	15589
43 Ca	50950	29410
44 Ca	42873	43858
31 -> 47 P	19877	8700
34-50 S corrected	12830	6011
51 V	11.63	34.76
52 Cr	35.21	116.24
53 Cr	36.12	117.45
55 Mn	1406.16	4507.23
56 Fe	5537.31	12348.10
59 Co	1.67	10.61
60 Ni	18.74	43.18
63 Cu	62.59	68.38
65 Cu	63.92	70.18
66 Zn	554.12	299.47
68 Zn	557.10	301.69
71 Ga	2.91	5.85
75 As	1.31	13.03
78 Se	0.95	1.15
82 Se	0.96	1.33
85 Rb	30.61	97.16
88 Sr	176.39	169.05
75 -> 91 As	1.05	14.63
78 -> 94 Se	0.36	0.58
95 Mo	8.57	6.07
82 -> 98 Se	0.00	0.50
105 Pd	0.03	0.05
107 Ag	0.00	0.10
110 Cd	0.36	0.11
111 Cd	1.44	0.39
114 Cd	1.45	0.43
118 Sn	15.45	83.64
137 Ba corrected	577.05	449.36
140 Ce	14.74	55.17
175 Lu	0.12	0.14
201 Hg corrected	0.11	0.02
205 Tl	0.29	0.27
206 Pb	4.91	15.32
207 Pb	4.73	15.05
208 Pb	4.80	15.02
235 U	1.65	3.07
238 U	0.62	1.15

5.4 Additional Considerations for Processing Wastes

The primary challenge associated with using wastes as a feedstock for a process which produces animal feed protein: potential contamination by pathogens and poisons. Paunch grass is generally considered to be contaminated by pathogens and vermin [31], and leafy green waste is a heterogeneous material which could contain poisonous plant material. Contamination by pathogens and poisons could make the resulting protein concentrate unfit for consumption by animals, but the nature of the protein extraction and recovery process mitigates these concerns. Low [32] notes that blood is not usually collected hygienically at abattoirs, and is thus contaminated by urine, hair, vomit, and wash water. Nevertheless, the processing steps (e.g., steam injection at 90°C, acid treatment) used during bloodmeal production ensure sufficient sterility for it to be used in animal feed [32]. The adjustment of the 85°C steam injection step used to coagulate leaf protein concentrate in this research should therefore also be sufficient to ensure sterility for animal feed.

Although it is possible that the leafy green waste could contain poisonous plant material, this will be kept to a minimum. Determination of the physical composition of the leafy green waste in Chapter 4 found that it was predominantly grass clippings and soft leafy weeds, which are generally not poisonous. Additionally, the process appears to only disrupt soft leaves (e.g., grasses and legumes); all other types of plant material are rejected with the fibre fraction. A more detailed study of the plants present in leafy green waste could be necessary before building a processing plant. The risks associated with the heterogeneity of the garden waste could also be mitigated by source-segregation and subsequent processing of the grass clippings, which dominate the physical composition of the green waste across all seasons.

The second challenge associated with processing paunch grass and leafy green waste is that the proteins present in the soft leaves of these materials have already been degraded by enzymatic and microbial action prior to processing [33]. Hence although paunch grass and leafy green waste potentially have similar protein recovery yields to fresh protein crops, the estimated yields require empirical verification. This verification was successfully completed and documented as per Sections 5.1 to 5.3 above.

5.5 Conclusion

The most effective method to extract leaf proteins from paunch grass and leafy green wastes is to macerate the feedstocks, pass them through a screw press, then wet and macerate the resulting press fibre before passing it through another screw press. Optimal protein recovery from the press liquor is achieved when the liquor is treated with hydrochloric acid minimise its Zeta potential prior to coagulation. The acidified press liquor should then be heated to 85°C by steam injection and held at that temperature for 30 seconds before the resultant slurry is centrifuged to separate the leaf protein concentrate from the waste process liquor.

The resulting paunch grass and leafy green waste leaf protein concentrates follow a similar essential amino acid profile to those published for other LPCs and fully meet the EAA levels specified in the most recent WHO EAA profile. They are slightly deficient in arginine and valine when compared with the NRC EAA profile for brown egg laying hens – this deficiency can be compensated for by blending with soy, canola, and alfalfa proteins. The PG-LPC and GW-LPC are deficient in arginine, leucine, lysine, and valine when compared with the NRC EAA profile for broiler hens. This can be compensated for by incorporating of dairy proteins or hydrolysed feather meal into a blended feed. Similarly, PG-LPC and GW-LPC can also be used to compensate for isoleucine, lysine, phenylalanine, methionine, cysteine, threonine, tyrosine and valine deficiencies in other animal feed proteins.

TGA studies suggest the optimal drying temperature for the PG-LPC and GW-LPC is approximately 70°C; and DSC studies suggest they can be extruded into pellets without the incorporation of a plasticising additive. However, ICP-MS profiles of PG-LPC and GW-LPC suggest that heavy metal contamination could limit the proportion of LPC that can be incorporated into an animal feed formulation. The fibre co-product could be used as a bulking agent for commercial composting operations dues to its low moisture content (ca. 45%), high carbon to nitrogen ratio (40-45 for mixed PG and GW fibres), and acceptable levels of heavy metals.

Key areas for further investigation work include: determining the scalability of results achieved with benchtop scale equipment; determining the degree of sterility achieved during steam injection; reducing the heavy metal content of the LPCs to enable greater incorporation into animal feeds; investigating the potential of the

press fibre as an anaerobic digestion feedstock, and developing a method for extruding LPC into animal feed pellets; and determining the palatability, digestibility, and EAA availability of PG-LPC and GW-LPC animal feed formulations.

5.6 References

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6 Technoeconomic Analysis

The technoeconomic analysis of leaf protein concentrate (LPC) production from soft leafy wastes (SLW) such as paunch grass (PG) and leafy green waste (GW) consisted of five main steps: estimation of feedstock qualities and quantities; the process mass balance; sizing and capital cost estimation for process equipment; operating cost estimation; and profitability analysis. Capital cost estimates were made for three plant designs (SP, BL, and IRP); three operational scenarios were then costed for each plant design (Figure 6.1).

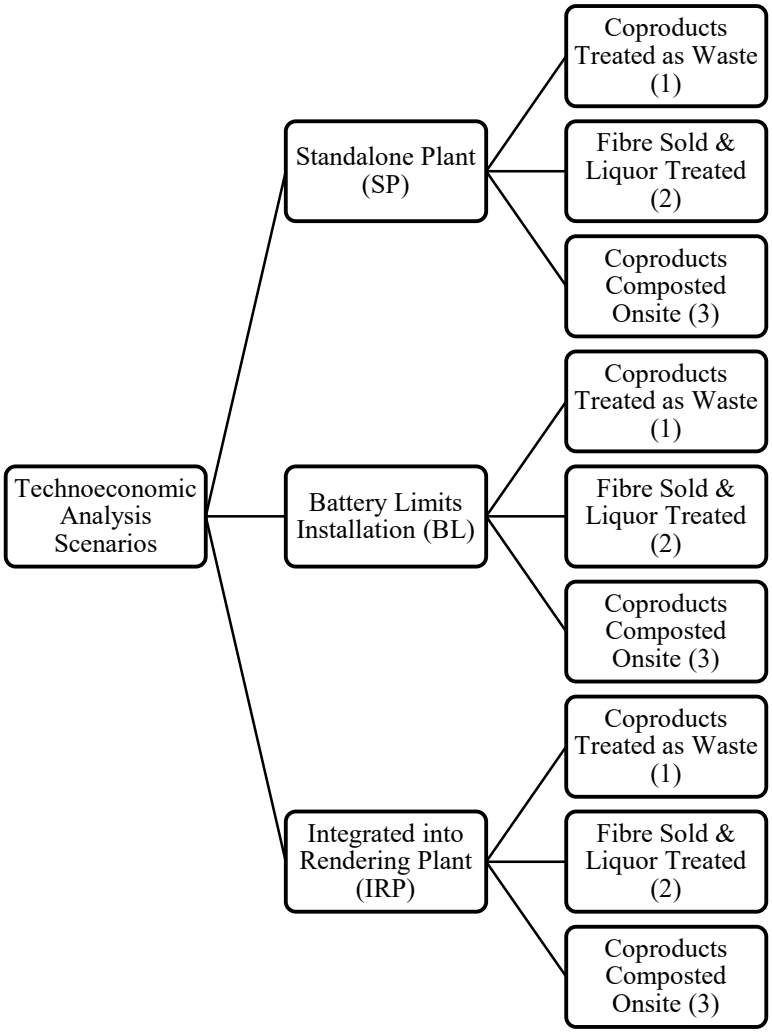

Figure 6.1: Plant designs and coproduct utilisation scenarios used for technoeconomic analysis of LPC production from PG and GW

The various configurations of the leaf protein concentrate production facility described in Figure 6.1 were designed to process all PG produced in the Upper North Island region of New Zealand (Figure 6.2) and all the GW produced in the Greater Waikato region of New Zealand (Figure 6.3).

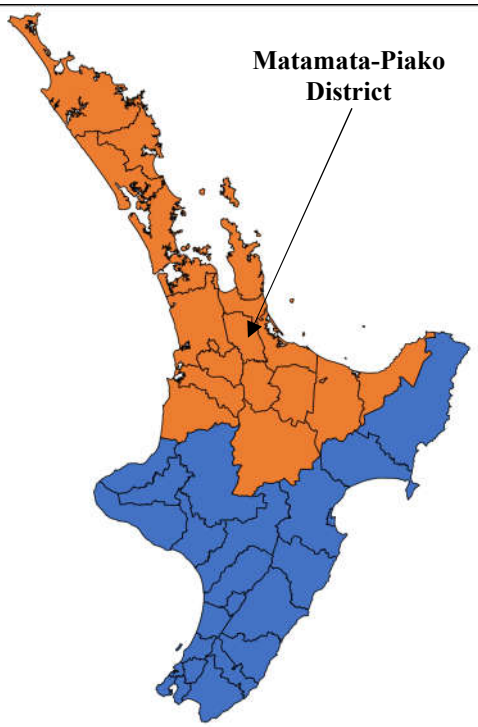

Figure 6.2: PG catchment area shaded orange and processing plant location in the Matamata-Piako District.

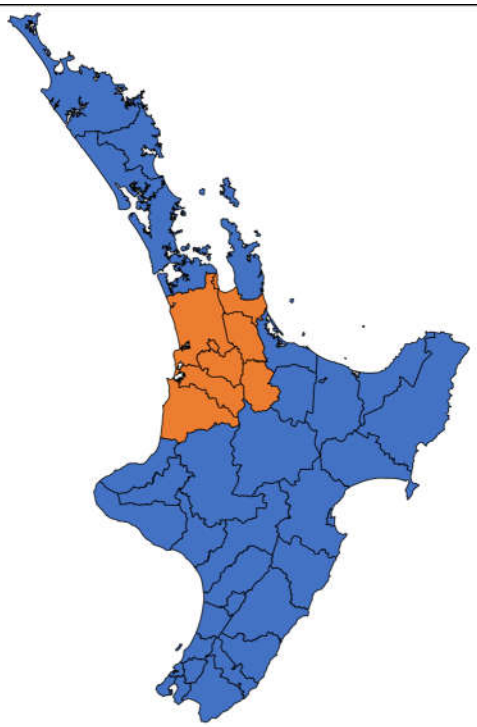

Figure 6.3: GW catchment area shaded orange

As PG is a regulated waste, it is viable to transport all the PG from the Upper North Island to a single location and process it as per Figure 6.2. Conversely, GW is not a regulated waste, so the catchment area was restricted to the Greater Waikato region as per Figure 6.3. This is the largest catchment that Hamilton Garden Bags and Red Lid Bins (the other commercial sponsor) of this research could service from their current base of operations on the outskirts of Hamilton. Collecting GW from areas further afield is unlikely to be viable due to transportation distances and competing GW disposal processes closer to where the GW is generated.

The processing facility is assumed to be in the Matamata-Piako district, near the geographic centre of the catchment area described in Figure 6.2. This location was driven by the needs of Wallace Corporation, one of the commercial sponsors of this research, who are interested in co-locating a leaf protein production facility with their existing rendering plant at Waitoa in the Matamata-Piako District. As PG is a regulated waste, it is viable to collect and process all the PG from the Upper North Island in a single location as per Figure 6.2. Additionally, the quantities of steam and wastewater treatment utility consumed by the processing facility are negligible

relative to the capacity of the utilities installed at the Waitoa rendering plant, allowing the process to be built and operated without upgrading utilities.

Since the process will be co-located with an existing rendering plant, a Battery Limits (BL) installation process design was considered as the baseline scenario. A BL plant had a positive net present value (NPV - Table 6.1) for Scenarios 2 & 3 over the 15-year depreciation period that is typical for most process equipment [2]. A further-integrated process which shares some process equipment (e.g., screw presses and dryers) with the existing rendering process was modelled as an Integrated into Rendering Plant (IRP) process design, which had positive NPVs for all scenarios. The NPVs for the IRP design exceeded the total permanent investment required (Table 6.2), and corresponding internal rates of return (IRR - Table 6.3) were well above the typical one year (2.47% pa) and five year (2.64% pa) term deposit rates available in New Zealand in December 2019 [3]. These results suggest that LPC production from PG and GW is financially viable, and large-scale laboratory or small pilot plant trials are justified.

Table 6.1: 15-year NPVs for the various LPC process designs and scenarios

	Net Present Value over 15 Years		
	Standalone Plant	**Battery Limits Installation**	**Integrated into Rendering Plant**
Scenario 1	-$44,810,354	-$21,958,772	$94,217,538
Scenario 2	-$20,759,985	$2,091,597	$118,267,907
Scenario 3	-$5,286,002	$12,590,938	$128,767,248

Table 6.2: Total permanent investments for the various LPC production facility process configurations and scenarios

	Total Permanent Investment		
	Standalone Plant	**Battery Limits Installation**	**Integrated into Rendering Plant**
Scenario 1	$16,819,986	$13,212,460	$6,777,123
Scenario 2	$16,819,986	$13,212,460	$6,777,123
Scenario 3	$15,249,363	$13,212,460	$6,777,123

Table 6.3: 15-year IRRs for the various LPC production facility process configurations and scenarios

	Internal Rate of Return over 15 Years		
	Standalone Plant	**Battery Limits Installation**	**Integrated into Rendering Plant**
Scenario 1	-	-25.66%	51.99%
Scenario 2	-	3.880%	57.64%
Scenario 3	-	9.973%	59.90%

6.1 Methodology

The technoeconomic analysis was performed by combining raw material characterisation, protein extraction and recovery, and product characterisation data with estimates of raw material quantities, likely process flow designs, and real-world prices for process inputs and output. This process consisted of five main steps:

i. Raw material quantities and properties estimation
ii. Mass balance around the protein extraction and recovery process
iii. Sizing of process equipment and capital cost estimation
iv. Operating cost estimation
v. Profitability analysis

6.2 Raw Material Quantities and Properties Estimation

The seasonal quantities of paunch grass generated in Upper North Island (Figure 6.4) region of New Zealand were estimated from livestock slaughter statistics collected between 2014 and 2019 [4]. These data were used to calculate the mean heads of cattle and sheep slaughtered each season during this five-year period. These averages were then multiplied by 62 kg of paunch grass per head of cattle and 5 kg of paunch grass per head of sheep to yield the amount of paunch grass available for processing each season [5; 6]. These data were combined with the seasonal chemical profiles presented in Chapter 3 to generate a seasonal mass and composition profiles for the regional supply of paunch grass (Table 6.4).

Table 6.4: Average seasonal masses and chemical compositions for paunch grass generated in the Upper North Island of New Zealand between 2014 and 2019

Description	Winter	Spring	Summer	Autumn
Average Cattle Slaughtered (Head)	237773	121409	92070	110999
Average Sheep Slaughtered (Head)	142163	153932	283266	174650
Estimated Paunch Grass Dump Mass (MT)	15450	8300	7120	7760
Average of Moisture Content (w/w%)	83.2%	84.0%	75.3%	81.2%
Average of Crude Protein (%DM)	16.9%	18.3%	16.6%	11.4%
Average of Lipids (%DM)	8.6%	11.5%	8.3%	10.8%
Average of Total Carbohydrates (%DM)	16.0%	19.0%	23.2%	22.1%
Average of Crude Fibre (%DM)	9.5%	8.6%	10.2%	7.2%
Average of Ash (%DM)	16.8%	15.4%	11.1%	10.9%
Average of Organic N Content (%Total N)	81.3%	82.8%	79.1%	82.3%

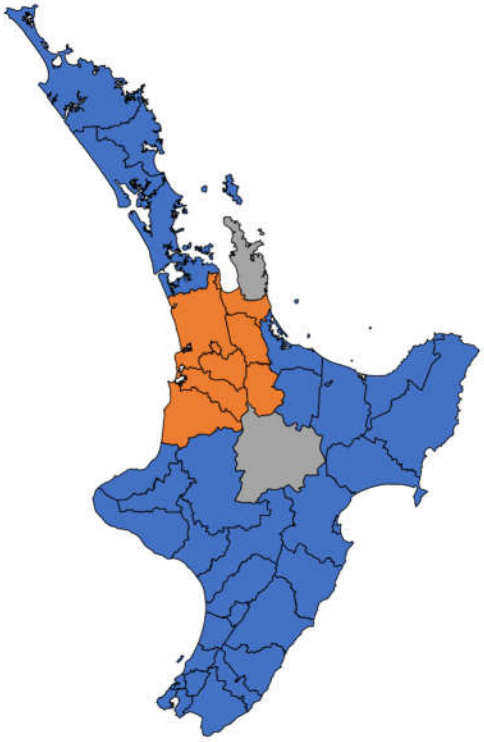

Figure 6.4: The Greater Waikato region is shaded orange. The Thames-Coromandel and Taupo Districts were shaded grey; they are part of the Waikato Regional Council area but were excluded from the analysis.

Adapted under license (CC BY-SA 4.0) from original work by Korakys [1]

A more complex calculation was required to estimate the seasonal quantity of leafy green waste generated in the Greater Waikato region of New Zealand (Figure 6.4). The most recent survey of leafy green waste generated in New Zealand was in 2007 [7]; this data needed to be adjusted for both the population of the Greater Waikato region in 2007 and population growth to 2018: the most recent year that detailed population data is available at a territorial authority level [8]. It was assumed that leafy green waste is solely generated by urban areas as leafy green waste generated in rural areas is likely to be disposed of within its generating property. Hence data from Statistics New Zealand [4], the World Bank [9], and van Bunnik, *et al.* [7] were used to calculate the amount of leafy green waste generated per head of urban population in New Zealand in 2007, which was assumed to remain constant through to 2018. The urban population of the Greater Waikato Region (Figure 6.4) was calculated by subtracting the populations of the Taupo District and Thames Coromandel District from that of the Waikato Regional Council area [8]. The remaining population was then multiplied by a factor of 75% (the fraction of urbanised population in the Waikato Regional Council area) [10]. These data were then combined with the seasonal dump mass distribution determined by Ministry

for the Environment [11] and the seasonal chemical profiles presented in Section 4.3 to calculate the total mass (Table 6.5) and seasonal masses of GW available for processing in the Greater Waikato Region (
Table 6.6).

Table 6.5: Total mass of leafy green waste generated in the Greater Waikato region of New Zealand in 2018

Description	Quantity	Source
Population of New Zealand in 2007	4,223,800	[4]
Population of New Zealand in 2018	4,824,600	[4]
Relative Urban Population of New Zealand in 2007	86.3%	[9]
Relative Urban Population of New Zealand in 2018	86.5%	[9]
Urban Population of New Zealand in 2007	3,645,100	
Urban Population of New Zealand in 2018	4,173,300	
Mass of Waste Sent to Landfill in NZ in 2007 (MT)	2,461,000	[11]
Leafy green waste content of landfill waste (w/w%)	6.02%	[11]
Leafy green waste produced in NZ in 2007 (MT)	148,100	
Leafy green waste produced per head of NZ urban population in 2007 (kg)	40.6	
Leafy green waste produced in NZ in 2018 (MT)	169,600	
Population of Waikato Regional Council Area	458,202	[8]
Urban Population Percentage of Waikato Region	75.0%	[10]
Population of Taupo District Council Area	37,203	[8]
Population of Thames-Coromandel District Council Area	29,895	[8]
Greater Waikato Population in 2018	*391,104*	
Greater Waikato Urban Population in 2018	*293,328*	
LEAFY GREEN WASTE PRODUCED IN THE GREATER WAIKATO IN 2018 (MT)	***11,900***	

***Italics* denote calculated quantities**

Table 6.6: Seasonal masses and chemical compositions of leafy green waste generated in the Greater Waikato region of New Zealand in 2018

Description	Winter	Spring	Summer	Autumn
Seasonal Proportion of Annual Dump Mass	19%	26%	33%	22%
ESTIMATED LEAFY GREEN WASTE DUMP MASS (MT)	2270	3080	3920	2630
Average of Moisture Content (w/w%)	73.6%	78.3%	67.7%	73.8%
Average of Crude Protein (%DM)	13.6%	18.9%	17.1%	15.4%
Average of Lipids (%DM)	13.7%	10.4%	11.1%	12.1%
Average of Total Carbohydrates (%DM)	15.1%	17.4%	20.3%	15.7%
Average of Crude Fibre (%DM)	5.0%	4.7%	5.6%	3.8%
Average of Ash (%DM)	14.7%	18.2%	14.3%	17.7%
Average of Organic N Content (%Total N)	68.5%	79.5%	79.9%	75.2%

The relative and absolute amounts of paunch grass and leafy green waste generated inside the catchment area are presented in Figure 6.5. During winter the total quantity of waste generated is significantly higher than during other seasons and the leafy green waste is generally difficult to separate from the other components of the garden waste streams [12]; hence the processing plant will only handle paunch grass during winter. This allows a design capacity of 125 metric tonnes per day to be assumed for the processing plant and enables calculation of seasonal average chemical compositions for the leafy green waste feedstock (Table 6.7).

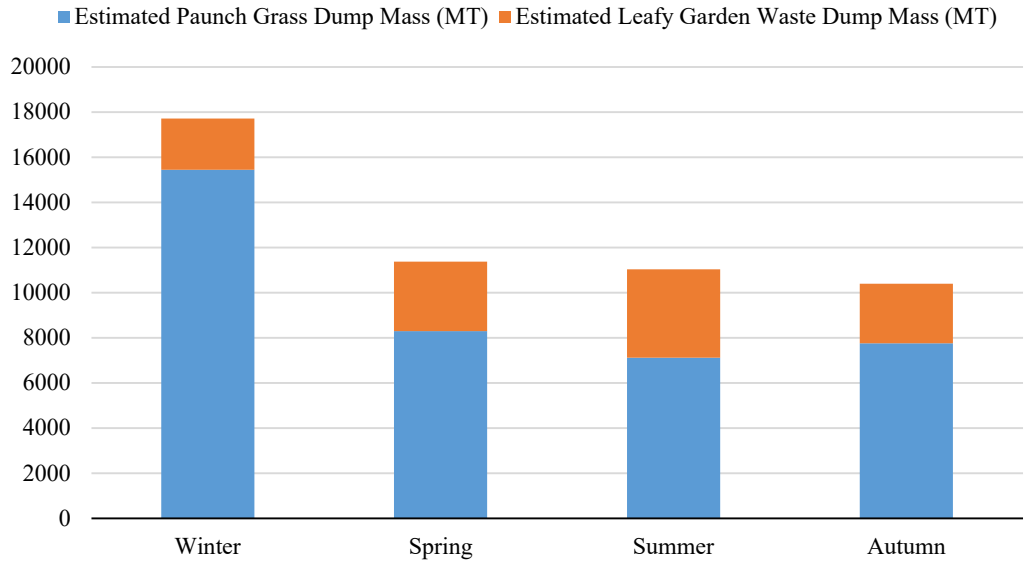

Figure 6.5: Seasonal process catchment area dump masses of leafy green wastes

Table 6.7: Annual and seasonal mean chemical compositions of leafy green wastes collected for leaf protein concentrate production

Description	Annual Mean	Winter	Spring	Summer	Autumn
Moisture Content (w/w%)	78.5%	83.2%	82.5%	72.6%	79.3%
Solids Content (w/w%)	21.5%	16.8%	17.5%	27.4%	20.7%
Crude Protein (%DM)	16.3%	16.9%	18.5%	16.7%	12.5%
Lipids (%DM)	10.0%	8.6%	11.0%	9.6%	11.4%
Total Carbohydrates (%DM)	18.4%	16.0%	18.2%	21.8%	18.9%
Crude Fibre (%DM)	7.7%	9.5%	6.8%	8.0%	5.6%
Ash (%DM)	15.3%	16.8%	16.7%	12.6%	14.2%
Organic Nitrogen Content (%Total N)	80.6%	81.3%	81.6%	79.4%	79.8%

6.3 Process Mass Balance

The process mass balance (Table 6.8) was performed according to the universal mass accounting equation (Equation 6.1) described by Holtzapple and Reece [13]. Unit operation mass balances were systematically performed for each step in the process block flow diagram (Figure 6.6). Each mass stream was broken down into component mass fractions (Figure 6.7, Table 6.9, & Table 6.10) which were used to calculate component mass flows and stream totals; the relevant stream totals were then used to calculate the overall process mass balance. All calculations were performed in a Microsoft Excel workbook, including iterative calculations to determine the mass flow rates of the recycle streams (Appendix C). Full stream tables can be found in Appendix D.

Table 6.8: Terms included in the process mass balance for leafy green waste protein concentrate production.

Mass In	Mass Out	Mass Generation	Mass Consumption
Leafy green waste	Press Fibre	*Protein in Solution [Screw Press 1]*	*Plant-Bound Protein [Screw Press 1]*
Process Water	Water Vapour	*Protein in Solution [Screw Press 2]*	*Plant-Bound Protein [Screw Press 2]*
30% HCl Solution	Liquor Effluent	*Coagulated Protein [Steam Injector]*	*Protein in Solution [Steam Injector]*
Steam	Leaf Protein Concentrate		

Equation 6.1: Universal mass accounting equation

$$\sum \dot{m}_{in} - \sum \dot{m}_{out} + \sum \dot{m}_{generation} - \sum \dot{m}_{consumption} = 0$$

The process model was based on the process published by Donnelly *et al.* [14]. Although McDonald [15] suggested the addition of a third screw press, this process follows the original two-press design – the investigations presented in Chapter 5 determined that triple-pressing is unnecessary for processing leafy green wastes.

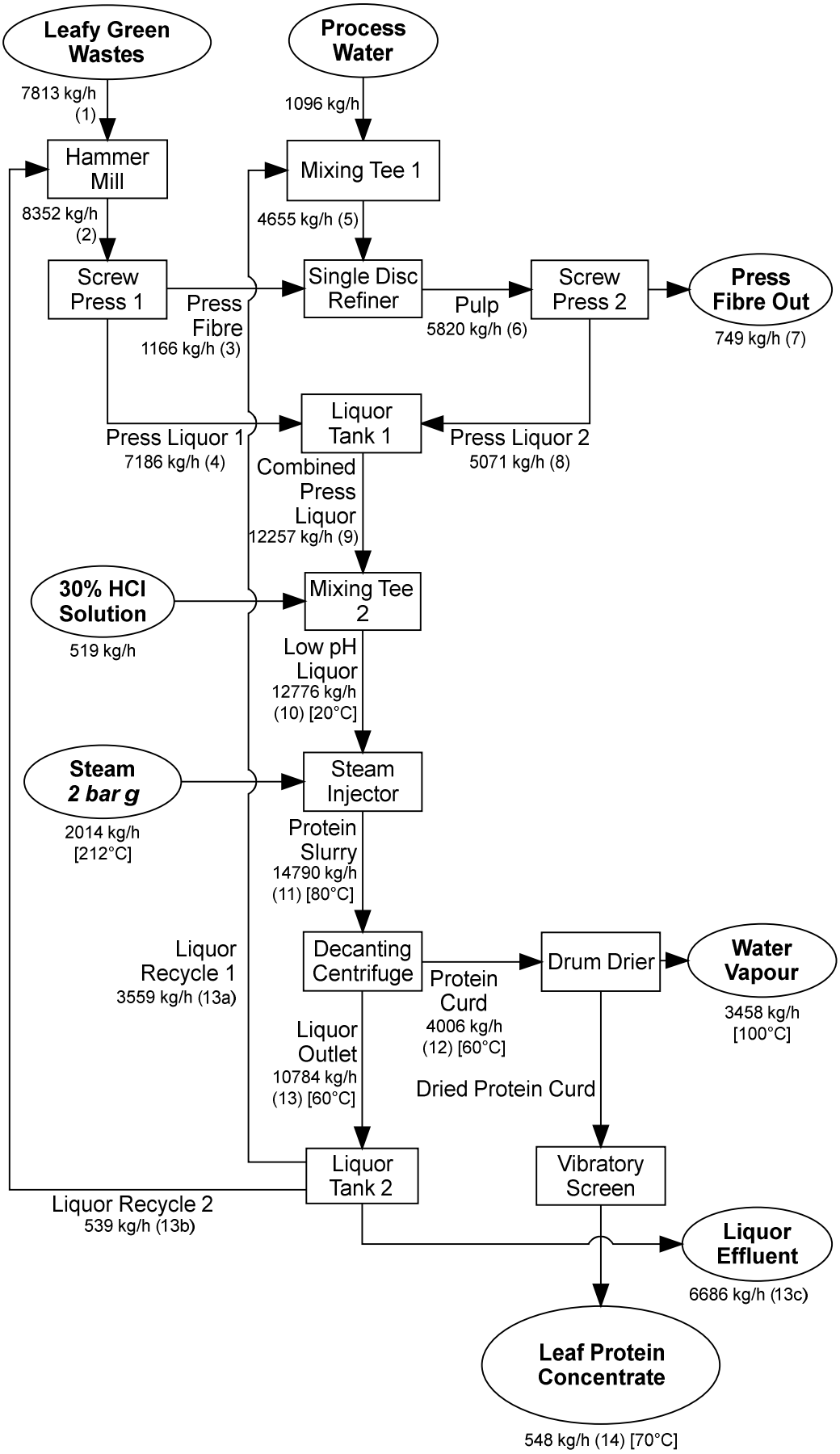

Figure 6.6: Block flow diagram of the soft leafy waste protein concentrate production process with mass flows and selected stream temperatures. Brackets identify streams in the composition table.

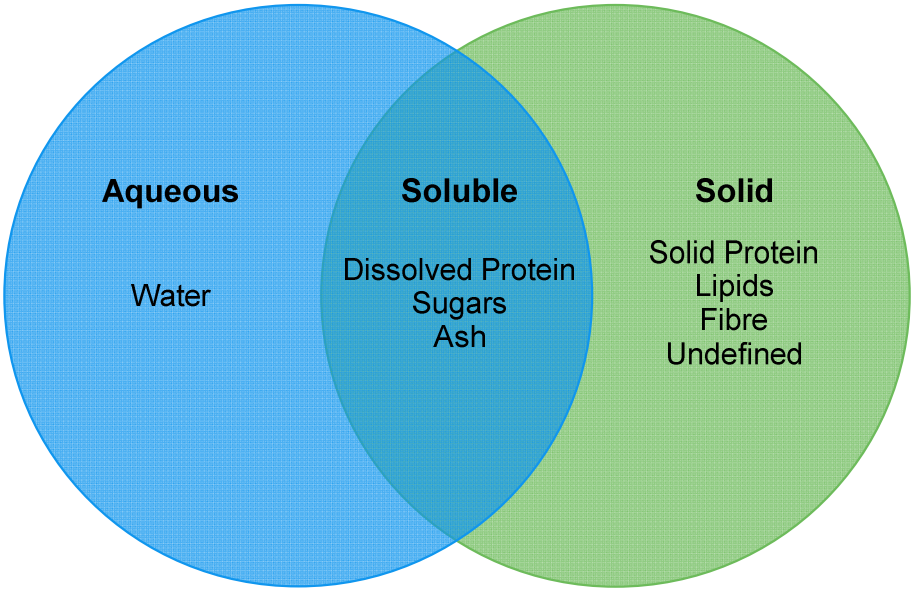

Figure 6.7: Mass stream component fractions modelled in the process mass balance.

Table 6.9: Mass composition of process mass balance streams 1 to 7.

Stream No.	1	2	3	4	5	6	7	8	9
Water	78.48%	78.88%	58.08%	82.26%	88.26%	82.21%	54.60%	86.29%	83.93%
Soluble components	84.09%	85.06%	64.00%	90.64%	94.04%	88.67%	59.22%	93.59%	91.86%
Solids	21.52%	21.12%	41.92%	17.74%	11.74%	17.79%	45.40%	13.71%	16.07%
Protein bound to plant material / protein solids	3.50%	2.83%	6.90%	0.00%	0.00%	0.73%	1.85%	0.00%	0.00%
Protein in solution	0.00%	0.50%	1.74%	2.46%	0.55%	1.43%	1.28%	2.02%	2.28%
Lipids	2.15%	2.10%	2.93%	1.96%	1.02%	1.40%	3.18%	1.14%	1.62%
Crude carbohydrates	2.32%	2.27%	1.67%	2.37%	1.23%	1.32%	0.88%	1.38%	1.96%
Fibre	1.65%	1.55%	11.07%	0.00%	0.00%	2.22%	17.24%	0.00%	0.00%
Ash	3.29%	3.41%	2.51%	3.56%	4.00%	3.71%	2.46%	3.89%	3.70%
Undefined	8.61%	8.47%	15.09%	7.39%	4.94%	6.97%	18.51%	5.27%	6.51%
Total (%)	***100.00***	***100.00***	***100.00***	***100.00***	***100.00***	***100.00***	***100.00***	***100.00***	***100.00***

Table 6.10: Mass composition of process mass balance streams 7 to 14.

Stream No.	10	11	12	13	13a	13b	13c	14
Water	83.18%	85.47%	87.69%	84.64%	84.64%	84.64%	84.64%	10.00%
Soluble components	88.80%	89.16%	91.47%	92.21%	92.21%	92.21%	92.21%	39.85%
Solids	16.82%	14.53%	12.31%	15.36%	15.36%	15.36%	15.36%	90.00%
Protein bound to plant material / protein solids	0.00%	1.17%	4.31%	0.00%	0.00%	0.00%	0.00%	31.54%
Protein in solution	2.19%	0.72%	0.74%	0.72%	0.72%	0.72%	0.72%	5.42%
Lipids	1.56%	1.34%	1.38%	1.33%	1.33%	1.33%	1.33%	10.08%
Crude carbohydrates	1.88%	1.63%	1.67%	1.61%	1.61%	1.61%	1.61%	12.19%
Fibre	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Ash	4.95%	4.27%	1.68%	5.24%	5.24%	5.24%	5.24%	12.25%
Undefined	6.25%	5.40%	2.54%	6.46%	6.46%	6.46%	6.46%	18.53%
Total (%)	***100.00***	***100.00***	***100.00***	***100.00***	***100.00***	***100.00***	***100.00***	***100.00***

6.3.1 Assumptions

Performing the process mass balance required a series of assumptions to be made regarding:

- The protein extraction ratios of the hammer mill, disc mill, and screw presses;
- The moisture, lipid, and fibre contents of the fibre streams exiting the screw presses;
- The relative quantity of centrifuge supernatant liquor recycled to the hammer mill and the single disc refiner;
- The relative quantity of water added to the single disc refiner;
- The properties and relative quantity of the hydrochloric acid solution used to acidify the press liquor;
- The pressure and mass addition ratio of the steam injected into the acidified press liquor;
- The protein recovery ratio during steam coagulation;
- The dry matter, crude protein, and ash contents of the protein curd ejected from the decanting centrifuge; and
- The final moisture content of the leaf protein concentrate exiting the process.

These assumptions were generally based on the empirical data presented in Chapter 5 with three exceptions:

- The water addition and liquor recycle ratios were based on iterative calculation trials that optimised process performance;
- All crude fibre fed into the screw presses was assumed to exit with the fibre streams; and
- The moisture content of the leaf protein concentrate was based on specifications for the alfalfa leaf protein concentrate already on the market [16].

Details of these assumptions are presented by process section (Figure 6.8).

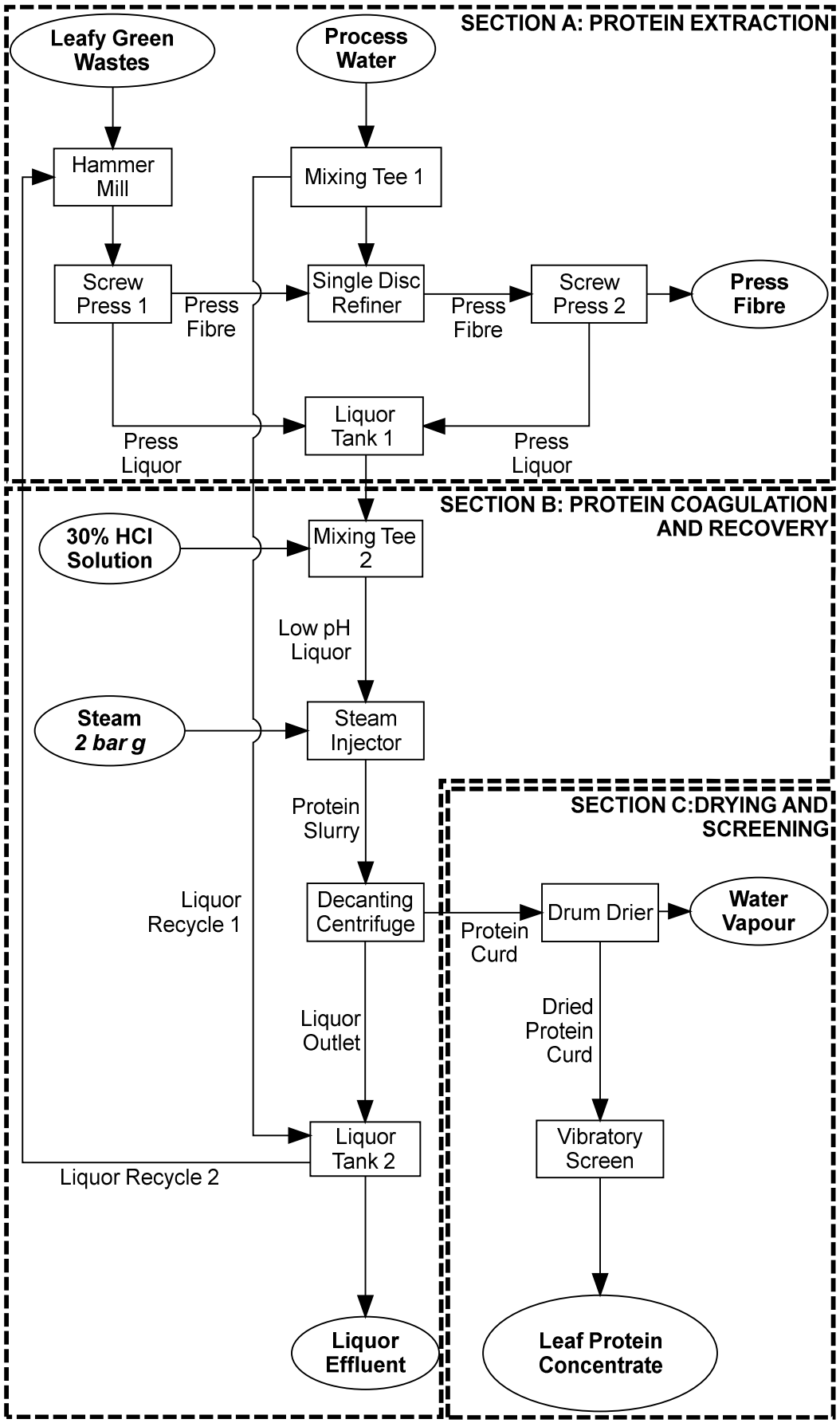

Figure 6.8: Process sections overlaid on the block flow diagram of the soft leafy waste protein concentrate production process

Section 1: Mills and Presses

The paunch grass and leafy green waste parameters assumed for the protein extraction section of the process are presented in Table 6.11. Each basis in the process model used a feedstock parameter calculated as an average of the PG and GW parameters weighted by the mass ratio of GW to PG in the process feedstock.

Table 6.11: PG and GW parameter assumptions for the protein extraction section of the leaf protein concentrate production process

Assumption	PG	GW
Protein extraction ratio of the hammer mill	0.1538	0.0743
Protein extraction ratio of the single disc refiner	0.1538	0.0743
Protein extraction ratio for Screw Press 1	0.6040	0.4261
Protein extraction ratio for Screw Press 2	0.0482	0.3328
Liquor recycle ratio to the hammer mill *(mass fraction of centrifuge supernatant)*	0.05	0.05
Liquor recycle ratio to the single disc refiner *(mass fraction of centrifuge supernatant)*	0.33	0.33
Water addition ratio to the single disc refiner *(mass fraction of press fibre exiting Screw Press 1)*	0.94	0.94
Moisture content of press fibre exiting Screw Press 1	0.5860	0.5600
Lipid content of press fibre exiting Screw Press 1	0.0774	0.0404
Moisture content of press fibre exiting Screw Press 2	0.5449	0.5505
Lipid content of press fibre exiting Screw Press 2	0.0774	0.0404
Mass recovery of crude fibre in the screw presses $\left(\frac{m_{FC}(Press\ Fibre)}{m_{FC}(Press\ Feed)}\right)$	1.00	1.00

The protein extraction ratios of the hammer mill, disc mill, and screw presses were determined by solving simultaneous equations (Equation 6.2 to Equation 6.4) for the protein extraction ratios achieved with different combinations of unit operations. Protein extraction efficiencies for processing paunch grass and leafy green waste were derived from the empirical data presented in Chapter 5 and substituted into the array and solved for η_M, η_{P1}, and η_{P2}. Iterative calculations in the process model were used to determine the respective liquor recycle ratios of centrifuge supernatant liquor fed into the hammer mill and the mixing tee above the single disc refiner. Similar calculations were used to determine the mass addition ratio for the process water used to dilute recycled liquor in the mixing tee above the single disc refiner. The moisture and lipid contents of the fibre exiting the screw presses were also derived from the empirical data presented in Chapter 5. All crude fibre fed into the screw presses was assumed to exit with the fibre stream, as the

crude fibre contents of the liquor streams were insignificant and could not be determined empirically.

Equation 6.2: Protein extraction ratio (η_a) yielded by a single maceration step and a single pass through the screw press	**Equation 6.3: Protein extraction ratio (η_b) yielded by a single maceration step and dual passes through the screw press**	**Equation 6.4: Protein extraction ratio (ηc) yielded by dual maceration steps and dual passes through the screw press**
$\eta_M + \eta_{P1} = \eta_a$	$\eta_M + \eta_{P1} + \eta_{P2} = \eta_b$	$2\eta_M + \eta_{P1} + \eta_{P2} = \eta_c$

Equation 6.5: Array of protein extractions equations with empirical paunch grass protein extraction ratios substituted for η_a, η_b, and η_c	**Equation 6.6: Array of protein extraction equations with empirical paunch grass protein extraction ratios substituted for η_a, η_b, and η_c**
$\left[\begin{array}{ccc\|c} 1 & 1 & 0 & 0.7578 \\ 1 & 1 & 1 & 0.8060 \\ 2 & 1 & 1 & 0.9598 \end{array}\right]$	$\left[\begin{array}{ccc\|c} 1 & 1 & 0 & 0.5004 \\ 1 & 1 & 1 & 0.5332 \\ 2 & 1 & 1 & 0.9075 \end{array}\right]$

Section 2: Protein Coagulation and Recovery

The paunch grass and leafy green waste parameters assumed for the protein coagulation and recovery section of the process are presented in Table 6.12. Each basis in the process model uses a feedstock parameter calculated as an average of the PG and GW parameters weighted by the mass ratio of GW to PG in the process feedstock. All parameters used in this section were derived from the empirical data presented in Chapter 5.

Table 6.12: PG and GW parameter assumptions for the protein coagulation and recovery section of the leaf protein concentrate production process

Assumption	PG	GW
Hydrochloric acid concentration (v/v%)	30%	30%
Hydrochloric acid concentration (w/w%)	34.5%	34.5%
Hydrochloric acid solution density (kg/L)	1.15	1.15
Hydrochloric acid addition ratio (kg-HCl added per kg-total-liquor-flow*) **total liquor flow from Screw Presses 1 & 2*	0.0905	0.0544
Steam pressure (bar g)	200	200
Steam injection ratio (kg-steam/kg-acidified-liquor-flow)	0.1576	0.1576
Protein recovery ratio $\left(\frac{m_{solid\ protein(protein\ slurry)}}{m_{dissolved\ protein(acidified\ liquor)}}\right)$	0.6460	0.5061
Centrifuge solids outlet dry matter content (w/w%)	11.66%	14.93%
Centrifuge solids outlet crude protein content (DM%)	37.72%	24.33%
Centrifuge solids outlet ash content (DM%)	10.23%	27.14%

Section 3: Drying and Screening

Only a single assumption was required for the drying and screening section of the process. The leaf protein concentrate exiting the drum drier had an assumed moisture content of 10% (w/w). This moisture content is consistent with the specifications for commercially available alfalfa leaf protein concentrates [16].

6.3.2 Conversion of Mass Stream Components

The mass balance includes several production and consumption terms due to changes that occur during key unit operations. The net change in mass around each of these process steps is zero, but mass can still be converted from one component to another. For example, the mass of protein in solution increases as the protein bound to plant material is liberated during hammer mill attrition, screw press extraction, and disc milling. During steam coagulation, the protein in solution is coagulated to recover protein solids.

6.3.3 Treatment of Soluble Components

The distribution of soluble components between wet solid streams and liquid streams is described by Equation 6.7; soluble and insoluble components are defined in Table 6.13 and Figure 6.7.

Equation 6.7: Calculation of the soluble components of wet solid streams where x refers to a mass fraction of the total stream and z refers to a mass fraction of the soluble components

$$\dot{m}_{soluble} = \frac{\dot{m}_{insoluble}}{\left(1 - x_{water} - \sum z_{i,soluble}\right)}$$

Table 6.13: Definition of soluble and insoluble components during the leaf protein production process

Component	Protein Coagulation and Centrifugation Step Solubility	Rest of Process Solubility
Water	✓	✓
Solid protein	×	×
Dissolved protein	✓	✓
Lipids	✓	×
Crude carbohydrates	✓	✓
Fibre	×	×
Ash	×	✓
Other	×	×

Table 6.14: Decanting centrifuge solid ash content parameters

Parameter	Paunch Grass Processing	Leafy Green Waste Processing	Weighted Average
Ash Content of Solids Exiting the Decanting Centrifuge (Dry Matter %)	10.23%	27.14%	13.61%

The exception is for the ash content during the steam coagulation and centrifugation step, where it is treated as an insoluble component. It is assumed that the solids ejected from the decanting centrifuge in Figure 6.9 have an ash content of 13.61% of dry matter (Figure 6.12), as this gives an ash content in the final leaf protein concentrate which is consistent with the results observed in Chapter 5.

6.4 Process Equipment Sizing and Capital Cost Estimation

The design basis for sizing process equipment and estimating capital cost assumes the plant processes 125 tonnes of feedstock per day over 16 hours in a semi-continuous operation mode. This allows the daily operational hours of the plant to be adjusted to suit the amount of feedstock available for processing. Process equipment was sized and its capital cost estimated according to the factorial method developed by Gerrard [17] (adapted for New Zealand by Bouman *et al.* [18]):

- Develop an inventory of main plant items (MPI) and calculate the relevant sizing characteristics for each main plant item (mass flow, volumetric flow, power consumption etc).
- Use these sizing characteristics to determine the uninstalled main plant item cost (MPIC) for each MPI.
- Adjust the MPICs to New Zealand Dollars (NZD) with the applicable exchange rates, standardise the MPICs at December 2000 prices with the applicable price indices, and convert all MPIC to a carbon steel basis ($MPIC_x$) using materials factors.
- Calculate the erected cost for each item (c_x) in December 2000 by applying factors to allow for various costs of installation then adjust the erected costs to December 2019 prices with the applicable price indices.
- Calculate the total equipment erected cost at December 2019 prices.
- Calculate the total permanent capital investment by applying installation overhead factors to the total erected cost.

6.4.1 Main Plant Item Inventory and Sizing

The design basis process mass balance was used to develop a process flow diagram (PFD) for the leaf protein concentrate production facility (Figure 6.9 & Appendix B). Three scenarios were considered when developing the inventory for the facility: standalone production facility; battery-limits installation; integrated production facility (i.e. integrated into rendering plant).

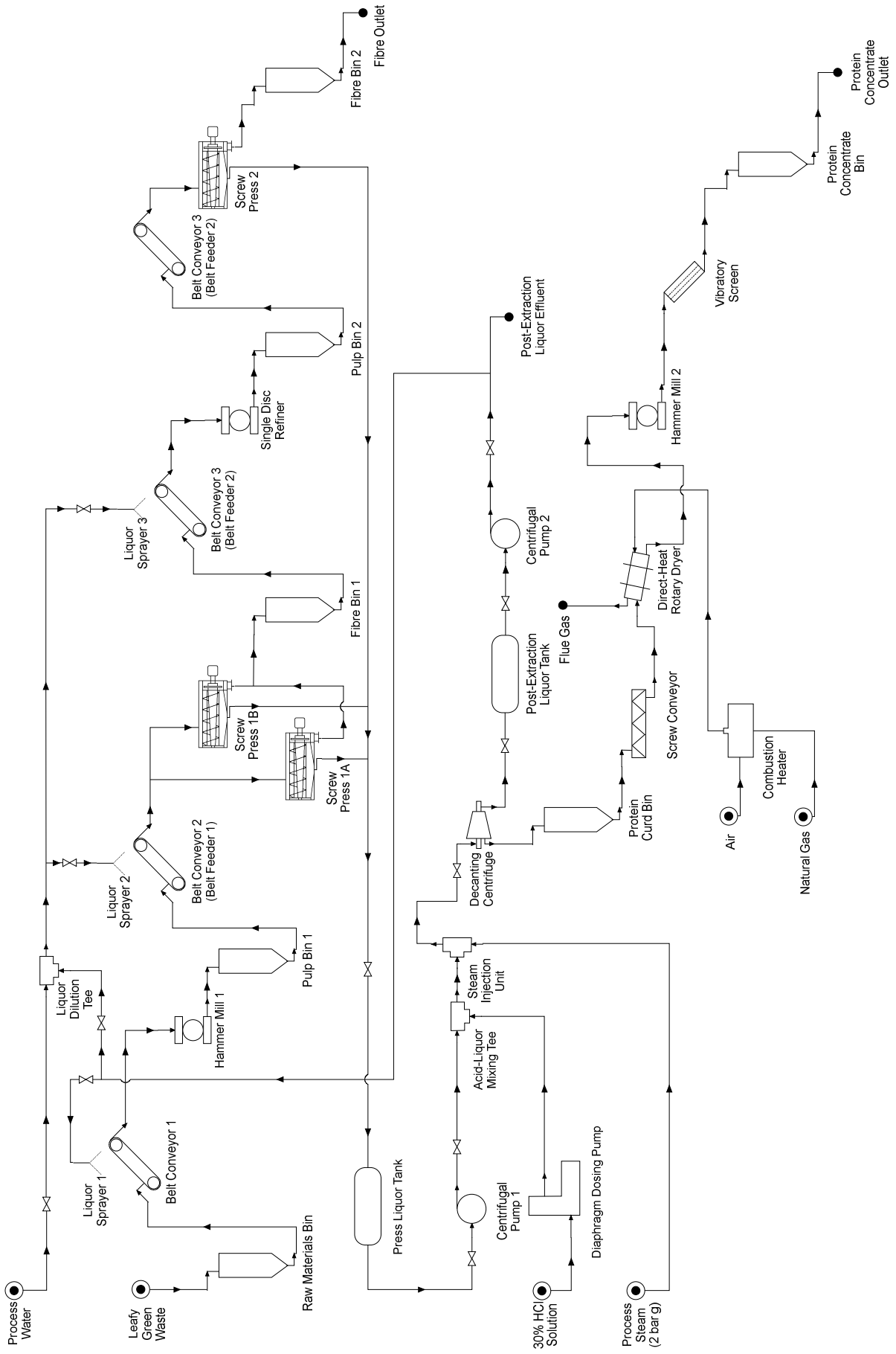

Figure 6.9: Process flow diagram for soft leafy waste protein concentrate production facility

The PFD and process mass balance was then used to develop an inventory of MPIs for a battery-limits installation (Table 6.15 and Table 6.16) based on sizing correlations published by Bouman, *et al.* [18] and Seider *et al.* [19]. A supplementary inventory was prepared for the utility plant in a standalone facility (Table 6.17). The characteristic values for each MPI were initially determined by the process mass flow rates; further characteristic values were calculated according to the assumptions in Table 6.18.

Table 6.15: Main Plant Items and their characteristic values for the protein coagulation and recovery & the drying and screening sections of a battery-limits installation soft leafy waste protein concentrate production facility

Main Plant Item	Primary Value				Secondary Value		Tertiary Value	
Raw Materials Bin	115.8	m^3	4088	ft^3				
Belt Conveyor 1	9.75	m	31.98	ft	14	in		
Belt Conveyor 1 motor and drive	0.110	kW	0.082	hp	4.78	lb/s	16.0	ft
Hammer Mill 1	8352	kg/h	9.21	Short ton/h	185	kW		
Belt Conveyor 2	9.86	m	32.36	ft	14	in		
Belt Conveyor 2 motor and drive	0.118	kW	0.088	hp	5.11	lb/s	16.2	ft
Pulp Bin 1	75.1	m^3	2651	ft^3				
Belt Feeder 1	9.4	m^3/h	331.4	ft^3/h				
Screw Press 1A	4176	kg/h	9206	lb/h	14.9	kW		
Screw Press 1B	4176	kg/h	9206	lb/h	14.9	kW		
Fibre Bin 1	45	m^3	1586	ft^3				
Belt Feeder 2	5.6	m^3/h	198.2	ft^3/h				
Belt Conveyor 3	7.48	m	24.54	ft	14	in		
Belt Conveyor 3 motor and drive	0.017	kW	0.013	hp	0.71	lb/s	12.3	ft
Single-Disc Refiner	85	cm	34	in	110	kW		
Pulp Bin 2	52.3	m^3	1847.6	ft^3				
Belt Feeder 3	6.5	m^3/h	231.0	ft^3/h				
Screw Press 2	5820	kg/h	12831	lb/h	14.9	kW		
Liquor Holding Tank	87.71	m^3	23170	US gal				
Belt Conveyor 4	6.13	m	20.12	ft	14	in		
Belt Conveyor 4 motor and drive	0.010	kW	0.007	hp	0.46	lb/s	10.1	ft
Fibre Bin 2	28.85	m^3	1019	ft^3				

Table 6.16: Main Plant Items and their characteristic values for the protein extraction section of a battery-limits installation soft leafy waste protein concentrate production facility

Main Plant Item	Primary Value				Secondary Value		Tertiary Value	
Centrifugal Pump 1	11.43	m^3/h	50.3	US gal /minute	45.6	m		
Centrifugal Pump 1 VSD	1.96	kW	3	hp				
HCl Diaphragm Dosing Pump	0.45	m^3/h	15.9	ft^3/h				
Direct Steam Injection Unit[*1]	0.32	m	1.04		0.3	m		
Decanting Centrifuge[*2]	0.597	kg/s	2.37	Short ton/h	42.9	kW		
Post-Extraction Liquor Holding Tank	77.2	m^3	20385	US gal				
Centrifugal Pump 2	9.65	m^3/h	42.5	US gal /minute	45.6	m		
Centrifugal Pump 2 VSD	1.65	kW	2	hp				
Protein Curd Bin 1	32.0	m^3	1130	ft^3				
Screw Feeder 1	4.000	m^3/h	141.3	ft^3/h				
Screw Feeder 1 VSD	0.084	kW	0.063	hp	2.45	lb/s	2	ft
Direct Heat Rotary Drier	104.7	m^2	1127	ft^2	7.7	ft	46.4	ft
Hammer Mill 2	1041.3	kg/h	1.15	Short ton/h	23	kW		
Vibratory Screen 1	3.0	m^2	32	ft^2	1.0	kW		
Protein Concentrate Bin	59.3	m^3	2093.8	ft^3				

[*1] Horizontal process vessel | [*2] Also called a continuous-scroll solid-bowl centrifuge

Table 6.17: Main Plant Items and their characteristic values for the utilities plant in a standalone soft leafy waste protein concentrate production facility

Main Plant Item	Primary Value		Secondary Value		Tertiary Value		Reference
Process Water Tank	8.76	m^3	2315	US gal			[19]
Centrifugal Pump 3 (Process Water)	1.10	m^3/h	4.82	US gal /minute	51.0	m	[18]
Centrifugal Pump 3 VSD	0.19	kW	0.252	hp			[18]
Natural Gas Fired Steam Boiler	2931	kW	10,000,000	Btu/h	1		[19]
Wastewater Treatment System	0.111	m^3/min	29.4	US gal /minute			[19]

Table 6.18: General assumptions for calculating secondary and tertiary characteristic values

Assumption	Value	Reference
Operational time capacity of bins and tanks	8 hours	[19]
Material of construction for bins and conveyors	Carbon Steel	[18; 19]
Conveyor incline	30° above horizontal	*Arbitrarily selected*
Material of construction for all other main plant items	Stainless Steel	[18; 19]
Pump pressure	500 kPa g	[20]
Electric motor efficiency, η_M	0.9	[19]
Centrifugal pump efficiency, η_P	0.9	[19]
Steam pressure	3 bar g	[Chapter 5]
Steam injector residence time	30 seconds	[Chapter 5]

In the absence of suitable empirical data, a conveyor incline of 30° above the horizontal was selected arbitrarily (Table 6.18), as this is the steepest angle a belt conveyor can operate at; steeper angles require buckets [21]. More detailed design assumptions were required to calculate the characteristic values for the disc refiner, direct-heat rotary drum drier, and vibratory screen. The disc refiner size (Table 6.19) was estimated by scaling up the diameter from the pilot plant described by McDonald [15] and rounding up to the nearest size offered by the equipment manufacturer [22].

Table 6.19: Assumptions for calculating the characteristic values of the single disc refiner

Assumption	Value	*Reference*
Disc refiner capacity is proportional to disc area	N/A	[22]
Ruakura Pilot Plant Disc Refiner Capacity	1000 kg/h	[15; 23]
Ruakura Pilot Plant Disc Refiner Diameter	12 inches	[15; 23]
Soft Leafy Waste Facility Disc Refiner Capacity	7800 kg/h	[22]
Soft Leafy Waste Facility Disc Refiner Diameter	34 inches 85 cm	[22]

Determining the assumptions for sizing the direct-heat rotary drum drier was a more complicated process (Table 6.20). Initial assumptions about the protein curd inlet temperature and water evaporation rate were derived from experimental data and the process mass balance, respectively. The drier was then iteratively optimised in an Excel spreadsheet using the guidelines published by Mani and Sokhansanj [24], sizing limits published by Seider*, et al.* [19]; and a psychrometric software package

incorporating a high-temperature psychrometric chart [25]; the latter was then used to determine the properties of the air streams entering and exiting the drier and the enthalpy change between them. The natural gas consumption was then calculated using the lower heating value published by Çengel and Boles [26], as this allowed the water vapour generated by the combustion of the natural gas to be disregarded.

Table 6.20: Assumptions for calculating the characteristic values of the direct-heat rotary drum drier

Assumption	Value		Reference
Protein curd inlet temperature	60°C		[Chapter 5]
Drying rate	2964	kg-H2O/h	*Calculated*
Drum drier water evaporation rate	3	lb/hr-ft^3	[19]
Inlet air DB T	10°C		[25]
Inlet air RH	90%		[25]
Inlet air enthalpy	28	kJ/kg-DA	[25]
Inlet air HR	7	g-H2O/kg-DA	[25]
Outlet air DB T	70°C		[25]
Outlet air RH	27.8%		[25]
Outlet air enthalpy	104.29 242.6	Btu/lb-DA kJ/kg-DA	[25]
Outlet air HR	409.8 903.5	g-H2O/lb-DA g-H2O/kg-DA	[25]
Outlet air specific volume	17.05 1.064	ft^3/lb-DA m^3/kg-DA	[25]
Air mass flow	7360	kg-DA/h	*Calculated*
Air volumetric flow	7833	m^3/h	*Calculated*
Drier diameter	7.7 2.36	ft m	[19; 24]
Air velocity	0.50	m/s	*Calculated*
Enthalpy change	1579197 439	kJ/h kW	*Calculated*
Thermal Efficiency	0.75		[24; 27]
Energy Demand	585	kW	*Calculated*
Natural Gas LHV	45000	kJ/kg	[26]
Natural Gas Flow Rate	0.0130 46.8	kg/s kg/h	*Calculated*

Refer to Appendix C for calculation details.

Selecting the assumptions to calculate the characteristic values of the vibratory screen (Table 6.21) simply involved selection of parameters according to the guidelines published by Seider, *et al.* [19] and the specifications published by WS Tyler [28].

Table 6.21: Assumptions for calculating the characteristic values of the vibratory screen

Assumption	Value		Reference
Screen Efficiency	0.8	ton/ft^2-hr-mm	[19]
Screen Size	32 18	ft^2 US mesh	[19; 28]

Several assumptions also had to be made to determine the characteristic values for a utility steam boiler for the standalone plant scenarios (Table 6.22). These were determined from thermodynamic data published by Çengel and Boles [26], and the process mass balance and efficiency data published by Cleaver Brooks [29]. The inlet water temperature was arbitrarily set at 15°C, as this was considered representative of the average temperature of mains water in the study area. The steam quality was also set to 1, as the steam supply was considered dry but not superheated; this is consistent with the operation of modern boiler systems which trap condensate and return it to the boiler [29].

Table 6.22: Assumptions for calculating the characteristic values of the utility steam boiler

Assumption	Value		Reference
Inlet Water T	15	°C	*Arbitrarily selected*
Water C_p	4.18	kJ/kg-K	[26]
Inlet Water Specific Enthalpy	62.7	kJ/kg	[26]
Steam flow rate	2014	kg/h	[26]
Steam Pressure	200 29.0 301.325	kPa g psi g kPa a	[26]
Steam T	212.38	°C	[26]
Sat Liq Specific Enthalpy	561.4	kJ/kg	[26]
Sat Vap Specific Enthalpy	2724.9	kJ/kg	[26]
Steam Quality	1		*Arbitrarily selected*
Specific Enthalpy Change	2662.2	kJ/kg	*Calculated*
Boiler Duty	1489	kW	*Calculated*
Boiler Thermal Efficiency	80%		[29]
Boiler Energy Consumption	1861 6351168	kW Btu/hr	*Calculated*
Characteristic Boiler Duty	10000000 10550560	Btu/hr kJ/hr	[19]
Characteristic Boiler Pressure	500	psi g	[19]

6.4.2 Main Plant Item Costs

The characteristic values for each Main Plant Item (MPI) in Section 6.4.1 were substituted into cost correlations estimate its uninstalled main plant item cost ($MPIC_a$) in New Zealand at 2004 prices [18] ; American cost correlations at 2006 prices [19] were used when no New Zealand correlation was available and converted to New Zealand Dollars with historical exchange rate data [3]. No cost correlations were available for the single disc refiner, so the main plant item cost was obtained directly from the manufacturer [22]. Most $MPIC_a$ were already estimated on carbon steel (CS) basis, but some were estimated on a stainless-steel (SS) basis and thus termed $MPIC_{a(x)}$. Each $MPIC_{a(x)}$ was thus divided by a materials factor (f_{mx}) to convert it to a carbon steel basis [18]; the Plant, Machinery, and Equipment Index (PMEI) published by Statistics New Zealand [4] and Equation 6.8 were then used to adjust all MPIC to January 2000 prices ($MPIC_{2000}$). Full inventories of $MPIC_a$ and $MPIC_x$ were hence prepared for a battery limits installation (Table 6.23 and Table 6.24) and a standalone utility plant (Table 6.25).

Equation 6.8: Conversion of various Main Plant Item Costs to a carbon steel basis at December 2000 prices (f_{mx}(CS) = 1.0 | f_{mx}(SS) = 2.0)

$$MPIC_a = \frac{MPIC_{a(x)}}{f_{mx}} \times \frac{I_{2000}}{I_a}$$

Table 6.23: Main Plant Item Costs at January 2000 carbon steel-basis prices for the battery-limits installation of the LPC production facility.

Main Plant Item	$MPIC_a$	f_{mx}	I_a	I_{2000}	$MPIC_{2000}$	Reference
Raw Materials Bin	$37,775	1.0	1389	1164	$31,656	[19]
Belt Conveyor 1	$13,914	1.0	1155	1053	$12,686	[19]
Belt Conveyor 1 motor and drive	$186	1.0	1044	1030	$184	[18]
Hammer Mill 1	$465,454	1.0	1050	1176	$521,308	[19]
Belt Conveyor 2	$14,081	1.0	1155	1053	$12,838	[19]
Belt Conveyor 2 motor and drive	$186	1.0	1044	1030	$185	[18]
Pulp Bin 1	$30,952	1.0	1389	1164	$25,938	[19]
Belt Feeder 1	$9,403	1.0	1155	1053	$8,573	[19]
Screw Press 1A	$532,955	2.0	1211	1064	$234,130	[19]
Screw Press 1B	$532,955	2.0	1211	1064	$234,130	[19]
Fibre Bin 1	$24,435	1.0	1389	1164	$20,477	[19]
Belt Feeder 2	$7,735	1.0	1155	1053	$7,052	[19]
Belt Conveyor 3	$10,678	1.0	1155	1053	$9,735	[19]
Belt Conveyor 3 motor and drive	$169	1.0	1044	1030	$167	[18]
Single-Disc Refiner	$25,816	1.0	1214	1242	$26,412	[19]
Pulp Bin 2	$26,214	1.0	1389	1164	$21,968	[19]
Belt Feeder 3	$8,197	1.0	1155	1053	$7,473	[19]
Screw Press 2	$691,042	2.0	1211	1064	$303,579	[19]

Table 6.24: Main Plant Item Costs at January 2000 carbon steel-basis prices for the battery-limits installation of the LPC production facility (continued).

Main Plant Item	$MPIC_a$	f_{mx}	I_a	I_{2000}	$MPIC_{2000}$	Reference
Liquor Holding Tank	$64,473	1.0	1389	1164	$54,029	[19]
Belt Conveyor 4	$8,756	1.0	1155	1053	$7,983	[19]
Belt Conveyor 4 motor and drive	$168	1.0	1044	1030	$166	[18]
Fibre Bin 2	$19,934	1.0	1389	1164	$16,705	[18]
Centrifugal Pump 1	$3,171	1.0	1070	1038	$3,076	[18]
Centrifugal Pump 1 VSD	$1,383	1.0	1044	1030	$1,365	[18]
HCl Diaphragm Dosing Pump	$1,240	1.0	1070	1038	$1,203	[18]
Direct Steam Injection Unit[*1]	$1,140	1.0	1389	1164	$955	[18]
Decanting Centrifuge[*2]	$215,608	2.0	1137	1242	$117,760	[18]
Post-Extraction Liquor Holding Tank	$60,397	1.0	1389	1164	$50,614	[19]
Centrifugal Pump 2	$2,945	1.0	1070	1038	$2,857	[18]
Centrifugal Pump 2 VSD	$1,331	1.0	1044	1030	$1,313	[18]
Protein Curd Bin 1	$20,908	1.0	1389	1164	$17,521	[19]
Screw Feeder 1	$13,593	1.0	1211	1064	$11,943	[19]
Screw Feeder 1 VSD	$407	1.0	1044	1030	$402	[18]
Direct Heat Rotary Drier	$425,014	2.0	1081	1056	$207,592	[19]
Hammer Mill 2	$91,748	1.0	1050	1176	$102,758	[19]
Vibratory Screen 1	$23,703	1.0	1050	1176	$26,547	[19]
Protein Concentrate Bin	$27,766	1.0	1389	1164	$23,269	[19]

[a] Horizontal process vessel | [2] Also called a continuous-scroll solid-bowl centrifuge PMEI values (I) from Statistics New Zealand [4]; and materials factors (f_{mx}) from Bouman, et al. [18]

Table 6.25: Main Plant Item Costs at January 2000 carbon steel-basis prices for the utility plant of a standalone soft leafy waste protein concentrate production facility

Main Plant Item	$MPIC_a$	f_{mx}	I_a	I_{2000}	$MPIC_{2000}$	Reference
Process Water Tank	$19,917	1.0	1389	1164	$16,691	[19]
Centrifugal Pump 3 (Process Water Pump)	$1,859	1.0	1070	1038	$1,804	[18]
Centrifugal Pump 3 VSD	$432	1.0	1044	1030	$426	[18]
Natural Gas Fired Steam Boiler	$343,604	1.0	1288	999	$266,506	[19]
Wastewater Treatment System	$1,108,172	1.0	1242	1099	$980,580	[19]

PMEI values (I) from Statistics New Zealand [4]; and materials factors (f_{mx}) from Bouman, et al. [18]

The Bare Module Cost at January 2000 prices ($c_{BM,2000}$) was calculated from Equation 6.9 for each Main Plant Item (Table 6.26 to Table 6.29); the calculation includes factors for: the delivered cost in a specified material (f_{my}); erection (f_{er}); piping, ducting, and chutes (f_p); instrumentation (f_i); electrical (f_{el}); civil work (f_c); surrounding structures and buildings (f_{sb}); and lagging (f_l). The factors for civil work were multiplied by 1.3 as foundations may need to be piled [18].

Equation 6.9: Calculation of the Bare Module Cost for a Main Plant Item at January 2000 prices. $f_{my}(CS) = 1.0$ | $f_{my}(SS) = 2.0$

$$c_{BM,2000} = MPIC_x\left(f_{my} + f_{er} + f_p + f_i + f_{el} + 1.3f_c + f_{sb} + f_l\right)$$

Table 6.26: Bare Module Costs at January 2000 prices for the battery-limits installation of a soft leafy waste protein concentrate production facility

Main Plant Item	$MPIC_{2000}$	f_{my}	f_{er}	f_p	f_i	f_{el}	f_c	f_{sb}	f_l	$c_{BM.2000}$
Raw Materials Bin	$31,656	1.00	0.08	0.28	0.24	0.10	0.22	0.05	0.00	$64,293
Belt Conveyor 1	$12,686	1.00	0.15	0.43	0.43	0.41	0.28	0.59	0.00	$42,801
Belt Conveyor 1 motor-drive	$184	1.00	0.38	0.00	0.75	0.60	0.35	0.74	0.00	$722
Hammer Mill 1	$521,308	1.00	0.10	0.05	0.13	0.25	0.21	0.24	0.00	$1,065,032
Belt Conveyor 2	$12,838	1.00	0.15	0.43	0.43	0.41	0.28	0.59	0.00	$43,314
Belt Conveyor 2 motor-drive	$185	1.00	0.38	0.00	0.75	0.60	0.35	0.74	0.00	$728
Pulp Bin 1	$25,938	1.00	0.08	0.28	0.24	0.10	0.22	0.50	0.00	$64,353
Belt Feeder 1	$8,573	1.00	0.20	0.59	1.00	0.60	0.28	0.85	0.00	$39,470
Screw Press 1A	$234,130	2.00	0.13	0.40	0.22	0.34	0.31	0.39	0.00	$909,128
Screw Press 1B	$234,130	2.00	0.13	0.40	0.22	0.34	0.31	0.39	0.00	$909,128
Fibre Bin 1	$20,477	1.00	0.08	0.28	0.24	0.10	0.22	0.50	0.00	$50,802
Belt Feeder 2	$7,052	1.00	0.48	0.59	1.00	0.60	0.35	0.74	0.00	$34,307
Belt Conveyor 3	$9,735	1.00	0.15	0.43	0.43	0.41	0.28	0.74	0.00	$34,306
Belt Conveyor 3 motor-drive	$167	1.00	0.38	0.00	0.75	0.60	0.35	0.74	0.00	$655
Single-Disc Refiner	$26,412	1.00	0.18	0.98	0.77	0.60	0.50	0.59	0.00	$125,984
Pulp Bin 2	$21,968	1.00	0.08	0.28	0.24	0.10	0.22	0.50	0.00	$54,502
Belt Feeder 3	$7,473	1.00	0.48	0.59	1.00	0.60	0.35	0.85	0.00	$37,181
Screw Press 2	$303,579	1.00	0.13	0.40	0.22	0.34	0.31	0.39	0.00	$875,219
Liquor Holding Tank	$54,029	2.00	0.08	0.98	0.69	0.10	0.22	0.05	0.00	$225,896
Belt Conveyor 4	$7,983	1.00	0.38	0.59	0.75	0.60	0.35	0.74	0.00	$36,041
Belt Conveyor 4 motor-drive	$166	1.00	0.38	0.00	0.75	0.60	0.35	0.74	0.00	$650
Fibre Bin 2	$16,705	1.00	0.08	0.43	0.43	0.13	0.28	0.59	0.00	$50,432
Centrifugal Pump 1	$3,076	2.00	0.38	1.76	1.00	1.00	0.35	0.08	0.00	$20,535
Centrifugal Pump 1 VSD	$1,365	2.00	0.38	0.00	1.00	1.00	0.35	0.08	0.00	$6,707
HCl Diaphragm Dosing Pump	$1,203	1.00	0.00	1.40	1.00	0.00	0.00	0.00	0.00	$4,090
Direct Steam Injection Unit[*1]	$955	2.00	0.48	1.94	1.14	0.19	0.85	0.74	0.38	$7,619
Decanting Centrifuge[*2]	$117,760	2.00	0.15	0.66	0.60	0.60	0.40	0.41	0.14	$598,219

Lang Factors from Bouman, *et al.* [18]

Table 6.27: Bare Module Costs at January 2000 prices for the battery-limits installation of a soft leafy waste protein concentrate production facility (continued)

Main Plant Item	$MPIC_{2000}$	f_{my}	f_{er}	f_p	f_i	f_{el}	f_c	f_{sb}	f_l	$c_{BM.2000}$
Centrifugal Pump 2	$2,857	2.00	0.38	1.76	1.00	1.00	0.35	0.08	0.00	$19,070
Centrifugal Pump 2 VSD	$1,313	2.00	0.38	0.00	1.00	1.00	0.35	0.08	0.00	$6,455
Protein Curd Bin 1	$17,521	2.00	0.08	0.43	0.43	0.13	0.28	0.69	0.31	$77,601
Screw Feeder 1	$11,943	2.00	0.20	0.43	0.65	0.51	0.60	0.69	0.31	$66,525
Screw Feeder 1 VSD	$402	2.00	0.38	0.00	1.00	0.51	0.85	0.85	0.38	$2,500
Direct Heat Rotary Drier	$207,592	2.00	0.40	0.10	0.43	0.34	0.31	0.31	0.08	$843,448
Hammer Mill 2	$102,758	1.00	0.15	0.56	0.13	0.33	0.40	0.48	0.00	$325,743
Vibratory Screen 1	$26,547	1.00	0.13	0.28	0.49	0.34	0.50	0.56	0.00	$91,589
Protein Concentrate Bin	$23,269	2.00	0.08	0.28	0.24	0.10	0.28	0.56	0.21	$89,096

*[*a] Horizontal process vessel | [*2] Also called a continuous-scroll solid-bowl centrifuge*
Lang Factors from Bouman, *et al.* [18]

Table 6.28: Bare Module Costs for the steam plant of a standalone soft leafy waste protein concentrate production facility

Main Plant Item	$MPIC_{2000}$	f_{my}	f_{er}	f_p	f_i	f_{el}	f_c	f_{sb}	f_l	$c_{BM.2000}$
Process Water Tank	$16,691	2.00	0.09	1.04	0.65	0.13	0.28	0.06	0.00	$72,338
Centrifugal Pump 3	$1,804	2.00	0.38	1.40	1.00	1.00	0.35	0.08	0.00	$11,389
Centrifugal Pump 3 VSD	$426	2.00	0.38	0.00	1.00	1.00	0.35	0.08	0.00	$2,094
Steam Boiler (NG Fired)	$266,506	1.00	0.40	0.61	0.43	0.03	0.14	0.39	0.08	$832,033

Lang Factors from Bouman, *et al.* [18]

Table 6.29: The Bare Module Cost for a wastewater treatment plant in a standalone soft leafy waste protein concentrate production facility

Main Plant Item	$MPIC_{2000}$	f_{my}	f_{er}	f_p	f_i	f_{el}	f_c	f_{sb}	f_l	$c_{BM.2000}$
Wastewater Treatment System	$980,580	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	$980,580

Lang Factors from Bouman, *et al.* [18]

All $c_{BM,2000}$ were then converted to December 2019 prices ($c_{BM,2019}$) using PMEI data from Statistics New Zealand [4] and Equation 6.10. Full inventories of $c_{BM,2019}$ are presented in Table 6.30 and Table 6.31.

Equation 6.10: Adjustment of a Bare Module Cost from January 2000 prices to December 2019 prices

$$c_{BM,2019} = c_{BM} \times \frac{I_{2019}}{I_{2000}}$$

Table 6.30: Bare Module Costs adjusted to December 2019 prices for the battery-limits installation of a soft leafy waste protein concentrate production facility

Main Plant Item	$C_{BM,2000}$	I_{2000}	I_{2019}	$C_{BM,2019}$
Raw Materials Bin	$64,293	1164	1740	$96,108
Belt Conveyor 1	$42,801	1053	1505	$61,173
Belt Conveyor 1 motor and drive	$722	1030	1196	$838
Hammer Mill 1	$1,065,032	1176	1192	$1,079,523
Belt Conveyor 2	$43,314	1053	1505	$61,907
Belt Conveyor 2 motor and drive	$728	1030	1196	$845
Pulp Bin 1	$64,353	1164	1740	$96,198
Belt Feeder 1	$39,470	1053	1505	$56,412
Screw Press 1A	$909,128	1064	1581	$1,350,876
Screw Press 1B	$909,128	1064	1581	$1,350,876
Fibre Bin 1	$50,802	1164	1740	$75,942
Belt Feeder 2	$34,307	1053	1505	$49,034
Belt Conveyor 3	$34,306	1053	1505	$49,032
Belt Conveyor 3 motor and drive	$655	1030	1196	$760
Single-Disc Refiner	$125,984	1242	1214	$123,144
Pulp Bin 2	$54,502	1164	1740	$81,472
Belt Feeder 3	$37,181	1053	1505	$53,140
Screw Press 2	$875,219	1064	1581	$1,300,490
Liquor Holding Tank	$225,896	1164	1740	$337,680
Belt Conveyor 4	$36,041	1053	1505	$51,512
Belt Conveyor 4 motor and drive	$650	1030	1196	$754
Fibre Bin 2	$50,432	1164	1740	$75,388
Centrifugal Pump 1	$20,535	1038	1511	$29,893
Centrifugal Pump 1 VSD	$6,707	1030	1196	$7,788
HCl Diaphragm Dosing Pump	$4,090	1038	1511	$5,954
Direct Steam Injection Unit[*1]	$7,619	1164	1740	$11,389
Decanting Centrifuge[*2]	$598,219	1242	1214	$584,732
Post-Extraction Liquor Holding Tank	$208,073	1164	1740	$311,036
Centrifugal Pump 2	$19,070	1038	1511	$27,760
Centrifugal Pump 2 VSD	$6,455	1030	1196	$7,495
Protein Curd Bin 1	$77,601	1164	1740	$116,002
Screw Feeder 1	$66,525	1064	1581	$98,849
Screw Feeder 1 VSD	$2,500	1030	1196	$2,903
Direct Heat Rotary Drier	$843,448	1056	1315	$1,050,317
Hammer Mill 2	$325,743	1176	1192	$330,175
Vibratory Screen 1	$91,589	1176	1192	$92,835
Protein Concentrate Bin	$89,096	1164	1740	$133,184

Table 6.31: Bare Module Costs adjusted to December 2019 prices for the utility plant of a standalone soft leafy waste protein concentrate production facility

Main Plant Item	$C_{BM,2000}$	I_{2000}	I_{2019}	$C_{BM,2019}$
Process Water Tank	$72,338	1164	1740	$108,134
Centrifugal Pump 3 (Process Water Pump)	$11,389	1038	1511	$16,579
Centrifugal Pump 3 VSD	$2,094	1030	1196	$2,432
Natural Gas Fired Steam Boiler	$832,033	999	1534	$409,230
Wastewater Treatment System	$980,580	1099	1214	$1,083,189

The Total Bare Module Cost of the plant ($C_{TBM,2019}$) is the arithmetic sum of all relevant $c_{BM,2019}$ for a given scenario (Equation 6.11). $C_{TBM,2019}$ for each plant design (see Figure 6.1) are presented in Table 6.32.

Equation 6.11: Calculation of the Total Bare Module cost (installed cost) of the waste leaf protein concentrate production facility

$$C_{TBM,2019} = \sum c_{BM,2019}$$

Table 6.32: Total Bare Module Costs at December 2019 prices for various designs of soft leafy waste protein concentrate production facility

Design	Total Bare Module Cost ($C_{TBM,2019}$)
Standalone Plant	$11,651,364
Battery Limits Installation	$9,163,414
Integrated into Existing Rendering Plant	$4,604,980

The Total Permanent Capital Investment (C_{TPI}) was calculated by two methods: Bouman*, et al.* [18] (Table 6.33) and Seider*, et al.* [19] (Table 6.34). The former method specifies a few general factors for New Zealand conditions; the latter is more detailed and uses multiple discretionary factors for each specific cost. Iteratively adjusting these factors brought the estimates from both methods into agreement and allowed the determination of Total Depreciable Capital (C_{TDC}) and Working Capital requirements for profitability analyses (see Section 0).

Table 6.33: Total Permanent Capital Investment at December 2019 prices for various designs of soft leafy waste protein concentrate production facility calculated by the method of Bouman*, et al.* [18]

Description	Factor	Standalone Plant	Battery Limits Installation	Integrated with Existing Plant
Total Bare Module Cost* ($C_{TBM,2019}$)	1.00	$11,651,364	$9,163,414	$4,604,980
Engineering Design and Supervision (C_{EDS})	0.15	$1,747,705	$1,374,512	$690,747
Management Overheads (C_M)	0.10	$1,165,136	$916,341	$460,498
Commissioning Costs (C_{comm})	0.05	$582,568	$458,171	$230,249
Working Capital (C_{WC})	0.15	$1,747,705	$1,374,512	$690,747
Total Permanent Capital Investment (C_{TPI})	**1.45**	**$16,894,478**	**$13,286,951**	**$6,677,220**

No capital was allocated for utility plants in the battery limits and integrated plant installations, as the utility demand was considered negligible when compared to the existing utility capacity.

Table 6.34: Total Permanent Capital Investment at December 2019 prices for various designs of soft leafy waste protein concentrate production facility calculated by the method of Seider, *et al.* [19]

Capital Investment	Factor	Standalone Plant	Battery Limits Installation	Integrated with Existing Plant
Total bare-module investment (C_{TBM})	1.00	$11,651,364	$9,163,414	$4,604,980
Cost of Site Preparation (C_{site})	0.10	$1,159,901	$545,688	$274,230
Cost of Service Facilities (C_{serv})	0.21	$2,446,786	$2,290,854	$1,151,245
Allocated Costs for Utility Plants and Related Facilities (C_{alloc})	-	-	-	-
Total Direct Permanent Investment (C_{DPI})	**-**	**$15,258,052**	**$11,999,956**	**$6,030,454**
Cost of Contractor's Fees (C_{cont})	0.03	$457,742	$359,999	$180,914
Total Depreciable Capital (C_{TDC})	**-**	**$15,715,793**	**$12,359,954**	**$6,211,368**
Cost of Land (C_{land})	0.02	$314,316	$247,199	$124,227
Cost of Plant Startup ($C_{startup}$)	0.06	$864,369	$679,797	$341,625
Total Permanent Capital Investment (C_{TPI})	**1.41**	**$16,894,478**	**$13,286,951**	**$6,677,220**

6.5 Operating Costs and Revenues Estimation

The operating costs and revenues were estimated using the textbook cost sheet method of Seider, *et al.* [19]. The operational costs and revenues generated by each process configuration and coproduct utilisation scenario (see Figure 6.1) are summarized in Table 6.35 to Table 6.37; details of the assumptions and the calculation process are presented in Sections 6.5.1 to 0. Graphical breakdowns of Total Production Cost are presented in Figure 6.58, Figure 6.12, Figure 6.62; the Utilities Costs are also broken down in Figure 6.59, Figure 6.61, and Figure 6.63. Each process design and coproduct utilisation scenario summarized in Table 6.35 to Table 6.37 generated positive earnings before interest and tax (EBIT); this justified the calculation of both approximate profitability measures (Section 6.6.1) and rigorous profitability measures (Section 6.6.2). Figure 6.58, Figure 6.12, and Figure 6.62 demonstrate that differences in the Total Cost of Production can be caused by both plant design and operational scenario, but the plant design has the larger impact. The difference in total cost of production between standalone plant and battery limits installation processes is approximately $1M per annum and can be mostly attributed to greater depreciation, maintenance-related operations and operating overhead costs arising from the slightly higher value of the capital equipment installed the standalone plant [19] (see Sections 6.4.2 and 0).

Table 6.35: Cost sheet for standalone plant, battery limits installation, and integrated into rendering plant process configurations when coproducts are treated as waste

Cost Sheet Item	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
Feedstock Costs	$1,460,272	$1,460,272	$1,460,272
Utilities Costs	$1,333,999	$1,047,885	$537,496
Labour-Related Operations (O)	$7,419,241	$7,419,241	$3,709,620
Maintenance-Related Operations (M)	$1,333,999	$1,047,885	$537,496
Operating Overheads	$1,194,065	$1,037,353	$526,100
Depreciation	$1,329,952	$1,044,706	$535,866
Cost of Manufacture (COM)	$14,105,297	$13,169,776	$7,894,477
General Expenses	$524,764	$524,764	$524,764
Total Production Cost (C)	$14,639,971	$13,684,746	$8,444,644
Sales	$3,723,988	$3,723,988	$3,723,988
Gate Fees	$9,464,350	$9,464,350	$9,464,350
Total Revenue (R)	$13,188,338	$13,188,338	$13,188,338
Earnings Before Interest and Tax (EBIT)	-$1,451,632	-$496,408	$4,743,694

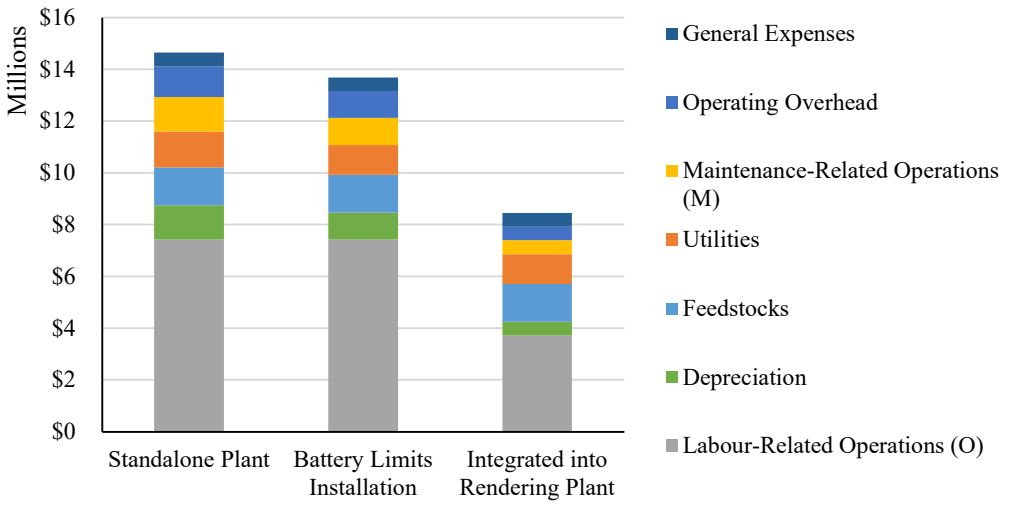

Figure 6.58: Breakdown of the total cost of production by process design when all coproducts are disposed as waste.

Utilities costs are also a factor - Figure 6.59, Figure 6.61, and Figure 6.63 indicate higher utilities costs (ca. $200k per annum) for the standalone plant process. These arise from the inefficiency of running an independent boiler below the minimum boiler capacity specified by Seider, *et al.* [19]; in contrast, the battery limits and

integrated into rendering plant processes purchase steam from the onsite utilities and therefore achieve greater energetic and financial efficiencies.

In addition to process design, the operational scenario also impacts the utilities costs. When comparing Figure 6.59 to Figure 6.61 and Figure 6.63, approximately $250k per annum is saved by not paying dump fees to dispose of the wet fibre coproduct. Similarly, when comparing Figure 6.63 to Figure 6.59 and Figure 6.61, approximately $280k per annum is saved by utilising the waste liquor in a co-located composting facility instead of paying wastewater treatment costs. Transferring the wet fibre and waste liquor to a co-located composting facility therefore saves approximately $530k when compared to disposing of the process coproducts as waste.

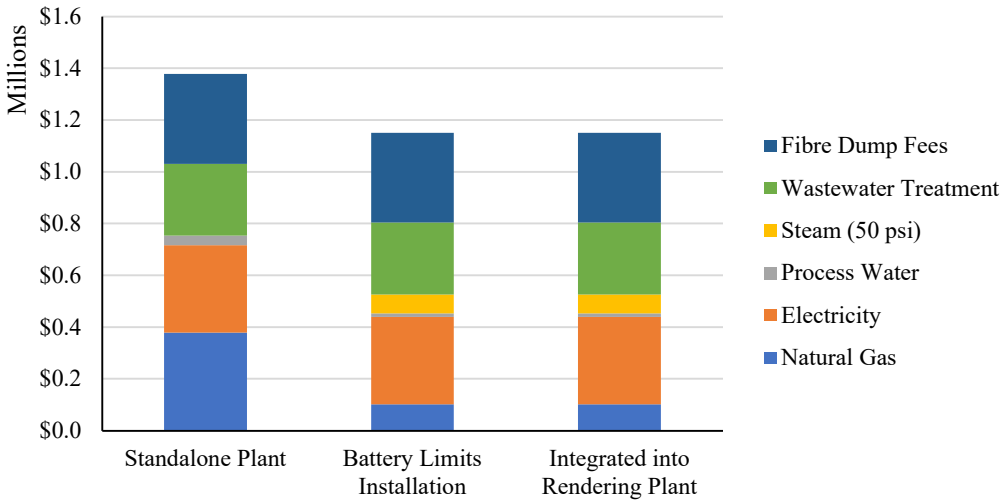

Figure 6.59: Breakdown of the utilities costs by process design when all coproducts are disposed as waste.

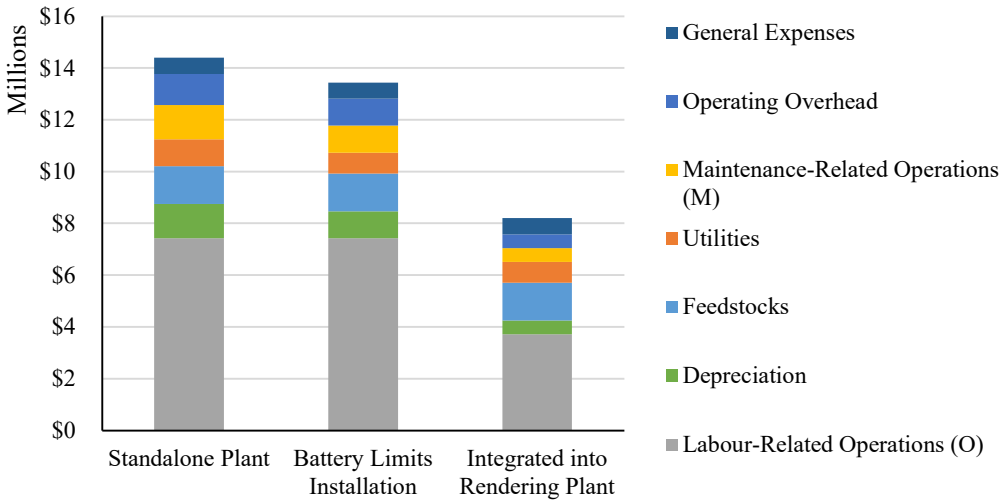

Figure 6.12: Breakdown of the total cost of production by process design when the fibre is sold as a composting aid.

Table 6.36: Cost sheet for standalone plant, battery limits installation, and integrated into rendering plant process configurations when the fibre is sold as a composting aid and the post-extraction liquor is sent to a wastewater treatment plant.

Cost Sheet Item	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
Feedstock costs	$1,460,272	$1,460,272	$1,460,272
Utilities costs	$1,030,801	$803,650	$803,650
Labour-Related Operations (O)	$7,419,241	$7,419,241	$3,709,620
Maintenance-Related Operations (M)	$1,333,999	$1,047,885	$537,496
Operating Overheads	$1,194,065	$1,037,353	$526,100
Depreciation	$1,329,952	$1,044,706	$535,866
Cost of Manufacture (COM)	$13,768,331	$12,813,106	$7,573,004
General Expenses	$627,668	$627,668	$627,668
Total Production Cost (C)	$14,395,998	$13,440,774	$8,200,672
Sales	$4,614,927	$4,614,927	$4,614,927
Gate Fees	$9,464,350	$9,464,350	$9,464,350
Total Revenue (R)	$14,079,277	$14,079,277	$14,079,277
Earnings Before Interest and Tax (EBIT)	-$316,721	$638,503	$6,367,553

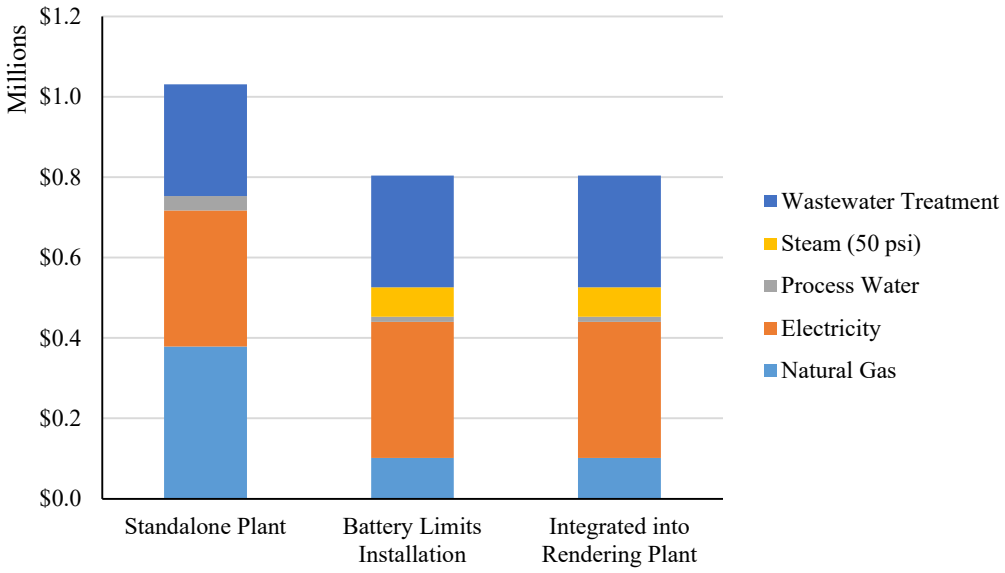

Figure 6.61: Breakdown of the utilities costs by process design when the fibre is sold as a composting aid.

The greatest reduction in total cost of production can be achieved by changing from a standalone plant process design to an integrated into rendering plant process design. Figure 6.58, Figure 6.12, and Figure 6.62 indicate this reduces the total cost

of production by \$5M to \$6M per annum across all operational scenarios. Approximately \$800k per annum of this comes from lower utility costs; this arises from the greater efficiency of drawing steam from a site boiler instead of running a small standalone boiler.

Table 6.37: Cost sheet for standalone plant, battery limits installation, and integrated into rendering plant process configurations when the fibre and treated liquor effluent are utilised in a co-located composting facility.

Cost Sheet Item	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
Feedstock costs	\$1,460,272	\$1,460,272	\$1,460,272
Utilities costs	\$753,072	\$525,920	\$525,920
Labour-Related Operations (O)	\$7,419,241	\$7,419,241	\$3,709,620
Maintenance-Related Operations (M)	\$1,333,999	\$1,047,885	\$537,496
Operating Overheads	\$1,135,623	\$1,037,353	\$526,100
Depreciation	\$1,205,764	\$1,044,706	\$535,866
Cost of Manufacture (COM)	\$13,307,970	\$12,535,377	\$7,295,274
General Expenses	\$439,258	\$439,258	\$439,258
Total Production Cost (C)	\$13,747,229	\$12,974,635	\$7,734,533
Sales	\$3,723,988	\$3,723,988	\$3,723,988
Gate Fees	\$9,464,350	\$9,464,350	\$9,464,350
Internal Transfers	\$913,747	\$913,747	\$913,747
Total Revenue (R)	\$14,102,085	\$14,102,085	\$14,102,085
Earnings Before Interest and Tax (EBIT)	\$354,857	\$1,127,450	\$6,367,553

A further \$3.7M per annum saving comes from the lower operating costs and the synergies created by allowing the steam injection and drying operations to be attended by operators of similar processes in the rendering plant. The remaining savings come from the lower depreciation, maintenance-related operations, and operating overhead associated with fewer new main plant items and hence a lower capital outlay for the process [19].

Total revenue for each operational scenario in Table 6.35, Table 6.36, and Table 6.37 remains constant across all three process designs: standalone plant (SP), battery limits (BL) installation, or integrated into rendering plant (IRP). The variation in total revenue presented in Figure 6.64 therefore arises between the

different operational scenarios: coproducts disposed of as waste (1); fibre sold as a composting aid (2); and fibre and liquor utilised in a co-located composting process (3). Coproduct sales increases total revenues by approximately $1M. When this additional revenue is aggregated with cost of production savings, EBIT increases by $2M to $8M per annum. This relationship between EBIT, process design, and operational scenario is presented in Figure 6.65.

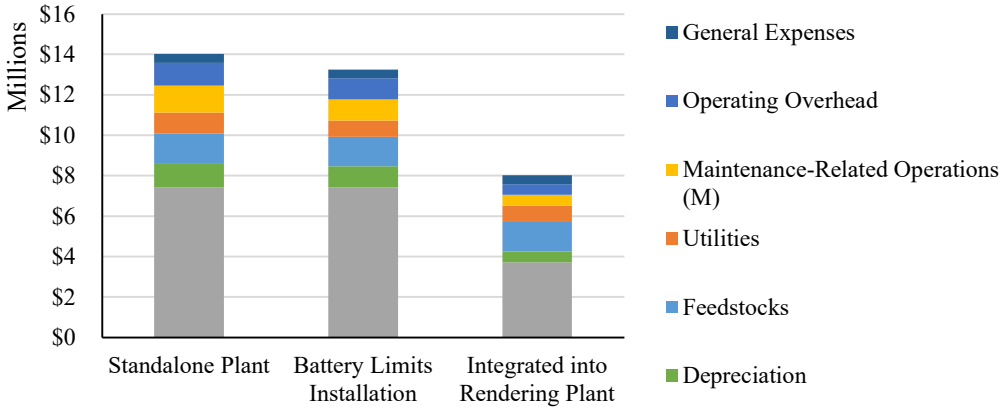

Figure 6.62: Breakdown of the total cost of production by process design when the fibre and waste liquor are used in a co-located composting facility.

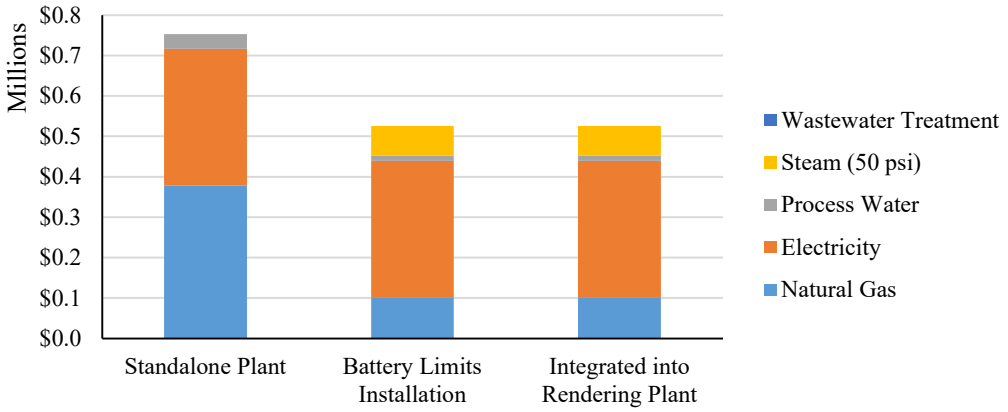

Figure 6.63: Breakdown of the utilities costs by process design when the fibre and waste liquor are used in a co-located composting facility.

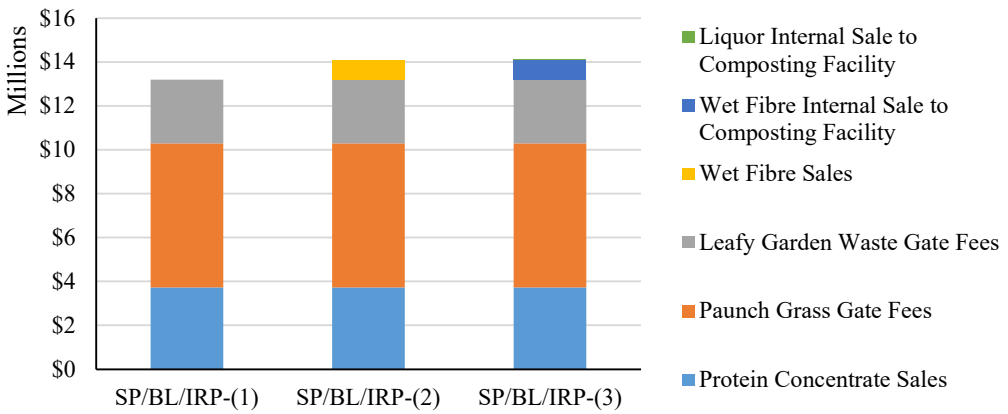

Figure 6.64: Breakdown of total revenue by operational scenario.

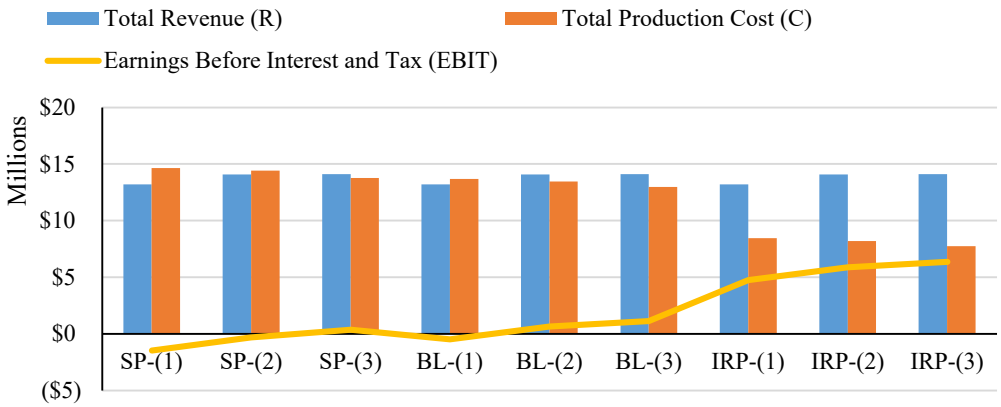

Figure 6.65: Breakdown total revenue, total production cost, and EBIT by process design and operational scenario.

6.5.1 Assumptions

Calculating the costs and revenues for each process design and operational scenario required assumptions for gate fees, utility costs, and chemical costs. Most of these assumptions were derived from real world price data (Table 6.38). Some price data were obtained in United States Dollars [30] and converted to New Zealand Dollars with exchange rates published by the Reserve Bank of New Zealand [3].

Table 6.38: Utility and chemical prices used in operating cost estimates

Description	Type	Price		Source
Electricity (Industrial Rates)	Utility Cost	$0.1367	per kWh	[31]
Natural Gas (Industrial Rates)	Utility Cost	$0.0241	per kWh	[31]
Steam (50 psi g)	Utility Cost	$5.92	per tonne	[19; 31]
Process Water	Utility Cost	$0.6174	per m^3	[32]
Hydrochloric Acid (30% solution)	Chemical Cost	$455.58	per metric tonne	[30]
Fibre Composting Charge	Utility Cost	$75.00	per metric tonne	[33]
Wastewater Treatment Charge (Tertiary Treatment)	Utility Cost	$0.824	per kg organic removed	[4; 19]

Real world price data was not publicly available for wastewater treatment and steam utilities. Hence costs were sourced from Seider, *et al.* [19] at 2006 prices and converted to New Zealand Dollars [3], and adjusted for inflation with price indices [4]. The wastewater treatment cost was indexed to the Producers Price Index (PPI) for water, sewerage, and waste services outputs [4]; and steam was indexed to the price of natural gas (Table 6.39) [31].

Equation 6.12: Adjustment of utility and labour prices from January 2000 prices to December 2019 prices

$$p_{2019} = p_{2006} \times \frac{I_{2019}}{I_{2006}}$$

Table 6.39: Index adjustments of utility and waste disposal costs

Utility	I_{2006}	I_{2019}	u_{2000}	u_{2019}	Unit Quantity
Steam (50 psi g)	1080	670	$9.54	5.92	per tonne
Wastewater Treatment	774	1337	$0.477	$0.824	per kg organic removed

Lab our prices (Table 6.40) were also obtained from Seider*, et al.* [19] but were not corrected for exchange rate as they were already consistent with New Zealand wages [4]. However, they were adjusted for inflation by indexing them to the PPI for fruit, oil, cereal and other food product manufacturing [4].

The plant is assumed to run on a semi-continuous basis, typically for 16 hours per day (Table 6.41). The standalone plant and battery limits installation scenarios have three operational process sections: the protein extraction (screw press) section; the protein coagulation and recovery section; and the drying and screening section. The integrated with rendering plant scenario only requires the former two sections, as it is assumed the and screening takes place outside the system boundary for the plant.

Table 6.40: Labour prices for soft leafy waste protein concentrate manufacturing

Labour Cost	I_{2006}	I_{2019}	Price 2006	Price 2019	Unit Quantity
Direct wages and benefits (DW&B)	885.6	1147.0	$35.00	$45.33	per operator-hr
Technical assistance to manufacturing	885.6	1147.0	$60,000	$77,712	per (operator/shift)-yr
Control laboratory	885.6	1147.0	$65,000	$84,188	per (operator/shift)-yr

Table 6.41: Operational hours for the soft leafy waste protein concentrate manufacturing facility

Parameter	Standalone Plant & Battery Limits Installation	Integrated Plant
Annual Plant Operating Hours	6177.28	6177.28
Daily Plant Operating Hours	16	16
Process sections	3	2
Operators per process section	4	3
Operator average work week (hours)	40	40
Shifts per week	4	4
Operators per shift	12	6

Gate fees and sale prices (Table 6.42) were all based on real-world data. Gate fees were obtained from the Matamata-Piako District Council [33] which collects green wastes in the envisioned operational area for the leaf protein concentrate production facility, and the leaf protein concentrate price was obtained from the sole global distributor of alfalfa leaf protein concentrate [34]. The price of wet fibre was based on that of twig mulch, the least expensive carbon source and bulking agent available [35]; most commercial composting processes typically use more expensive wood chip mulch [36]. The price of liquor internally transferred to a co-located composting facility is based on that of raw untreated water in the envisioned region of construction [32].

Table 6.42: Gate fees and sale prices used in revenue estimates

Description	Type	Price		Source
Paunch Grass Gate Fee	Revenue from negative-value feed	$170	per metric tonne	[33]
Leafy Green Waste Gate Fee	Revenue from negative-value feed	$75	per metric tonne	[33]
Leaf Protein Concentrate Sales	Revenue from product	$1100	per metric tonne	[34]
Wet Fibre Sales	Revenue from co-product	$40	per cubic metre	[35]
Wet Fibre Internal Sale to Composting Facility	Revenue from internal transfer	$40	per cubic metre	[35]
Liquor Internal Sale to Composting Facility	Revenue from internal transfer	0.62	per cubic metre	[32]

6.5.2 Cost of Manufacture

The three process configurations – standalone plant (SP), battery limits installation (BLI), and integrated into rendering plant (IRP) – have a fixed cost of manufacture (COM) irrespective of how the coproducts are utilised (see Figure 6.1). The COM is made up of costs for feedstock, utilities, labour operations, maintenance operations, operating overheads, and depreciation. These costs make up the first section of the cost sheet in the method of Seider*, et al.* [19]. Each component of the COM (excluding depreciation) is presented in Table 6.43 to Table 6.47.

Table 6.43: Feedstock costs for soft leafy waste protein concentrate manufacturing

Feedstocks	Unit Price	Annual Quantity (All Designs)	Cost (All Designs)
Hydrochloric Acid	$ 455.58 per metric tonne	3205	$1,460,272
SUBTOTAL		**3205**	**$1,460,272**

Table 6.44: Utilities costs for soft leafy waste protein concentrate manufacturing

Utilities	Unit Price		Annual Quantity (SP)	Annual Quantity (BLI/IRP)	Cost (SP)	Cost (BLI/IRP)
Natural Gas	$ 0.0241	per kWh	15712219	4214178	$378,664	$101,562
Direct Heat Rotary Drier			*4214178*	*4214178*	*$101,562*	*$101,562*
Steam Boiler			*11498042*		*$277,103*	*$0*
Electricity	$ 0.1367	per kWh	2541000	2539839	$347,355	$347,196
Conveyor and Feeder Drives			*2095*	*2095*	*$286*	*$286*
Decanting Centrifuge			*264974*	*264974*	*$36,222*	*$36,222*
Disc Refiner			*679501*	*679501*	*$92,888*	*$92,888*
Hammer Mills			*1288275*	*1288275*	*$176,107*	*$176,107*
Pumps			*23483*	*22322*	*$3,210*	*$3,051*
Screw Presses			*276495*	*276495*	*$37,797*	*$37,797*
Vibratory Screen			*6177*	*6177*	*$844*	*$844*
Process Water	$1.89	per m^3	8781	6768	$16,597	$12,791
Steam (50 psi)	$5.92	per tonne	-	12439	-	$73,619
Wastewater Treatment	$0.8240	per kg organic removed	337053	337053	$277,730	$277,730
Fibre Dump Fees	$75.00	per tonne	4625	4625	$346,876	$346,876
SUBTOTAL					**$1,377,677**	**$1,150,526**

Table 6.45: Labour operations costs for soft leafy waste protein concentrate manufacturing

Labour Cost	Unit Price or Factor		Annual Quantity (SP/BLI)	Annual Quantity (IRP)	Cost (SP/BLI)	Cost (IRP)
Direct wages and benefits (DW&B)	$45.33	per operator-hr	99840	49920	$4,525,974	$2,262,987
Direct salaries and benefits	15.00%	of DW&B	-	-	$678,896	$339,448
Operating supplies and services	6.00%	of DW&B	-	-	$271,558	$135,779
Technical assistance to manufacturing	$77,712	per (operator/shift)-yr	12	6	$932,550	$466,275
Control laboratory	$84,188	per (operator/shift)-yr	12	6	$1,010,262	$505,131
SUBTOTAL					**$7,419,241**	**$3,709,620**

Table 6.46: Maintenance operations costs for soft leafy waste protein concentrate manufacturing

Maintenance Operations	Factor	Cost (SP)	Cost (BLI)	Cost (IRP)
Wages and benefits (MW&B)	5% of C_{TDC}	$580,000	$455,602	$233,694
Salaries and benefits	25% of MW&B	$145,000	$113,901	$58,423
Materials and services	100% of MW&B	$580,000	$455,602	$233,694
Maintenance overhead	5% of MW&B	$29,000	$22,780	$11,685
SUBTOTAL		$1,333,999	$1,047,885	$537,496

Table 6.47: Operating overheads for soft leafy waste protein concentrate manufacturing

Operating Overheads	Factor	Cost (SP)	Cost (BLI)	Cost (IRP)
General plant overhead	7.10% of M&O-SW&B	$421,021	$409,980	$205,513
Mechanical department services	2.40% of M&O-SW&B	$40,211	$36,479	$18,416
Employee relations department	5.90% of M&O-SW&B	$106,973	$99,267	$49,999
Business services	2% of C_{TDC}	$312,930	$245,813	$126,086
Property taxes and insurance	2% of C_{TDC}	$312,930	$245,813	$126,086
SUBTOTAL		**$1,194,065**	**$1,037,353**	**$526,100**

Depreciation

Annual depreciation was calculated from Equation 6.13 using the parameters in Table 6.48 and the total depreciable capital figures presented in Table 6.34 [19]. The capital value of allocated plant was zero (see Section 0).

Equation 6.13: Calculation of annual depreciation (D)

$$D = d(C_{TDC}) - 1.18C_{alloc}$$

d = annual straight-line depreciation rate; C_{TDC} = total depreciable capital; C_{alloc} = allocated capital

Table 6.48: Depreciation parameters

Parameter	Rate	Source
Straight line depreciation (%pa) [d]	8.50%	[2]
Plant lifespan* (years) [T_d]	15	[2]

***Rounded down to nearest year**

Table 6.49: Depreciation costs for soft leafy waste protein concentrate manufacturing

Depreciation Item	Factor		Cost (SP)	Cost (BLI)	Cost (IRP)
Direct plant	8.5%	of C_{TDC} - $1.18C_{alloc}$	$1,329,952	$1,044,706	$535,866
Allocated plant	6.0%	of $1.18C_{alloc}$	-	-	-
SUBTOTAL			**$1,329,952**	**$1,044,706**	**$535,866**

6.5.3 General Expenses and Revenues

Unlike the COM, general expenses and revenues vary depending on how coproducts are utilised, as this generates additional revenue streams from coproduct sales or internal transfers and general expenses are directly proportional to sales revenue [19]. Three main coproduct utilisation scenarios were considered:

1. Both the fibre and liquor effluent are both treated as waste.
2. The fibre is sold as a composting aid and the liquor effluent is discharged through a wastewater treatment plant.
3. The fibre is transferred to a co-located composting facility and the treated liquor effluent is sprayed onto compost windrows to regulate their moisture contents.

The general expenses and revenues are therefore broken down for each coproduct utilisation scenario in Table 6.50 to Table 6.55. Gate fees for accepting both paunch grass and leafy green waste are a critical source of revenue which makes the process viable. These were based on real world prices which are already charged for accepting these waste materials for disposal [33]. This forms a sufficient baseline for the technoeconomic analysis, but it is noted that some discounting of gate fees could be required to incentivise waste generators to dispose of their material at the processing site. This has been explored further by the sensitivity analysis under Section 6.6.3.

Table 6.50: General expenses for soft leafy waste protein concentrate manufacturing when fibre and liquor effluent are both treated as waste

General Expenses	Factor		Cost (SP/BLI/IRP)
Transfer expense	1.00%	of gate fees	$94,644
Selling expense	3.00%	of sales	$111,720
Direct research	4.80%	of sales	$178,751
Allocated research	0.50%	of sales	$18,620
Administrative expense	2.00%	of sales	$74,480
Management incentive compensation	1.25%	of sales	$46,550
SUBTOTAL			**$524,764**

Table 6.51: Revenues for soft leafy waste protein concentrate manufacturing when fibre and liquor effluent are both treated as waste

Source	Unit Price	Quantity (SP/BLI/IRP)	Revenue (SP/BLI/IRP)
Protein concentrate sales	$1100 per tonne	3385	$3,723,988
Paunch grass gate fees	$170 per tonne	38630	$6,567,100
Leafy green waste gate fees	$75 per tonne	9630	$2,897,250
SUBTOTAL			**$13,188,338**

Table 6.52: General expenses for soft leafy waste protein concentrate manufacturing when the fibre is sold as a composting aid and the liquor effluent is discharged through a wastewater treatment plant

General Expenses	Factor	Cost (SP/BLI/IRP)
Transfer expense	1.00% of gate fees	$94,644
Selling expense	3.00% of sales	$138,448
Direct research	4.80% of sales	$221,516
Allocated research	0.50% of sales	$23,075
Administrative expense	2.00% of sales	$92,299
Management incentive compensation	1.25% of sales	$57,687
SUBTOTAL		**$627,668**

Table 6.53: Revenues for soft leafy waste protein concentrate manufacturing when the fibre is sold as a composting aid and the liquor effluent is discharged through a wastewater treatment plant

Source	Unit Price	Quantity (SP/BLI/IRP)	Revenue (SP/BLI/IRP)
Protein concentrate sales	$1100 per tonne	3385	$3,723,988
Wet fibre sales	$40 per cubic metre	22273	$890,939
Paunch grass gate fees	$170 per tonne	38630	$6,567,100
Leafy green waste gate fees	$75 per tonne	9630	$2,897,250
SUBTOTAL			**$14,079,277**

Table 6.54: General expenses for soft leafy waste protein concentrate manufacturing when the fibre and treated liquor effluent are utilised in a co-located composting facility

General Expenses	Factor	Cost (SP/BLI/IRP)
Transfer expense	1.00% of gate fees	$9,137
Selling expense	3.00% of sales	$111,720
Direct research	4.80% of sales	$178,751
Allocated research	0.50% of sales	$18,620
Administrative expense	2.00% of sales	$74,480
Management incentive compensation	1.25% of sales	$46,550
SUBTOTAL		**$439,258**

Table 6.55: Revenues for soft leafy waste protein concentrate manufacturing when the fibre and treated liquor effluent are utilised in a co-located composting facility

Source	Unit Price	Quantity (SP/BLI/IRP)	Revenue (SP/BLI/IRP)
Protein concentrate sales	$1100 per tonne	3385	$3,723,988
Wet fibre internal sale to composting facility	$40 per cubic metre	22273	$890,939
Liquor internal sale to composting facility	$0.62 per cubic metre	36943	$22,808
Paunch grass gate fees	$170 per tonne	38630	$6,567,100
Leafy green waste gate fees	$75 per tonne	9630	$2,897,250
SUBTOTAL			**$14,102,085**

6.6 Profitability Analysis

The *Integrated into Rendering Plant* design has positive Earnings Before Interest and Tax (EBIT) for all operational scenarios (i.e. IRP-1, IRP-2, and IRP-3) and the *Battery Limits* Installation design has positive EBIT for both coproduct utilisation scenarios (i.e. BL-2 & BL-3) as per Table 6.56. More detailed profitability analysis is therefore justified. The tax, discount rate, commercial lending rate, and reasonable return on investment assumptions presented in Table 6.57 were used to calculate annual interest costs (Table 6.58) on the total permanent investment (Table 6.59), net earnings after tax (Table 6.60), and net earnings after interest and tax (Table 6.61). The reasonable return on investment is defined as the discount rate plus 5 percent per annum [19]. These calculations clearly indicate that BL-2, BL-3, IRP-1, IRP-2, and IRP-3 design-operation configurations will turn an annual profit, even if the leaf protein concentrate production facility is built with borrowed capital. This justifies further analysis using both approximate and rigorous profitability measures.

Table 6.56: EBIT for each leaf protein concentrate plant configuration and operational scenario

Operational Scenario	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
(1) Fibre and liquor effluent treated as waste	-$1,451,632	-$496,408	$4,743,694
(2) Fibre sold as composting aid & liquor effluent treated as waste	-$316,721	$638,503	$5,878,605
(3) Fibre and treated liquor utilised in co-located composting facility	$354,857	$1,127,450	$6,367,553

It is also important to note that the cost of capital is at a historical low [3]. The results for earnings after interest and tax presented in Table 6.61 could become materially less favourable should interest rates return to their historical norms.

Table 6.57: Taxation, borrowing, and return on investment assumptions

Parameter	Rate	Source
Company tax rate [t] (%pa)	28%	[37]
Discount rate – 1 year term deposit rate, Dec-2019 [r] (%pa)	2.47%	[3]
Corporate borrowing rate [b] (%pa)	4.59%	[38]
Reasonable return on investment [i_{min}] (%pa)	7.47%	[3; 19]

Table 6.58: Annual interest costs on the total permanent investment for each leaf protein concentrate plant configuration and operational scenario

Operational Scenario	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
(1) Fibre and liquor effluent treated as waste	$804,554	$652,611	$368,061
(2) Fibre sold as composting aid & liquor effluent treated as waste	$807,416	$655,473	$370,923
(3) Fibre and treated liquor utilised in co-located composting facility	$801,470	$650,225	$365,675

Table 6.59: Total permanent investment for each leaf protein concentrate plant configuration and operational scenario

Operational Scenario	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
(1) Fibre and liquor effluent treated as waste	$17,547,514	$14,233,610	$8,027,497
(2) Fibre sold as composting aid & liquor effluent treated as waste	$17,609,941	$14,296,036	$8,089,924
(3) Fibre and treated liquor utilised in co-located composting facility	$17,480,272	$14,181,580	$7,975,468

Table 6.60: Net earnings after tax for each leaf protein concentrate plant configuration and operational scenario

Operational Scenario	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
(1) Fibre and liquor effluent treated as waste	-$1,451,632	-$496,408	$3,415,460
(2) Fibre sold as composting aid & liquor effluent treated as waste	-$316,721	$459,722	$4,232,596
(3) Fibre and treated liquor utilised in co-located composting facility	$255,497	$811,764	$4,584,638

Table 6.61: Net earnings after interest and tax for each leaf protein concentrate plant configuration and operational scenario

Operational Scenario	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
(1) Fibre and liquor effluent treated as waste	-$1,624,454	-$827,294	$3,150,456
(2) Fibre sold as composting aid & liquor effluent treated as waste	-$809,379	-$12,219	$3,965,531
(3) Fibre and treated liquor utilised in co-located composting facility	-$321,562	$343,602	$4,321,352

6.6.1 Approximate Profitability Measures

Several approximate profitability measures were calculated to determine the preliminary profitability of producing leaf protein concentrate from soft leafy wastes: the return on investment (ROI; Equation 6.14), venture profit (VP; Equation 6.15), and simple payback period (SPBP; Equation 6.16 and Equation 6.17), which were adapted for New Zealand from Seider, *et al.* [19]. ROI and SPBP were calculated for direct investment of company funds and borrowed capital scenarios.

Equation 6.14: Calculation of return on investment (ROI)

$$ROI = \frac{(1-t)(R-C)}{C_{TPI}}$$

Equation 6.15: Calculation of venture profit (VP)

$$VP = (1-t)(R-C) - i_{min}(C_{TPI})$$

Equation 6.16: Calculation of simple payback period (SPBP)

$$SPBP = \frac{C_{TDC}}{(1-t)(R-C)+D}$$

Equation 6.17: Calculation of simple payback period for borrowed capital (SPBP)

$$SPBP_I = \frac{C_{TDC}}{(1-t)(R-C-I)+D}$$

t = NZ business income tax rate;
R = annual revenues from gate fees, product sales, and coproduct transfers;
C = annual production cost; C_{TPI} = total permanent capital investment;
C_{TDC} = total depreciable capital;
I = annual interest cost; i_{min} = reasonable return on investment;

The BL-2, BL-3, IRP-1, IRP-2, and IRP-3 all had positive direct investment ROI were positive. However only the IRP plant designs exceeded the reasonable return on investment (i_{min}; 7.47%) in Table 6.57. This is confirmed by the mostly positive annual venture profits presented in Table 6.63. Similarly, borrowed capital ROI (Table 6.65) also exceeded i_{min}, indicating that it is viable to borrow capital to build an IRP design processing plant. This is supported by simple payback periods that

generally suggest that an IRP process producing leaf protein concentrate from soft leafy wastes can pay for itself within five years and is financially viable.

Proceeding with a BL plant design is not advisable from a financial perspective but may still be viable as an environmentally conscious investment. However, such a plant should not be built with borrowed capital. The BL-2 and BL-3 design-operation configurations could take up to nine years to pay for themselves and have negative venture profits.

Table 6.62: Direct investment ROI for each leaf protein concentrate plant configuration and operational scenario

Operational Scenario	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
(1) Fibre and liquor effluent treated as waste	-	-	42.55%
(2) Fibre sold as composting aid & liquor effluent treated as waste	-	3.22%	52.32%
(3) Fibre and treated liquor utilised in co-located composting facility	1.46%	5.72%	57.48%

Table 6.63: VP for each leaf protein concentrate plant configuration and operational scenario

Operational Scenario	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
(1) Fibre and liquor effluent treated as waste	-$2,762,432	-$1,559,659	$2,815,806
(2) Fibre sold as composting aid & liquor effluent treated as waste	-$1,632,184	-$608,192	$3,628,279
(3) Fibre and treated liquor utilised in co-located composting facility	-$1,050,280	-$247,600	$3,988,870

Table 6.64: Borrowed capital ROI for each leaf protein concentrate plant configuration and operational scenario

Operational Scenario	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
(1) Fibre and liquor effluent treated as waste	-	-	39.25%
(2) Fibre sold as composting aid & liquor effluent treated as waste	-	-	49.02%
(3) Fibre and treated liquor utilised in co-located composting facility	-	2.42%	54.18%

Table 6.65: Direct investment SPBP for each leaf protein concentrate plant configuration and operational scenario

Operational Scenario	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
(1) Fibre and liquor effluent treated as waste	-128.59 years	22.42 years	1.60 years
(2) Fibre sold as composting aid & liquor effluent treated as waste	15.44 years	8.17 years	1.32 years
(3) Fibre and treated liquor utilised in co-located composting facility	9.71 years	6.62 years	1.23 years

Table 6.66: Borrowed capital SPBP for each leaf protein concentrate plant configuration and operational scenario

Operational Scenario	Standalone Plant	Battery Limits Installation	Integrated into Rendering Plant
(1) Fibre and liquor effluent treated as waste	-53.13 years	56.53 years	1.71 years
(2) Fibre sold as composting aid & liquor effluent treated as waste	30.06 years	11.90 years	1.40 years
(3) Fibre and treated liquor utilised in co-located composting facility	16.04 years	8.85 years	1.30 years

6.6.2 Rigorous Profitability Measures

Two rigorous profitability measures – Net Present Value (NPV; Equation 6.18) and Investor's Rate of Return (IRR; Equation 6.19) – were calculated from the annual cashflows (CF) over the 15-year lifespan of the leaf protein concentrate production facility in an Excel spreadsheet with iterative calculation enabled. CF during the normal operation, construction, and start-up of the process plant were calculated using Equation 6.20 to Equation 6.22 [19]. It was assumed construction is completed during the year 2020, start-up during 2021, and normal operations occur from 2022 to 2035, and the dilapidated process plant has no salvage value. The results for each process configuration and coproduct utilisation scenario are presented in Table 6.67and Table 6.68, and an example calculation spreadsheet is presented in Table 6.69. The NPV for the BL-2, BL-3, IRP-1, IRP-2, and IRP-3 design-operation configurations are positive; the IRR for BL-3, IRP-1, IRP-2, and IRP-3 configurations also exceed the reasonable rate of return of (7.47%) in Table 6.57. This suggests that the BL-3, IRP-1, IRP-2, and IRP-3 configurations and coproduct utilisation scenarios are profitable and attractive investments. The BL-2

configuration may still be an attractive waste management solution or environmentally conscious investment.

Equation 6.18: NPV over lifespan of the process plant	**Equation 6.19: IRR over the lifespan of the process plant**
$$\boldsymbol{NPV = \sum_{y=2020}^{2035} CF_y}$$	Solve $NPV\{r\} = 0$ for r *(iterative calculation)*
Equation 6.20: Annual cashflow during normal operation of the process plant	**Equation 6.21: Annual cashflow during construction of the process plant**
$$CF = (1-t)(R-C) + D$$	$$CF = -C_{TDC} - C_{land}$$
Equation 6.22: Annual cashflow during start-up of the process plant	
$$CF = (1-t)(R-C) + D - C_{WC} - C_{startup}$$	

Table 6.67: 15-year NPVs for each process configuration and operating scenario

	Net Present Value over 15 Years		
Operational Scenario	**Standalone Plant**	**Battery Limits Installation**	**Integrated into Rendering Plant**
(1) Fibre and liquor effluent treated as waste	-$44,810,354	-$21,958,772	$94,217,538
(2) Fibre sold as composting aid & liquor effluent treated as waste	-$20,759,985	$2,091,597	$118,267,907
(3) Fibre and treated liquor utilised in co-located composting facility	-$5,286,002	$12,590,938	$128,767,248

Table 6.68: 15-year IRRs for each process configuration and operating scenario

	Internal Rate of Return over 15 Years		
Operational Scenario	**Standalone Plant**	**Battery Limits Installation**	**Integrated into Rendering Plant**
(1) Fibre and liquor effluent treated as waste	-	-	51.99%
(2) Fibre sold as composting aid & liquor effluent treated as waste	-	3.880%	57.64%
(3) Fibre and treated liquor utilised in co-located composting facility	-	9.973%	59.90%

Table 6.69: NPV and IRR analysis for Battery Limits Plant, Scenario 2

Year	Investment			Operating Income			Net Earnings	Annual Cash Flow	NPV Analysis			IRR Analysis	
	Depreciable Capital	Working & Start-Up Capital	Land	Depreciation	Total Production Cost Excluding Depreciation	Revenue			NPV	$2,091,5967		IRR	3.880%
									CF PV	Cum. PV		CF PV	Cum. PV
2020	-$12,290,660		-$245,813					-$12,536,473	-$12,536,473	-$12,536,473		-$12,536,473	-$12,536,473
2021		-$3,126,369		-$1,044,706	-$12,396,068	$14,079,277	$459,722	-$2,028,144	-$1,978,049	-$14,514,522		-$1,949,452	-$14,485,925
2022				-$1,044,706	-$12,396,068	$14,079,277	$459,722	$1,098,225	$1,044,643	-$13,469,879		$1,014,656	-$13,471,269
2023				-$1,044,706	-$12,396,068	$14,079,277	$459,722	$1,098,225	$1,018,840	-$12,451,038		$975,287	-$12,495,981
2024				-$1,044,706	-$12,396,068	$14,079,277	$459,722	$1,098,225	$993,675	-$11,457,363		$937,446	-$11,558,535
2025				-$1,044,706	-$12,396,068	$14,079,277	$459,722	$1,098,225	$969,131	-$10,488,232		$901,073	-$10,657,462
2026				-$1,044,706	-$12,396,068	$14,079,277	$459,722	$1,098,225	$945,194	-$9,543,038		$866,112	-$9,791,350
2027				-$1,044,706	-$12,396,068	$14,079,277	$459,722	$1,098,225	$921,847	-$8,621,191		$832,506	-$8,958,844
2028				-$1,044,706	-$12,396,068	$14,079,277	$459,722	$1,098,225	$899,078	-$7,722,113		$800,205	-$8,158,639
2029				-$1,044,706	-$12,396,068	$14,079,277	$459,722	$1,098,225	$876,871	-$6,845,243		$769,157	-$7,389,482
2030				-$1,044,706	-$12,396,068	$14,079,277	$459,722	$1,098,225	$855,212	-$5,990,031		$739,314	-$6,650,168
2031				-$1,044,706	-$12,396,068	$14,079,277	$459,722	$1,098,225	$834,088	-$5,155,943		$710,628	-$5,939,540
2032				-$798,893	-$12,396,068	$14,079,277	$636,708	$1,521,024	$1,126,665	-$4,029,278		$946,021	-$4,993,519
2033				$0	-$12,396,068	$14,079,277	$1,211,911	$2,895,120	$2,091,527	-$1,937,751		$1,730,793	-$3,262,726
2034				$0	-$12,396,068	$14,079,277	$1,211,911	$2,895,120	$2,039,866	$102,115		$1,663,638	-$1,599,088
2035				$0	-$12,396,068	$14,079,277	$1,211,911	$2,895,120	$1,989,482	$2,091,597		$1,599,088	$0

6.6.3 Sensitivity Analysis

The financial performance of any process plant is sensitive to changes in price for its inputs and outputs. A full breakdown of these prices can be found in Section 6.5. Sensitivity analysis seeks to confirm whether a process is still financially attractive if there is a significant change in the price of an input or output. The sensitivity analysis was performed on a battery-limits installation process configuration which sells the wet fibre as a composting aid but sends the liquor effluent to a wastewater treatment plant (BL-2). This was the least profitable straightforward combination (i.e. no integration or co-location required) of the process design and operation presented in Table 6.67 and thus the most sensitive to price changes

A visual breakdown of the process input costs is presented in Figure 6.66. Labour-related operations are the largest input cost, and the costs of hydrochloric acid and utilities may also vary. A visual breakdown of utility costs is presented in Figure 6.67, where electricity and wastewater treatment are clearly dominant. Of these, only electricity has significant variation [31]. Hence direct wages and benefits, hydrochloric acid, and electricity will be included in the sensitivity analysis.

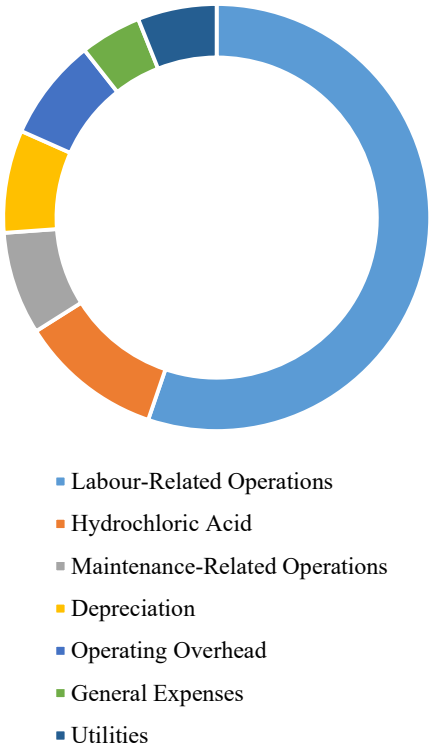

Figure 6.66: Relative costs of process inputs into a battery limits facility processing soft leafy waste into leaf protein concentrate.

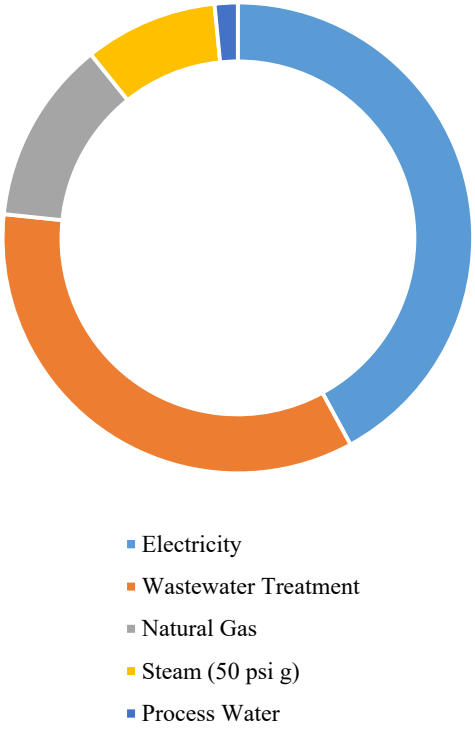

Figure 6.67: Relative costs of utility inputs into a battery limits facility processing soft leafy waste into leaf protein concentrate.

Variation in output revenues should also be considered; a visual breakdown of them is presented in Figure 6.68. Leaf protein concentrate sales and paunch grass gate fees dominate process revenue and will also be included in the sensitivity analysis.

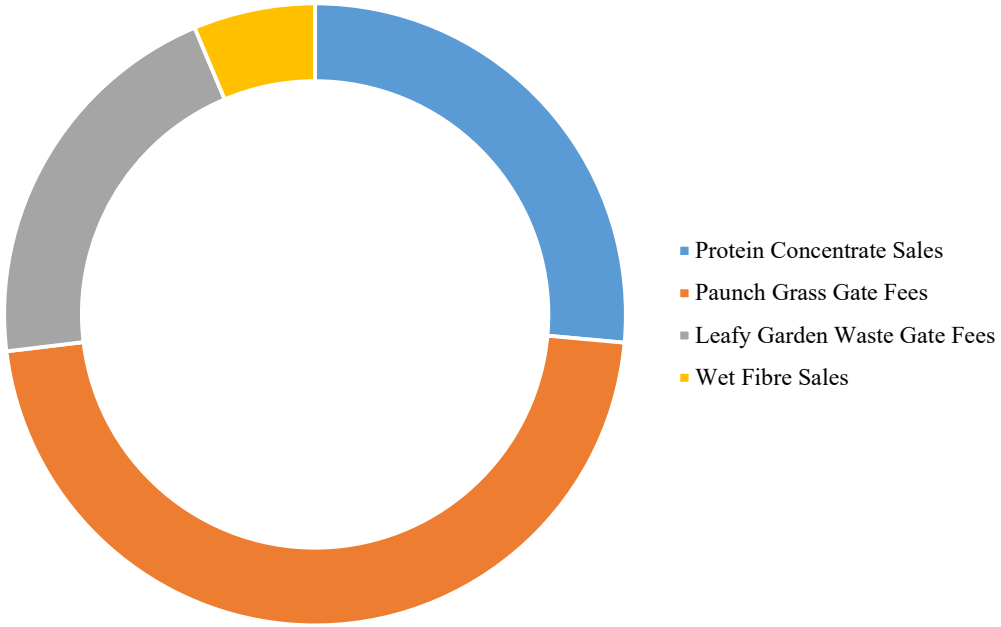

Figure 6.68: Relative values of revenue streams from a battery limits facility producing leaf protein concentrate from soft leafy wastes

Each price in the sensitivity analysis was increased and decreased by 5, 10, and 20 percent, respectively (Table 6.70). These percentages correspond to minor, moderate, and major changes in prices, respectively. The adjusted values were then entered into the spreadsheets used to calculate NPV and IRR (see Section 6.6.2) to determine whether a change in input or output price could make manufacturing leaf protein concentrate from soft leafy wastes no longer financially viable.

Table 6.70: Sensitivity analysis price changes for key process inputs and outputs

	Change in Parameter Value						
Parameter	**-20%**	**-10%**	**-5%**	**0%**	**5%**	**10%**	**20%**
Protein Concentrate Sale Price (NZD per metric tonne)	$880.00	$990.00	$1,045.00	$1,100	$1,155.00	$1,210.00	$1,320.00
Paunch Grass Gate Fees (NZD per metric tonne)	$136.00	$153.00	$161.50	$170	$178.50	$187.00	$204.00
Direct Wages and Benefits (NZD per operator-hr)	$36.27	$40.80	$43.07	$45.33	$47.60	$49.87	$54.40
Hydrochloric Acid Price (NZD per metric tonne)	$364.46	$410.02	$432.80	$456	$478.36	$501.14	$546.70
Electricity Price (NZD per kWh)	$0.11	$0.12	$0.13	$0.14	$0.14	$0.15	$0.16

All changes to the input and output pricing have a major effect on NPV (Table 6.71) and IRR (Table 6.72). Changes of 5% in most input and output prices are sufficient

to cause a negative NPV or IRR. This indicates that the financial viability of manufacturing leaf protein concentrates from soft leafy wastes using a BL-2 configuration is marginal and sensitive to input and output costs.

Table 6.71: Effect of changes in process input and output costs on NPV

Parameter	Net Present Value (Millions)						
Protein Concentrate Sale Price	-$11.830	-$4.869	-$1.389	$2.092	$5.572	$9.052	$16.013
Paunch Grass Gate Fees	-$25.426	-$11.667	-$4.788	$2.092	$8.971	$15.851	$29.610
Direct Wages and Benefits	$27.192	$14.642	$8.367	$2.092	-$4.183	-$10.459	-$23.009
Hydrochloric Acid Price	$8.344	$5.218	$3.655	$2.092	$0.529	-$1.034	-$4.161
Electricity Price	$3.533	$2.812	$2.452	$2.092	$1.731	$1.371	$0.650
	-20%	**-10%**	**-5%**	**0%**	**+5%**	**+10%**	**+20%**
	Parameter Change						

Table 6.72: Effect of changes in process input and output costs on IRR

Parameter	Internal Rate of Return						
Protein Concentrate Sale Price	-	-	1.482%	3.880%	6.051%	8.044%	11.620%
Paunch Grass Dump Fees	-	-	--	3.880%	8.001%	11.549%	17.554%
Direct Wages and Benefits	16.68%	10.99%	7.676%	3.880%	-	-	-
Hydrochloric Acid Price	7.669%	5.845%	4.882%	3.880%	2.834%	1.740%	-
Electricity Price	4.805%	4.347%	4.115%	3.880%	3.643%	3.404%	2.917%
	-20%	**-10%**	**-5%**	**0%**	**+5%**	**+10%**	**+20%**
	Parameter Change						

The effect of changes to input and output price parameters on NPV and IRR are presented graphically in Figure 6.69 to Figure 6.72, respectively. These plots clearly indicate that the direct wages and benefits (DW&B) is the input cost with the largest effect on NPV and IRR; and both output prices (leaf protein concentrate and wet fibre) affect NPV and IRR at a similar magnitude to direct wages and benefits over most of the range of variation tested. Hence DW&B, the leaf protein concentrate

price, and the wet fibre price have the largest effect on the financial performance of the process.

These plots also indicate that changes to parameters have a near-linear effect on NPV within the range of variation tested. However, IRR is non-linear at higher levels of variation (i.e. 10% or more). Hence day to day volatility in prices of process inputs and outputs will not cause disproportionate changes in financial viability, but larger changes may have a disproportionate impact.

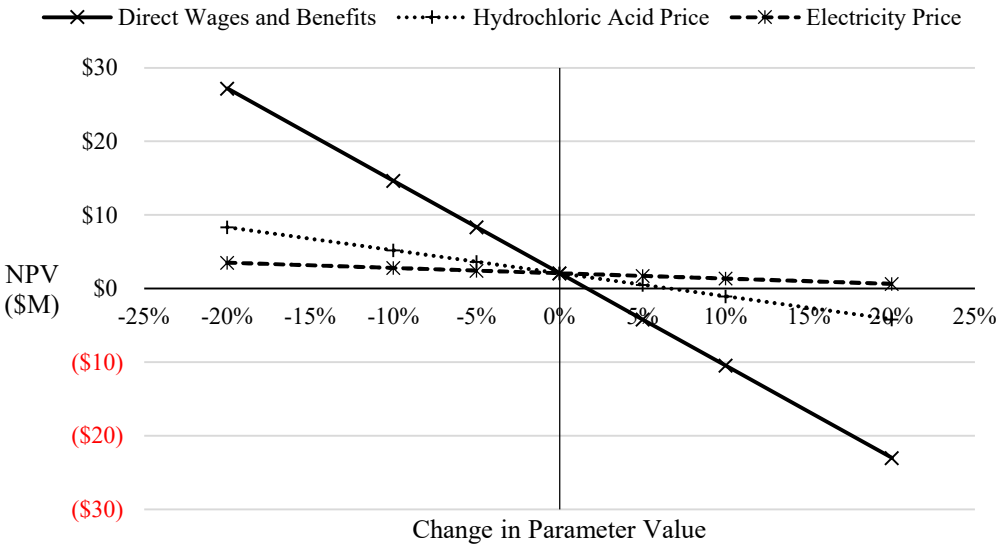

Figure 6.69: Effect of changes in input cost parameters on process NPV

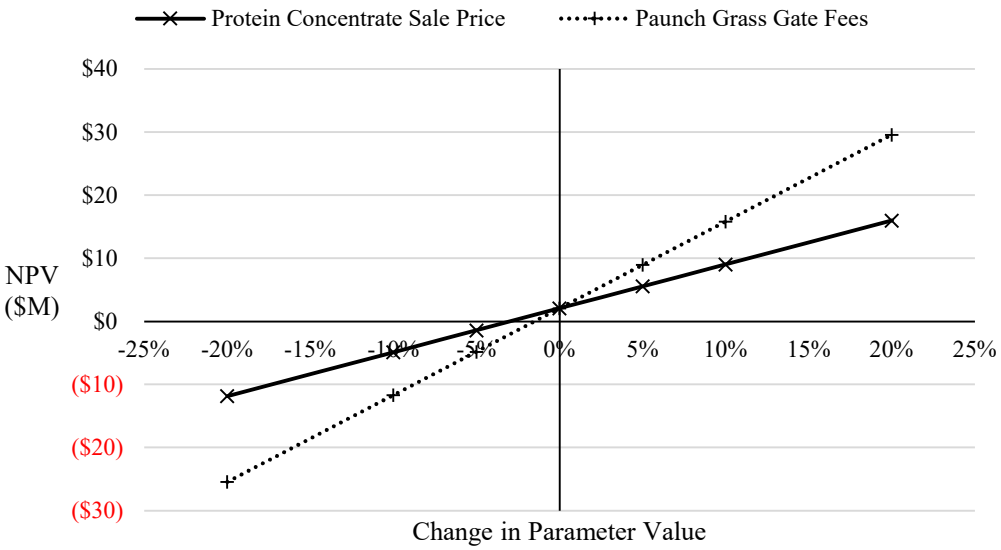

Figure 6.22: Effect of changes in output price parameters on process NPV

Another key result is the impact of variations in the gate fees charged for accepting paunch grass. As discussed in Section 0, some discounting of this gate fee may be

required to incentivise meat processors to dispose of their paunch grass at the leaf protein processing facility; however, the results presented in Figure 6.22 indicate this is not financially viable for the BL-2 design-operation combination. If discounting of gate fees is necessary, the process must use a more profitable design-operation combination (refer to Table 6.67 and Table 6.68). The economics of gate fees were out of scope for this study, but could be an interesting area of future research.

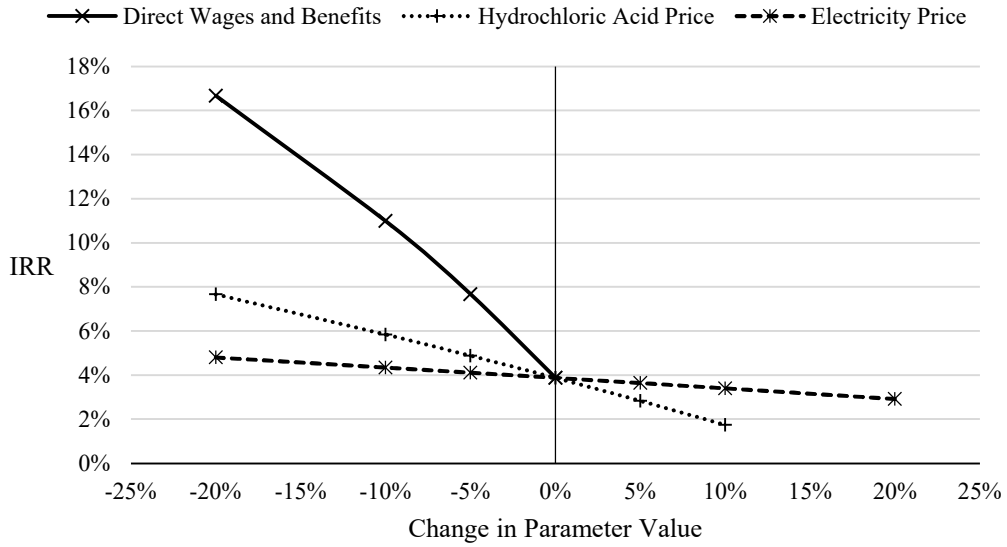

Figure 6.23: Effect of changes in input cost parameters on process IRR

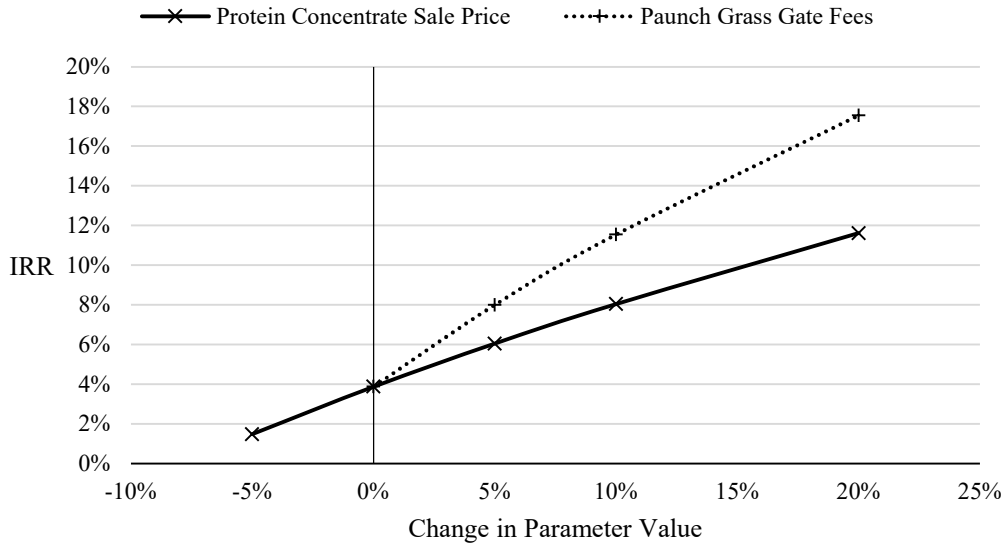

Figure 6.72: Effect of changes in output price parameters on process IRR

6.6.4 Comparison to Fresh Crop Processing

The first-generation process technology for manufacturing leaf protein concentrates (LPC) peaked during the 1980s, when processing plants were operating in France, Hungary, the USA, and New Zealand [15; 39; 40]. However, the economic viability and financial performance of the first-generation process technology was limited by several factors: it was optimised to produce dehydrated alfalfa fibre for animal feed instead of the more valuable LPC [15; 39]; supplying energy to dry the fibre required large amounts of natural gas or fuel oil [15; 34; 39]; and the protein yield was approximately half of that achievable with second-generation process technology [14; 15]. Only a single plant operated by Desialis in France remains operational today, coproducing alfalfa LPC and dehydrated alfalfa fibre for animal feed applications [15; 41]. A comparatively recent study by Dale *et al.* [42] in 2009 noted that *"the economics are simply not competitive with other protein sources when leaf protein is the sole product."* It is therefore essential to coproduce leaf protein concentrate with other products to develop an economically attractive process.

High oil prices during the 1970s, 1980s, and late 2000s lead to research into producing cellulosic ethanol from alfalfa fibre as an alternative to petroleum-based liquid transport fuels with alfalfa LPC as a coproduct [41; 42; 43]. Unfortunately the price of bioethanol produced from the alfalfa fibre could not be made competitive with petroleum-based fuels [44]. McDonald [15] similarly noted in 2012 that *"with the current still-low cost of land, fuel, feed and food, and the lack of effective price signals to discourage greenhouse gas emissions, pollution, soil damage and water depletion, leaf proteins are unlikely to be able to compete with soy."* The fermentation technologies required to produce bioethanol from the press fibre are also neither mature nor economic at this stage [15; 42]. However these technologies have great potential fully developed: modelling by Laser *et al.* [45] concluded that coproduction of a switchgrass LPC alongside ethanol and Rankine-cycle power generation can at minimum pay for its own production costs at a historically low protein price of USD 310 per tonne, enabling ethanol to be sold at USD 0.19 per litre which is competitive with gasoline [45]. When the LPC price increases to USD 440 per tonne (the historical average) or USD 660 per tonne (the historical high), LPC production subsidises ethanol production and allows the price of ethanol to be reduced to USD 0.17 or 0.14 per litre, respectively. Hence

coproduction of LPC and ethanol from soft leafy wastes could be an interesting area of research as the technology matures.

Most fresh leaf crops are only available on a seasonal basis [45; 46]. These seasonal peaks of feedstock availability require fresh crop LPC processes to have larger process equipment than that required to process the same tonnage of feedstock produced consistently throughout the year, leading to higher capital costs and the process plant sitting idle during the winter months. The fresh crops must also be grown, purchased, and transported to the processing plant [43; 45]. McDonald*, et al.* [43] estimated that one tonne of fresh alfalfa would cost NZD 50 per dry tonne in 1981; this corresponds to $197 per dry tonne or $31.50 per wet tonne when adjusted to December 2019 prices using the Food Price Index (the Producers Price Index starts in 1994) [4]. Using the technology envisioned in this study to process fresh alfalfa would result in slightly higher yields [14; 15], but would also eliminate revenue from gate fees and incur a purchase cost for the feedstock. Considering that gate fees constitute approximately two thirds of the process revenue, processing fresh crops into LPC is unlikely to be economically attractive.

Facilities processing soft leafy wastes into LPC will not be subject to the same limitations as those processing alfalfa or other fresh crops. Feedstock is available year-round, enabling smaller process equipment to be used to process largely consistent feedstock volumes. The feedstock is also negative value (i.e. suppliers will pay for it to be taken away) allowing it to be acquired by charging gate fees instead of paying a purchase price, greatly increasing process revenue and enabling the feedstock to be processed profitably. This is the critical difference between processing fresh crops and soft leafy wastes into LPC. Unlike fresh crops, soft leafy wastes can also be profitably processed into LPC without the need to coproduce ethanol and power. This leads to a much simpler process plant, lower operating costs, and no need to incorporate immature technologies or novel steps, substantially decreasing capital costs [19]. The technologies to make fresh crop LPC processing viable are under development but are not yet commercially available. This essentially makes processing soft leafy wastes into LPC a straightforward waste valorisation process that can be performed economically with presently available technologies. Conversely, fresh crop LPC production is

essentially a complex biorefinery process that relies upon speculative assumptions about the performance of unproven and immature technologies.

6.7 Conclusion

The technoeconomic analysis of leaf protein concentrate (LPC) manufacturing from soft leafy wastes demonstrates that this process is technologically and financially viable. The battery limits plant design was only viable under coproduct valorisation scenarios. The baseline design-operation configuration is a battery limits installation adjacent to a facility processing meat or abattoir wastes, as it is straightforward to implement and requires minimal integration or co-location with other processes. This facility requires a total permanent investment of $11,932,680 and would process all paunch grass generated in the Upper North Island of New Zealand and all municipal leafy green waste generated in the Greater Waikato region within this area to coproduce 3,385 and 22,273 tonnes per annum of LPC and wet press fibre, respectively. Annual revenues would be $3,723,988 from leaf protein concentrate sales ($1,100 per tonne), $890,939 from selling the wet press fibre as a composting aid ($40 per cubic metre), and $9,464,350 per annum from gate fees for disposing of negative-value leafy green wastes. This corresponds to a total production cost of $13,440,774 per annum and $638,503 per annum of earnings before interest and tax; net earnings are $459,722 for a company that can make the total permanent investment with its own capital. It is not viable to borrow capital to build the plant. During its 15-year lifetime, this facility generates a net present value of $2,091,597, an internal rate of return of 3.880 percent, and achieves simple payback within 12 years.

The most attractive scenario is a process plant integrated with an existing rendering facility and co-located with a composting process. This facility requires a total permanent investment of $6,777,123 and would process all paunch grass generated in the Upper North Island of New Zealand and all municipal leafy green waste generated in the Greater Waikato region within this area to coproduce 3,385 and 22,273 tonnes per annum of LPC and wet press fibre, respectively. Annual revenues would be $3,723,988 from leaf protein concentrate sales ($1,100 per tonne), $890,939 from selling the wet press fibre as a composting aid ($40 per cubic metre), $22,808 per annum from providing waste liquor to the compost process, and $9,464,350 per annum from gate fees for disposing of negative-value leafy green

wastes. This corresponds to a total production cost of $7,734,533 per annum and $6,367,553 per annum of earnings before interest and tax; net earnings are $4,584,638 for a company that can make the total permanent investment with its own capital or $4,321,352 for company using borrowed capital and paying 4.59% interest per annum. During its 15-year lifetime, this facility generates a net present value of $128,767,248, an internal rate of return of 59.903 percent, and achieves simple payback within 2 years.

This is a stark contrast to manufacturing leaf protein concentrate from fresh crops, which simply is not viable when leaf protein concentrate is the sole product. Although a single processing plant in France continues to coproduce leaf protein concentrate and dehydrated alfalfa fibre for animal feed, the numerous other plants that were operating around the world in the 1970s and 1980s have been shut down. This is due to both limitations of the first-generation processing technology they used as well as economic considerations. The technological limitation were much lower protein yields that are approximately half of those achieved by the second-generation technology considered in this study, high energy requirements and costs for fibre dehydration, and lack of mature fermentation technologies to convert the wet press fibre into ethanol or microbial oil coproducts. The latter technological limitation is particularly problematic from an economic standpoint, as LPC production from fresh crops is only viable when it is coproduced with another valuable product.

The great economic strength of processing soft leafy wastes into LPCs is that it is a waste valorisation process, and gate fees can therefore be charged for accepting paunch grass and leafy green waste for disposal. These fees constitute approximately 54 percent of the revenue and are essential for offsetting the cost of production. The BL-2 baseline design-operation scenario is marginal, and even a 5 percent drop in gate fee revenue will compromise the financial viability of the process. The BL-3, IRP-1, IRP-2, and IRP-3 design-operation scenarios are more profitable and are thus expected to remain financially viable when gate fees decrease.

Stable gate fee revenue also makes the process economically robust in the face of other major cost and revenue variations: it remains profitable even when input and

output prices shift by up to 20 percent or the wet fibre coproduct is commercially composted instead of sold. Conversely, fresh crop LPC processes must purchase fresh alfalfa or other leaf crops from farmers that must grow, harvest, and deliver them profitably to a processing facility. In New Zealand this would cost approximately $31.50 per wet tonne of fresh alfalfa (at December 2019 prices). Purchasing leaf crop feedstocks not only increases the cost of manufacture; it also eliminates the revenue stream that makes the process economically robust and removes the need for LPC to be coproduced with another valuable product. It is this latter requirement that in turn introduces technological limitations that makes new fresh crop LPC production unviable.

To summate, manufacturing LPC from paunch grass and leafy green wastes is technologically and financially viable because it is a waste valorisation process. The fees collected for accepting the negative-value feedstock for disposal largely offset the cost of manufacture and allow where the LPC can be the sole product. This in turn allows a simple and relatively inexpensive process plant to be established using mature technologies by eliminating the costs and risks associated with coproduction of biofuels or nutraceuticals using experimental or immature biotechnologies.

6.8 References

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7 Lifecycle Assessment

The lifecycle assessment of the leaf protein concentrate production from soft leafy wastes such as paunch grass and leafy green waste determined the environmental impacts of feedstock transportation and LPC manufacturing as per Figure 7.1.

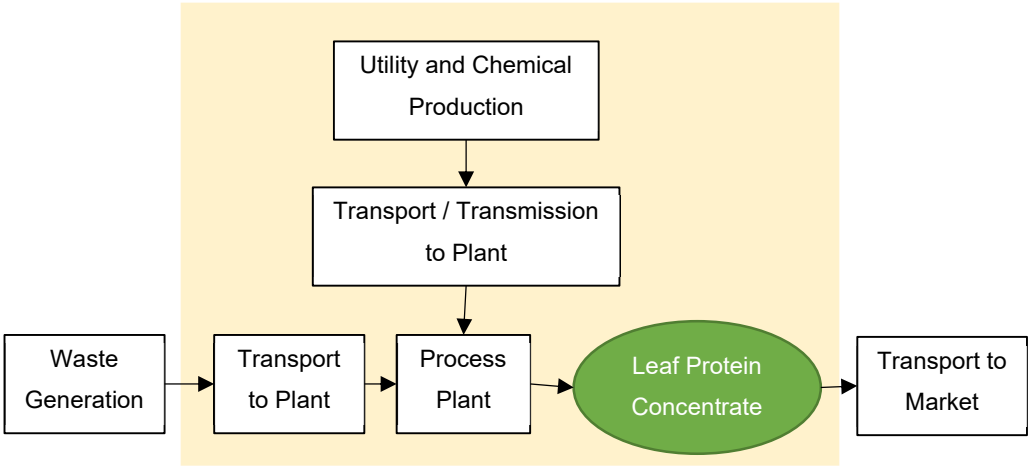

Figure 7.1: Lifecylce assessment system boundary

For transportation of feedstock and inputs, this included the energy consumption, carbon dioxide emissions, and consumptive water use associated with transporting the PG and GW feedstock to the processing facility. The key impacts are presented in Table 7.1.

Table 7.1: Greenhouse gas emission and product water footprint impacts of LPC produced from PG and GW in the Upper North Island of New Zealand.

Summary Statistic	Greenhouse Gas Emissions Footprint (kg CO_2-e /MT-LPC)	LPC Product Blue Water Footprint, WF_{prod}[LPC] (m^3-H_2O/MT-LPC)
Mean	2171.3	42.4
Median	2093.5	39.3
Max	5658.6	135.8
Min	1122.3	17.8
Standard Deviation	559.4	13.67

The PG feedstock transportation inventory was estimated from transportation distances and payloads; the GW transportation inventory was estimated from the data of Farzaneh *et al.* [1]. The LPC manufacturing process impacts included the electric power use, natural gas consumption, total energy use, nitric oxide emissions, carbon dioxide emissions, and consumptive water use. These were then used to determine carbon and water footprints for the leaf protein concentrate exiting the

process. The LPC manufacturing process inventory was based upon the process modelling performed in Chapter 6. Where appropriate, emissions factors from the literature were applied to mass, power, and mileage data to generate emissions inventories.

Data quality assumptions were then used to determine 95% confidence bounds; linear distribution Monte Carlo simulations were performed between these limits and the data used to generate summary statistics for each impact. The impacts calculated from the LCA were then compared with those of compost processes and those of other animal feed proteins found in the literature.

7.1 LCA Goal

This attributional analysis was performed to determine the greenhouse gas emission and consumptive water use impacts associated with manufacturing LPC from PG and GW as per the manufacturing process subjected to technoeconomic analysis in Chapter 6, as well as the impacts of transporting PG and GW to the manufacturing site.

The intended audience of the analysis were the sponsors of this research: Wallace Corporation; Hamilton Garden Bags and Red Lid Bins; as well as environmental scientists and engineers. The analysis results will allow these audiences to compare the greenhouse gas emission and consumptive water use impacts of LPC manufactured from PG and GW with those other animal feed protein ingredients and compost. In turn, this enables a preliminary assessment of whether the process to convert PG and GW waste into LPC has a positive environmental impact, as well as being technologically and financially viable.

7.1.1 Choice of Attributional Analysis

An attributional analysis was performed even though a comparison of products such as LPC, animal feed proteins, and compost would usually entail a consequential analysis. However, the primary objective of the lifecycle assessment was to determine the environmental impacts of producing leaf protein concentrate from paunch grass and leafy green waste, and thus verify its environmental value and complement the technoeconomic analysis presented in Chapter 6. An attributional

analysis is sufficient for these purposes. Although the relative environmental value of LPC can be gauged by comparison with the impacts of other animal feed proteins and compost, determining the impacts of these products was outside the scope of this study. The impacts of comparator products were thus obtained from the literature and compared to those of the leaf protein concentrate.

A consequential analysis is required for a robust comparison of: LPC production from PG and GW; compost production from PG and GW; and other animal feed protein sources. However, this requires all processes to deliver the same services: protein production; and the provision of nutrients and water retention to soils. This, in turn, entails a system expansion to account for protein produced in the form of meat to permit a common functional unit for both processes (i.e., tonne of protein produced). The provision of nutrients and water retention to soils would be provided by compost and the fibre co-product, respectively. Analysing the expanded system would have required time and resources that were not available during this study. Nevertheless, a consequential comparison of LPC, compost, and other animal feed products would make an interesting topic for further investigation.

7.2 LCA Scope

The LCA analyses two main processes: the transportation of PG and GW feedstock to the processing facility from slaughterhouses and the kerbside, respectively; and the manufacturing process which converts these feedstocks into LPC, fibre, and waste liquor. The analysis does not account for temporal effects, as these variations are captured by the Monte Carlo simulation limits. The transportation process analyses use an energy-based approach: impacts are calculated by simulating payload, distance, and specific fuel consumption functions of the transportation processes. Their native functional unit is tonne of feedstock delivered to the processing facility; this is then converted to tonne of LPC produced from the feedstock. The manufacturing process analysis studies the electricity use, natural gas use, and water use functions of the manufacturing process. Its native functional unit is plant operating hour, as this was the basis on which the process mass balance was performed. This basis was then converted to tonne of LPC produced by the manufacturing process. This enables the cumulative impacts of both feedstock

transportation and manufacturing to be calculated, and for them to be compared with the impacts of other animal feed proteins.

7.2.1 General Methodology

The analysis was based on experimental data collected in Chapter 4: *Feedstock Characterisation* and Chapter 5: *Process Optimisation and Product Characterisation*. These data subsequently form the basis of the Chapter 6: *Technoeconomic Analysis* process model which in turn forms the Life Cycle Inventory for the manufacturing process. Conversely, the Life Cycle Inventories for the transportation processes are mostly based on data collected from literature sources. All impact factors were also obtained from literature sources. All LCA data sources are summarised in Table 7.2.

Table 7.2: Data sources used in the LCA of LPC production from PG and GW.

Data Type	Description	Source(s)
Empirical	Feedstock chemical composition	Chapter 4
Empirical	Protein extraction efficiency	Chapter 5
Empirical	Protein recovery efficiency	Chapter 5
Empirical	Leaf protein concentrate chemical composition	Chapter 5
Empirical	Fibre chemical composition	Chapter 5
Empirical	Process water use	Chapters 5 & 6
Empirical	Steam use	Chapters 5 & 6
Empirical	Hydrochloric acid use	Chapters 5 & 6
Empirical	Effluent discharge	Chapters 5 & 6
Empirical	Paunch grass delivery weights	Confidential
Literature	Paunch grass delivery truck fuel consumption	[2]
Literature	Paunch grass delivery truck driving distances	[3; 4]
Literature	Garden waste delivery weights Garden waste truck fuel consumption Garden waste delivery truck driving distances Garden waste truck carbon dioxide emissions	[1]
Literature	Diesel fuel impact factors	[5; 6]
Literature	Grid electricity impact factors	[7; 8; 9]
Literature	Natural gas impact factors	[7; 10]
Literature	Bunker fuel impact factors	[6; 7]
Literature	Hydrochloric acid impact factors	[11]
Literature	Process water supply and treatment impact factors Wastewater treatment impact factors	[7; 8; 9; 12]
Literature	Water footprint catchment weighting factors	[13; 14]

A 10,000 run linear-distribution Monte Carlo simulation was performed for each function of each process. The simulated functions were calculated in Microsoft Excel using the RANDBETWEEN() function and the limits defined in Table 7.3. The impacts were then calculated using the impact factors in Table 7.4 and Equation 7.1 and Equation 7.4 as per the methods of the Ministry for the Environment [7]; CEFIC and ECTA [5]; and Hoekstra *et al.* [15]. The ratios in Table 7.5 were then used to convert the impacts from their native functional units into the final tonne of LPC produced functional unit (Equation 7.5 to Equation 7.8).

Table 7.3: Monte Carlo simulation functions and their limits

Simulation Function	Upper Simulation Limit	Median	Lower Simulation Limit	Source
Net Weight of PG Delivery (kg)	22440	11520	2840	Confidential
PG Roundtrip Delivery Distance (km)	704	226	26	[3; 4]
PG Truck Diesel Fuel Consumption (L/100 km)	53.5	36.2	26.7	[2]
GW Roundtrip Delivery Distance (km)	72.0	67.0	60.0	[1]
GW Truck Diesel Fuel Consumption (L/100 km)	105.8	94.4	79.8	[1]
GW Truck CO_2 emissions (kg CO_2km^{-1})	2.86	2.67	2.05	[1]
Electric Power Use (kWh Electrical Energy)	689	411	246	Appendix C
Natural Gas Consumption (kWh Thermal Energy)	4100	2450	1460	Appendix C
30% HCl Use (kg)	870	519	309	Appendix C
Process Water Use (m^3)	5.21	3.11	1.86	Appendix C
Wastewater Treated (m^3)	11.2	6.69	3.99	Appendix C
Grid Electricity Specific Consumptive Water Use (mL/kWh)	1640	-	1580	[8; 16] (See §7.2.3)
Natural Gas Specific Consumptive Water Use (mL/kWh)	9.6	-	9.3	[10] (See §7.2.3)

The individual impacts are reported as both summary statistics and histograms. Individual greenhouse gas impacts in the inventory were summated as per Equation 7.9 to determine an overall carbon footprint for the LPC [7]. Similarly, consumptive water uses were summated (Equation 7.10) as per the methods of Hoekstra*, et al.* [15] and Boulay*, et al.* [13] to determine an global blue water footprint for the LPC. Green Water is not applicable to this analysis, as none of the process inputs require

natural precipitation for their production. Similarly, Grey Water is not considered in this analysis as it is assumed that the wastewater treatment system performs primary, secondary, and tertiary treatments of the process effluent to reduce contaminant concentrations to a level compliant with applicable environmental standards and no further dilution in the natural environment is required [15].

Table 7.4: Factors used to calculate impacts from Monte Carlo simulation functions

Description	Value	Units	Source
Well To Wheel CO_2 Emissions of Diesel Fuel	2.9	g CO2/L	[5]
Well To Wheel Consumptive Water Use of Diesel Fuel	154	L/GJ	[6]
NZ Grid Electricity GHG Emissions (Generation)	0.101	kg CO_2-e/kWh (electrical energy)	[7]
NZ Grid Electricity GHG Emissions (Transmission Losses)	0.00870	kg CO_2-e/kWh (electrical energy)	[7]
Well To Flue GHG Emissions of Natural Gas	0.195	kg CO_2-e/kWh (thermal energy)	[7]
Manufacturing GHG Emissions of HCl	0.907	kg H_2O/kg HCl	[11]
Manufacturing Consumptive Water Use of HCl (Process Water)	28.0	kg H_2O/kg HCl	[11]
Manufacturing Consumptive Water Use of HCl (Cooling Water)	28.7	kg H_2O/kg HCl	[11]

Table 7.5: Functional unit conversion factors derived from the process mass balance.

Conversion Factor	Value	Units
PG to LPC ratio (f_m)	11.4	kg PG / kg LPC
GW to LPC ratio (f_m)	2.85	kg GW / kg LPC
LPC to plant operating hour ratio (f_t)	0.548	kg LPC / Plant operating-hour

f_m = material basis conversion factor | f_t = time basis conversion factor

Equation 7.1: Calculation of diesel fuel use as a function of specific fuel consumption and distance travelled.

$$q = \mu d$$

Equation 7.2: Calculation of greenhouse gas emissions as a function of energy, fuel, or material use.

$$GHG_q = \sum_{i=1}^{n} f_{GHG(i)}\, q$$

Equation 7.3: Calculation of greenhouse gas emissions from freight as a function of mass and distance

$$GHG_x = \sum_{j=1}^{n} f_{GHG(j)}\, md$$

Equation 7.4: Calculation of consumptive water use as a function of fuel, energy, or material use.

$$WC_x = \sum_{k=1}^{n} f_{WC(k)} q$$

q = quantity of fuel, energy, or material | μ = specific fuel consumption d = distance travelled | f_{GHG} = greenhouse gas emissions factor f_{WC} = water consumption factor

Equation 7.5: Conversion of feedstock GHG impact from mass delivered basis to product mass basis	**Equation 7.6: Conversion of feedstock WC impact from mass delivered basis to product mass basis**
$GHG_y = GHG_x \times f_m$	$WC_y = WC_x \times f_m$
Equation 7.7: Conversion of manufacturing GHG impact from time basis to product mass basis	**Equation 7.8: Conversion of manufacturing WC impact from time basis to product mass basis**
$GHG_y = \frac{GHG_x}{f_t}$	$WC_y = \frac{WC_x}{f_t}$
Equation 7.9: Product GHG impact as a function of transportation and manufacturing process impacts	**Equation 7.10: Product WC impact as a function of transportation and manufacturing process impacts**
$GHG_Y = \sum_{l=1}^{l} GHG_y$	$WC_Y = \sum_{l=1}^{l} f_{cw} \times WC_y$
f_{cw} = catchment weighting factor*	
***Can be a Water Stress Index (WSI) factor [14] or Available Water Remaining (AWARE) factor [13]**	

7.2.2 Transportation Process Considerations

At a prima facie level, the transportation of PG and GW to the processing facility are both trucking processes; however, in practice they have very different operating modes. The PG transportation process can be considered a long-distance trucking operation from just 11 sites to the processing plant located at Waitoa, Waikato, New Zealand (Table 7.6). For this process, both energy-based and activity-based approaches to the LCA should produce comparable outcomes.

Conversely, the GW transportation is a stop-start collection process which operates mostly in urban areas; the trucks only perform long-distance driving once they are full and are enroute to the processing facility. The activity-based emissions and fuel consumption factors which apply to long-distance trucking operations are inconsistent with those used for GW collection and transportation [1; 17]; it is therefore critical to use an energy-based approach. Similarly, the two processes use different models of truck with very different specific fuel consumption profiles [2; 17]. Transportation of PG and GW feedstocks to the processing facility are therefore two very different processes which must be analysed separately.

Table 7.6: Roundtrip driving distances between Waitoa LPC Manufacturing Plant and Meat Processing Facilities [3; 4].

Meat Processing Facility	Roundtrip Driving Distance from Waitoa Manufacturing Plant (km)
Greenlea Morrinsville	26
Silver Fern Farms Te Aroha	28
Greenlea Hamilton	94
AFFCO Horotiu	102
Universal Beef Packers	226
AFFCO Rangiuru	226
Te Kuiti Meat Processors	228
Auckland Meat Processors	252
Crusader Meats	296
Silver Fern Farms Dargaville	620
AFFCO Moerewa	704

Delivery of Paunch Grass to the Processing Facility

The PG delivery system boundary includes all activities involved with the transportation and transfer of bulk PG from the source facility to the LPC manufacturing facility as per Figure 7.2.

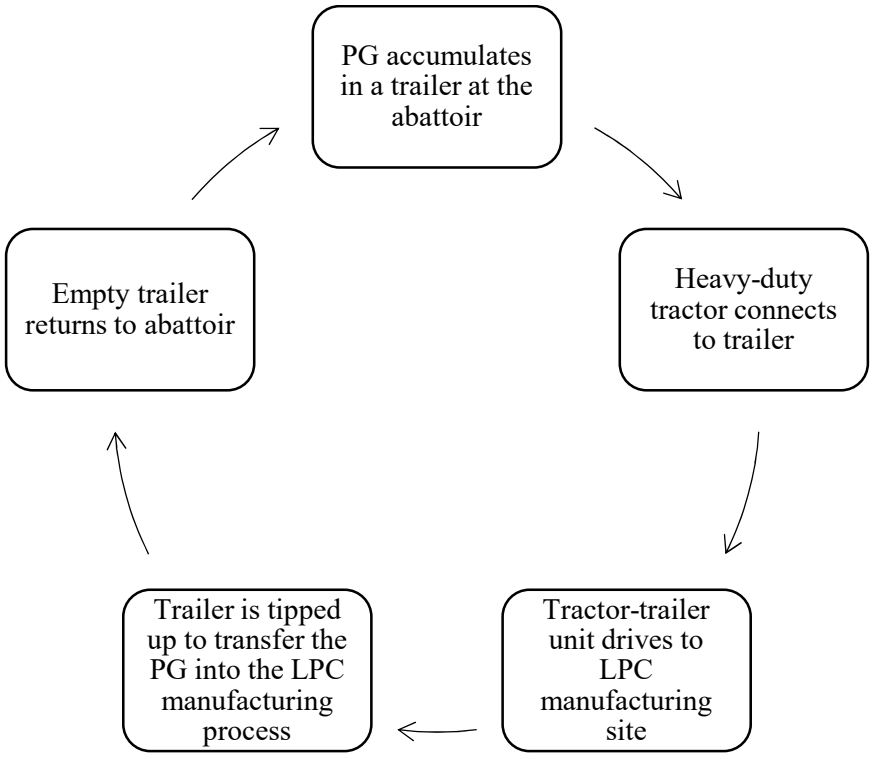

Figure 7.2: Process steps required to transport PG from a meat processing facility (abattoir) to the LPC manufacturing process.

Movements of PG within the source facility are therefore considered to be out of scope; but unloading the truck at the LPC manufacturing facility is within scope. As delivered PG is the only product from this process, all greenhouse gas emission and consumptive water use impacts are therefore allocated to it as kg-CO_2 and m^3-H_2O per tonne PG delivered. Transportation impacts are also considered as a component of the LPC product impact; this requires converting the functional unit to a product basis (kg-CO_2 or m^3-H_2O per tonne LPC produced).

Assumptions and Data

The analysis makes six key assumptions:

- The roundtrip distances and PG delivery weights are linearly distributed.
- The PG is hauled to the processing facility in heavy-duty trucks with linearly-distributed fuel economies ranging between 26.7 L/100 km and 53.5 L/100 km [2].
- The distance of each journey is between 26 km and 704 km. This corresponds to real-world driving distances between the assumed location of the processing plant at Waitoa and the meat processing facilities in the Greater Waikato region (Table 7.6).
- The PG payload on each journey is linearly distributed between 2.8 tonnes and 22.4 tonnes.
- Greenhouse gas emissions arise from a Well to Wheel (WTW) emissions factor is 2.9 g-CO_2 per litre of diesel fuel [5].
- Consumptive water use arises from WTW Water Consumption Footprint (WCF) of 154 L/GJ of diesel fuel with a LHV of 513 MJ/L [6].

Limitations and Data Quality

There are some limitations to this approach. In practice it is unlikely that the trucks are completely dedicated to paunch grass transfer and transportation: LCA guidance indicates that trucks run empty 25% of the time [5]. For example, they may travel to a source facility after delivering another bulk material to another facility nearby; this would reduce the roundtrip distance and reduce the diesel fuel consumption and resultant impacts associated with the PG delivery. The variation in emissions

arising from different degrees of freight capacity utilisation or empty running is therefore likely to be captured by the Monte Carlo simulation limits.

Actual fuel consumption will depend on payload, road conditions, traffic, and terrain. The analysis used industry-wide data from the USA, however this is unlikely to be significantly different from the study area; a recent review of fuel consumption by heavy duty trucks in the USA and European Union found that the respective fleets had comparable and overlapping ranges of fuel consumption [18]. It is likely that a comparison between New Zealand and American truck fleets would produce a similar result. The inaccuracies in emissions estimates arising from geographical differences are therefore likely to be captured by the Monte Carlo simulation limits.

Delivery of Leafy Green Waste to the Processing Facility

The GW delivery system boundary includes all activities associated with collection, transport, and transfer of GW to the LPC manufacturing facility (Figure 7.3). Movements of GW within the source property (such as gathering and transferring GW into a collection bin) are therefore considered to be out of scope; but unloading the truck at the LPC manufacturing facility is within scope. As delivered GW is the only product from this process, all transportation impacts are allocated to it: litres of diesel fuel per tonne of GW delivered; and kg-CO_2 per tonne GW delivered. Transportation impacts are also interpreted as a component of the LPC product impact; this requires converting the functional unit to a product basis.

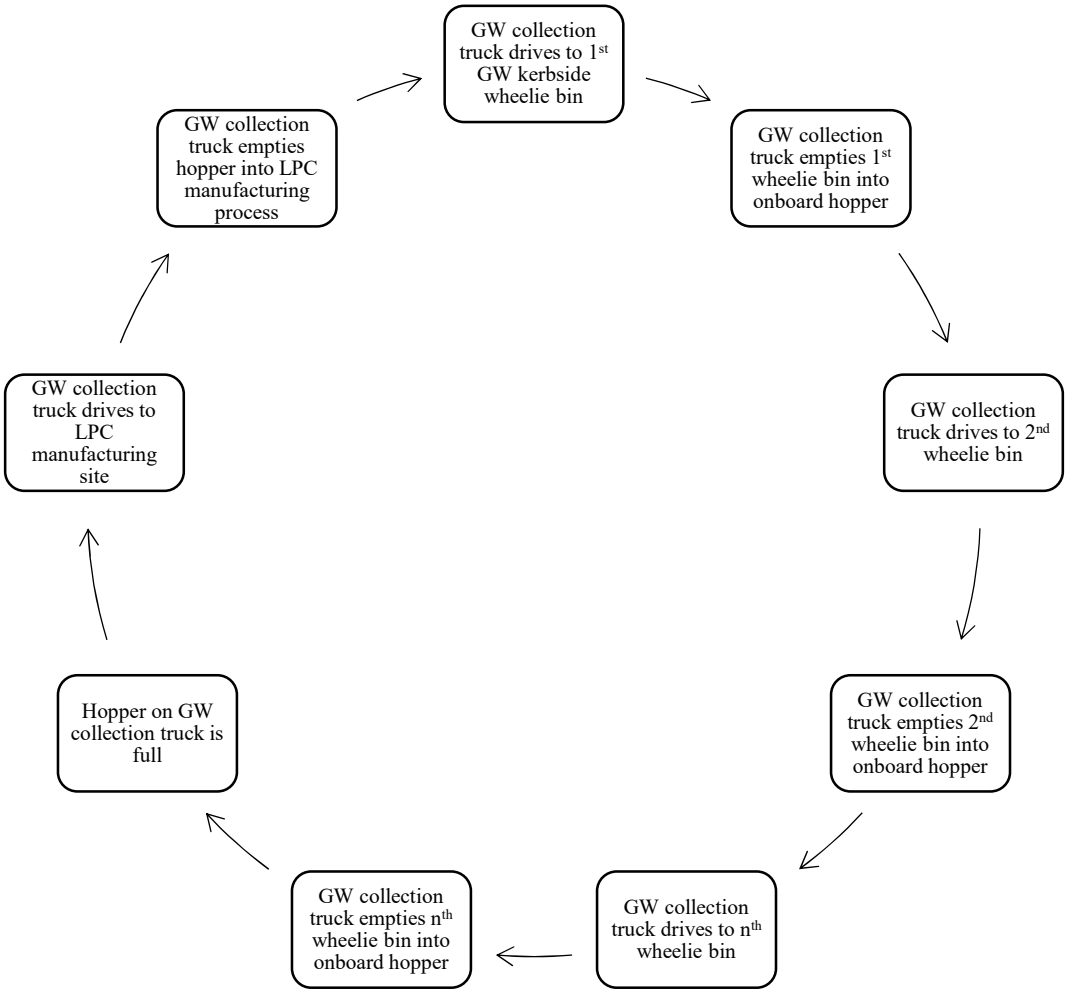

Figure 7.3: Process steps required to transport GW from a kerbside collection bin to the LPC manufacturing process.

Assumptions and Data

The analysis makes three key assumptions:

- The trucks operating in the GW transportation systems are linearly distributed across the 2002, 2004, and 2006 model years manufactured by McNelius Truck and Manufacturing [19].
- Each truck body can hold 16.8 m^3 of GW [20]. The mass capacity of the truck body (5.5 metric tonnes) is derived from its volumetric capacity and the bulk density of GW used in the process model.
- The trucks operating in the GW transportation system do not proceed to the LPC manufacturing site until fully laden with GW and follow a linear distribution of the driving profiles described by Farzaneh*, et al.* [1].

Limitations and Data Quality

The limitations to this approach arise from the dataset used for the LCA being taken from a geographical location and time that is different from that of the GW transportation system. The dataset originates from El Paso, Texas and was applicable to the years 2004 to 2009. El Paso is 1111 metres higher above sea level than Hamilton, the largest city in the Waikato region of New Zealand [21]; using a dataset from a significantly higher elevation will lead to fuel consumption and CO_2 emissions from the trucks being overestimated by approximately 10 percent [22; 23].

Similarly, the trucks in the dataset are approximately 5-10 years older and of a different make from those that would be used in the GW transportation system. Trucks manufactured in more recent model years would likely be more fuel efficient and meet more stringent emissions standards [24; 25]. Again, this misalignment between the LCA dataset and actual system conditions will cause diesel fuel consumption and CO_2 emissions to be overestimated.

A further limitation arises from assuming the driving profiles from Farzaneh*, et al.* [1] are directly applicable to the GW transportation systems. This requires the driving distances between the urban GW collection area and the dump site (i.e., the LPC manufacturing facility) to be similar to those of the reference system. At this stage there is no evidence to suggest that these distances are aligned, this therefore leads to greater uncertainty in the analysis.

7.2.3 Manufacturing Process

The manufacturing process aspect of the lifecycle assessment focuses on the function of producing LPC from PG and GW. As the mass balance and process model for the manufacturing process are performed on an hourly basis, the first functional unit in the analysis is impact per plant operating hour. To allow comparisons with other protein concentrate products, impacts are then converted to impact per metric tonne of LPC as per and . The entire manufacturing facility (including utilities) is considered part of the LPC product system as per Figure 7.4. All impacts are allocated to the LPC as it is the primary product of the process and orders of magnitude more valuable than the coproducts. The LPC product impact

is the sum of component impacts studied listed in Table 7.7 as per Equation 7.9 and Equation 7.10.

Table 7.7: Components of LPC product impacts.

Components of Greenhouse Gas Emissions (kg CO_2-e)	Components of Global Water Footprint (m^3 global-H_2O)
Diesel Used by PG Freight Trucks	Diesel Production for PG Freight Trucks
Diesel Used by GW Collection and Delivery Trucks	Diesel Production for GW Collection and Delivery Trucks
Electricity Generation	Process Water Use / Steam Generation
Electricity Transmission	Electricity Generation for Process Operation
Natural Gas Use	Electricity Generation for Process Water Supply
30% HCl Manufacturing	Electricity Generation for Wastewater Treatment
30% HCl Shipping	30% HCl Manufacturing
	Bunker Fuel Production for 30% HCl Sea Freight
	Diesel Production for 30% HCl Road Freight

Assumptions and Data

The analysis is based on mass and energy calculations in Chapter 6. In addition to the assumptions in Chapter 6, the analysis assumes:

- That the manufacturing process operates at steady state; and
- The stream values calculated from the process model are representative of process median values.

Process-Specific Methodology

Lifecycle inventories were obtained from the mass balance and equipment sizing calculations in Chapter 6. A Data Pedigree Matrix (Table 7.8) was then manually generated for each simulation variable as per the methods of Weidema *et al.* [26]; corresponding uncertainty factors (Table 7.9) were then generated as per the methods of Ciroth *et al.* [27] and used to calculate the geometric standard deviation for each process input as per Equation 7.11 [27].

Table 7.8: Data Pedigree Matrix for manufacturing process inputs

Manufacturing Process Input	Reliability (U_1)	Completeness (U_2)	Temporal Correlation (U_3)	Geographical Correlation (U_4)	Further Technological (U_5)	Sample Size (U_6)
Electricity (kWh)	4	5	1	2	4	5
Natural Gas (kWh)	4	5	1	2	4	5
Process Water (m^3)	4	5	1	2	4	5
Wastewater Treated (m^3)	4	5	1	2	4	5
30% v/v HCl (kg)	4	5	1	2	4	5

Table 7.9: Uncertainty factors and geometric standard deviations for manufacturing process inputs.

Manufacturing Process Input	Reliability (U_1)	Completeness (U_2)	Temporal Correlation (U_3)	Geographical Correlation (U_4)	Further Technological (U_5)	Sample Size (U_6)	Baseline Uncertainty (U_b)	Geometric Standard Deviation (SD_{g95})
Electricity (kWh)	1.2	1.2	1	1.01	1.5	1.2	1.05	1.68
Natural Gas (kWh)	1.2	1.2	1	1.01	1.5	1.2	1.05	1.68
Process Water (m^3)	1.2	1.2	1	1.01	1.5	1.2	1.05	1.68
Wastewater Treated (m^3)	1.2	1.2	1	1.01	1.5	1.2	1.05	1.68
30% v/v HCl (kg)	1.2	1.2	1	1.01	1.5	1.2	1.05	1.68

The geometric standard deviation was then used to determine 95% Confidence Limits (Table 7.3) for each process input as per Equation 7.12 and Equation 7.13 [28; 29]. The study used the most recent impact factors available for the area under study [2; 5; 14; 27], but overall accuracy was limited by the process model being based on laboratory data and theoretical calculations.

Equation 7.11: Geometric Standard Deviation as a function of uncertainty factors.

$$SD_{g95} = \sigma^2 = exp\sqrt{(\ln U_1)^2 + (\ln U_2)^2 + (\ln U_3)^2 + (\ln U_4)^2 + (\ln U_5)^2 + (\ln U_6)^2 + (\ln U_b)}$$

Equation 7.12: 95% lower confidence limit as a function of Geometric Standard Deviation and median.

$$CL_{95,Lower} = \frac{\tilde{x}}{SD_{g95}}$$

Equation 7.13: 95% upper confidence limit as a function of Geometric Standard Deviation and median.

$$CL_{95,Upper} = \tilde{x} \times SD_{g95}$$

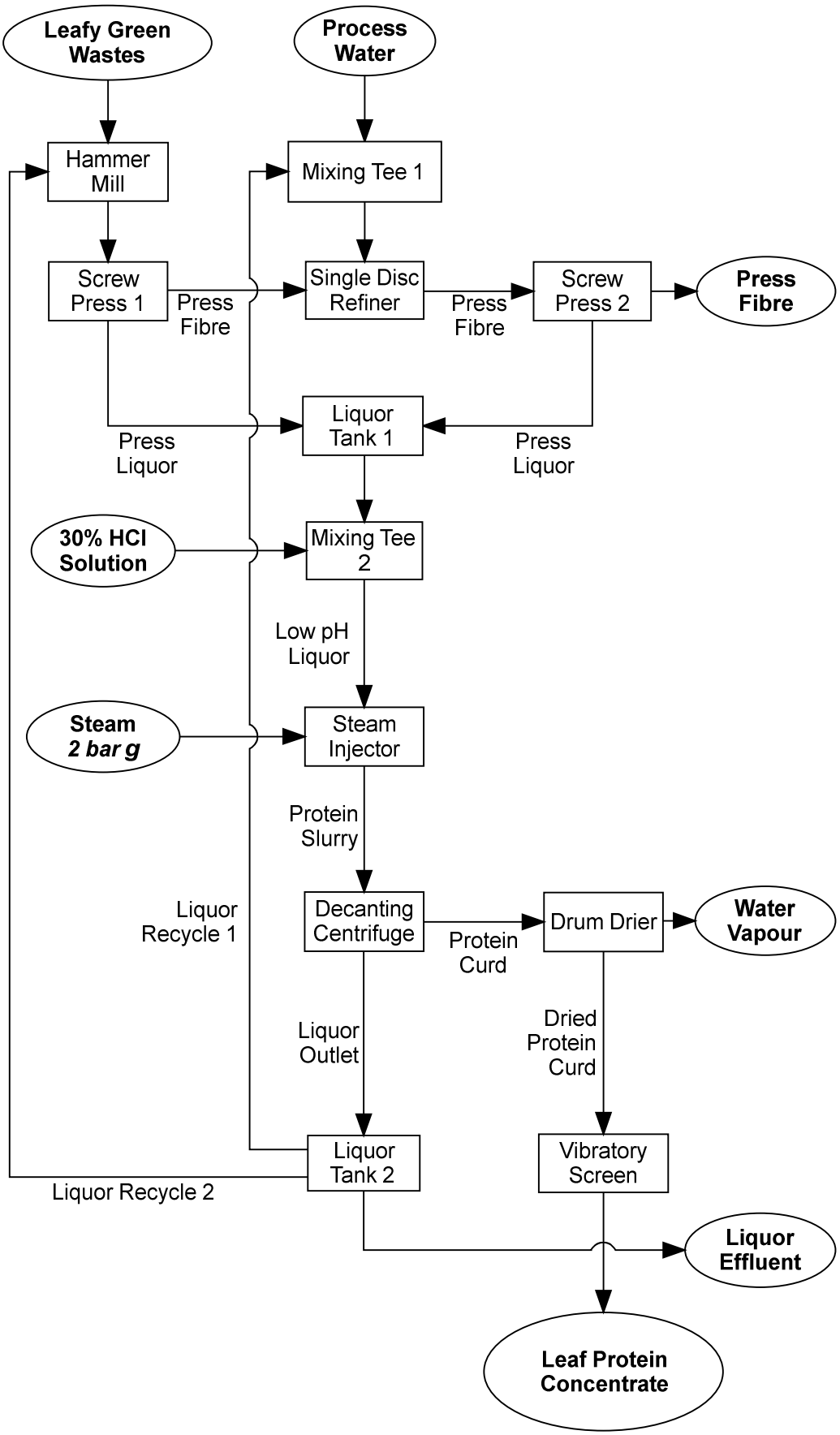

Figure 7.4: Block flow diagram of the soft leafy waste protein concentrate production process

7.2.4 Data Pedigree and Uncertainty

There were no data quality requirements for the analysis, which was performed using the empirical data from Chapter 4 and Chapter 5 and process model calculations from Chapter 6; data from the scientific literature was used when the first two data sources proved insufficient. No further datasets were collected for LCA purposes. The data pedigree matrices and uncertainty factors for each variable were therefore determined by the methods of Althaus *et al.* [30], Ciroth*, et al.* [27], and Weidema and Wesnæs [31] and are presented in Table 7.10 to Table 7.12. In some cases, the only data available in the scientific literature was from the USA; collecting this data for the Upper North Island of New Zealand should be an important focus area for future studies.

Table 7.10: Data Pedigree Matrix for Transportation Simulation Variables.

Simulation Variable	Data Pedigree (U_1,U_2,U_3,U_4,U_5,U_6)	Rationale
Net Weight of PG Delivery (kg)	(1,3,2,1,1,1)	Simulation limits were calculated using actual weighbridge data from a paunch grass collection facility.
PG Roundtrip Delivery Distance (km)	(1,1,1,1,1,1)	Simulation limits were determined using current real-world driving distances [3; 4].
PG Truck Diesel Fuel Consumption (L/100 km)	(2,3,2,5,1,2)	Fuel consumption limits were taken from a 2016 survey of US heavy-duty truck fleets [2].
GW Roundtrip Delivery Distance (km)	(2,4,3,5,3,1)	Simulation limits were taken from a 2009 study of refuse trucks in El Paso, Texas [1].
GW Truck Diesel Fuel Consumption (L/100 km)	(2,4,3,5,3,1)	
GW Truck CO_2 emissions (kg CO_2km^{-1})	(2,4,3,5,3,1)	

Table 7.11: Data Pedigree Matrix for Manufacturing Simulation Energy Variables.

Simulation Variable	Data Pedigree (U_1,U_2,U_3,U_4,U_5,U_6)	Rationale
Electric Power Use (kWh Electrical Energy)	(4,5,1,2,4,5)	Simulation median was taken from the Chapter 6 process model which is based on laboratory-scale data from Chapter 4 and Chapter 5.
Natural Gas Consumption (kWh Thermal Energy)	(4,5,1,2,4,5)	
Process Water (m^3)	(4,5,1,2,4,5)	
Wastewater Treated (m^3)	(4,5,1,2,4,5)	
30% v/v HCl (kg)	(4,5,1,2,4,5)	

U_1 = Reliability | U_2 = Completeness | U_3 = Temporal Correlation | U_4 = Geographical Correlation U_5 = Further Technological | U_6 = Sample Size

Table 7.12: Data Pedigree Matrix for Manufacturing Simulation Water Variables.

Simulation Variable	Data Pedigree (U_1,U_2,U_3,U_4,U_5,U_6)	Rationale
Grid Electricity Specific Consumptive Water Use (mL/kWh Electrical Energy)	(1,1,3,5,1,3)	Simulation limits were calculated using the electricity generation mix for NZ during the study period and water consumption from a 2011 US review of consumptive water use in electricity generation [32; 33].
Natural Gas Specific Consumptive Water Use (mL/kWh Thermal Energy)	(3,2,3,5,1,3)	Simulation limits taken from a 2013 US study of consumptive water use in conventional* onshore natural gas production [10]. *without hydraulic fracturing

U_1 = Reliability | U_2 = Completeness | U_3 = Temporal Correlation | U_4 = Geographical Correlation U_5 = Further Technological | U_6 = Sample Size

7.3 Lifecycle Impact Analysis

The overall greenhouse gas and water product footprints of leaf protein concentrate (LPC) are presented in Table 7.13; their globally-weighted water consumption footprints are presented in

These overall greenhouse gas and water consumption footprints are the arithmetic sums of footprints generated from the feedstock transportation and manufacturing process inventories described in Sections 7.3.1 and 7.3.2. It is also clear that the results from the two methods for calculating the Water Consumption Footprint yield disagree by orders of magnitude (Table 7.14). This is not unexpected, as the Water Stress Index (WSI) method is based on the maximum amount of water that humans could hypothetically withdraw from a catchment minus current human water use [34; 35]; by contrast, the Available Water Remaining (AWARE) method also accounts for the water requirements of the local ecosystem [13; 36]. The AWARE method gives a better indication of the true water consumption impact but has only been in use since 2018. Water footprints were therefore calculated using both to allow comparisons with as many studies as possible.

Table 7.14Table 7.14. Distribution plots are presented in Figure 7.5 to Figure 7.8. The GHG emission impact appears to be a Weibull distribution, the product water footprint appears to be a Poisson distribution, and the weighted water footprints appear to be linear distributions.

Table 7.13: Greenhouse gas emission and product water footprint impacts of LPC produced from PG and GW in the Upper North Island of New Zealand.

Summary Statistic	Greenhouse Gas Emissions Footprint (kg CO_2-e /MT-LPC)	LPC Product Blue Water Footprint, WF_{prod}[LPC] (m^3-H_2O/MT-LPC)
Mean	2182.5	42.4
Median	2111.2	39.3
Max	5919.0	135.9
Min	1039.0	0.2
Standard Deviation	562.5	13.54

These overall greenhouse gas and water consumption footprints are the arithmetic sums of footprints generated from the feedstock transportation and manufacturing process inventories described in Sections 7.3.1 and 7.3.2. It is also clear that the results from the two methods for calculating the Water Consumption Footprint yield disagree by orders of magnitude (Table 7.14). This is not unexpected, as the Water Stress Index (WSI) method is based on the maximum amount of water that humans could hypothetically withdraw from a catchment minus current human water use [34; 35]; by contrast, the Available Water Remaining (AWARE) method also accounts for the water requirements of the local ecosystem [13; 36]. The AWARE method gives a better indication of the true water consumption impact but has only been in use since 2018. Water footprints were therefore calculated using both to allow comparisons with as many studies as possible.

Table 7.14: WSI and AWARE-weighted water consumption footprint impacts of LPC produced from PG and GW in the Upper North Island of New Zealand.

Summary Statistic	Total WSI-Weighted Global Water Consumption Footprint (m^3 H_2O/MT-LPC)	Total AWARE-Weighted Global Water Consumption Footprint (m^3 H_2O/MT-LPC)
Mean	10.1	356
Median	10.1	355
Max	66.5	607
Min	5.3	184
Standard Deviation	2.75	86.8

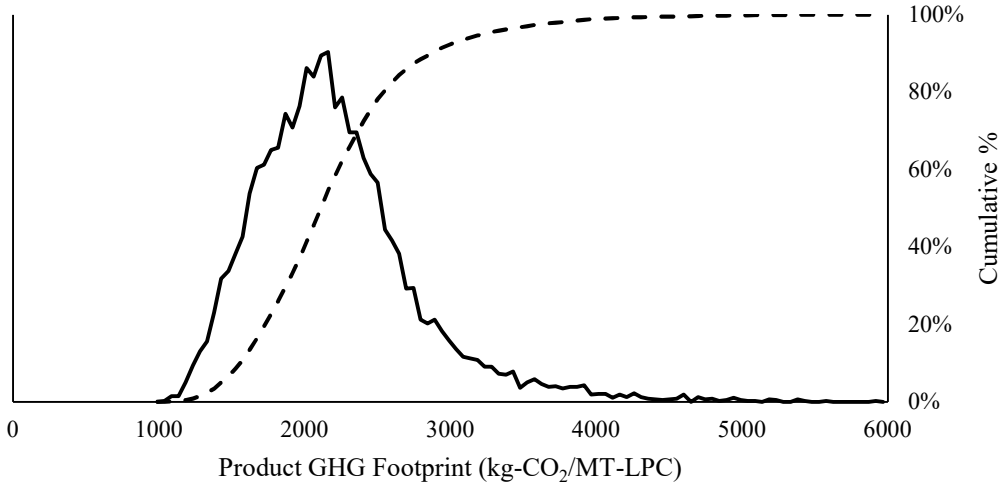

Figure 7.5: Distribution plot of LPC product GHG footprint.

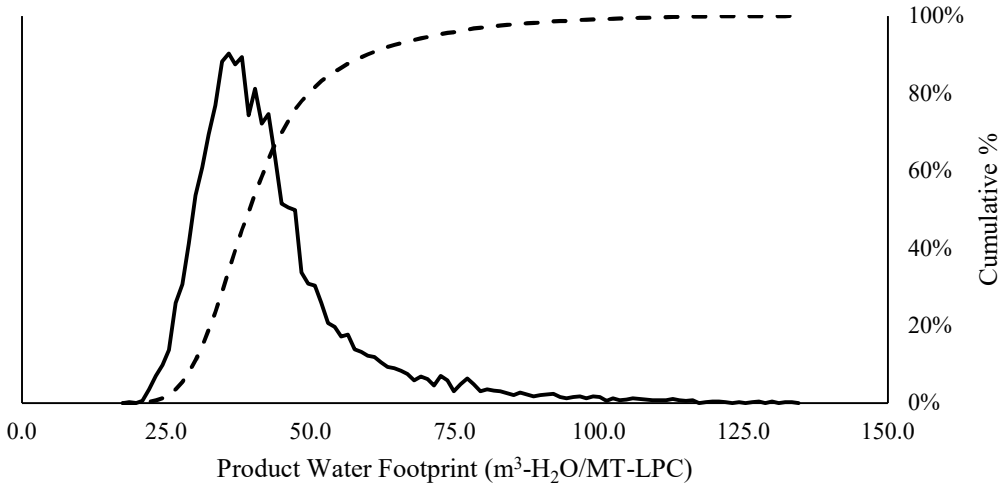

Figure 7.6: Distribution plot of LPC Product Water Footprint.

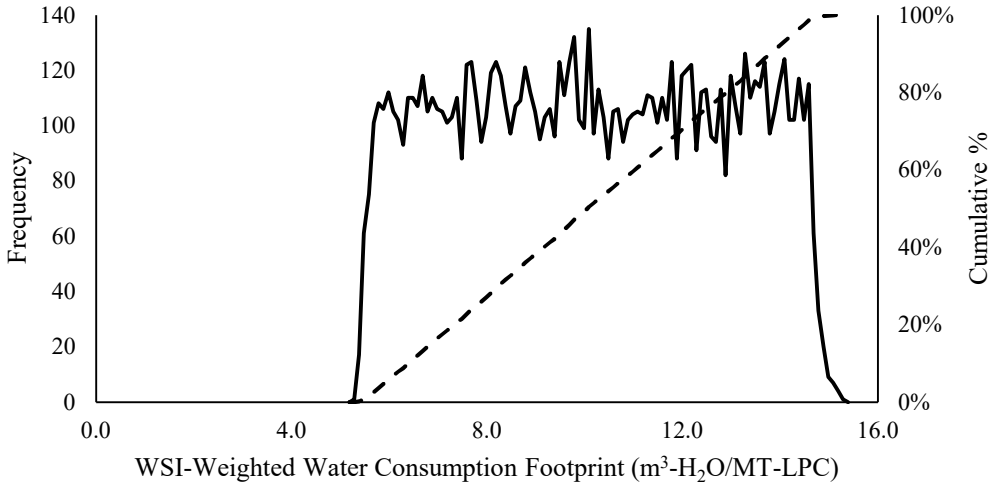

Figure 7.7: Distribution plot of LPC WSI-weighted Water Consumption Footprint.

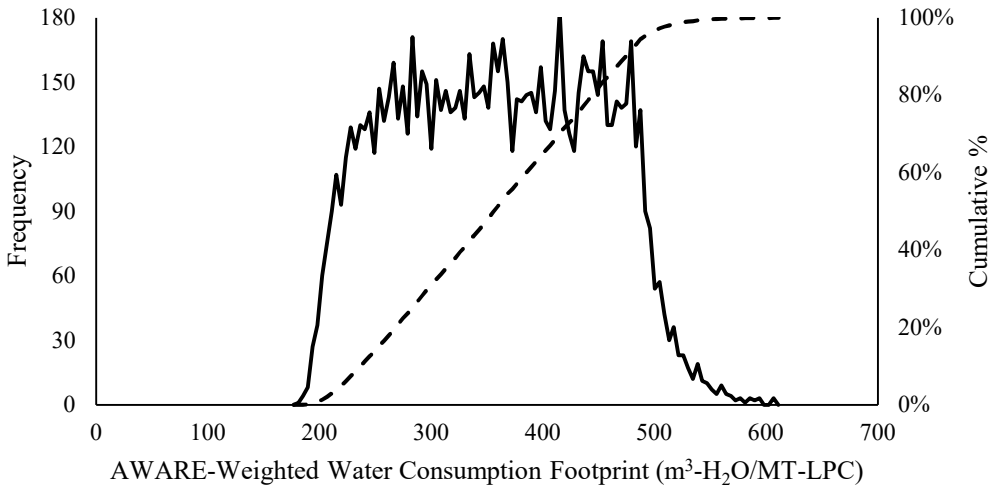

Figure 7.8: Distribution plot of LPC AWARE-weighted Water Consumption Footprint.

7.3.1 Feedstock Transportation

The overall impacts of feedstock transportation are a weighted average of the PG and GW transportation impacts (Table 7.15, Figure 7.9 to Figure 7.12). Surprisingly, the means for these impacts are within one standard deviation of each other, as the typical transportation distances for each feedstock compensates for an order of magnitude difference in fuel consumption per unit distance. PG is typically travels a much greater distance to the manufacturing facility than GW; but the GW collection trucks have an order of magnitude greater diesel fuel consumption per unit distance [1; 2]. The overall impacts of feedstock transportation are therefore determined by the ratio of PG:GW (4:1) rather than the unit impacts of transporting either feedstock (Table 7.16). The different distributions for PG and GW impacts can be attributed to the different driving profiles for PG and GW delivery (Table 7.6 and Farzaneh*, et al.* [1]).

Table 7.15: Impacts of PG and GW delivery to the LPC manufacturing facility.

Simulated Lifecycle Impact Variable	Mean	Median	Max	Min	Standard Deviation
PG GHG Footprint (kg CO_2/MT PG delivered)	44.7	32.8	336	1.11	42.1
GW GHG Footprint (kg CO_2/MT GW delivered)	32.1	32.0	47.7	16.2	4.18
PG Diesel Fuel Consumption (L/MT PG delivered)	7908	5810	59356	196	7442
GW Diesel Fuel Consumption (L/MT GW delivered)	5710	5693	7067	4487	567
PG Mass Fraction of Feedstock Transportation CO_2 Emissions (%)	75.5%	80.4%	97.8%	10.4%	16.9%
PG Mass Fraction of Feedstock Transportation Diesel Fuel Use (%)	75.4%	80.2%	97.8%	10.5%	16.9%

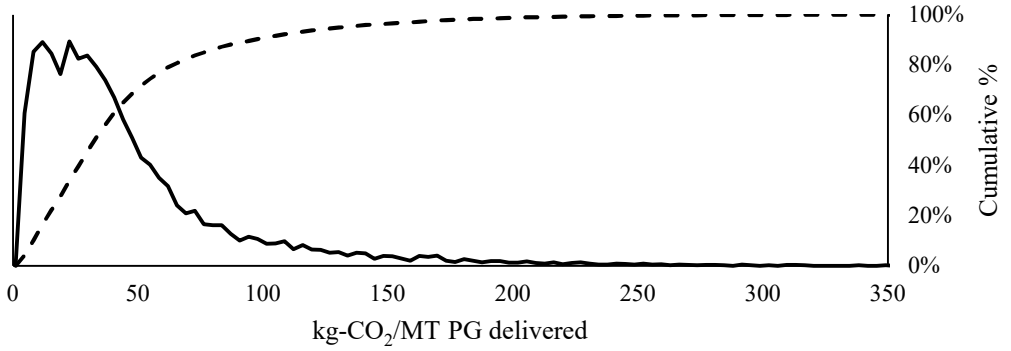

Figure 7.9: Distribution plot for GHG footprint of PG deliveries.

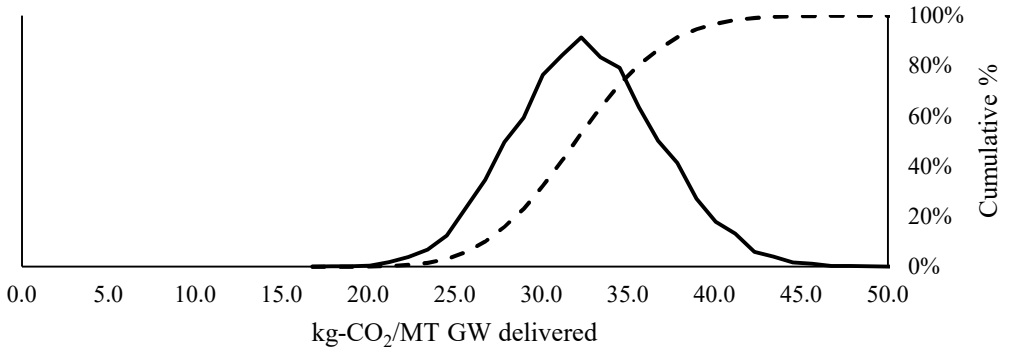

Figure 7.10: Distribution plot for GHG footprint of GW deliveries.

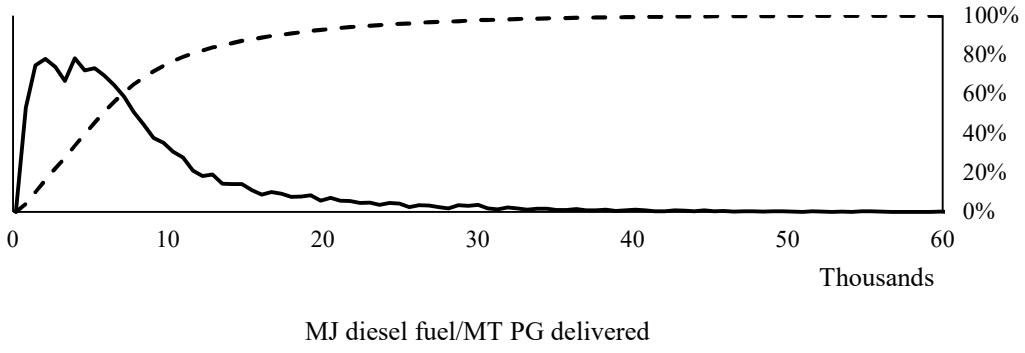

Figure 7.11: Distribution plot for diesel fuel footprint of PG deliveries.

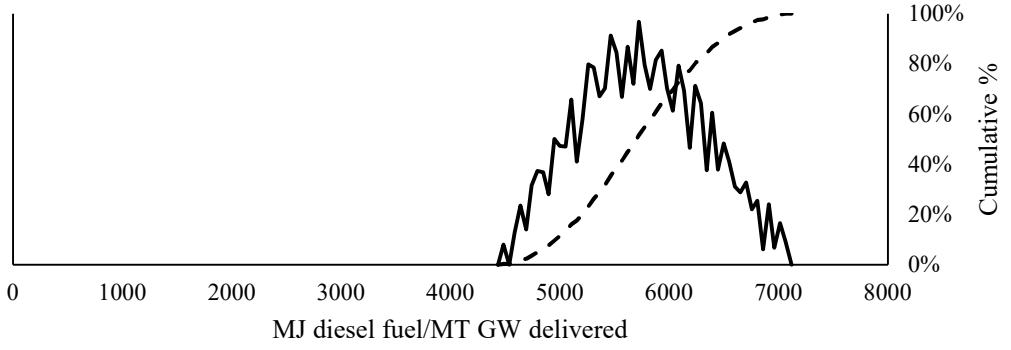

Figure 7.12: Distribution plot for diesel fuel footprint of GW deliveries.

The impacts of transporting PG and GW to the LPC manufacturing facility are presented in Table 7.16 and have Poisson distributions (Figure 7.13 to Figure 7.15).

GHG emissions arising from feedstock transportation make up approximately one quarter of overall LPC GHG emissions. The water consumption footprint of feedstock transportation is relatively small – on average it is less than 5% of the global water consumption footprint of LPC.

Table 7.16: Impacts of feedstock transportation processes per unit of LPC.

Simulated Lifecycle Impact Variable	Mean	Median	Max	Min	Standard Deviation
Feedstock Transport CO_2 Emissions (kg/MT-LPC)	601	466	3916	85	480
Feedstock Transport Diesel Fuel Use (MJ/MT-LPC)	106468	82310	693090	16524	84859
Well to Wheel Water Footprint of Diesel Fuel used for Feedstock Transport (m^3/MT-LPC)	16.40	12.68	106.74	2.54	13.07
Feedstock Transportation Component of LPC CO_2 Emissions (Mass%)	25.4%	23.0%	75.9%	4.6%	13.1%
Feedstock Transportation Component of LPC WSI-Water Footprint (%)	1.812%	1.359%	16.45%	0.198%	1.529%
Feedstock Transportation Component of LPC AWARE-Water Footprint (%)	7.88%	6.28%	48.4%	0.96%	5.77%

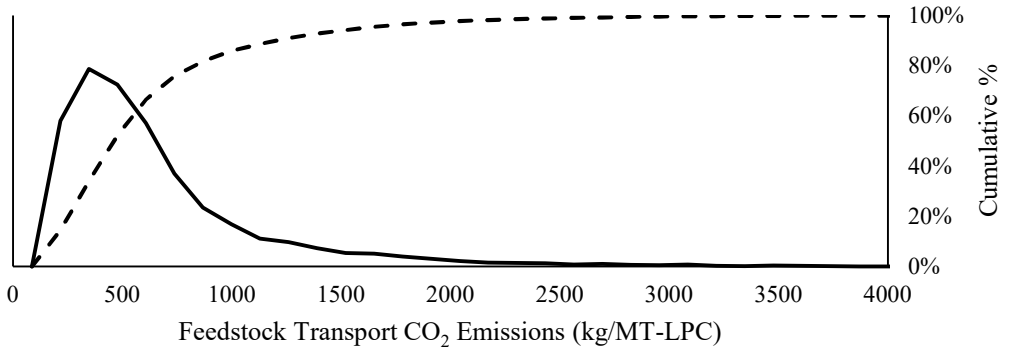

Figure 7.13: Distribution plot of feedstock transportation CO_2 emissions.

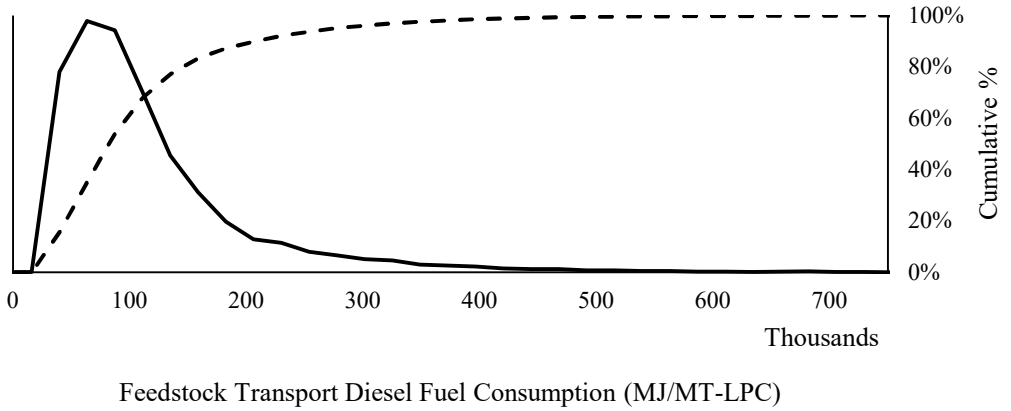

Figure 7.14: Distribution plot of feedstock transportation diesel fuel consumption.

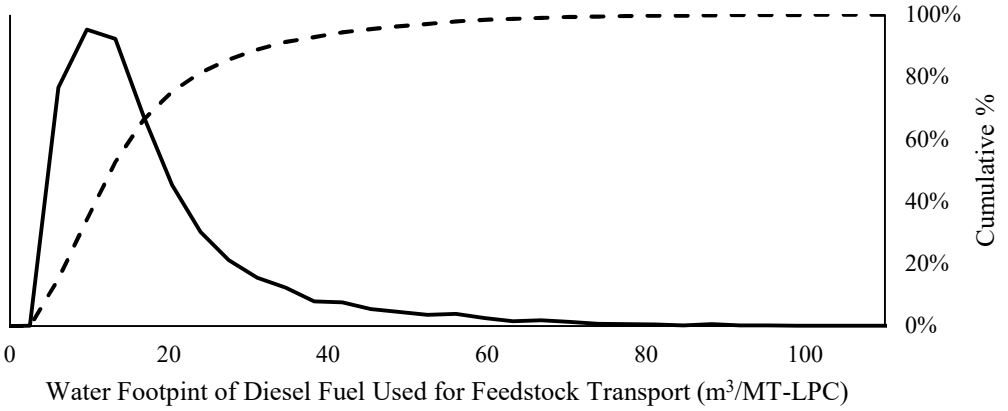

Figure 7.15: Distribution plot of well to wheel water footprint of diesel fuel used for feedstock transportation.

7.3.2 Manufacturing Process

The manufacturing process lifecycle inventory is summarised in Table 7.17. The impacts arising from this the lifecycle have been broken down into three parts:

- GHG Emissions for LPC manufacturing process operations (Table 7.18);
- Consumptive Water Use for LPC manufacturing process operations (Table 7.19); and
- GHG Emissions and Consumptive Water Use (Table 7.20) for manufacturing 30% HCl in China and transporting it to the manufacturing process.
- Distribution plots for the key items in the LPC manufacturing process lifecycle inventory and the impacts arising from them are presented in Figure 7.16 to Figure 7.23. These plots demonstrate that all the simulated variables and impacts in the manufacturing follow a linear distribution.

Table 7.17: Summary of the Lifecycle Inventory for the LPC manufacturing process.

Lifecycle Inventory Item	Mean	Median	Max	Min	Standard Deviation
Electricity Use (kWh-electrical/MT LPC)	830	832	1224	436	229
Natural Gas Use (GJ-thermal/MT LPC)	18.9	18.9	28.0	9.97	5.22
Process Water Use (m^3/MT LPC)	6.42	6.39	9.51	3.39	1.76
Process Effluent Sent to Wastewater Treatment Plant (m^3/MT-LPC)	13.79	13.75	20.44	7.28	3.78
Process Water Supply and Treatment Energy Use (kWh-electrical/MT-LPC)	0.1989	0.1982	0.2947	0.1050	0.0544
Wastewater Treatment Plant Energy Use (kWh-electrical/MT-LPC)	12.45	12.41	18.46	6.57	3.42
Billable Energy Use* (kWh/MT-LPC)	6100	6081	8984	3231	1469
30% HCl (MT-30%-HCl/MT-LPC)	1.079	1.078	1.586	0.566	0.296

***Billable Energy is the sum of metered electric power and natural gas use.**

Table 7.18: Summary of LPC manufacturing process GHG emissions.

LPC Manufacturing Process Impact	Mean	Median	Max	Min	Standard Deviation
Grid Electricity Generation (kg CO_2-e/MT-LPC)	83.9	84.0	123.7	44.0	23.2
Grid Electricity Transmission (kg CO_2-e/MT-LPC)	7.2	7.2	10.7	3.8	2.0
Natural Gas Well to Chimney (kg CO_2-e/MT-LPC)	1025	1023	1516	540	282
Process Water Supply and Treatment GHG (kg CO_2-e/MT-LPC)	0.199	0.198	0.295	0.105	0.054
Wastewater Treatment (kg CO2-e/MT-LPC)	6.302	6.282	9.343	3.327	1.729
Total Manufacturing Process (kg CO_2-e/MT-LPC)	1123	1120	1651	597	284

Table 7.19: Summary of LPC manufacturing process Consumptive Water Use.

Water Footprint Component	Mean	Median	Max	Min	Standard Deviation	WSI Catchment (Factor)	AWARE Catchment (Factor)
Process Water (m^3/MT LPC)	6.42	6.39	9.51	3.39	1.76	Waikato (0.010459)	Waikato (0.4)
Natural Gas (m^3-WTC/ MT-LPC)	0.189	0.189	0.280	0.100	0.052	Taranaki (0.010409)	Waikato (0.4)
Manufacturing Grid Electricity (m^3/MT-LPC)	1.337	1.340	1.999	0.690	0.370	Waikato (0.010459)	New Zealand (1.689)
Process Water Supply and Treatment Grid Electricity (m^3/MT-LPC)	0.156	0.156	0.234	0.0811	0.0428	Waikato (0.010459)	New Zealand (1.689)
Wastewater Treatment Grid Electricity (m^3/MT-LPC)	20.0	20.0	30.2	10.43	5.51	Waikato (0.010459)	New Zealand (1.689)

WTC = Well to Chimney (i.e. full lifecycle impact)

Table 7.20: Summary of 30% HCl manufacturing and transportation carbon footprint.

Carbon Footprint Component	Mean	Median	Max	Min	Standard Deviation
Manufacturing GHG Emissions (kg CO_2-e/ MT-LPC)	292	291	431	154	80
Freight GHG Emissions (kg CO_2-e/ MT-LPC)	152	151	223	79	41
Total Carbon Footprint (kg CO_2-e/ MT-LPC)	**443**	**444**	**653**	**236**	**91**

Table 7.21: Summary of 30% HCl manufacturing and transportation water footprint.

Water Footprint Component	Mean	Median	Max	Min	Standard Deviation	WSI Catchment (Factor)	AWARE Catchment (Factor)
Water Content (m^3/MT-LPC)	0.65	0.65	0.97	0.34	0.18	Qingdao (0.919387)	Qingdao (81.1)
Process Water (m^3/MT-LPC)	8.99	8.98	13.30	4.74	2.48	Qingdao (0.919387)	Qingdao (81.1)
Cooling Water (m^3/MT-LPC)	9.22	9.21	13.64	4.86	2.54	Qingdao (0.919387)	Qingdao (81.1)
Bunker Fuel for Sea Freight (WTW_1) (m^3/MT-LPC)	0.420	0.419	0.621	0.221	0.116	Qingdao (0.919387)	China (27.081)
Diesel Fuel Road Freight (WTW_2) (m^3/MT-LPC)	0.172	0.172	0.254	0.0906	0.0474	Marsden Point (0.010459)	Marsden Point (0.4)

WTW_1 = Well to Wake | WTW_2 = Well to Wheel

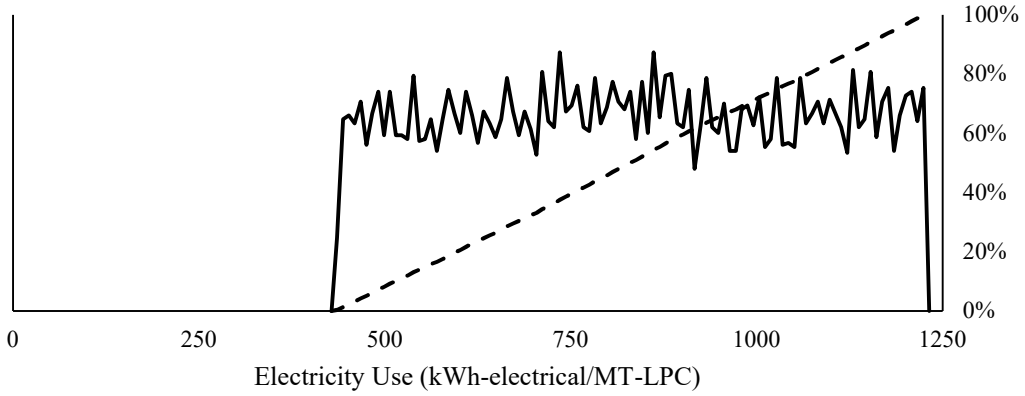

Figure 7.16: Electricity use of the process plant at the LPC manufacturing facility.

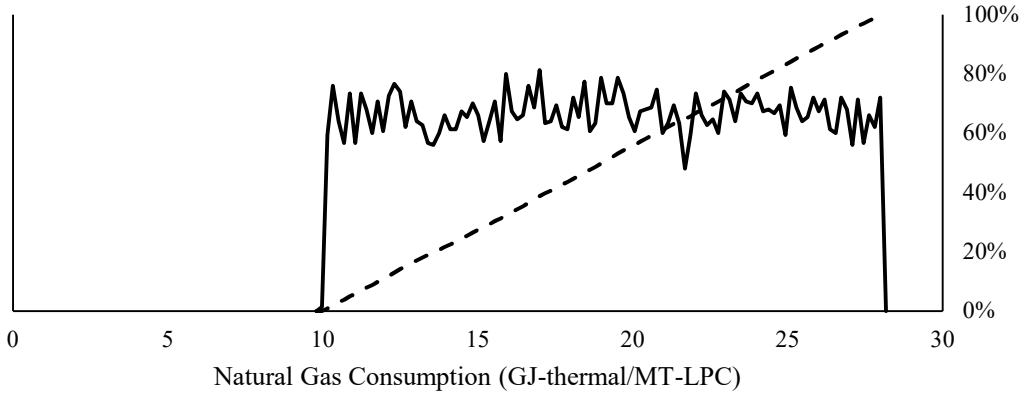

Figure 7.17: Natural gas use of the process plant at the LPC manufacturing facility.

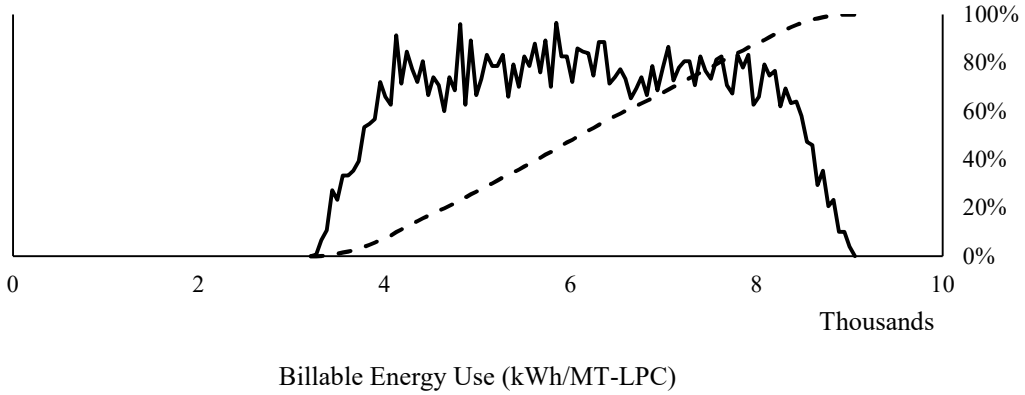

Figure 7.18: Billable energy use of the LPC manufacturing facility.

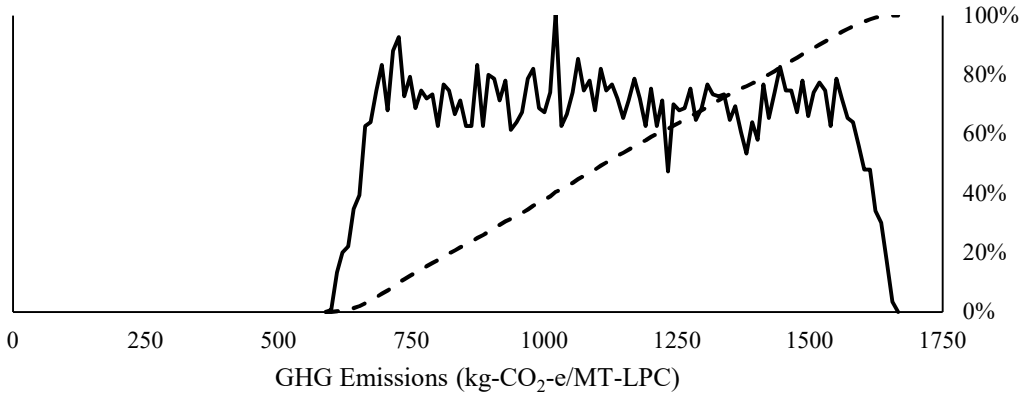

Figure 7.19: GHG emissions from the LPC manufacturing facility.

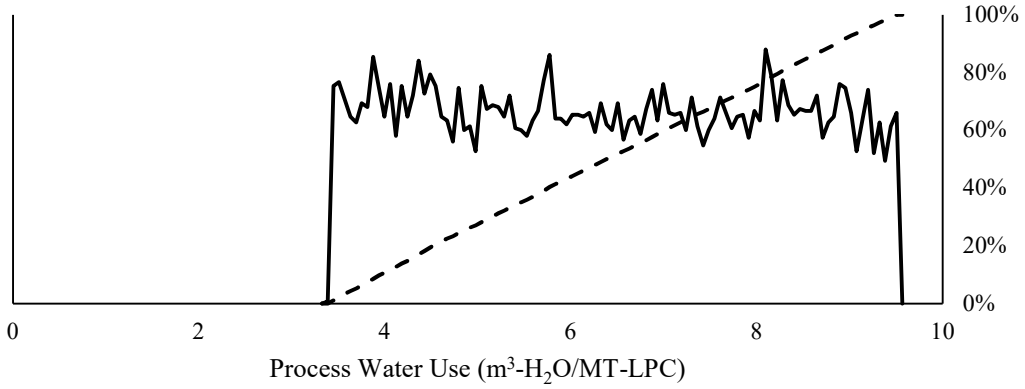

Figure 7.20: Process water use in LPC manufacture.

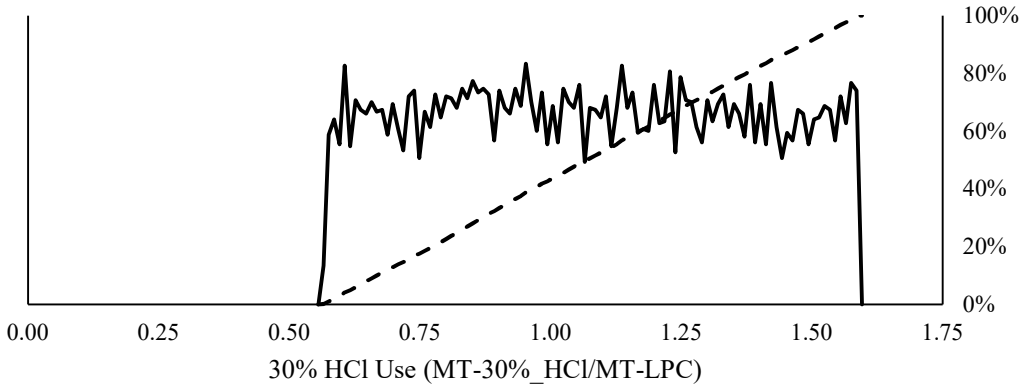

Figure 7.21: 30% HCl used in LPC manufacture.

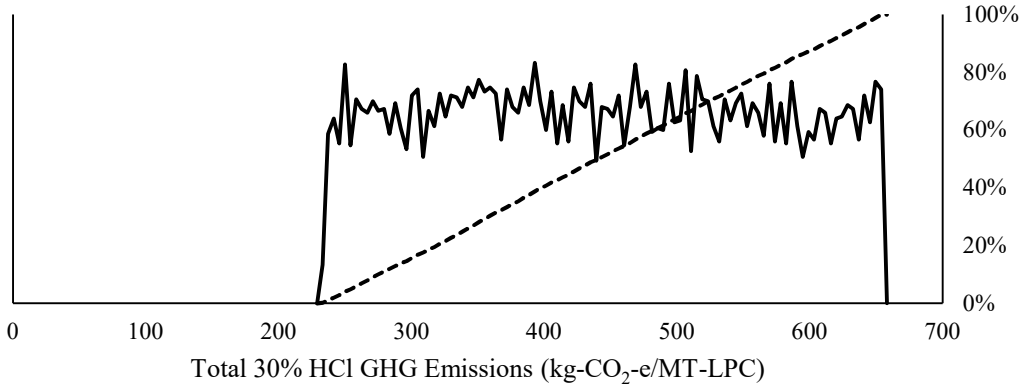

Figure 7.22: GHG Emissions from manufacture and transportation of 30% HCl to the LPC manufacturing facility.

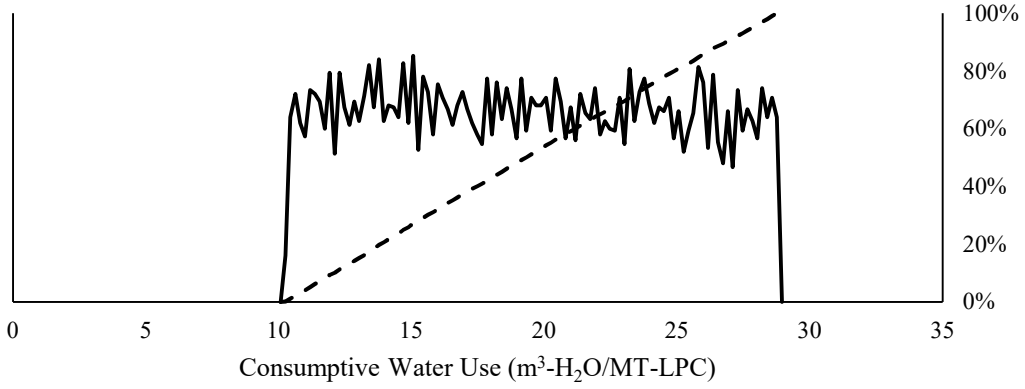

Figure 7.23: Consumptive Water Use from manufacture and transportation of 30% HCl to the LPC manufacturing facility.

7.4 Interpretation and Discussion

The greenhouse gas emission and consumptive water use impacts of leaf protein concentrate manufactured from paunch grass and garden waste compare favourably with those of competing animal feed protein products as well as compost, the product which results from current PG and GW disposal practices at Wallace Corporation and Red Lid Bins. This demonstrates that producing LPC from PG and GW is attractive from both an economic and sustainability perspective and worthy of further investigation.

7.4.1 Comparison with Animal Feed Products

The global warming potential (GWP) of LPC made from PG and GW is greater than those of crop-based animal feeds but less than those of alternative protein sources and milk powder (Table 7.22). This suggests that the baseline carbon-intensive process for manufacturing LPC from PG and GW is already an acceptable alternative for supplementary protein source from a climate change perspective. As per Table 7.18, the GHG emissions from the manufacturing process are dominated by emissions from the supply and combustion of natural gas (~91% of manufacturing emissions) to generate heat for steam generation and drying. In turn, the supply and combustion of natural gas accounts for approximately 48% of the LPC product GHG emissions (Table 7.13 and Table 7.18). This impact could be partially or fully mitigated by drying the fibre co-product from the LPC manufacturing process and using it for heat recovery instead of its current end-use a composting aid or waste stream for disposal (Chapter 6). An alternative could be gasification and combustion in a combined cycle turbine as proposed for alfalfa by Brushwood *et al.* [37]. This could have a large impact on the both the product GHG emissions and the overall profitability of the manufacturing process. It is therefore an important area of future work.

The Blue Water Footprint (BWF) of the LPC is compared with those of crop-based animal feeds in Table 7.23. Unlike its GHG emissions impact, the BWF of the LPC is lower than the global averages for most animal feeds. This indicates that LPC from PG and GW is more sustainable than most existing animal feed products from a Blue Water perspective when considering the global average. However, it is important to take regional variation into account.

Table 7.22: Global Warming Potentials (GWPs) of common animal feed products.

Product	GWP (Wet Basis) kg CO_2-e / MT	GWP (CP Basis) kg CO_2-e / kg CP	GWP (DM Basis) kg CO_2-e / kg DM	DM kg / MT Product	CP kg / MT Product	Source
Corn silage	47	25.00	0.200	235	2	[38; 39]
Alfalfa silage	55	0.94	0.180	308	59	[38; 39]
Winter Wheat - USA	127	3.91	0.430	295	32	[38; 39]
Alfalfa hay	152	0.93	0.170	894	163	[38; 39]
Poultry meat and bone meal - Europe	326	0.707	0.341	957	461	[40]
Poultry meat meal - Europe	326	0.562	0.343	950	580	[40]
Corn grain	337	4.43	0.390	863	76	[38; 41]
Soybeans - USA	349	0.97	0.390	895	360	[38; 39]
Soybean Meal - USA	429	0.98	0.460	932	438	[38; 41]
Soybean Meal - Netherlands	500	1.08	0.573	873	464	[40]
Defatted algae - low GWP	544	1.18	0.618	880	460	[40]
Soybean Meal - Ukraine	600	1.29	0.687	873	464	[40]
Distiller's dry grains with solubles from maize, wet mill - USA	600	2.82	0.670	896	213	[38; 41]
Soybean Meal - South America	622	1.34	0.712	873	464	[40]
Oats - USA	745	7.92	0.850	876	94	[38; 41]
Distiller's dry grains with solubles from maize, dry mill - USA	815	3.83	0.910	896	213	[38; 41]
High Protein Sunflower Seed Meal	860	1.87	0.977	880	460	[40]
Distiller's dry grains from maize - Europe	895	3.43	0.993	901	261	[40]
Defatted algae, high GWP	975	2.12	1.11	880	460	[40]
Bacterial single cell protein, low emissions	1850	2.96	2.10	880	625	[40]
LPC from PG & GW - NZ	**2093**	**6.64**	**2.33**	**900**	**315**	**§7.3**
Mealworms	3260	7.56	3.70	880	431	[40]
Bacterial single cell protein, high emissions	5001	8.00	5.68	880	625	[40]
Milk powder, confinement system - Europe	7716	13.0	8.00	965	595	[42; 43]
Milk powder, grass-based system - Europe	8937	15.0	9.26	965	595	[42; 43]

An example is given in Table 7.24, which compares the BWF of LPC with wheat produced in different countries around the world: the LPC has a larger BWF than wheat from countries that require low levels of irrigation, and a smaller BWF than wheat from countries that require high levels of irrigation. As high-irrigation countries have a larger share of global production, the LPC BWF is less than the global average BWF for wheat [44].

Similarly, Table 7.25 demonstrates the value of weighting water footprints to account for the relative abundance of water resources in different geographical locations as advocated by Pfister*, et al.* [35] and Boulay*, et al.* [13].

Table 7.23: Blue Water Footprints of LPC and animal feed products.

Product	**Blue Water Footprint, WF_{prod} (m^3-H_2O/MT)**
Cassava	0
Sweet potatoes	5
Sugar beet	26
Fodder crop average	27
Potatoes	33
Leaf protein concentrate from PG & GW	**39.3**
Millet	57
Soybean oilcake	58
Sunflower seed oilcake	60
Soy beans	70
Maize groats and meal	72
Oat groats and meal	72
Barley	79
Maize	81
Soybean flour and meals	83
Sorghum	103
Rape seed oilcake	114
Buckwheat	144
Potato flour and meal	165
Oats	181
Cotton-seed oilcake	279
Rice, paddy	341
Wheat	343
Milk powder	398
Rice, groats and meal	454
Rice, flour	535

Sources: Mekonnen and Hoekstra [45] for crop-based feed; and Mekonnen and Hoekstra [46] for milk powder.

Here it can be seen that consumptive water use associated with LPC production in New Zealand has a greater impact on Blue Water resources than producing skim milk powder, as Blue Water is relatively abundant and only a small quantity of it is required for dairy factory processing; the dairy cows are fed with pasture which is produced entirely from Green Water [47]. Conversely, NZ LPC has a much smaller impact on Blue Water resources than skim milk powder produced in Heilongjiang, China, or California, USA. This is because Blue Water is relatively scarce in these locations, and is required for dairy feed production as well as dairy factory processing [47].

Table 7.24: Blue Water Footprints of LPC and wheat from selected countries.

Product	Blue Water Footprint, WF_{prod} (m^3-H_2O/MT)
Wheat - Germany	0
Wheat - France	1
Wheat - Canada	5
Wheat - Denmark	6
Wheat - Australia	18
Wheat - Ukraine	21
Wheat - Russia	31
Leaf protein concentrate from PG & GW – New Zealand	**39.3**
Wheat - USA	92
Wheat - Uzbekistan	101
Wheat - World	**343**
Wheat - China	466
Wheat - India	1173
Wheat - Pakistan	1478

Source: Mekonnen and Hoekstra [44]

Table 7.25: WSI-Weighted Water Consumption Footprints of LPC and skim milk powders produced in different countries.

Product	WSI-Weighted WCF (m^3-H_2O/MT-LPC)
Skim milk powder - New Zealand	0.6
Leaf protein concentrate from PG & GW – New Zealand	**10.0**
Skim milk powder - Heilongjiang, China	75.9
Skim milk powder - California, USA	3221.8

Source: Huang, et al. [47]

The importance of geography in determining globally-weighted water impacts is further demonstrated in Table 7.25 where the LPC Water Stress Index-Weighted Water Consumption Factor is dominated by water use in Qingdao, China, a very

water-stressed region [14; 36]. This water use arises solely from the manufacture and shipment of hydrochloric acid for use in the LPC manufacturing process. Finding a New Zealand manufacturer of hydrochloric acid or sourcing it from a less water-stressed region is therefore the best opportunity to reduce the WSI-weighted and AWARE-weighted WCFs of the LPC. To the best of the author's knowledge at the time of writing, all other studies utilising the WSI and AWARE methods in New Zealand focus on fresh milk rather than animal feed products and cannot be compared with this study [34; 48]. Nevertheless, the weighted WCFs in Table 7.26 will be a useful reference once comparable WCFs for NZ-produced animal feeds become available.

Table 7.26: Geographical components of the LPC product WSI-Weighted WCF.

WSI-Weighted WCF Component (m^3-H_2O/MT-LPC)	Mean	Median	Max	Min	Standard Deviation
Waikato, NZ	0.333	0.297	1.36	0.129	0.140
Taranaki, NZ	0.00108	0.00109	0.00160	0.000569	0.000297
Qingdao, China	9.7	9.8	14.4	5.13	2.67
Total	10.1	10.1	15.2	5.30	2.68

Substituting LPC for animal feed crops is likely to have a positive impact on the Consumptive Water Consumption Footprint (WCF) and the Total Water Footprint of animal feeds. This is because its Blue Water Footprint is relatively small (Table 7.13) and unlike animal feed crops it does not have a Green Water Footprint. This study assumed that the Grey Water Footprint of the LPC was zero; and in practice it should be small due to process effluents passing through wastewater treatment processes before it is discharged to the environment. Constraints on water resources are one of the main factors limiting further agricultural production [49; 50; 51; 52; 53]. Even though the LPC has a high Global Warming Potential, its low Total Water Footprint may make it an attractive substitute for animal feed crops and their derivatives in water-stressed regions. This is because sustainability is ultimately a nexus of carbon, energy, and water; climate change mitigations and adaptations must therefore carefully consider water scarcity to avoid adverse environmental outcomes [54; 55].

Overall, LPC produced from PG and GW in New Zealand has a smaller impact on Blue Water resources than most other animal feed products when compared to the

global average BWFs for these products. However, regional differences are significant and could alter the relative sustainability of the LPC BWF compared to other crops. There is a dearth of globally weighted WCF data for New Zealand animal feed products, making it difficult to ascertain the sustainability of consumptive water use in LPC production relative to production of other animal feed products within New Zealand. Similarly, the globally weighted WCF of LPC from NZ should be considered when it is evaluated against animal feed products produced in other countries. These are both important areas that should be investigated during future work.

7.4.2 Comparison with Composting

In addition to supplying a relatively sustainable source of animal feed protein, producing LPC from PG and GW is more sustainable than composting them in an open-windrow system at a *prima facie* level. Drawing comparisons with compost impacts from the literature was complex, as these studies typically did not use finished compost as their functional unit [56; 57; 58]. Composting impacts from the literature and the LPC impacts from this study were therefore standardised to functional unit of one metric tonne of wet waste for comparison in Table 7.27. Compost impacts from Lundie and Peters [58] were also adjusted to remove the impacts arising from the production of capital equipment and facilities construction, as these were outside the system boundary for all other studies in the comparison. Lundie and Peters [58] was also the only study in the literature which presented a Blue Water Footprint.

Table 7.27 indicates that producing LPC from PG and GW has an impact on GHG emissions in a similar order of magnitude to composting GW and food waste (FW) in open windrows. To the contrary, the BWF of LPC is an order of magnitude smaller than that of composting. It is also important to note that transportation of wet waste to the composting site is either excluded from these studies in this comparison [56], or occurs across relatively short distances [57; 58]. This is consistent with the urban organic waste collection and disposal systems that were analysed. These studies also did not allocate the full impact of the composting process to the functional unit of wet waste; impacts were allocated to other inputs such as carbon sources and bulking agents. Both factors minimise impacts of the

composting a functional unit of wet waste; the results from using the wet waste functional unit are therefore inappropriate for deriving the impacts of finished compost products.

Only one study by Saer *et al.* [59] used finished compost as the functional unit; this was also the only study of a composting facility which processed organic material transported over a long distance (~100 km). Hence the LPC and compost products are compared on a product basis in Table 7.28, which clearly shows that LPC has a much smaller GHG emission impact than compost. LPC produced from PG and GW hence has a GHG emissions impact similar to that of open windrow compost on a wet waste basis, and significantly less on a product basis. The BWF of LPC is significantly smaller than that of open windrow compost on a wet waste basis, but this finding is based on a single study in the literature [58]. To the best of the author's knowledge, no product BWF has ever been calculated for compost.

Table 7.27: LPC production from PG and GW and composting impacts on a wet waste basis.

Product	Global Warming Potential kg CO_2-e/MT-WW	Includes Wet Waste Transport Emissions	Blue Water Footprint m^3/MT-WW	Source
Compost from GW, Low Emissions - Aarhus, Denmark	66	No	-	[56]
Compost from GW and FW - Brisbane, Australia	67	Yes	-	[57]
Leaf Protein Concentrate from PG & GW, Low Emissions	76	Yes	1.39	§7.3
Compost from GW, Typical Emissions - Aarhus, Denmark	89	No	-	[56]
Compost from GW, High Emissions - Aarhus, Denmark	108	No	-	[56]
Compost from FW - South Korea	123	Yes	-	[47]
Leaf Protein Concentrate from PG & GW, Median Emissions	148	Yes	2.76	§7.3
Compost from GW and FW - Sydney, Australia	369	Yes	144	[58]
Leaf Protein Concentrate from PG & GW, High Emissions	428	Yes	9.76	§7.3

WW = Wet Waste | FW = Food Waste

One of the key benefits of composting organic wastes is that it results carbon sequestration over the medium-term when the compost product is used as a soil conditioner as demonstrated by numerous consequential studies performed on a wet waste basis [60; 61; 62; 63; 64]. On a product basis typically used in attributional studies, open windrow composting has a much higher GHG emissions impact than LPC from PG and GW; however the compost product still results in net negative GHG emissions over the medium-term when used as a soil conditioner [59; 65; 66]. This is usually due to the compost replacing peat or other soils which release GHGs during their extraction and use [59]. The amount of carbon sequestered over the medium-term is usually greater than the GWP of compost production, resulting in net negative GHG emissions. However over the long-term, most of the carbon sequestered in the soil is returned to the atmosphere [65; 66; 67; 68]. In this case, sequestered carbon does not offset the carbon emissions from compost production and the LPC becomes the lower-emitting product as per Table 7.28.

Table 7.28: Global Warming Potential of LPC and compost on a product basis.

Product	Global Warming Potential (kg CO_2-e/MT-product)	Includes Raw Material Transport Emissions	Source
Leaf Protein Concentrate from PG & GW, Low Emissions	1078	Yes	§7.3
Leaf Protein Concentrate from PG & GW, Median Emissions	2104	Yes	§7.3
Leaf Protein Concentrate from PG & GW, High Emissions	6100	Yes	§7.3
Compost from GW and FW, Low Emissions - Pennsylvania, USA	61900	Yes	[59]
Compost from GW and FW, Typical Emissions - Pennsylvania, USA	221000	Yes	[59]
Compost from GW and FW, High Emissions - Pennsylvania, USA	970000	Yes	[59]

FW = Food Waste

As discussed in Section 7.4.1, the large amounts of natural gas combusted to generate heat for steam generation and drying give the LPC product a GHG emissions impact that larger than most animal feed products, making the offsetting of GHG emissions by substitution impossible. Using the fibre co-product for heat recovery should reduce emissions and allow offsetting when LPC is substituted for some high-GWP animal feed products. Using the fibre for heat recovery will

therefore make the GHG emissions impact of LPC from PG and GW more attractive relative to open windrow composting from both attributional and consequential perspectives.

Most studies of open windrow composting to date are consequential analyses with a wet waste functional unit. Only one study by Saer*, et al.* [59] has performed an attributional analysis with a finished compost functional unit. Lundie and Peters [58] is also the only study to date which has considered consumptive water use. This makes it difficult to know the complete relative impacts of LPC manufacturing and open windrow composting of PG and GW. All literature data for the impacts of windrow composting was also collected from outside New Zealand and may or may not be applicable to NZ climate conditions and wet wastes. There are thus two gaps in the literature that should be filled during future work:

- Attributional analysis of open windrow composting accounting for consumptive water usage in New Zealand conditions and using a finished compost functional unit; and
- Consequential analysis of LPC production, open windrow composting, and other disposal options for PG and GW in New Zealand conditions, accounting for consumptive water usage, and using both wet waste and finished product functional units.

In summary, production of leaf protein concentrate from paunch grass and garden waste has lower GHG emissions impacts than composting when carbon sequestration is considered over the long-term. However, the GHG emissions from LPC production are too high to be offset by using LPC as a substitution for animal feed crops. Although the LPC has less Global Warming Potential than compost at the end of production process, it ultimately still increases GHG emissions by the end of its product lifecycle; conversely, compost will usually reduce GHG emissions and sequester carbon over the medium-term when it is used as a soil conditioner, but in the long-term this carbon is oxidised and returned to the atmosphere. This makes composting PG and GW more attractive from a climate change perspective in the medium-term but less attractive over the long-term.

The Blue Water Footprint of LPC appears to be orders of magnitude lower than that of compost, but this assessment is based on a comparison with a single study which

used a wet waste functional unit and did not capture the full impacts of the composting process. There is therefore insufficient data available to make a definitive assessment of the relative attractiveness of LPC production and composting from a Blue Water Footprint perspective.

7.5 Limitations and Future Investigations

There are several limitations to this study and its interpretation. The study limitations arose from the quality and quantity of the data used; the interpretation limitations arose from the quality and quantity of comparative data in the literature. Further work beyond the scope of this study is required to fill these gaps.

7.5.1 Data Quality

A major limitation of this study is that it was based on data from benchtop-scale experiments and literature review. Without scalable data from a pilot-plant, it was impossible to accurately estimate the water and energy consumption of the manufacturing process; similarly hydrochloric acid consumption may also vary with scale. As a result, the Monte Carlo simulations had to assume a linear distribution and simulation variables had equal probabilities of falling anywhere between the simulation limits. This creates an unnecessarily large uncertainty in the simulation, limits the accuracy of the results, and may result in overestimation of impacts which do not follow a linear distribution in practice.

One approach to resolve this issue is to assume all simulation variables are normally distributed. Although this reduces the uncertainty in the simulation, it still runs the risk of overestimating impacts, as simulation limits were calculated from assumed medians using a geometric standard deviation derived from the data pedigree matrix. This approach is therefore not recommended, as it will result in artificially high impacts with artificially low uncertainties.

The preferred approach to resolve this issue is to obtain data from similar processes, such as the fresh alfalfa leaf protein facility in France [69], and researchers working on larger-scale alfalfa protein extraction technologies [70; 71]. Pulp and paper mills employ similar technologies to leaf protein production facilities but handle a material which is much richer in lignin and more difficult to break down [72].

Process data could be obtained from process operator interviews and process control software databases (where available). Either approach will improve the Further Technological pedigree of the data used in the process model. At a minimum, this would enable an applicable distribution to be selected for the Monte Carlo simulations between the limits derived from the technoeconomic analysis in Chapter 6. More detailed data would then allow the simulation limits to be narrowed to reflect industrial-scale processing and distribution characteristics to be estimated. Both improvements would reduce the uncertainty and increase the accuracy of the Monte Carlo simulations and hence the impacts of the LPC product. This will be an important area for future investigations.

7.5.2 Data Gaps

The interpretation and discussion of the Lifecyle Impact Analysis in Section 7.4 identified several gaps in both this study and the literature. They limit both the accuracy of the existing attributional impact assessment as well as the potential to perform consequential assessments of PG and GW disposal and alternative LPC manufacturing process configurations. Hence there are several key topics for future work.

- Attributional and consequential analysis of drying the fibre co-product from the LPC manufacturing process and using it for heat recovery to minimise or eliminate natural gas use;
- Water Footprint Assessment of other animal feed products at a national or regional resolution and consequential analysis of using LPC as a substitute for those animal feed products;
- Attributional analysis of open windrow composting accounting for consumptive water usage in New Zealand conditions and using a finished compost functional unit; and
- Consequential analysis of LPC production, open windrow composting, and other disposal options for PG and GW in New Zealand conditions, accounting for consumptive water usage, and using both wet waste and finished product functional units.

The other key gap in the study data was wastewater characteristics of the process effluent. This arose from the lack of pilot plant data Not only does this create

uncertainties around the intensity and nature of the wastewater treatment process, it also makes it impossible to determine several of the impacts that would normally be included in an LCA as per the ISO 10400 standards [73; 74; 75; 76]: aquatic eco-toxicity potential, acidification potential, and eutrophication potential; these are also necessary to determine the grey water footprint. The lack of data combined with the presence of a wastewater treatment utility allowed zero grey water footprint to be assumed in this study, but in reality even treated wastewater from still has a small grey water footprint [15]. Although it is expected to be small, calculating the grey water footprint of the LPC will allow important comparisons to be drawn with other animal feed products, many of which have considerable grey water footprints [44; 45; 46; 77]. Similarly, all process outputs should also be assessed for their human toxicity potential as this will align with similar studies and enable comprehensive consequential assessments to be made.

7.6 Conclusion

Leaf protein concentrate produced from paunch grass and garden waste had a median Global Warming Potential of 2093.5 kg CO_2-equivalent per metric tonne; a Blue Water Footprint of 39.3 m^3 of H_2O per metric tonne; and a Water Stress Index-weighted Water Consumption Factor of 10.1 m^3 of H_2O per metric tonne. The GWP of LPC is high relative to animal feed crops and their derivative products, but low relative to alternative proteins such as mealworms and single cell proteins, and milk powder. It is also comparable to the GWP of compost production; however, it becomes significantly larger when the carbon sequestration benefits of compost soil-conditioning are considered. Natural gas supply and combustion accounts for approximately 48% of the LPC product GHG emissions so using the fibre product as a fuel for process heat could significantly reduce the LPC GWP and make it a more attractive animal feed from a climate change perspective. This is the first key area of future investigation.

The Lifecycle Impact Analysis also suffers from high uncertainties arising from linear distribution assumptions in the Monte Carlo simulations. This is due to a reliance on benchtop-scale experimental data in the process model used to build the Lifecycle Inventory. Improving the Further Technological pedigree of the data in the process model using pilot plant data or industrial plant data from similar

processes will allow a data distribution to be estimated for the Monte Carlo simulations, leading to increased accuracy and reduced uncertainty of LPC impacts. This is the second key area of future investigation.

The relatively high GWP of LPC from PG and GW is compensated for by its relatively low Blue Water Footprint and Water Stress Index-weighted Water Consumption Factor. The former is below the global average for most animal feed products; the latter is below the global average for milk powders produced in water-stressed regions. LPC also does not have a Green Water Footprint. This makes LPC an attractive animal feed substitute in an increasingly water-scarce world. The sensibility of LPC substitution will vary with national and regional water stress levels. There is insufficient data in the literature to perform consequential analysis of LPC substitution at this resolution and filling this data gap is the third key area of future investigation.

Similarly, there is very limited data available on consumptive water use in compost production or its Grey Water Footprint. This study also did not determine the Grey Water Footprint of LPC. Determining the full Water Footprint of compost, the Grey Water Footprint of LPC, and performing product functional unit Water Footprint and Water Consumption Footprint comparisons is the fourth key area of future investigation.

7.7 References

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8 Conclusion

The chemical compositions of paunch grass (PG) and leafy green waste (GW) have been characterised in New Zealand for the first time at both annual and seasonal resolution. Their protein contents (15.2 to 16.2 DM%) are comparable to those of fresh leaf crops, making them suitable feedstocks for leaf protein extraction. The optimal method to extract leaf proteins into a protein rich liquor from PG and GW feedstock is double screw-pressing with intermediary maceration; the most effective recovery method acidifies the liquor with hydrochloric acid then coagulates the protein by steam injection. This was proven to be more effective than the LPC manufacturing process currently used in the animal feed industry. Technoeconomic analysis confirmed that industrial-scale manufacture of LPC from PG and GW is technologically and financially viable when: it is co-sited with a meat processing facility or rendering plant; and the fibre fraction is treated as a valuable co-product instead of a waste stream. The resulting LPC has a crude protein content and essential amino acid profile that is comparable to other plant protein products used for animal feed and has sufficient thermal stability to be used in animal feed manufacture. The environmental impacts (2094 kg CO_2-e per MT and 39.3 m^3-H_2O per MT) are comparable to other animal feed ingredients.

8.1 Feedstock Characterisation

PG and GW are scientifically feasible feedstocks for leaf protein extraction. On a dry basis, they contain less protein than fresh leaf crops, but also have a higher dry matter content (Table 8.1).

Table 8.1: Annual mean levels of PG and GW biochemical parameters.

Parameter	PG Annual Mean	GW Annual Mean
Moisture (w/w%)	81.1 %	64.7 %
Crude protein (DM %)	15.2 %	16.2 %
Crude lipids (DM %)	10.4 %	11.9 %
Total carbohydrates (DM %)	20.4 %	17.1 %
Crude fibre (DM %)	23.1 %	15.6 %
Ash (DM %)	13.1 %	16.1 %
C:N ratio (DM %)	19.0	17.2

This study has also confirmed that leafy green waste and paunch grass should not be composted without a supplementary carbon source and bulking agent due to their

low carbon to nitrogen ratios and high moisture contents. The variations in the annual mean chemical compositions of paunch grass and leafy green waste are statistically significant; there are also statistically significant seasonal variations within the chemical compositions for each material.

8.2 Optimisation of Leaf Protein Concentrate Extraction and Recovery

The most effective method to extract leaf proteins from paunch grass and leafy green wastes is to macerate the feedstocks, pass them through a screw press, then wet and macerate the resulting press fibre before passing it through another screw press. Optimal protein recovery (45.9 to 62.0 mass percent) from the press liquor is achieved when the liquor is treated with hydrochloric acid minimise its Zeta potential prior to coagulation. Steam should then be injected into the acidified press liquor to heat it to 85°C. The resulting slurry should be kept at 85°C for 30 seconds before it is centrifuged to separate the LPC from the waste process liquor.

The resulting LPCs have crude protein contents of 33 to 38 percent of dry matter, have similar essential amino acid profiles to those published for other LPCs and fully meet the EAA levels specified in the most recent WHO EAA profile. They are slightly deficient in arginine, leucine, lysine, and valine when compared with the NRC EAA profiles used for poultry feed. These deficiencies can be overcome by blending with soy, canola, alfalfa, and dairy proteins; or blending with hydrolysed feather meal. PG-LPC and GW-LPC can also be used to compensate for isoleucine, lysine, phenylalanine, methionine, cysteine, threonine, tyrosine, and valine deficiencies in other animal feed proteins. However, heavy metal contamination could limit the amount of LPC that can be included in animal feed formulations.

8.3 Technoeconomic Analysis

Manufacturing LPC from paunch grass and leafy green wastes is technologically and financially viable because it is a waste valorisation process. The fees collected for accepting the negative-value feedstock for disposal largely offset the cost of manufacture and keep the process profitable even when LPC is the sole product. This in turn allows a simple and relatively inexpensive process plant to be

established using mature technologies by eliminating the costs and risks associated with coproduction of biofuels or nutraceuticals using experimental or immature biotechnologies.

However, the battery limits plant design was only viable under coproduct valorisation scenarios. The baseline design-operation configuration is a battery limits installation adjacent to a facility processing meat or abattoir wastes, as it is straightforward to implement and requires minimal integration or co-location with other processes. This facility would process all paunch grass generated in the Upper North Island of New Zealand and all municipal leafy green waste generated in the Greater Waikato region within this area to coproduce 3,385 and 22,273 tonnes per annum of LPC and wet press fibre, respectively During its 15-year lifetime, this facility generates a net present value of $2,091,597, an internal rate of return of 3.880 percent, and achieves simple payback within 12 years. The values are not attractive enough to justify borrowing capital to build the processing plant.

The most attractive design is a process plant integrated with an existing rendering facility and co-located with a composting process. This facility would also process all paunch grass generated in the Upper North Island of New Zealand and all municipal leafy green waste generated in the Greater Waikato region within this area to coproduce 3,385 and 22,273 tonnes per annum of LPC and wet press fibre, respectively. During its 15-year lifetime, this facility generates a net present value of $128,767,248, an internal rate of return of 59.903 percent, and achieves simple payback within 2 years for a direct investment. It is also viable to build this the processing plant with capital borrowed at 4.59% interest per annum.

8.4 Lifecycle Assessment

The Global Warming Potential of LPC from PG and GW (2094 kg CO_2-e / MT-LPC) is high relative to animal feed crops and their derivative products, but low relative to alternative proteins such as mealworms and single cell proteins, and milk powder. It is also comparable to the GWP of compost production; however, it becomes significantly larger when the carbon sequestration benefits of compost soil-conditioning are considered. However, the high GWP of the LPC is compensated for by its relatively low Blue Water Footprint (39.3 m^3-H_2O/MT-LPC)

which is below the global average for most animal feed products. LPC also does not have a Green Water Footprint. This makes LPC an attractive animal feed substitute in an increasingly water-scarce world.

8.5 Future Work

There are several important areas for future work. There are several aspects to the fibre co-product that should be investigated further. Its low moisture content (ca. 45%), high carbon to nitrogen ratio (40-45 for mixed PG and GW fibres), mechanical pre-treatment, and acceptable levels of heavy metals could make it a potent bulking agent for compost systems or anaerobic digestion feedstock. In the latter case, the biogas from the anaerobic digester could be used to provide heat and power for the leaf protein concentrate manufacturing process. Another use would be heat recovery by combustion in a solid fuel boiler. As the use of natural gas in the manufacturing process accounts for approximately 48% of the LPC product GHG emissions, this could significantly reduce the GWP of the LPC and bring it into a similar range to other plant-based animal feed proteins.

Another important area of future work focuses on lifecycle assessment and environmental impacts. The first step be determining the Grey Water Footprint, aquatic eco-toxicity potential, acidification potential, and eutrophication potential of the LPC. The full lifecycle impacts of competing animal feed proteins and compost products should then be determined by attributional analysis. This will then inform consequential determinations of net environmental impacts of LPC use in animal feeds and its sensibility at national and regional resolutions.

Finally, the processability of the LPC is presently unknown. Thermal analysis suggests that it should be dried at 70°C, and that it can be extruded into pellets without the incorporation of a plasticising additive. Further knowledge of LPC processability will be required before this research can be commercialised.

Appendix A

Statistical analyses of feedstock characterisation data (see supporting files)

WGPM AllVariables Summary.xlsx

WGPM AllVariables Stat Analysis.xlsx

Appendix B

High resolution process flow diagram (see supporting files)

PFD_LGW-PER-process_05-APR-2020_stream-labels.pdf

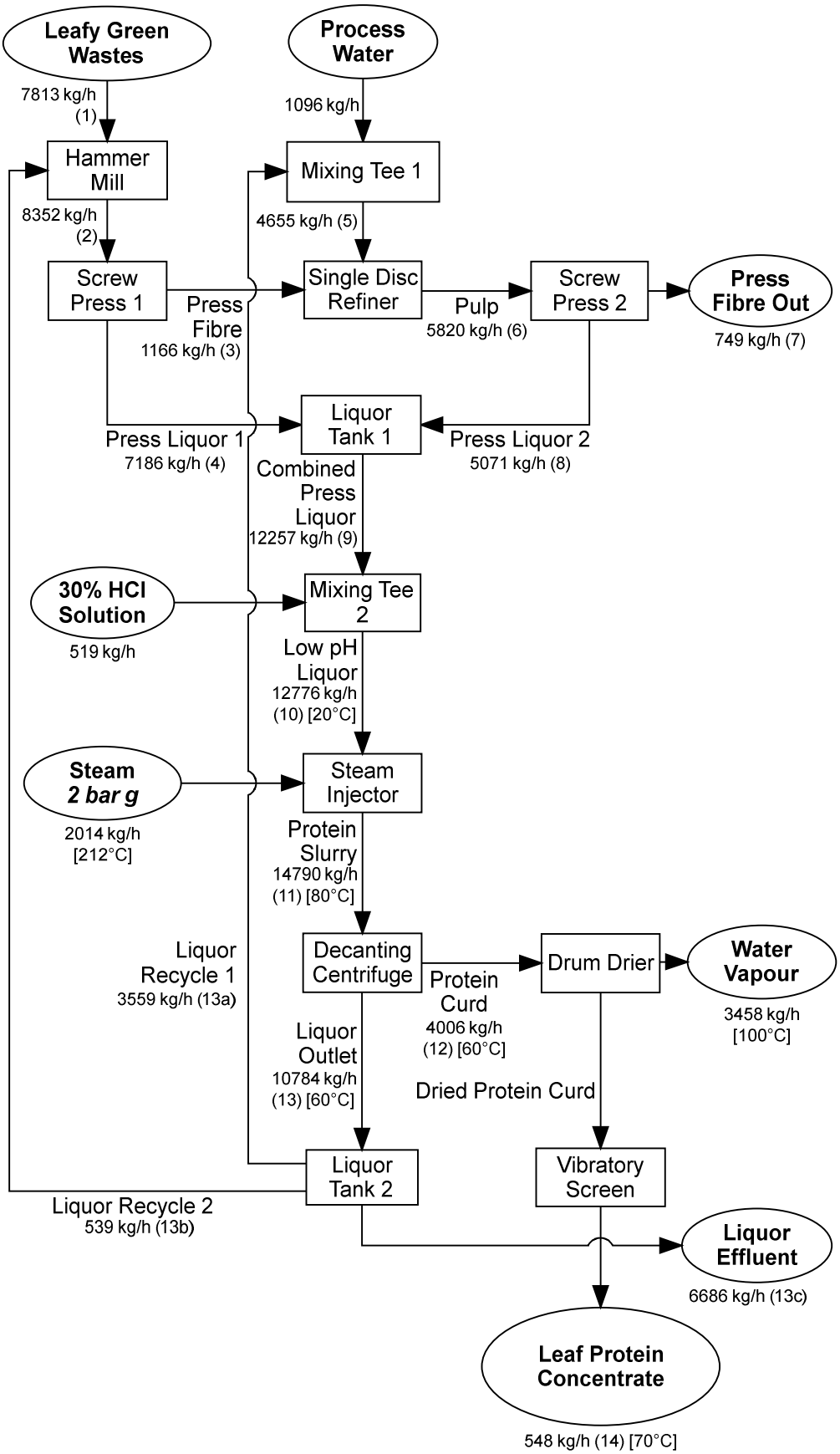
Appendix C

Excel spreadsheet of mass balance, technoeconomic analysis, and lifecycle assessment (see supporting files).

LP recovery process model 10-2022 (RecycleDilution).xlsx

Appendix D

Process flow diagram and component-level stream tables.



Stream 1: Leafy Green Wastes Entering Hammer Mill

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (MT/day)	125			
Total Stream (kg/h)	7813	100.00%		
Water	6132	78.48%		93.34%
Soluble Components	6569	84.09%		100.00%
Solids	1681	21.52%	100.00%	
Protein bound to plant material	274	3.50%	16.29%	
Protein in solution	0	0.00%	0.00%	0.00%
Lipids	168	2.15%	10.00%	
Sugars	181	2.32%	10.77%	2.75%
Fibre	129	1.65%	7.68%	
Ash	257	3.29%	15.27%	3.91%
Undefined	672	8.61%	40.00%	
CHECKSUM	*7813*	*100.00%*	*100.00%*	*100.00%*

Stream 2: Pulp Exiting Hammer Mill

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	8352	100.00%		
Water	6588	78.88%		92.73%
Soluble Components	7104	85.06%		
Solids	1764	21.12%	100.00%	
Protein bound to plant material	236	2.83%	13.38%	
Protein in solution	42	0.50%	2.36%	0.59%
Lipids	175	2.10%	9.94%	
Sugars	190	2.27%	10.75%	2.67%
Fibre	129	1.55%	7.32%	
Ash	285	3.41%	16.16%	4.01%
Undefined	707	8.47%	40.09%	
CHECKSUM	*8352*	*100.00%*	*100.00%*	*100.00%*

Stream 3: Fibre Exiting Screw Press 1 / Entering Disc Refiner

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	1166	100.00%		
Water	677	58.08%		90.74%
Soluble Components	746	64.00%		100.00%
Solids	489	41.92%	100.00%	
Protein bound to plant material	80	6.90%	16.46%	
Protein in solution	20	1.74%	4.15%	2.72%
Lipids	34	2.93%	7.00%	
Sugars	19	1.67%	3.99%	2.61%
Fibre	129	11.07%	26.41%	
Ash	29	2.51%	5.99%	3.93%
Undefined	176	15.09%	36.00%	
CHECKSUM	*1166*	*100.00%*	*100.00%*	*100.00%*

Stream 4: Liquor Exiting Screw Press 1

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	7186	100.00%		
Water	5911	82.26%		90.74%
Soluble Components	6514	90.64%		100.00%
Solids	1275	17.74%	100.00%	
Protein bound to plant material	0	0.00%	0.00%	
Protein in solution	177	2.46%	13.88%	2.72%
Lipids	141	1.96%	11.06%	
Sugars	170	2.37%	13.34%	2.61%
Fibre	0	0.00%	0.00%	
Ash	256	3.56%	20.05%	3.93%
Undefined	531	7.39%	41.66%	
CHECKSUM	*7186*	*100.00%*	*100.00%*	*100.00%*

Stream 5: Liquor Entering Disc Refiner

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	4654	100.00%		
Water	4108	88.26%		93.85%
Soluble Components	4377	94.04%		100.00%
Solids	547	11.74%	100.00%	
Protein bound to plant material	0	0.00%	0.00%	
Protein in solution	25	0.55%	4.66%	0.58%
Lipids	47	1.02%	8.67%	
Sugars	57	1.23%	10.48%	1.31%
Fibre	0	0.00%	0.00%	
Ash	186	4.00%	34.11%	4.26%
Undefined	230	4.94%	42.08%	
CHECKSUM	*4654*	*100.00%*	*100.00%*	*100.00%*

Stream 6: Pulp Exiting Disc Refiner / Entering Screw Press 2

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	5820	100.00%		
Water	4785	82.21%		92.72%
Soluble Components	5161	88.67%		
Solids	1035	17.79%	100.00%	
Protein bound to plant material	43	0.73%	4.12%	
Protein in solution	83	1.43%	8.07%	1.62%
Lipids	82	1.40%	7.88%	
Sugars	77	1.32%	7.42%	1.49%
Fibre	129	2.22%	12.47%	
Ash	216	3.71%	20.84%	4.18%
Undefined	406	6.97%	39.21%	
CHECKSUM	*5820*	*100.00%*	*100.00%*	*100.00%*

Stream 7: Press Fibre Exiting Process

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	749	100.00%		
Water	409	54.60%		92.20%
Soluble Components	443	59.22%		100.00%
Solids	340	45.40%	100.00%	
Protein bound to plant material	14	1.85%	4.08%	
Protein in solution	10	1.28%	2.82%	2.16%
Lipids	24	3.18%	7.00%	
Sugars	7	0.88%	1.93%	1.48%
Fibre	129	17.24%	37.96%	
Ash	18	2.46%	5.42%	4.16%
Undefined	139	18.51%	40.78%	
CHECKSUM	*749*	*100.00%*	*100.00%*	*100.00%*

Stream 8: Liquor Exiting Screw Press 2

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	5071	100.00%		
Water	4376	86.29%		92.20%
Soluble Components	4746	93.59%		100.00%
Solids	695	13.71%	100.00%	
Protein bound to plant material	0	0.00%	0.00%	
Protein in solution	103	2.02%	14.77%	2.16%
Lipids	58	1.14%	8.31%	
Sugars	70	1.38%	10.10%	1.48%
Fibre	0	0.00%	0.00%	
Ash	197	3.89%	28.38%	4.16%
Undefined	267	5.27%	38.44%	
CHECKSUM	*5071*	*100.00%*	*100.00%*	*100.00%*

Stream 9: Liquor Entering Mixing Tee (Acidification Unit)

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	12257	100.00%		
Water	10287	83.93%		91.36%
Soluble Components	11260	91.86%		100.00%
Solids	1970	16.07%	100.00%	
Protein bound to plant material	0	0.00%	0.00%	
Protein in solution	280	2.28%	14.19%	2.48%
Lipids	199	1.62%	10.09%	
Sugars	240	1.96%	12.20%	2.13%
Fibre	0	0.00%	0.00%	
Ash	453	3.70%	22.99%	4.02%
Undefined	799	6.51%	40.53%	
CHECKSUM	*12257*	*100.00%*	*100.00%*	*100.00%*

Stream 10: Low pH Liquor Entering Steam Injector

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	12776	100.00%		
Water	10627	83.18%		93.66%
Soluble Components	11346	88.80%		100.00%
Solids	2149	16.82%	100.00%	
Protein bound to plant material	0	0.00%	0.00%	
Protein in solution	280	2.19%	13.01%	2.46%
Lipids	199	1.56%	9.25%	1.75%
Sugars	240	1.88%	11.18%	2.12%
Fibre	0	0.00%	0.00%	
Ash	632	4.95%	29.40%	
Undefined	799	6.25%	37.15%	
CHECKSUM	*12776*	*100.00%*	*100.00%*	*100.00%*

Stream 11: Protein Slurry Exiting Steam Injector / Entering Decanting Centrifuge

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	14790	100.00%		
Water	12640	85.47%		95.86%
Soluble Components	13187	89.16%		100.00%
Solids	2149	14.53%	100.00%	
Protein bound to plant material	173	1.17%	8.04%	
Protein in solution	107	0.72%	4.97%	0.81%
Lipids	199	1.34%	9.25%	1.51%
Sugars	240	1.63%	11.18%	1.82%
Fibre	0	0.00%	0.00%	
Ash	632	4.27%	29.40%	
Undefined	799	5.40%	37.15%	
CHECKSUM	*14790*	*100.00%*	*100.00%*	*100.00%*

Stream 12: Wet Protein Curd Exiting Decanting Centrifuge / Entering Drum Dryer

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	4006	100.00%		
Water	3512	87.69%		95.86%
Soluble Components	3664	91.47%		100.00%
Solids	493	12.31%	100.00%	
Protein bound to plant material	173	4.31%	35.04%	
Protein in solution	30	0.74%	6.02%	0.81%
Lipids	55	1.38%	11.20%	1.51%
Sugars	67	1.67%	13.54%	1.82%
Fibre	0	0.00%	0.00%	
Ash	67	1.68%	13.61%	
Undefined	102	2.54%	20.59%	
CHECKSUM	*4006*	*100.00%*	*100.00%*	*100.00%*

Stream 13: Liquor Exiting Decanting Centrifuge

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	10784	100.00%		
Water	9128	84.64%		91.80%
Soluble Components	9944	92.21%		100.00%
Solids	1656	15.36%	100.00%	
Protein bound to plant material	0	0.00%	0.00%	
Protein in solution	77	0.72%	4.66%	0.78%
Lipids	144	1.33%	8.67%	
Sugars	174	1.61%	10.48%	1.75%
Fibre	0	0.00%	0.00%	
Ash	565	5.24%	34.11%	5.68%
Undefined	697	6.46%	42.08%	
CHECKSUM	*10784*	*100.00%*	*100.00%*	*100.00%*

Stream 13a: Liquor Recycled to Mixing Tee at Disc Refiner Inlet

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	3559	100.00%		
Water	3012	84.64%		91.80%
Soluble Components	3281	92.21%		100.00%
Solids	547	15.36%	100.00%	
Protein bound to plant material	0	0.00%	0.00%	
Protein in solution	25	0.72%	4.66%	0.78%
Lipids	47	1.33%	8.67%	
Sugars	57	1.61%	10.48%	1.75%
Fibre	0	0.00%	0.00%	
Ash	186	5.24%	34.11%	5.68%
Undefined	230	6.46%	42.08%	
CHECKSUM	*3559*	*100.00%*	*100.00%*	*100.00%*

Stream 13b: Liquor Recycled to Hammer Mill

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	539	100.00%		
Water	456	84.64%		91.80%
Soluble Components	497	92.21%		100.00%
Solids	83	15.36%	100.00%	
Protein bound to plant material	0	0.00%	0.00%	
Protein in solution	4	0.72%	4.66%	0.78%
Lipids	7	1.33%	8.67%	
Sugars	9	1.61%	10.48%	1.75%
Fibre	0	0.00%	0.00%	
Ash	28	5.24%	34.11%	5.68%
Undefined	35	6.46%	42.08%	
CHECKSUM	*539*	*100.00%*	*100.00%*	*100.00%*

Stream 13c: Liquor Effluent

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	6686	100.00%		
Water	5659	84.64%		91.80%
Soluble Components	6165	92.21%		100.00%
Solids	1027	15.36%	100.00%	
Protein bound to plant material	0	0.00%	0.00%	
Protein in solution	48	0.72%	4.66%	0.78%
Lipids	89	1.33%	8.67%	
Sugars	108	1.61%	10.48%	1.75%
Fibre	0	0.00%	0.00%	
Ash	350	5.24%	34.11%	5.68%
Undefined	432	6.46%	42.08%	
CHECKSUM	*6686*	*100.00%*	*100.00%*	*100.00%*

Stream 14: Dried Leaf Protein Concentrate Exiting Process

Component	Mass Flow (kg/h)	Wet Mass Fraction	Dry Mass Fraction	Soluble Mass Fraction
Total Stream (kg/h)	548	100.00%		
Water	55	10.00%		25.09%
Soluble Components	218	39.85%		100.00%
Solids	493	90.00%	100.00%	
Protein bound to plant material	173	31.54%	35.04%	
Protein in solution	30	5.42%	6.02%	13.59%
Lipids	55	10.08%	11.20%	
Sugars	67	12.19%	13.54%	30.58%
Fibre	0	0.00%	0.00%	
Ash	67	12.25%	13.61%	30.73%
Undefined	102	18.53%	20.59%	
CHECKSUM	*548*	*100.00%*	*100.00%*	*100.00%*