Energy partition and nitrogen utilization lambs fed Willow (*Salix alba*)

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Abstract

Willow (*Salix* spp.) has gained attention in recent years for its potential to enhance livestock nutrition and mitigate environmental impacts, particularly in the context of reducing methane (CH_4) emissions from ruminants. This study investigated energy metabolism and nitrogen (N) utilization of Romney lambs under two treatments: summer pasture and willow + summer pasture. All lambs were placed in metabolic crates for three days where feed intake, faecal excretion, live weight changes were measured, and feed, faecal, and urine samples were analysed for nutrient composition. The contents of dry matter (DM), organic matter (OM), crude protein (CP), fat and lignin in willow were higher than those in summer pasture (366.8 g/kg willow vs. 357.5 g/kg pasture; 918.0 vs. 905.8 g/kg DM; 133.6 for 100g/kg DM; 44.8 vs. 34.8g/kg DM; 91.2 vs. 50.8 g/kg DM; respectively), while the ash, neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were lower than those of summer pastures (82 vs. 94.2 g/kg DM; 303.2 vs. 562.6 g/kg DM; 223.7 vs. 324.4 g/kg DM; respectively). The apparent digestibility of willow (DM: 63.7%; OM: 65.1%; CP: 50.8%; fat: 61.6%; NDF: 51.6%) was higher than that of summer pasture (DM:57%; OM: 59.1%; CP: 50.2%; fat; 54.5%; NDF: 51.2%). It was found that the use of willow as a supplementary feed in summer resulted in improved apparent digestibility, energy utilization and N balance in lambs. Willow supplemented lambs had lower urine N loss. However, willow supplementation did not reduce the live weight loss of lambs, which may be related to the insufficient proportion of willow in the mixed diet, the inadequate adaptation of rumen fermentation to willow and the detoxification process caused by secondary compounds in willow. Although willow supplementation led to an increase in faecal N, and an increase in estimated CH_4 emissions in the predicted model, willow reduced the proportion of energy loss in the faeces, urine, and methane. Willow is a potential feed that provides a higher metabolizable energy (ME) intake and apparent digestibility during the summer months, reducing energy loss and improving energy utilization and N balance in lambs.

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Abbreviations

ADF	Acid detergent fibre
ADG	Average daily gain
AFRC	Agricultural Food Research Council
Ammonia	NH_3
Amino acid	AA
AOAC	Association of Official Analytical Chemists.
BCS	Body condition scores
C	Carbon
CH_4	Methane
CP	Crude protein
CT	Condensed tannin
DM	Dry matter
DMI	Dry matter intake
DMD	Dry matter digestibility
DE	Digestible energy
DOMD	Proportion of digestible OM in the DM
FE	Faecal energy
FAO	Food and Agriculture Organisation
GE	Gross energy
GHG	Greenhouse gas
GWP	Global warming potential
H; H_2	Hydrogen
IPCC	Intergovernmental Panel on Climate Change
IPPU	Industrial Processes and Product Use
LULUCF	Land Use, Land-Use Change, and Forestry
LW	Live weight

ME	Metabolisable energy
NADH	Reduced nicotinamide adenine dinucleotide
N_2O	Nitrous oxide
NDF	Neutral detergent fibre
NSC	Non-structural carbohydrate
OM	Organic matter
OMI	Organic matter intake
OMD	Organic matter digestibility
SAS	Statistical Analysis System.
SCFA	Short-chain fatty acids
SE	Standard error
SF_6	Sulphur hexafluoride
UE	Urine energy
UNFCCC	United Nations Framework Convention on Climate Change
VFA	Volatile fatty acid
WSC	Water-soluble carbohydrates
Y_m	Methane emission factor

1. Chapter 1 General introduction

Sheep farming is a cornerstone of New Zealand's agricultural economy, contributing significantly to global lamb exports and wool production. However, the sector faces increasing scrutiny due to its environmental impact, particularly greenhouse gas (GHG) emissions, with methane (CH_4) from enteric fermentation being a major contributor. Methane has 28 times the GHG potency of carbon dioxide (CO_2) (Mizrahi et al., 2021). As New Zealand works towards ambitious emission reduction targets (Ministry for the Environment, 2023), innovative solutions are needed to balance environmental sustainability with agricultural productivity.

During the dry summer months, the capacity of pastures to provide adequate energy and protein as a ruminant diet is reduced. The high fibre structure and low digestibility of diets limit the utilization of energy by ruminants (Jung et al., 1995). Therefore, how to modify pasture-based ruminant diets to provide more energy needs to be considered.

Willow has gained attention due to its potential to enhance livestock nutrition and mitigate environmental impacts, particularly in the context of reducing CH_4 emissions from ruminants. The tannins present in willow leaves act as natural inhibitors of methanogenesis by reducing hydrogen (H) availability, thereby limiting the formation of CH_4 in the rumen (Puchala et al., 2005; Waghorn, 2007). When lambs consume willow, the slow degradation of tannins in the rumen minimizes the accumulation of intermediates that could negatively affect fermentation efficiency or animal health, offering a balanced approach to feed supplementation. In addition to its CH_4-reducing properties, willow serves as a valuable source of protein and fibre, making it a suitable supplement in grazing systems where pasture quality fluctuates seasonally (Athanasiadou et al., 2004; Min et al., 2003). This multifunctionality makes willow a sustainable feed alternative that not only improves lamb performance, e.g. growth rates, but also supports environmental goals by reducing CH_4 emissions and improving land use efficiency in pastoral farming systems (Waghorn et al., 2002; Min et al., 2015).

However, current knowledge about willow's chemical properties and its impact on GHG emissions when used as a feed supplement for sheep is limited. This gap highlights the need for robust studies to evaluate willow's nutritional characteristics and its effects on sheep digestion, metabolism and CH_4 production, critical information needed to accurately predict GHG using the New Zealand inventory framework (Hickeyet al., 2022).

The objective of the present study is to address this knowledge gap by chemically characterizing willow's properties and assessing its impact on sheep in a controlled metabolism trial. By combining a detailed nutritional analysis with an animal model, this study aims to provide critical data to describe the role of willow in sustainable sheep farming systems and its potential contribution to GHG mitigation strategies. The experiment was conducted by rearing lambs in indoor metabolic crates, collecting and weighing fresh feed offered, refusal feed, faeces and urine samples every day, then conducting nutritional analyses, and combining this information of the growth performance of lamb to obtain scientific results. The digestion of nutrients, energy utilization and nitrogen (N) balance are the main variables determined in this study.

2. Chapter 2 Review of Literature

2.1. Overview of New Zealand sheep production systems

As small ruminants, sheep provide a large amount of high-quality protein for humans, and New Zealand accounts for two-fifths of the world's lamb exports (FAO, 2008). New Zealand's unique landscape supports efficient grazing sheep production systems. The majority of sheep are reared on extensive grasslands, constituted by uplands, hills and rolling countryside (Morris, 2013). These areas are shared with beef production, while dairy production occupies the flat and more fertile land able to grow plenty of higher quality grass. It is estimated that approximately 5 million sheep in New Zealand are distributed across approximately 9,000 farms, categorized into 8 classes based on factors such as geographical location, topography, and production systems (Figure 2.1). The sheep industry in New Zealand is estimated to earn billions of dollars a year from lamb exports. (Beef + Lamb New Zealand, 2020). New Zealand is also a major exporter of wool, divided into coarse, fine, medium and hybrid wool, exporting around 100,000 tonnes of wool around the world each year (FigureNZ. 2024).

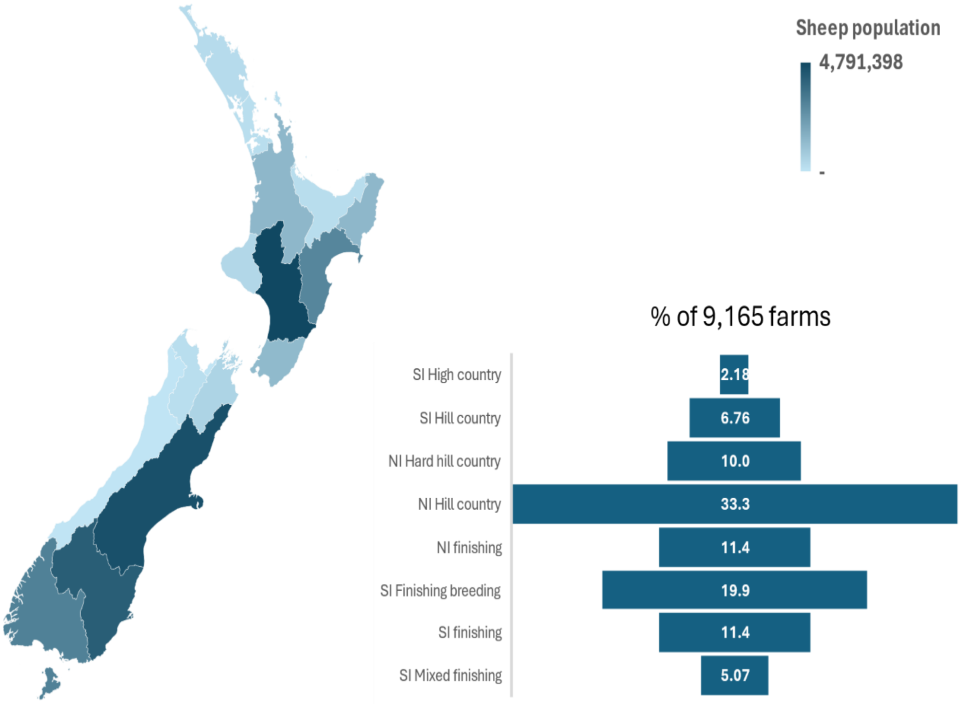

Figure 2.1: The number of sheep in New Zealand distributed by region (darker colours indicate higher sheep density; Stats NZ, 2023) alongside the percentage of farms within each class from

a total of 9,165 estimated farms (Beef + Lamb NZ, 2020; NI – North Island; SI – South Island).

In New Zealand, for example, sheep and cattle are often raised on the same farm, which helps to improve feeding flexibility and balance grazing pressure to maintain the sward utilization efficiency of hill farm (Hodgson et al., 2019). The production, composition and plant diversity of sheep and cattle farms varies greatly from season to season (White and Hodgson, 1999). There are eight farm types in New Zealand with the lowest stocking rates being at least two units of livestock per hectare and the highest stocking rates being at least 15 stock units (Figure 2.2). The Stocking Unit ratio represents how much space is being taken up by cattle and sheep. Although cattle consume more pasture than sheep, sheep are the most abundant grazing animal in New Zealand throughout the country and are concentrated in high densities in Canterbury, Otago in the South Island and Manawatu-Whanganui in the North Island (Figure 2.2).

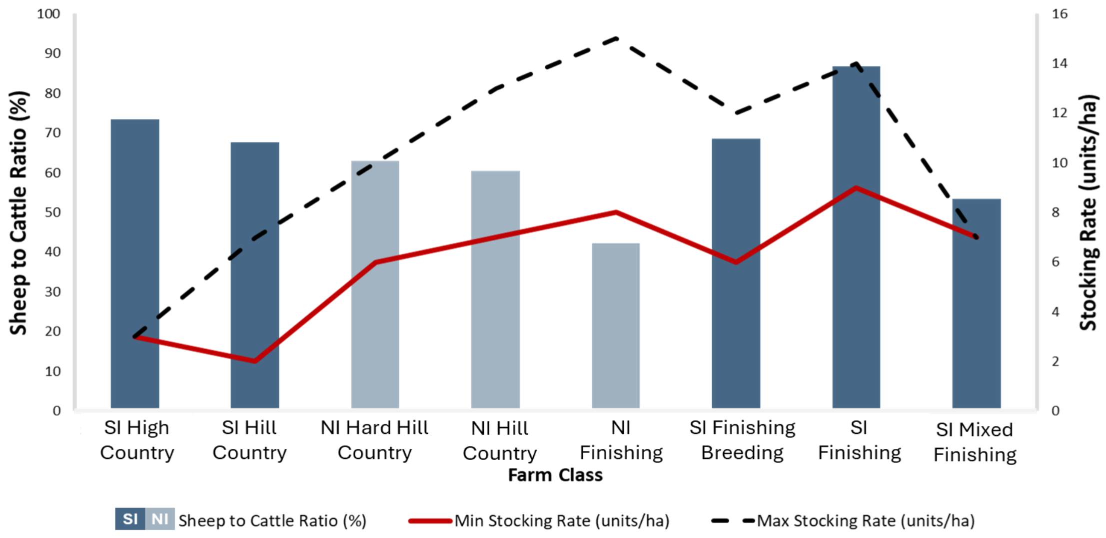

Figure 2.2: Stocking rates and sheep-to-cattle ratios for eight New Zealand farms classes (Adapted from Farm classes, n.d).

2.2. The connection between methane emissions in New Zealand and sheep

New Zealand has a unique GHG emissions profile, with approximately half of its total emissions originating from agriculture—a significantly higher proportion compared to most developed countries. From the 1990s to the early 2000s, the country's agricultural emissions grew by 1% annually (Leslie et al., 2008). Globally, CH_4 has gained increasing attention due to its rising contribution to the greenhouse effect. Since 2013, CH_4 emissions have grown at a faster rate compared to the slower growth of CO_2 and the relatively stable levels of nitrous oxide (N_2O) (Quere et al., 2016).

Methane is a colourless, odourless gas that dissolves easily in organic solvents but is insoluble in water (Broucek, 2014). It is the most abundant organic trace gas in the atmosphere and has a half-life of approximately 12.4 years (Hill et al., 2015). In the troposphere, CH_4 undergoes oxidation reactions with hydroxyl radicals, producing compounds such as formaldehyde and carbon monoxide (Lelieveld et al., 2008). These oxidation reactions also influence atmospheric chemistry by interfering with ozone levels in the stratosphere and accelerating the formation of water vapor, which, in turn, enhances CH_4's radiative forcing effects. This contributes to CH_4's role as a potent GHG. Collins et al. (2013) reported that CH_4 has a global warming potential (GWP) of 34, whereas more recent findings by Mizrahi et al. (2021) estimate CH_4's GWP at 28, indicating that each kilogram of CH_4 has the equivalent warming effect of 28 kilograms of CO_2, which is used in the New Zealand inventory. These properties underscore the critical role of CH_4 in driving climate change and highlight the importance of understanding and mitigating CH_4 emissions.

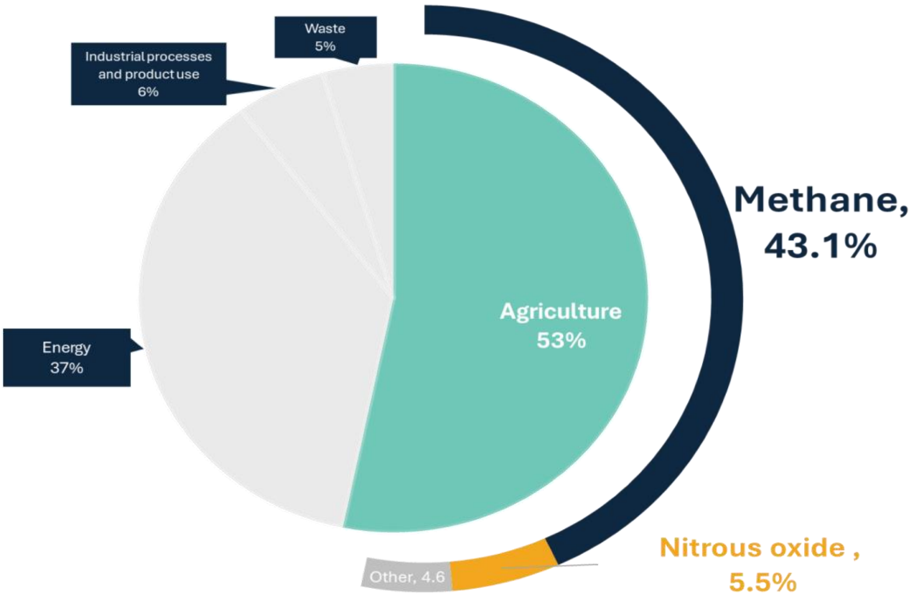

Figure 2.3: Greenhouse gas emissions from different industries in New Zealand in 2022 (Adapted from Ministry for the Environment, 2024).

Although total CH_4 emissions increased by 2% over the past three decades, they stabilized between 2006 and 2019 and have gradually declined since. This reduction aligns with significant changes in New Zealand’s agricultural landscape. For instance, the national sheep population has decreased by more than 40% since the late twentieth century, which has contributed to reduced CH_4 emissions from agriculture (Ministry for the Environment, 2023). Additionally, the land area used for sheep farming declined by nearly 1.7 million hectares (approximately 30%) between 2002 and 2019 (Stats NZ, 2021). However, the sheep industry still plays an important role in New Zealand where emissions from enteric fermentation constitute about one-third of the country’s total CH_4 emissions (UNFCCC, 2008), with sheep enteric CH_4 accounting for 11.4% of total GHG emissions (Ministry for the Environment, 2024).

2.3. Rumen fermentation and methanogenesis

In ruminants, CH_4 production is a natural byproduct of enteric fermentation. Microbes in the rumen break down feed enabling the animal to process structural carbohydrates, through fermentation. Before the nutrients in the feed are absorbed, the H in the gut is converted to CH_4 after the fermentation stage. This process not only contributes to GHG emissions but also represents an energy loss for the animal, with 2–12% of gross energy (GE) intake lost as CH_4 (Chagunda et al., 2008; Benchaar and Greathead, 2011).

As a ruminant animal, sheep are also a major contributor to enteric CH_4 emissions in New Zealand as previously discussed. Ruminants possess a specialised rumen-based digestive system, which allows them to have a higher fibre utilisation than other herbivorous animals (Millen et al., 2016). The rumen’s unique structure and function, developed through thousands of years of evolution, features a layered keratinized epithelium without glands, which supports fermentation as the primary mode of digestion. The rumen also plays an important role in the sheep's digestive system by being able to store ingested feed for the time necessary for its fermentation. This adaptation allows ruminants to symbiotically utilize microorganisms to break down structural carbohydrates and convert them into usable energy sources. The rumen epithelium plays a multifaceted role, protecting the organ, absorbing nutrients, and metabolizing volatile fatty acids (VFA), essential for the animal's energy supply (Baldwin and Connor, 2017).

The rumen contains a highly complex microbial community that provides approximately 70% of the sheep's daily energy needs through VFA (Flint and Bayer, 2008). This anaerobic ecosystem efficiently degrades plant material, producing fermentation end-products such as VFA, CO_2, H, and CH_4 (Broucek, 2014). Rumen metabolism can be divided into three main parts (Figure 2.4). In the first part, plant cellulose is colonized by rumen microorganisms and converted into various monomers in soluble forms under the action of different glycosyl hydrolases (Morais and Mizrah, 2019). Microorganisms then use soluble sugar to metabolise through a variety of pathways such as the pentose phosphate pathway (Hackmann et al., 2017). In the next stage, since the rumen is a large anaerobic environment, soluble sugars begin to ferment and produce volatile fatty acids, which are direct energy suppliers (McAllister and Newbold, 2008), as well as CH_4-producing substrates such as H ion and CO_2 (Martin et al., 2009). Cellulolytic bacteria, such as *Ruminococcus albus*, are fundamental to interactions between rumen microorganisms and the animal for nutrient digestion and energy transport in the rumen, converting plant polysaccharides into short-chain fatty acids and CH_4 precursors (Mosoni et al., 2007). Organic acids and CH_4 (H disposal) are the final products.

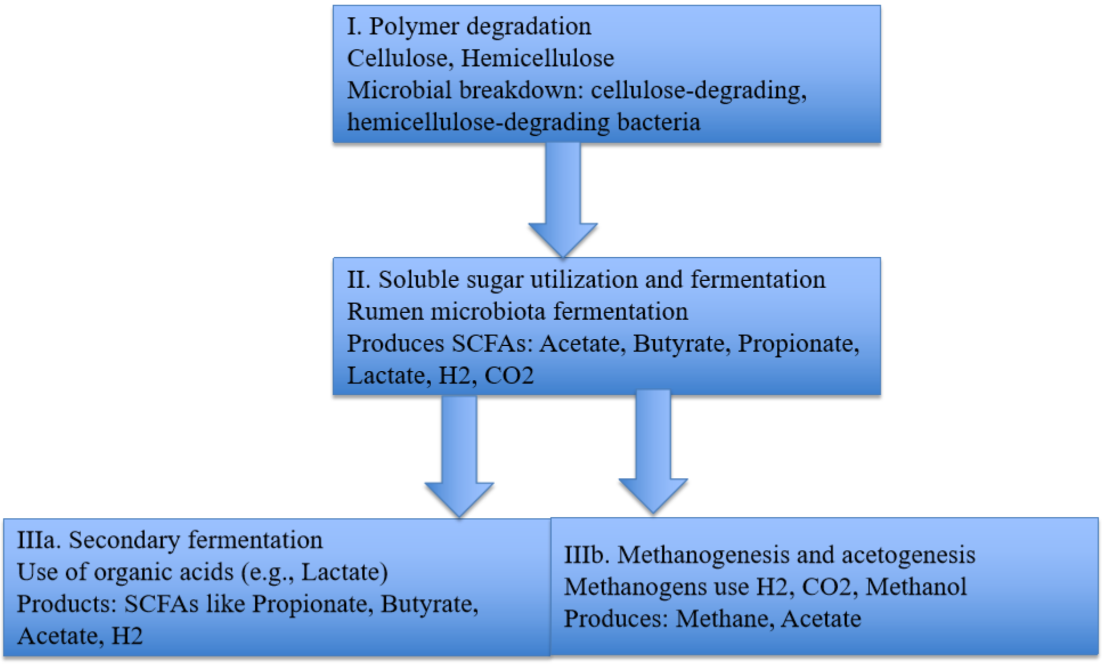

Figure 2.4: Trophic level in rumen metabolism. Adapted from Mizrahi et al., 2021.
H_2: hydrogen; CO_2: carbon dioxide; SCFA: short-chain fatty acids.

Excess H ions are expelled from the ruminal chamber through CH_4 formation and ensures digestive efficiency by preventing the inhibition of microbial enzymes, such as reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase, by increased H partial pressure (Millen et al., 2016; Morgavi et al., 2010). Disrupted H ion transfer among rumen microbes can inhibit fermentation by altering redox reactions and coenzyme reoxidation (Wolin et al., 1997). Therefore, methanogenesis is critical for maintaining rumen pH and digestive efficiency.

Methanogens are the sole producers of CH_4 in the rumen microbiome. While not relatively abundant, they are found in almost all foregut animals and are considered core microorganisms in ruminant CH_4 emissions (Henderson et al., 2015). The methanogen community composition is influenced by environmental factors such as feed (Friedman et al., 2017). Methane-producing bacteria are divided into several species, with four major groups identified (Leahy et al., 2019). These methanogens, similar to those in other anaerobic environments, rely on specific enzymes for metabolism (Leahy et al., 2019). For instance, sheep raised on ryegrass farms in New Zealand have rumen contents containing between 10^9 and 10^{10} methanogens per gram (Attwood et al., 2011).

Methanogens interact closely with other rumen microbes, including cellulolytic bacteria and anaerobic fungi, to enhance cellulose degradation and CH_4 production. For instance, *Ruminococcus albus* and methanogens metabolize fermentation intermediates to produce acetate and CH_4 (Wolin et al., 1997). Conversely, some relationships are competitive. Lactic acid bacteria, for instance, reduce rumen pH and compete with methanogens for H, thereby inhibiting CH_4 production (Moraïs and Mizrahi, 2019). Methanogens use substrates like CO_2 and H, but they can also metabolize formate and acetic acid from carbohydrate fermentation and methanol from pectin fermentation to produce CH_4. However, experimental evidence supporting these pathways remains limited (Rea et al., 2007; Qiao et al., 2014).

Methane accounts for approximately 27% of gases produced in the rumen, second only to CO_2 (Cunningham, 2008). Most CH_4 produced in the rumen is expelled through exhalation, accounting for about 89% of total CH_4 emissions, while a smaller fraction (approximately 2%) originates from the posterior intestine and is absorbed into the bloodstream before being exhaled with other respiratory gases (Murray et al., 1999; Pinares-Patiño et al., 2009). This highlights the reticulorumen as the primary site of CH_4 production. While CH_4 production is an inevitable part of rumen fermentation, its environmental impact and energy loss underscore the importance of developing mitigation strategies to reduce emissions without compromising the efficiency of ruminant digestion.

2.4. Factors influencing the production of enteric methane

For CH_4 production to occur, specific rumen conditions are required, including the presence of methanogenic microorganisms, substrates such as CO_2 and H_2, and a pH range of 6.6 - 7.6. Any deviation from these conditions can influence methanogenesis. Factors such as feed quantity and quality, physiological conditions, environmental stresses, and the microbial ecology of the rumen play crucial roles in modulating CH_4 emissions. Overall, feed intake and type are the dominant factors for enteric CH_4 emission in ruminants (Broucek, 2014, McAllister et al. 1996).

2.4.1. Feed quality – Dry matter intake

In New Zealand, due to the unique characteristics of the agricultural production system, emission factors between CH_4 production and dry matter intake (DMI) were designed. Methane emission factor (Y_m) is a constant CH_4 production factor and is recommended by the International Panel on Climate Change as one of the parameters for estimating CH_4 emissions from sheep. However, CH_4 production, which is more strongly related to intake, was directly used to predict CH_4 emissions from sheep in New Zealand (Muetzel and Clark, 2015).

Muetzel and Clark (2015) conducted a study on the relationship between different feeding levels and different forage quality and the CH_4 emissions of sheep of different ages. They concluded that 80% of daily CH_4 emissions could be explained by daily DMI. Methane emissions from lambs younger than one year also nearly doubled when DMI increased from 0.35 kg to 0.87 kg per day. Meanwhile, the experiment of Molano and Clark (2008) also found that the relationship between daily DMI and daily CH_4 emission was strong with an R-square value of 0.83, indicating that the two factors were highly correlated.

2.4.2. Feed quality

Since the microbial community involved in digestion in the rumen requires various nutrients, the composition of forage is related to CH_4 emission (Moss et al., 2000). For instance, increased protein levels in the feed led to decreased CH_4 production, but fibre levels were positively correlated with CH_4 levels (Shibata and Terada, 2009). Increased levels of non-cellulosic sugars can promote propionate fermentation leading to loss of H, which is one of the feedstocks for CH_4 formation (Archimede et al., 2014). Moreover, CH_4 emissions were directly proportional to feeding level and forage digestibility, but inversely proportional to dietary fat content, while carbohydrate seems to have no effect (Chianese et al., 2009). High digestibility often means low fibre content and high protein levels, which also induce propionic acid fermentation to reduce rumen pH and inhibit methanogenic bacteria activity (Hook et al., 2011). Alternatively, the concentration of soluble sugar in forage is unable to reduce rumen environmental pH below 5.5 (Lettat et al., 2010).

However, some experiments also found that feed quality had no decisive effect on CH_4 emissions (Muetzel and Clark, 2015; Molano and Clark, 2008). Nevertheless, the influence of feed quality on CH_4 emission seems to be variable under different conditions (van Gastelen et al., 2019).

2.4.3. Age and physiological stage

It is difficult to determine whether age of animal influences CH_4 emissions. Knight et al (2008) stated that CH_4 emissions from lambs less than one year old differed in only one of the four experiments. In contrast, physiological stages seem to have a more significant impact on CH_4 emissions. According to the data of Molano and Clark (2008), the DMI of sheep at different physiological stages explains the CH_4 emission to different degrees and the slope of the regression equation for dry ewes and lambs is similar but different from that of lactating ewes (Figure 2.5). In some experiments, the types of methanogens in the rumen were found to be different at different physiological stages (Friedman et al., 2017). For example, newborn lambs contain more methylotrophs while most rumen methanogens in mature sheep belong to hydrogenotrophs (Furman et al., 2020). This may be the reason why researchers have always considered the role of age in CH_4 emissions.

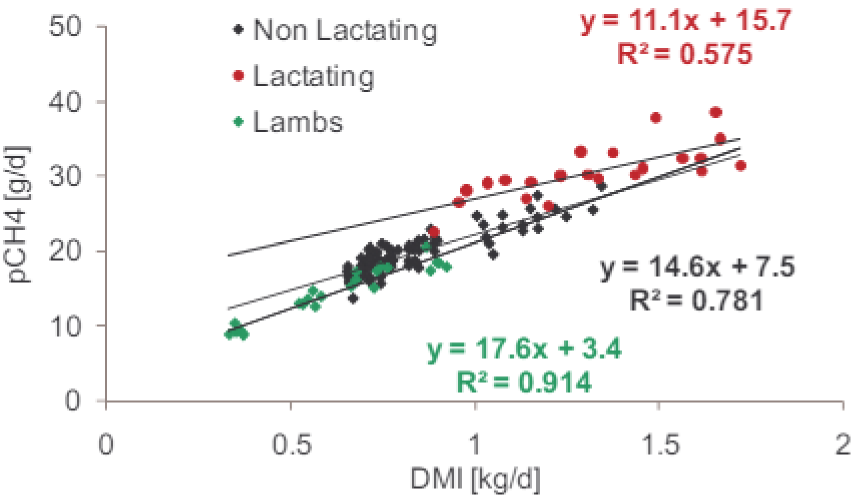

Figure 2.5: Effects of dry matter intake on methane emissions in sheep at different physiological stages (Molano and Clark, 2008).

2.5. The New Zealand's greenhouse gas inventory framework

New Zealand has established a comprehensive GHG inventory framework to accurately monitor, report, and manage emissions in line with international obligations under the United Nations Framework Convention on Climate Change (UNFCCC) and the Paris Agreement. This inventory is integral to understanding emission sources, developing effective mitigation strategies, and tracking progress toward emission reduction targets. The framework divides GHG emissions into five key sectors: agriculture, energy, industrial processes and product use (IPPU), waste, land-use change, and forestry (LULUCF). Specifically, the sheep industry within New Zealand employs a Tier 2 approach for agricultural emissions, incorporating detailed, country-specific data on livestock populations, feed quality, and production systems. This methodology ensures greater accuracy compared to Tier 1 estimates, which rely on global default values.

For sheep, CH_4 emissions have primarily been estimated using the Sulphur hexafluoride (SF_6) tracer technique, respiration chambers, and GreenFeed Systems. These are examples of animal-based CH_4 measurement technologies (Tedeschi et al., 2022), each varying significantly in methodology, application, cost, labour intensity, and accuracy (Table 2.1). No single method is universally ideal due to the wide variation in production systems, management strategies, and the inherent assumptions of each technique (Tedeschi et al., 2022). For policy-making and large-scale monitoring, satellite imagery, drone measurements, and aircraft-mounted sensors are often the best choices (Tedeschi et al., 2022). New Zealand’s approach to estimating GHG emissions incorporates country-specific data to reflect the unique characteristics of its sheep farming practices, which predominantly rely on year-round outdoor grazing on ryegrass and clover pastures. The accuracy of New Zealand's GHG inventory depends heavily on reliable activity data, such as animal population statistics derived from the Agricultural Production Census and annual surveys. These datasets provide detailed categorization of sheep into subgroups, including breeding ewes, lambs, and wethers (Pickering et al., 2022).

Table 2.1: Summary of on animal-based methane measurement methods.

Method	**Cost**	**Labour Intensity**	**Accuracy**	**Precision**	**Scalability**	**Advantages**	**Disadvantages**	**Reference**
Respiration Chambers	High	High	High	High	Low	Gold standard for precision and accuracy; controlled environment.	Expensive, labour-intensive, limited scalability may not represent natural conditions.	Pinares-Patiño and Waghorn (2014), Johnson and Johnson (1995), Goopy et al. (2016)
Polytunnels	Moderate	Moderate	High	Moderate	Low	Simulates semi-natural grazing; captures group emissions.	Limited to smaller studies, expensive setup.	Gaviria-Uribe et al. (2020), Goopy et al. (2016), Murray et al. (2001)
Hood and/or headbox systems	High / Moderate	Moderate	Moderate	High	Moderate	Portable, less invasive than chambers. Scalable, practical for large herds; works in grazing and confined systems	Limited to spot sampling Requires animal training; bait feed may influence dietary trials. Include Greenfeed.	Pinares-Patiño and Waghorn (2014), Hristov et al. (2016), Alemu et al. (2017)
SF_6 Tracer Technique	Moderate	Moderate	High	Moderate	Moderate	Suitable for grazing animals.	Technical expertise required; potential environmental concerns due to SF_6 gas. Less precise due to variability in air mixing and muzzle positioning	Pinares-Patiño and Clark (2008), Johnson and Johnson (1995)
Open-Path Laser	High	Low	Moderate	Low	Medium	Good for large herds; works in grazing and intensive settings.	Expensive equipment, influenced by microclimatic conditions.	Tomkins and Charmley (2015), Van Well et al. (2005)
***In Vitro* Techniques**	Low	Low	Low	Moderate	Low	Effective for evaluating feed additives; replicable conditions.	May not fully represent in vivo conditions.	Tomkins and Charmley (2015), Van Well et al. (2005) Yáñez-Ruiz et al. (2016)

Cost: Includes both setup and operational costs. Labour Intensity: Evaluates the human resources required to implement and maintain the technology. Accuracy: Reflect the method's ability to measure CH_4 emissions correctly. Precision: Highlights the repeatability and consistency of measurements. Scalability: How easily the method can be implemented on a larger scale.

Methane emissions are directly linked to DMI, which is calculated using metabolisable energy (ME) requirements for maintenance, growth, and reproduction (Figure 2.6). Seasonal variations in pasture quality, including fluctuations in neutral detergent fibre and protein content, are accounted for in ME calculations. Data from Beef + Lamb New Zealand further enhance these estimations by providing insights into regional variations in stocking rates and pasture quality.

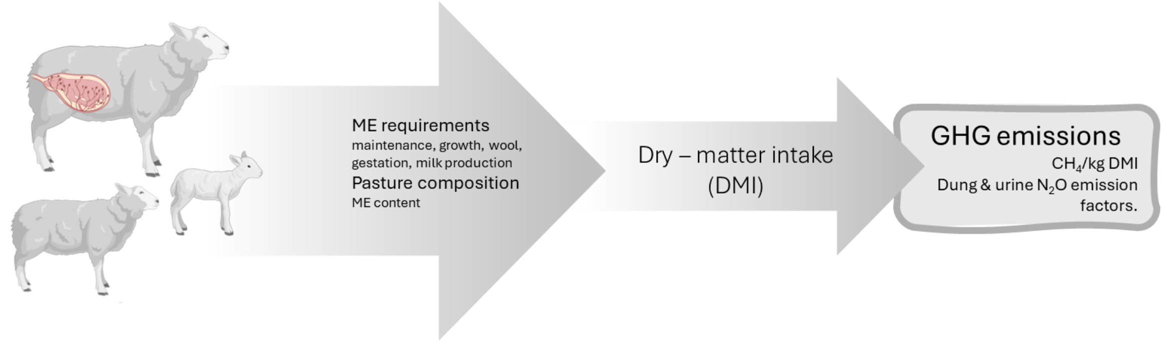

Figure 2.6: Overview of New Zealand's greenhouse gas (GHG) inventory framework.

All of this information is integrated with country-specific emission factors to produce precise CH_4 estimates (Pickering et al. 2022). However, the accuracy of these estimations is highly dependent on the quality of the input data. Knowing the ME values of different feedstuffs beyond ryegrass and clover, such as shrubs like willow, is essential for accurate DMI estimations and, consequently, precise GHG reporting. Incorporating diverse feed sources into the inventory could improve the robustness of New Zealand's emissions data and better support its GHG reduction targets.

Swainson et al. (2016) designed a CH_4 emission prediction model that best fits New Zealand lambs under one year of age (Equation 2.1). All the data for this equation comes from a wide range of sources in New Zealand and both CH_4 emissions and DMI are accurately measured.

Equation 2.1: Prediction model of metabolisable energy and dry matter intake on methane in lambs under one year old (Swainson et al., 2016)

$$\ln(pCH_4) = 0.734\times\ln(DMI)+0.05\times ME+2.46$$

where $\ln = \log_e$

2.6. Ruminant digestibility, energy utilization and nitrogen cycle

2.6.1. Ruminant digestibility and plant cell wall composition

The digestibility of each nutrient in feed is related to feed energy. Faecal nutrient loss is the most important digestibility factor (Kitessa et al., 1999). Metabolisable energy is related to digestibility and is further lost due to nutrient loss in urine and CH_4. Metabolisable energy is usually calculated by multiplying the digestible organic matter (OM) in DM (g/kg DM) (DOMD) by 0.016 (AFRC 1993). To improve the digestibility detection efficiency and reduce the experimental cost, many experimenters have adopted *in vitro* methods for prediction (Table 2.2).

Table 2.2: *In vitro* prediction of digestibility of ruminants (Adapted from Kitessa et al. 1999)

Method	Advantage	Disadvantage
Prediction equation based on chemical composition of feed	The measurement process has high efficiency and low cost.	Nutrients are not considered as a source of digestibility variation in the body.
Rumen liquor-pepsin	It is not easily affected by changes in chemical indicators.	The change of feed particle size has great influence on the prediction accuracy. And the animal cost is high, the efficiency is low
Faeces liquor-pepsin	Animal welfare is maintained and the cost of feeding intubated animals is reduced	Lack of rigorous testing
Enzymatic methods	Rumen fluid is no longer necessary for the donor animal; Short processing time; Strong repeatability	The predicted digestibility of low-quality feed may be low
Fermentation products	Less time consumed; The error is avoided in the process of washing and filtering.	Differences in the measurement of gas production and modeling processes tend to result in lower digestibility predictions than traditional methods.
In situ/in sacco method	Closer to the actual rumen digestion environment	Lack of standardization of procedures leads to large variations between laboratories

Increased feeding levels lead to an increase in the speed at which feed passes through the gastrointestinal tract, reducing the time spent on the feed by enzymes, which reduces digestibility (Kitessa et al., 1999). Furthermore, when the diet is a mixture of two feeds, the digestibility coefficient of the mixed diet is different from the average digestibility coefficient of the mixed diet because of synergistic or antagonistic effects between the different feeds (Weiss, 1994). The feed type and the proportion of different feeds in the mixed diet will affect the interaction between different feeds. In terms of animal species, sheep have a higher efficiency in the digestion of grain feed while cattle have a higher efficiency in the digestion of low-quality feed (McDonald et al. 1995). Most scholars believe that the cell wall composition of plants is the main influence on digestibility because the digestibility of the cell contents is close to complete (Kitessa et al., 1999). Lignin is the main component of the plant cell wall (Jung and Deetz, 1993). and, although lignin concentration is negatively correlated with feed digestibility, it may only reduce NDF digestibility, but not dietary organic matter digestibility (OMD) (Smith et al., 1972; Van Soest, 1993).

Plant cell wall concentration is inversely proportional to DMI and energy density, and improving the digestibility of the cell wall is conducive to improving the energy utilization rate of ruminants (Jung et al., 1995). Slower fermentation and elimination of fibre components from thereticulorumen results in reduced DMI in ruminants (Jung et al., 1995). In previous studies, it was found that the correlation between neutral detergent fibre (NDF) and acid detergent fibre (ADF) and DMI was 0.65 and 0.53, respectively (Van Soest, 1965).

2.6.2. Nitrogen metabolism in ruminants

The digestive tract of ruminants is the main site for energy metabolism and nitrogen (N) metabolism (Reynolds, 1995). Unique rumen physiological mechanism of ruminants enables the utilization of N from different sources in the diet (Zurak et al., 2023). Rumen N metabolism is divided into two stages: N source required by bacteria comes from protein degradation followed by microbial protein synthesis (Bach et al., 2005). Different rumen bacteria attach to undigested feed pellets. Some rumen microorganisms have proteolytic enzyme activity and degrade proteins through symbiosis. This process provides peptides and amino acids (AA) (Prins et al., 1983). The degradation rate and degree are related to the proteolytic activity of rumen microorganisms and the type of protein (Bach et al., 2005). In addition, peptides and AA generated from the hydrolysis of proteins outside rumen cells are transported by microbial cells. This process is related to energy. When there is sufficient energy, AA is used for microbial protein synthesis; when there is insufficient energy, the carbon skeleton of AA is fermented into volatile fatty acids (VFA). When the rate of protein degradation is higher than the rate of energy supply, a large amount of N will be lost in the form of NH_3. And when the energy supply is faster than protein degradation, microbial protein synthesis will decrease (Nocek and Russell, 1988).

In addition, ruminants absorb nitrogen in their feed in the form of NH_3, and their livers detoxify the NH_3 and convert it into urea (Reynolds, 1992). 40% to 60% of the urea produced by the livers of ruminants is returned to the digestive tract, which is conducive to the synthesis and supply of AA by microorganisms (Huntington, 1989). Urea in the digestive tract is converted into NH_3 under the action of microbial urease, and this NH_3 is synthesized by microorganisms into AA (Reynolds, 1992). The catabolism of nitrogen-containing compounds such as amino acids can also form NH_3 (Reynolds, 1992). Therefore, there is a non-protein nitrogen cycle between the liver and the digestive tract.

2.7. Willow and greenhouse gases

Ramirez-Restrepo et al. (2009) reported that lambs grazing on willow feed blocks exhibited up to 20% lower CH_4 emissions compared to those grazing on ryegrass/white clover pastures by the end of summer. However, this reduction diminished as the season transitioned to autumn, when CH_4 emissions were generally lower across all diets compared to summer.

The effect of willow on rumen fermentation have been studied since the 20th century. Researchers observed that the higher cellulose content in willow and other woody feeds increased the production of VFA while shifting fermentation toward a higher propionate-to-CH_4 production ratio (Van Hoven and Boomker, 1983). This shift may be attributed to the presence of CT in willow, which bind with lignin and other fibre complexes, reducing fibre degradation (McSweeney et al., 2001). As a result, H is preferentially directed toward propionate synthesis, leaving less available for CH_4 production.

Condensed tannin in willow also negatively affects key rumen microorganisms, including methanogens and cellulolytic bacteria (Kumar et al., 2013). The structure and molecular weight of CT are critical in this interference (Lowry et al., 1996). For instance, CT inhibit the growth of microbes like *Vibrio cellobutyricoides* (Jones et al., 1994). Condensed tannin also exhibits enzyme inhibition and substrate deprivation properties, forming CT-enzyme complexes that limit microbial activity (Scalbert, 1991). Additionally, CT bind to cellulase and peroxidase, inhibiting microbial enzymes, and sequester essential metal ions, creating deficiencies in the rumen microbiome (Scalbert, 1991).

While CT do not significantly alter rumen pH in most studies, willow intake has been associated with a reduction in rumen pH, potentially inhibiting methanogen activity (Van Kessel and Russell, 1996; Van Nevel and Demeyer, 1996). This suggests that compounds other than CT might also play a role in reducing CH_4 production. Supporting this, Bhatta et al. (2009) found an inverse relationship between the concentration of CT in the feed and the population of methanogens. These findings suggest that willow feed blocks could serve as an effective dietary strategy for reducing CH_4 emissions in sheep farming systems while maintaining rumen fermentation efficiency.

2.7.1. Non-nutritional uses of willow

Farmers in eastern coastal areas of New Zealand have successfully used willows and poplars as supplemental feed (Charlton et al., 2003), but willow has other non-nutritional uses as well (Figure 2.7). Willow adaptability to various soil types and climates makes it an ideal candidate for agroforestry systems designed to complement conventional pasture-based sheep farming (Roberts, 2017). Willows have a role in resisting soil erosion and providing shade to ruminants in hilly areas in New Zealand (Wilkinson, 1999). Willows increase the pH in the soil and increase the concentration of calcium ions in the soil (Pitta, 2007). Falling willow leaves decompose at the soil surface to compensate for OM and reduce the loss of pasture productivity (Kemp et al., 2001). Furthermore, the roots of the willow tree can bind to the soil and transpiration in the deeper soil, which ensures the health of the soil (Pitta, 2007). Trees with long roots, such as willows, stabilize the soil and reduce the impact of rain and wind (Figure 2.7). Rain and wind will force the soil exposed to the ground to move, while the soil protected by the willow root system is difficult to carry away (Roundy, 2023).

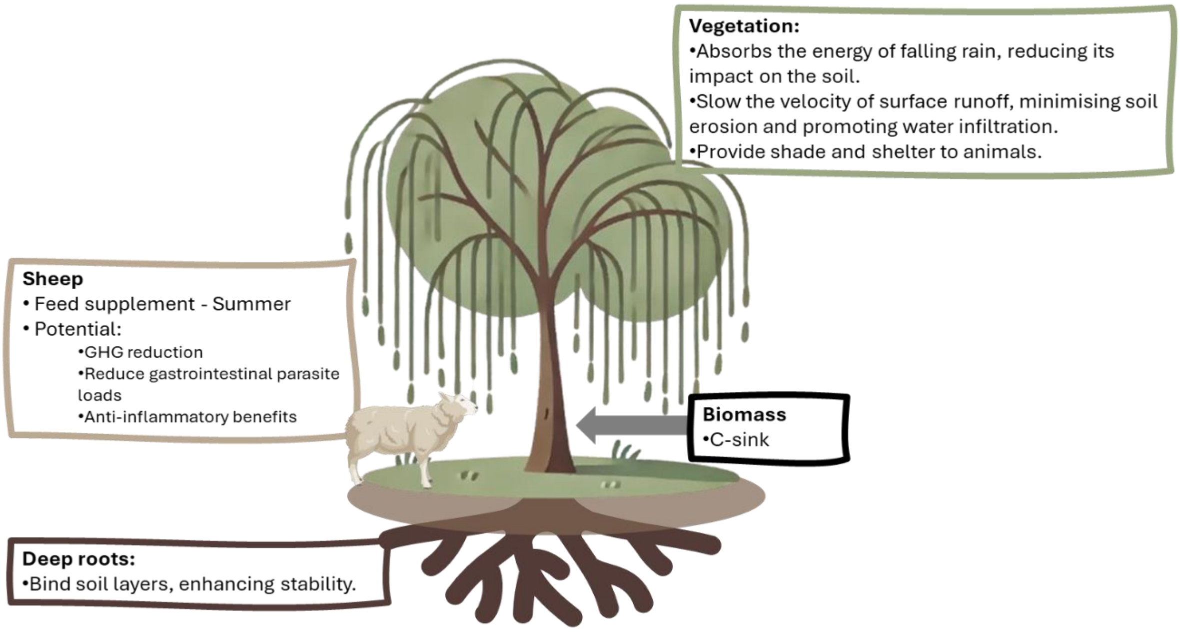

Figure 2.7: Potential of *Salix* spp. on farm.

Ruminants need to maintain a constant body temperature within a small range to reduce energy expenditure for warmth or cooling (Palmer et al., 2003). Willow can be used as a shelter to protect grazing animals from adverse effects during harsh summers and winters (Gregory, 1995). For example, sheep under the shelter of willow trees can maintain a certain growth rate and ovulation rate even in the face of cold (Lynch and Donelly, 1980). Moreover, miscarriage rates and lamb mortality at low temperatures are also reduced by the presence of willow trees (Alexander et al., 1980).

2.7.2. Willow as a tool towards the carbon emission reduction targets.

New Zealand's domestic targets aim to reduce biogenic CH_4 emissions by 10% by 2030 and 24–47% by 2050 (relative to 2017 levels) under the Split-Gas Approach (Ministry for the Environment, 2024). Within this framework, the use of Salix spp. offers significant potential as a mitigation tool. Willow contributes to these goals by providing high-quality feed during summer, enhancing digestion efficiency, and reducing CH_4 intensity - lowering enteric CH_4 emissions per unit of product. Additionally, willow serves as a sustainable alternative to high-emission supplemental feeds like concentrates and may improve the N-retention.

Under New Zealand's Zero Carbon Act in the Climate Change Response Act 2002, which distinguishes CH_4 from long-lived gases, willow plantations further support carbon sequestration by increasing tree cover on farmland, particularly in riparian zones and paddocks, enabling long-term carbon storage in biomass and soils. Willow plantations are estimated to be capable of sequestering 3.5–4.0 mg carbon (C)/ha in biomass and 0.4–0.5 mg C/ha in soils per annum, making it a valuable tool for farmers to mitigate atmospheric CO_2 levels and enhancing long-term carbon storage (Ritter et al., 2011). This aligns with the government's afforestation initiatives and carbon credit policies (Beef + Lamb New Zealand, 2024).

Moreover, *Salix* spp. enhance farm sustainability and resilience by reducing heat stress through shade provision, improving water retention in soils, and serving as a drought-tolerant forage option. These contributions directly support the objectives of the Emissions Reduction Plan (ERP2) to lower emissions while maintaining agricultural productivity.

2.7.3. Influence of willow on volatile nitrogen emissions from ruminants

Nitrogen in the faeces and urine excreted by grazing livestock from pasture is close to 26% of the anthropotable nitrogen emissions from agriculture, mainly in the forms of ammonia (NH_3) and nitrous oxide (Beusen et al., 2008; FAO, 2021). Insufficient utilization of N in ruminants is mainly caused by excessive absorption of NH_3. The entire intestine is a site for the generation of NH_3, and the sources of NH_3 mainly come from dietary nitrogen and the urea cycle (see Remond et al., 1993). Nitrogen loss in ruminants can change due to various factors such as dietary type. CT in willow improves the liver urea N cycle by inhibiting rumen protein digestion and reducing NH_3 (Müller-Kiedrowski et al., 2025). In addition, CT has an inhibitory effect on the transformation of urea to NH3 in the soil and increases the excretion of hippuric acid in urine, which is conducive to inhibiting the formation of Nitrous oxide (N_2O) in the soil (Powell et al., 2011; van Cleef et al., 2022). Salicylate in willow leaves forms salicylic acid after being ingested by ruminants. Salicylic acid forms strong molecular bonds with urea, thereby inhibiting urea hydrolysis and preventing NH3 emissions (Silva et al., 2020). The experiment of Muller-Kiedrowski et al. (2025) used concentrated feed, willow leaves and concentrated feed, alfalfa to feed calves respectively. The experimental results revealed that calves fed a diet containing willow leaves had a 20% reduction in urea excretion but a 20% increase in faecal N. However, 91% of N_2O and 87% of NH_3 in the excreta of ruminants come from urine N excretion (Laubach et al., 2013; Voglmeier et al., 2019).

2.8. Willow as a feed Supplement

As a common plant in rural settings, willow (*Salix* spp.) is widely distributed throughout the world and belongs to the largest woody plant in the Salicaceae (Lauron-Moreau et al., 2015). Willows can adapt to most climates so they can be found even in semi-arid regions. The main reason is that willow trees have strong drought resistance, and the transpiration rate is very fast, so willows are not sensitive to soil water saturation (Kuzovkina et al., 2004). In addition, willow has strong regenerative capacity, so it can grow new branches quickly after harvest, and it can adapt to intensive planting (Muklada et al., 2017). These are desirable features that make willow a potential supplemental feed for ruminants.

A mix of perennial ryegrass and white clover forms the basis of the sheep farming system in New Zealand (McWilliam et al., 2005). This grazing system not only saves costs but also contributes to the development of animal welfare. However, due to the special geographical location, the seasons have a huge impact on the pasture. For example, the eastern coastal areas are prone to drought in the summer and autumn, which leads to a decline in pasture quality, which means that the productivity of sheep farming is reduced (McWilliam et al., 2005).

Willow species commonly found in New Zealand include weeping willow, crack willow, golden willow and globe willow (Pitta, 2007). The two willows that are most used in New Zealand pastures to supplement sheep feed are hybrid willow and Kinuyanagi willow. Hybrid willows are characterized by strong wind resistance and retain many low branches, thus providing shade and soil stabilization (Hathaway, 1986). In addition, it has a very strong drought tolerance and can maintain growth in short-term rotation systems (Hathaway, 1986).

2.8.1. Feeding value

There are many ways to provide willow feed to livestock, the most common is to prune the lower branches and provide the resulting leaves and branches to livestock or to use grazing feed blocks (Pitta, 2007). Willow branches at different stages of growth or widely spaced are often cut off as supplemental feed for ruminants (Douglas et al., 2003). Rows of willows planted near the pasture are of practical value. Initially, root cuttings were used in a wide range of planting patterns, but in order to consider economic costs, rootless cuttings became a better alternative

(Zsuffa et al., 1992). Fodder blocks are usually established by vertical planting of willow cuttings (Van Kraayenoord, 1980). Willow cuttings are usually more than one meter long, and longer cuttings contain higher growth potential and therefore more yield (Douglas et al., 2003). During the dry summer months these willow cuttings will be used as browse plants for ruminants such as sheep (Oppong et al., 1996).

Studies have shown that lambs fed tannin-rich browse plants like willow demonstrate improved growth rates and N utilization compared to animals on conventional grass-only diets (Athanasiadou et al., 2004; Min et al., 2003). The N-fixing properties of tannins also improve protein absorption by binding to dietary proteins, which are released for digestion in the abomasum, thus enhancing the animal's overall N retention and protein synthesis (Barry and McNabb, 1999; Min and Solaiman, 2018). Moreover, willow's high content of secondary compounds such as salicylates contributes to its anti-inflammatory properties, which may improve animal health and resilience under grazing conditions. In light of its beneficial properties, the primary aim of research has been to evaluate the potential of willow as a regular component of lamb diets. Researchers have examined its impact on lamb growth, feed conversion efficiency, and CH_4 emissions, with promising results showing reduced CH_4 production without compromising lamb health or productivity (Tavendale et al., 2005; Mueller-Harvey, 2006). In addition, willow is more readily available and more cost-effective than providing supplementary feed to sheep grazing hilly pastures (Moore et al., 2003).

Calculations based on DM content show willow branches provide 55% of the biomass, with the remaining 45% coming from willow leaves (Muklada et al., 2017). In addition, willow also contains a variety of secondary compounds such as peroxides, analgesics and metabolites with anti-insect properties (Boeckler et al., 2013). These secondary compounds are mainly divided into two groups: phenolic compounds and phenolic glycosides. Phenolic acids, flavonoids and condensed tannin (CT) belong to phenolic compounds, while salicylic acids with analgesic and anti-inflammatory functions belong to phenolic glycosides (Saller et al., 2008; Tyśkiewicz et al., 2019).

The nutritional value of the willow tree exceeds that of lower quality forage in the summer, and it contains a crude protein (CP) concentration of 165 to 210 grams of CP per kilogram of DM (Pitta, 2007). Douglas et al. (1996) found that willow trees contained approximately 17 to 37 grams of N per kilogram of DM (equivalent to approximately 106 grams to 231 grams of CP. The ratio of easily fermentable carbohydrates to structural carbohydrates in the willow-based supplemental feed was lower than that of white clover but higher than that of summer forage (McWilliam et al., 2005). Additionally, fresh willow leaves have an OMD of about 50% and a ME of about seven to eight megajoules per kilogram of DM (Kemp et al., 2001). Willow contains high lignin, about 180 grams of lignin per kilogram of DM, which limits the OMD to a certain extent (Pitta, 2007). Livestock generally prefer to eat willow leaves because of their palatability and higher nutritional value than branches but branches less than 5 mm in diameter are also preferred by grazing animals (Kemp et al., 2001). The main nutritional values of willow trees are summarized in Table 2.3.

Table 2.3: Overview of the Nutritional value of willow.

Trait	**Reference**
Willows contain higher concentrations of fermentable carbohydrates and CP than those in summer pastures	Pitta, 2007; McWilliam et al., 2005
Higher ME than those in summer pastures	Kemp et al., 2001
Willow contains a variety of secondary compounds such as CT, etc. These secondary compounds are beneficial for N fixation in ruminants, reducing CH_4 energy loss and resisting parasites, etc.	Tavendale et al., 2005; Min and Solaiman, 2018; Mupeyo et al., 2010
Contains a variety of trace elements essential for ruminants	(Kendall et al., 2021
Contains enzymes related to N storage	Cooke and Weih, 2005

2.8.2. Nitrogen supplement

Willow tends to have greater leaf growth in the summer, and as a result is the time of the year when willow is a source of protein for animals. By the end of summer, the stems start developing and leaf content decreasing, and the N level in leaves decreases (Brereton et al., 2013). In addition to the higher N content, the secondary metabolites of willow may also have beneficial effects on N utilization. An enzyme called rubisco is present in willow leaves, which is one of the main proteins in willow leaves and functions as a form of N storage (Cooke and Weih, 2005). Plant protein is also bound to CT in willow feed to prevent plant protein degradationin the rumen, which facilitates the consumption and absorption of plant protein in the abomasum and small intestine (Norton, 2000). Condensed tannins in willows increase the proportion of amino acids in the small intestine and thus improve protein uptake by lambs (McWilliam et al., 2002).

2.8.3. Effects on live weight and body condition score of ewes during mating period

In the experiment of McWilliam et al. (2005), Romney ewes were herded on dry pastures for a total of 87 days from late summer to autumn, and the willow treatment group ewes received 1.3kg of willow trimmings as supplements every day. The willow varieties were Tangoio and Moutere. These willows trimming from coppiced stools were cut every three days. Feeding willow to ewes during mating has been shown to increase reproductive rate by approximately 25% while reducing live weight (LW) loss (McWilliam et al., 2005). In addition, ewes with long-term willow supplementation had higher LW and body condition scores (BCS) than ewes grazing on dry pastures and ewes with short willow supplementation cycles. The main reason is that microbial protein synthesis requires rumen-degradable N and soluble carbohydrates, both of which are present in willow (McWilliam et al., 2004). In addition, CT contained in willow can form insoluble complexes with proteins and thus reduce the protein breakdown in sheep rumen (Patra and Saxena, 2010). Condensed tannins not only protect degradation of proteins by the microbes, but CT also increase rumen escape protein levels, which means more N sources and energy available to sheep (Bunglavan and Dutta, 2013). At the same time, the CP concentrations in willows can support the demand for metabolisable protein in ewes (Preston and Leng, 1987).

2.8.4. Trace elements

Willow can provide important trace minerals to ruminants such as sheep. Willow trees, for example, contain higher levels of zinc and cobalt than sheep require (Kendall et al., 2021). The contents of these two elements are mainly determined by the species of willow and are basically unaffected by season and geographical location, while the concentrations of selenium and iodine are affected by location and season, respectively (Kendall et al., 2021). Among them, cobalt is highlighted as the most important element in lamb growth. Sheep require cobalt as a mineral during growth because the rumen microbiome requires this trace element in the synthesis of vitamin B12 (Wang et al., 2010). Cobalt deficiency in weaned lambs not only reduces daily gain but also suppresses non-specific immunity (Silk, 2016).

2.8.5. Parasitic pressure

Plants with CT can alleviate parasitic stress in ruminants (Waghorn, 2007). Dry matter of willow leaves contains about 30 to 50 grams of CT per kilogram (Wilkinson, 1999; Campbell et al., 2018). Mupeyo et al. (2010) conducted two experiments raising lambs in indoor individual pens, comparing diets based on alfalfa and chopped willow. Their results showed that lambs supplemented with willow had significantly lower gastrointestinal parasite burdens. This suggests that willow supplementation may help mitigate the impact of parasites—such as nematodes—that impair protein utilization in ruminants (Mupeyo et al., 2010).

The interference mechanism of CT on intestinal parasites can be divided into direct and indirect parts. In an indirect mechanism, CT bind to leaf proteins and prevent the complex from being degraded in the rumen at neutral pH. The binding body then breaks down when the environment in the abomasum is acidic and releases proteins through the small intestine to promote protein absorption in sheep (Barry et al., 2001; Jones and Mangan, 1977). The direct mechanism of CT may act directly on proteins on the parasite's surface, thus impeding its physiological function (Hoste et al., 2006).

2.8.6. Willow species and nutritional value

Main willow varieties in early New Zealand were *Salix fragilis* (crack willow) and *Salix. alba* var. *vitellina* (golden willow). However, these two types of willows grow relatively slowly, and their branches are prone to breakage, so they are not suitable for wide cultivation (Hathaway, 1977). The cloning of *Salix alba* was introduced from Italy and hybridized with *Salix* *matsudana* from China, resulting in *Salix matsudana* Koidz. *x alba* L, which grew faster and was drought-tolerant (Hathaway, 1977). This hybrid willow often appears as supplementary feed in studies designed by New Zealand scholars. In addition, *Salix kinuyanagi* Kimura (Kinuyanagi willow), which was introduced from the UK, has also become one of the common willow varieties in New Zealand nowadays (OPPONG et al., 2001). Organic matter digestibility of *Salix matsudana × alba* was 64%-72%, which was higher than 40%-65% of *Salix kinuyanagi* and willow varieties in other regions (Table 2.4). The CP concentration of Salix matsudana × alba from the same study was higher than that of Salix kinuyanagi, but the NDF was lower than or equal to that of Salix kinuyanagi (Table 2.4).

Table 2.4: Nutritional composition of various willow varieties in the past related studies

Cultivars	**Season**	**OMD[2] (%)**	**CP (g/kg DM)[3]**	**NDF[4] (g/kg DM)**	**ADF[5] (g/kg DM)**	**Reference**
Salix matsudana × alba	Summer	64	137.5	330	259	Douglas et al., (1996)
Salix kinuyanagi		40	131.25	365	303	
Salix matsudana × alba	Summer/Autumn	71.4	132	381[5]	264[5]	Kemp et al., (2003)
Salix kinuyanagi		65	71	381[5]	264[5]	
Salix acmophylla	Autumn	51.95	136	449	362	Muklada et al., (2018）
Salix matsudana × alba	Summer/Autumn	70	100			Lira et al., (2007)
Salix matsudana × alba	Summer/Autumn	72	100	370		Pitta et al., (2006)
Salix matsudana × alba	Summer/Autumn	70.7	143.75			Ramírez-Restrepo et al., (2010)

Salix alba[1]	Summer	56.46	228.3	636.7	286.1	Gürsoy, (2024)

[1]：Only leaves do not contain stems；[2]: OMD = organic matter digestibility; [3]: CP = crude protein, DM = dry matter; [4]: NDF = neutral detergent fibre; [4]: ADF = acid detergent fibre; [5]: Average values of NDF and ADF of two kinds of willows.

Metabolisable energy of *Salix alba* leaves were measured by Oruç and Avcı (2018) at 8.8 to 8.95 MJ per kg of DM. Another study of the same willow species in the same area found that the ME value of willow leaves was about 5.4 MJ per kg of DM (Özdemir and Kaya, 2020). Leaf digestibility of *Salix alba* from Turkey in Table 2.4 is much lower than that of *Salix matsudana* × *alba* in New Zealand. Moreover, *Salix acmophylla* from Israel (add ref from table) had lower digestibility and ME than the New Zealand hybrid willow, which may be related to species and climate factors (Table 2.5). Willows in the Middle East have a higher degree of lignification and at the time of measurement the Israeli willows were measured on samples taken in the autumn and therefore had stems larger than 5 mm (Muklada et al., 2018). The nutritional value of willow varies with growing area, variety, season, age and detection method.

Table 2.5: Metabolisable energy of *Salix matsudana* × *alba* and pasture in New Zealand during summer and autumn

Cultivars	**Season**	**ME (MJ/kg DM)**	**Reference**
Salix matsudana × *alba*	Summer/Autumn	9.3	Moore et al. (2003)
Pasture		8.4	
Salix matsudana × *alba*	Summer/Autumn	10.7	Lira et al. (2007)
Pasture		9.7	
Salix matsudana × *alba*	Summer/Autumn	10.36	Ramírez-Restrepo et al. (2009)
Pasture		9.17	
Salix matsudana × *alba*	Summer/Autumn	10.7	Pitta et al. (2006)
Pasture		7.5	
Salix matsudana × *alba*	Summer/Autumn	10.1	McWilliam et al. (2005)
Pasture		7.5	
Salix matsudana × *alba*	Summer/Autumn	10.4	McWilliam et al. (2005)
Pasture		7.2	
Salix matsudana × *alba*	Summer	10.11	Pitta et al. (2008)
Pasture		8.31	
Salix matsudana × *alba*	Summer/Autumn	9.87	Pitta et al. (2007)
Pasture		8.16	

2.9. Conclusion of literature review

Reducing CH_4 emissions by improving digestive efficiency, reducing N waste by improving N utilization, supporting carbon sequestration by increasing tree cover, and enhancing farm sustainability and resilience with drought-tolerant, nutrient-rich feed, willow offers a potentially versatile solution to New Zealand's mitigation goals. Although studies of willow growth performance and energy metabolism in ruminants in New Zealand have existed since the end of the last century, most of them appeared before 2010 and used hybrid willow varieties. In addition, past studies have mostly used *in vitro* digestion techniques that may differ from more realistic metabolic conditions. In terms of feeding patterns, previous experiments mostly adopted the feeding pattern of grazing on different feed blocks and the purpose of the previous studies was not to determine feed take or under the digestibility of willow DM and its nutrients.

This study used *Salix alba* L., a willow variety that has not been used in previous studies and adopted a metabolic crate feeding model to determine energy and N metabolism of willow.

The experiment hypothesized that willow trees can improve the energy and N utilization of lambs and improve their growth performance during the summer. Another major hypothesis of the study is that willows reduce the proportion of GE that lambs use to produce CH_4.

The aim of this experiment was to compare the effects of feeding willow supplement diet (referred to hereafter as the ryegrass pasture + willow diet) on energy balance and N utilization of growing Romney lamb under summer drought conditions, and to provide insights for further research on the effects of ryegrass pasture + willow diet on improving animal performance and reducing CH_4 emissions in ruminants.

3. Chapter 3 Method and Material

3.1. Experimental design

The study was conducted at Massey University in Palmerston North, New Zealand (40° 22’39”S, 175°36’26”E), during the summer of 2024. All procedures were conducted in accordance with the Massey University Animal Ethics Committee (protocol AEC 06/119). The experiment used a completely randomized design with two feeding treatments: control (ryegrass pasture) and experimental (ryegrass pasture + willow).

3.2. Animal Management

The study utilised twelve (12) Romney wether lambs, approximately 5 months of age (32.6 ± 4.28 kg LW). The lambs were randomly assigned, using random number generation in Excel. Sheep with randomly generated even numbers were assigned to the control diet, while those with randomly generated odd numbers were assigned to the ryegrass pasture + willow diet.

3.2.1. Management in outdoor paddock

All the lambs were grazed in a paddock adjacent to the indoor facility for three days at the start of the experiment. In addition to grazing, lambs were also offered approximately 6 kg of freshly cut willow branches daily, to familiarise them with the willow forage prior to the trial.

3.2.2. Management in indoor pens

After acclimatisation in the outdoor paddock, the lambs were moved to indoor individual pens (1.94 m^2). They were housed in these pens for three days to aid in the subsequent transition and adaptation to the metabolism crates. Upon entry to the indoor pens, lambs were allocated to one of two feeding treatments: either 100% fresh pasture (Control), or an approximate mix of 70% fresh pasture and 30% willow leaves (Experimental: ryegrass pasture + willow). Feed was added to the feeder daily at 10:00 a.m., 1:00 p.m., and 4:00 p.m. All lambs were weighed before entering the individual pens. During this period, the feed offered and refused was weighed every day to determine the daily feed intake of each lamb.

3.2.3. Management in the metabolism crates

After the adaptation to indoor, individual pens, lambs were placed into individual, indoor metabolism crates with an area of 0.465 m^2 for three consecutive days. Each crate was equipped with separate feed and water containers. The metabolism crates were positioned so that the lambs could see but not touch each other. All lambs were weighed before entering the metabolism crates. Lambs were held in the metabolism crates for three days and fed their allocated feeding treatment. During this period total faecal and urine collection was conducted, and daily feed samples were collected.

3.3. Metabolism trial

The Control feeding treatment was 100% fresh cut pasture, which consisted of perennial ryegrass (*Lolium perenne L.*) and a small amount of white clover (*Trifolium repens L.*). The Experimental treatment was diet of approximately 30% fresh willow leaves and 70% fresh pasture (ryegrass pasture + willow).

A sickle-bar mower was used to harvest fresh pasture daily. This was delivered to the experimental site between 7:00 and 8:30 am every day. Willow branches were hand cut fresh each day from a tree block adjacent to the experimental site. The willow leaves were stripped from the harvested willow branches, leaving only leaf material and some thinner branches (less than 5 mm in diameter) to be fed to the lambs (Figure 3.1).

Figure 3.1. Illustration of the stripped willow (*Salix alba*) branches (upper image) that were discarded and the leaf material and thinner stems (lower image) that were fed to the lambs.

3.4. Feeding regime and access to water

The daily amount of DM offered to each sheep was calculated as 5% its LW prior to being housed in both the indoor individual pens and the metabolism crates. The total daily feed allowance for each lamb was weighed out in the morning and the feed was then offered over three feeding times throughout the day at 10:00 am, 1:00 pm and 4:00 pm, each time providing roughly a third of the total daily feed allowance. If a lamb was observed to be consuming all or most of its feed at the 10:00 am or 1:00 pm feeding times (i.e., leaving little feed residual), an additional portion of the lamb’s total daily allocation of feed was added to the feed bin to ensure that feed was available at all times. If a lamb was observed to be leaving little residual, the subsequent day their total daily feed allowance was increased by 10% of their previously estimated DM intake.

In the indoor, individual pens, water was available *ad libitum*. In the metabolism crates, each lamb was initially provided water at 5% of its LW. This was monitored on the first day of the metabolism crate period by collecting and weighing the refused water to ensure lambs were being offered sufficient water. Ultimately each lamb was provided with a total of 3 litres of water daily for the second and third days of the metabolism crate period. Water offered and water remaining was weighed daily. Fresh water was provided daily.

3.5. Metabolism crate sample collection

3.5.1. Feed samples

During the three consecutive days of the metabolism crate period, daily willow and pasture samples were collected and frozen for subsequent nutritional analysis. Each day of the metabolism crate period 100 grams of fresh pasture was sampled and was manually sorted each day into ryegrass, white clover, dead matter and weeds to determine an approximate botanical composition of the pasture offered.

Feed refusal was measured daily for each lamb. Refusals were collected daily for pasture only, as there was no refusal for the willow component offered in the ryegrass pasture + willow treatment. The feed refusals were pooled together across day and animals, and one sub-sample was sent for chemical analysis.

3.5.2. Faecal and urine samples

Faecal and urine volumes were measured and recorded daily at 9:00 am. The collection of urine and faeces was done through a container located below the metabolic crate. Faeces were collected into plastic bags and weighed, with approximately 20% subsequently subsampled and frozen at -20°C for later chemical analysis.

The urine produced by each lamb was transferred to plastic buckets through a sloped stainless-steel chute under the metabolism crate's mesh floor. Each day, when collected, the urine was acidified with 50 mL of 10% sulfuric acid, weighed and then sub-sampled into labelled bottles. All samples were stored in the freezer at -20°C for later chemical analysis.

3.6. Sample preparation and analysis

The pasture and willow offered, the feed refusal samples, and faecal samples were freeze-dried in a Cuddon 0610 freeze drier (WGG Cuddon Ltd, Blenheim, New Zealand). All samples were weighed prior to and after freeze-drying to obtain a DM percentage. The dried sample was ground through a 1 mm screen using a Wiley mill and stored prior to being sent to the laboratory for nutritional analyses.

The feed offered, feed refusal and faecal samples were all analysed at Massey University's Nutrition Laboratory for DM, ash, OM, N, CP, fat, NDF, ADF, lignin and GE. Urine samples were measured for N. Laboratory method AOAC 942.05 was used to determine ash content. Samples were held at 550ºC for up to 16 hours to determine OM, and an adiabatic bomb calorimeter (Gallenkamp, Loughborough, Leics, United Kingdom) was used to determine GE.

Sample moisture was analysed by AOAC (1994) methods 925.10, 930.15, and the fat was acid hydrolysed using the Mojonnier method (AOAC 922.06). The Dumas method AOAC 968.06 (Leco Corporation, St Joseph, MI, United States) was used to determine total N. The total N was estimated by pyrolysis combustion and then multiplied by 6.25 to determine the CP concentration. NDF was determined by AOAC (1994) 2002.04 method, and ADF and lignin were determined by AOAC (1994) 973.18 method.

3.7. Calculations

The initial DM percentage can be obtained by comparing the weight of the sample after freeze-drying treatment with the weight before treatment, which is conducive to calculating the DMI of lambs and the DM loss of faeces after nutritional analysis. It is necessary to determine the proportion and GE of each nutrient in the pasture and willow to calculate the nutrient and energy intake and nutrient digestibility of each lamb.

For each lamb, each day the DMI was calculated as follow:

a. DMI pasture (kg/day) = Pasture fresh weight (kg/day) × DM × residual DM – refusal fresh weight × DM × residual DM

b. DMI willow (kg/day) = willow fresh weight (kg/day) × DM × residual DM *c.* DMI total (kg/day) = DMI pasture + DMI willow*

(*for the Control feeding treatment that contained pasture only, DMI willow is 0).

The daily intake for the different analytes: organic matter, ash, GE, N, NDF, ADF and lignin were calculated as:

d. AnalyteI (kg/day) = DMI pasture (kg/day) × Analyte pasture + DMI willow (kg/day) × Analyte willow.

The daily faecal DM output (DM_{FO}) was calculated as:

e. DM_{FO} = Fresh faecal weight (kg/day) × DM × residual DM

The daily analyte's of the faecal output (FO) was calculated as:

f. $Analyte_{FO} = DM_{FO} \times Analyte_{FO}$

The daily GE and N excreted in the urine output (UO) were calculated as:

g. GE_{UO} (MJ/day) = urine quantity (L/day) × GE in urine (MJ/L)

h. N_{UO} (g/day) = urine quantity (L/day) × N in urine (g/L)

i. N_{FO} (g/day) = DM_{FO} × N in faeces (g/kg DM)

j. N balance (g/day) = $NI - N_{UO} - N_{FO}$

The daily apparent total tract digestible intake (AI) of each analyte was calculated as.

k. $AI = (AnalyteI - Analyte_{FO})$

The energy losses in CH_4 were estimated as:

l. CH_4 (g/d) = $21.977 \times (\text{DMI total})^{0.765}$ (Swainson et al, 2016)

m. GE-CH_4 (MJ/day) = CH_4 (g/d) × 0.05565 (IPCC, 2006)

The metabolisable energy intake was calculated as:

n. MEI (MJ/day) = (GEI – GE_{fo} – GE_{uo} – $GECH_4$)

The digestible and metabolisable content of the willow alone was calculated as follows for each analyte:

o. The ratio of willow DM intake (Pwillow) in the Expeimental diet was calculated as:

Digestible Analyte in willow (kg/kg DM) = [(digestible Analyte in pasture+willow diet) – ((1-Pwillow) × digestible Analyte in pasture)] / Pwillow feed digestibility coefficient (FDC) (%) = (DMI- DM_{fo})/DMI

Average daily gain (ADG) = (Final LW- Initial LW)/ Experimental days

Feed conversion rate (FCR) (kg/kg DMI) = DMI/ADG

Feed conversion efficiency (FCE) (kg/kg DMI) = ADG/DMI

Where:

DM= percentage of DM of feed divided by 100;

residual DM= percentage of DM obtained at nutrient analysis (after the sample had been freeze-dried and ground) divided by 100;

Analyte = percentage of analyte dived by residual DM percentage ;

I = intake;

FO = faecal output;

UO = urine output;

N = protein concentration in feed divided by 6.25. Faecal N and urine N is measured directly by laboratory analysis.

3.8. Statistical analysis

Data analysis was performed in SAS (version 9.4; SAS Institute Inc., Cary, NC) using a one-way analysis of variance (ANOVA). Average daily gain, apparent digestibility, DMI, FCR, energy utilization efficiency, feed digestibility coefficient, energy, N balance, under different feeding treatments were analysed using a linear model with diet as a fixed effect (PROC GLM) The same model was used for nutrient composition and energy analysis of diet, faeces and urine.

$Y_{ij}= \mu+A_i+e_{ij}$

Where:

Y_{ij}: is the jth observation of the ith treatment group;

μ: a general mean;

A_i: the fixed effect of treatment group i;

e_{ij}: random residual.

The cut-off level of significance was set at 0.05.

4. Chapter 4 Result

In this experiment, one lamb from the Control group was excluded due to low DMI. The data of this lamb was deleted from the data set prior to analysis.

The OM, CP, lignin, fat and GE levels of willow were significantly higher than those of pasture ($P < 0.05$), while the concentrations of ADF, ash and NDF were significantly lower than those of pasture ($P < 0.05$) (Table 4.1).

Table 4.1: Nutrient composition of pasture and willow samples after drying and grinding treatment and laboratory analysis.

	Treatments			
Item (Mean ± SE)[1]	Pasture	Willow[2]	SE	P-value
Samples for analysis	3	3		
Freeze dried DM[2] (%)	37.3	38.6	0.0037	0.0714
Nutritional analysis DM (%)	95.9	95.1	0.0047	0.3094
Organic matter	905.8	918.0	0.0019	0.0103
Ash	94.2	82.0	0.0019	0.0103
Crude protein	100.0	133.6	0.0032	0.0016
Fat	34.8	44.8	0.0021	0.0256
Neutral detergent fibre	562.6	303.2	0.0033	<0.0001
Acid detergent fibre	324.4	223.7	0.0051	0.0001
Lignin	50.8	91.2	0.0042	0.0024
Gross energy (MJ/kg DM)	17.7	18.7	0.0271	<0.0001

[1]:g/kg DM (Dry matter) unless otherwise stated; SE = standard error [2]: Consists of willow leaves and stems smaller than 5 mm.

Through manual dissection of fresh pasture samples, it was found that the botanical composition of pasture during the metabolic crates experiment (3 days) was as follows: ryegrass (46.7%), clover (3.8%), dead material (38.1%), weeds (11.4).

Dry matter intake of willow and pasture in the diet of lambs fed the experimental treatment was 38.5% and 61.5%, respectively. Table 4.3 shows that the apparent dry matter digestibility (DMD) and OMD of lambs in the ryegrass pasture + willow diet (experimental) is higher than that of lambs fed only pasture (control) ($P < 0.05$). The digestibility of CP, fat and NDF of lambs in the experimental group was higher than that of lambs in the control group, but the digestibility of ADF and lignin of lambs in the control group was lower, and the differences were significant ($P < 0.05$). The digestible content of OM, CP, fat and lignin per kg DM in the experimental group was higher, and the digestible content of NDF and ADF was lower ($P < 0.05$, Table 4.2).

Table 4.2: Apparent digestibility and digestible content of nutrients and energy in diets of lambs fed a control (pasture) and experimental (pasture + willow) diet.

Parameter	Treatment			
	Control	Experimental	SE	P-value
	Digestibility (%)			
Dry matter	57.0	63.7	0.015	0.0140
Organic matter	59.1	65.1	0.015	0.0187
Crude protein	50.2	50.8	0.031	0.8999
Fat	54.5	61.6	0.033	0.1639
Neutral detergent fibre	51.2	51.6	0.020	0.9000
Acid detergent fibre	51.3	50.3	0.024	0.7727
Lignin	27.0	26.1	0.049	0.8956
Feed digestibility coefficient[1]	57.2	64.0	0.015	0.0135
Energy utilisation efficiency	44.2	50.5	0.015	0.0183
	Diet digestible content (kg or MJ/kg DM)			
Organic matter	0.526	0.585	0.014	0.0152
Crude protein	0.054	0.060	0.004	0.2569
Fat	0.019	0.024	0.001	0.0383
Neutral detergent fibre	0.280	0.234	0.011	0.0187
Acid detergent fibre	0.162	0.141	0.009	0.1009
Lignin	0.013	0.017	0.003	0.4103
Digestible energy	10.131	11.315	0.273	0.0137
Metabolisable energy	7.892	9.183	0.280	0.0102
Metabolisable energy correct[2]	6.668	7.968	0.280	0.0097

[1]: Digestible nutrients and energy in each kilogram of DM in pasture were obtained, and

digestible amounts of each nutrient in each kilogram of dry matter of willow were calculated according to the DMI ratio of willow and pasture in mixed diet. There is only one value and therefore no statistical analysis is performed. [2]: Metabolisable energy The ME value obtained after subtracting the energy lost due to methane emissions.

The available contents of digestible energy (DE), ME and ME per kilogram of DM present in the experimental diet were higher than those in the control diet ($P < 0.05$). The digestible contents of OM, CP, fat, lignin and energy of willow were higher than those of pasture, while the digestible contents of NDF and ADF were lower. The lambs receiving ryegrass pasture + willow diet (Experimental) had higher FDC and energy use efficiency, and the differences were significant ($P < 0.05$).

During the course of the experiment all the lambs across both treatments lost. Romney lambs fed pasture and pasture + willow both had an average weight loss of 8.3% (i.e. 33.9 versus 31.3 and 32.2 versus 29.3) (Table 4.3). There were no significant differences in all parameters related to growth performance of lambs ($P > 0.05$).

Table 4.3: Growth performance of lambs fed a control (pasture) and an experimental diet (pasture + willow) over 3 days in metabolism crates.

Parameter	Treatment			
	Control	Experimental		
	Mean	Mean	SE[1]	P-value
Initial live weight (kg)	33.9	32.2	1.85	0.5135
Ultimate live weight (kg)	31.3	29.3	1.94	0.4873
Daily weight gain (kg/d)	-0.4	-0.5	0.07	0.7474
Dry matter intake (kg/d)	0.8	0.9	0.05	0.6068

[1] SEM = Standard error.

Lambs that received the willow supplement with pasture (Experimental) had a higher N intake ($P > 0.05$), and while faecal N loss was higher than that of lambs that only ate pasture (control) ($P > 0.05$), urine N loss was significantly lower ($P < 0.05$) (Table 4.3). Nitrogen balance of lambs in the control group was significantly lower than that in experimental group ($P < 0.05$). The proportion of faecal N loss in the experimental group was slightly higher than that in the control group ($P > 0.05$), but the proportion of urinary N loss and N balance was significantly higher than that in the control group ($P < 0.05$).

The GE intake and DE (15.36 MJ/d; 9.60 MJ/d) of lambs fed the experimental diet was higher than that of lambs fed the control diet (14.45 MJ/d; 8.22 MJ/d) ($P > 0.05$) (Table 4.4). The faecal energy (FE), urine energy (UE) loss was lower than that of lambs fed pasture fed alone (5.75 MJ/d vs. 6.23 MJ/d; 1.80 MJ/d vs. 1.81 MJ/d) ($P > 0.05$). The residual energy of the experimental group after subtracting FE and UE from GE intake was also higher than that of the control group (7.81 MJ/d vs. 6.41 MJ/d) ($P > 0.05$), and the energy loss for CH_4 generation was lower than that of the control group (0.44 MJ/d vs. 0.37 MJ/d) ($P < 0.05$). Except for UE, there was a significant difference in the proportion of other energy to GE intake between the two treatments ($P < 0.05$) (Table 4.4).

Table 4.4: Energy and N balance of lambs in the control group (Pasture) and experimental group (Pasture + Willow).

Parameter	Treatment			
	Control	Ryegrass pasture + willow	SE	P value
	Nitrogen			
N intake, g/d	13.8	15.9	0.0	0.1281
Faecal N, g/d	6.9	7.8	0.0	0.3683
Urinary N, g/d	7.9	5.7	0.0	0.0399
N balance, g/d	-1.0	2.4	0.0	0.0230
Faecal N, %	49.5	50.0	3.1	0.9013
Urinary N, %	57.3	35.9	4.1	0.0051
N balance (%)	-6.8	15.1	6.0	0.0310
	Energy			
GE intake, MJ/d	14.45	15.36	0.87	0.4792
Faecal energy, MJ/d	6.23	5.75	0.43	0.4591
Urinary energy, MJ/d	1.81	1.80	0.21	0.9732
Digestible Energy, MJ/d	8.22	9.60	0.57	0.1213
Faecal energy, %	43.09	37.47	1.54	0.0302
Urinary energy, %	12.54	11.60	1.10	0.5648
Retained energy, %	44.37	50.93	1.61	0.0186
Estimated CH_4 energy loss, MJ/d	0.37	0.44	0.02	0.0162

[1]: The ratio of faecal N output, urinary N output and N balance to N intake

[2]: Gross energy minus FE and UE [3]: The percentage of each energy in GE intake

5. Chapter 5 Discussion

The purpose of this study was to determine and compare the energy and utilisation of lambs fed pasture + willow and lambs fed pasture. Secondly, the digestibility and LW change of lambs in different treatment groups was measured. The trial also predicted ME intake and CH_4 production. The main finding was that supplementation of willow with pasture in lambs increased GE, ME, N intake, N balance and decreased urinary N loss and FE loss, but increased faecal N, CH4 energy loss and LW loss.

5.1. Nitrogen

In the present experiment, the protein concentration of the willow supplement was higher than that of the pasture fed. As a result of this, faecal N content increased but urinary N content decreased in the Experimental lambs compared to the Control lambs, indicating better use of N within the body. This may be due to the proportion of non-structural carbohydrates (NSC) in willow being higher than in summer pastures (43.6% vs. 20.8%; Calculation: NSC% = ((1000-NDF-CP-Fat-Ash)/1000), which enhances rumen N utilisation efficiency. Non-structural carbohydrate supplementation has been shown to reduce rumen ammonia levels while promoting microbial protein synthesis (Chamberlain et al., 1985). The primary factors influencing N use efficiency in ruminants are rumen metabolism and dietary CP content (Tamminga, 1992; Huhtanen and Hristov, 2009). Ammonia utilisation and microbial protein synthesis are optimised when energy and protein supply are synchronised (Johnson, 1976), and this synchronicity is influenced by fluctuations in carbohydrate and N content in feed (Hristov and Pfeffer, 2005). The higher the concentration of NDF and the lower the concentration of CP in feed, but the higher the N use efficiency of ruminants (Delevatti et al., 2019). Some studies have found that increased dietary CP levels lead to increased urinary N loss (Castillo et al., 2001) whereas Nielsen et al. (2003) stated that feeding high-protein pasture increased faecal N excretion.

The inclusion of willow as a supplemental feed increased faecal N content (Table 4.4) without significantly affecting CP digestibility (Table 4.3). One theory is that CT in willow, can alter N partitioning, shifting urinary N excretion to faecal N excretion, thereby maintaining N retention (Theodoridou et al., 2010). However, it is more likely linked to the high protein content of willow leaves (Table 4.2), which may have exceeded the lambs' N requirements, leading to increased faecal N excretion as the proportion of N lost as faeces did not differ between treatments (Table 4.4)

When the CP content of dietary DM exceeds 10%, it is generally sufficient to meet the nitrogen requirements for maintenance (Hynd, 2019). However, to support growth in lambs, the CP level needs to reach approximately 16% of dietary (Haddad et al., 2001). In this experiment, the CP content of summer pasture and willow was 10% and 13.4%, respectively. This indicates that the control group met only the maintenance requirement, while the willow-supplemented group exceeded it, yet neither diet provided sufficient CP to meet the requirements for optimal lamb growth.

Previous studies have demonstrated that CT supplementation in growing ruminants can enhance N retention (Naumann et al., 2017). When ruminants consume forages without CT, such as ryegrass, rumen microbes degrade approximately 75% of the protein into ammonia (Barry et al., 2001). While most N-containing compounds are eventually synthesised into microbial proteins, a portion of absorbed N is excreted as urea in urine when ruminants are fed fresh forage (Min et al., 2003). Consequently, urinary N excretion was higher in lambs fed only summer pasture compared to those supplemented with willow.

5.2. Energy

5.2.1. Metabolisable energy

In this study, both DE and predicted ME intake of lambs in the experimental group were higher than those in the control group because CP, water-soluble carbohydrates (WSC) (Water-soluble carbohydrates are inversely proportional to NDF) and fat were higher in the experimental diet (Table 4.1) and these are directly proportional to ME and DE (Stergiadis et al., 2015). As CH_4 emissions were not measured in the present study ME was predicted using the Inventory framework (Ministry of Primary Industries, 2016). The ME required for lamb production, grazing, and gestation was assumed as 0 due to the lamb being in metabolic crates and no LW increase. The equation 5.1 was used to predict the maintenance ME of lambs in this experiment.

Equation 5.1: Maintain metabolisable energy prediction model (Ministry of Primary Industries, 2016)

$$ME_m = K \times S \times (0.28LW^{0.75} \times e^{-0.03A})/km + 0.1 \times ME_p$$

Where ME_m: Maintenance metabolisable energy K: difference coefficient of fasting heat production, lamb is 1; S: The difference coefficient of basal metabolic rate of different sex was 1 for castrated lamb; LW: Lamb weight before experiment; A: Age; $e^{-0.03A}$: close to 1 in lambs less than one year old; MEp: ME required for production.

The experimental group dietary ME intake was higher than maintenance requirement, and the control group's dietary ME intake was lower than maintenance requirement (control: 6.67 *vs.* 7.89; experimental: 7.97 *vs.* 7.22). However, LW loss was observed in all lambs in this study, indicating that ME intake was insufficient to meet the need to maintain ME requirements.

In this study, the calculated ME of willow and pasture were 10.04 MJ/kg DM and 6.67 MJ/kg DM respectively. Metabolisable energy of willow was similar to that of other studies in Table 2.5, but the ME of pasture was lower than that of other studies. The willow used in this study, as one of the maternal varieties of New Zealand hybrid willows, shares similar chemical properties (Table 2.4; Table 2.5). Furthermore, a possible reason is that most of the pastures in other studies were collected in summer and autumn, and the quality of pasture improves in autumn, thus increasing the average ME (Waghorn and Clark, 2004). This study was conducted in late summer, which is the stage of higher pasture maturity leading to lower ME. Therefore, the lower DMI and pasture ME might be the main reasons for the LW loss.

5.3. Digestibility

5.3.1. Neutral detergent fibre and Acid detergent fibre

Apparent digestibility of lambs in the experimental group was 10% greater than that of lambs in the control group (Table 4.3). This study is consistent with the results of several recent studies, which found that increasing dietary NDF content decreases the digestibility of dietary DM, CP and NDF (Molavian et al., 2020; de Paula Carlis et al., 2021). A ratio of NDF to DM of less than 30% or more than 70%, and a ratio of ADF to DM of less than 20% or more than 50% will lead to a decrease in digestibility (Hynd, 2019). The levels of NDF and ADF in the summer pasture and willow in the experiment were within the acceptable range, suggesting that fibre content may not have significantly influenced the results. Instead, the higher CP and WSC content in the experimental diet likely contributed to the greater digestibility observed. Two other studies found that apparent digestibility increased when NDF content was between 20% and 30% (Wang et al., 2016; Oh et al., 2016). Therefore, the results of this study may indicate that the apparent digestibility is inversely proportional to the NDF content when the NDF content is higher than 30%, but the effect of dietary type should be considered.

Previous studies have found that OMD and digestible OM in DM declined at a lower rate in lambs fed willow than in the same period of pasture (McWilliam et al., 2005). Neutral detergent fibre and ADF are lower in willow leaves than in willow stems and are therefore easier to digest (Muklada et al., 2018). New leaves located on the upper branches of willows have less NDF and ADF and higher digestibility (Muklada et al., 2018). Furthermore, intake of secondary metabolites in willow can promote digestion of NDF (Salem et al., 2016). Concentration of ADF and NDF in willow was lower than that in the summer pasture fed, but the fermentable carbohydrate was higher. This may be due to willow having a phenolic glycoside content of about 18%, which supplements additional NSC (Kemp et al., 2003). There was a positive association between soluble carbohydrates and DMD in fresh diets (Miller et al., 2001).

The willow feed had significantly lower NDF (303.2 g/kg DM) and ADF (223.7 g/kg DM) compared to the pasture (NDF: 562.6 g/kg DM, ADF: 324.4 g/kg DM). This aligns with results from a study evaluating *Salix matsudana* × *alba* by Pitta et al. (2006) (NDF: 370 g/kg DM) and Ramírez-Restrepo et al. (2010), which suggests willow species tend to have moderate NDF and ADF values. The *in vivo* digestibility found in the present study for the experimental diet was lower but is within the range of OMD reported in Table 5.2. This highlights the potential of willow as a nutritional supplement for lambs, particularly during summer, when pasture quality tends to decline.

5.3.2. Lignin

The cell wall of the forage partly determines feed digestibility because the digestion of soluble substances in neutral detergents is close to complete (Weisbjerg et al., 2004). Hemicellulose in NDF is digestible while the ADF and lignin are difficult to digest (Hynd, 2019). The lignin content in willows was higher than that in summer pastures (Table 4.1). This may be because the rigidity of willow leaves is mainly derived from lignin (Gonzalez-Zurdo et al., 2016). However, with an increase in age of willow leaves, the increase of structural components such as NDF, especially lignin, leads to a decrease in cellular soluble material, resulting in a decrease in digestibility (Van Soest, 1995). Therefore, it is best to choose younger willow leaves as a supplementary diet for ruminants in summer.

5.3.3. Organic matter digestibility in vitro and organic matter digestibility in vivo

Apparent OMD of the willow treatment in this study was similar to that of other New Zealand willow treatment used in summer experiments but lower than that used in experiments that spanned summer and autumn (Table 2.4). Kemp et al. (2003) stated that the value of OMD *in vitro* increased by 10% compared to the digestibility of OM *in vivo*. Therefore, the ME predicted by *in vitro* OMD will also be high because for most experiments the in *vitro* digestibility is used and ME is calculated by the DOMD. In Table 2.4 and Table 2.5, the results of some previous studies on the nutritional composition of willow in the summer and autumn dry seasons were summarised. It was found that the average *in vitro* OMD of willow (*Salix matsudana*×*alba*) was 69.6%, and the average ME was 10.2MJ/kg DM. Although *in vitro* OMD in past studies was higher than that of willow (69%) in this study, ME is similar to the willow in this study, and the difference between OMD did not reach 10%, which was different from the situation described by Kemp et al. (2003). However, the ratio of willow leaves in this experiment may be higher than in most studies because only the willow leaves and stems at the tip of willow branches were used as supplementary feed. The digestibility of willow in other experiments mostly came from multiple collection in summer and autumn, and the ratio of willow leaves to branches was lower than that in this experiment, so the OMD should be lower than that in this study. As a result, OMD *in vitro* may indeed be somewhat overestimated compared to OMD *in vivo*. There may also be differences due to different willow species. Multiple simultaneous *in vitro* and *in vivo* digestibility measurements may be required to further validate this hypothesis.

5.4. Live Weight change

There was no difference in LW change between treatments. Moore et al. (2003) recommends using low proportions of willow as a supplement because including high amounts of willow in the diet does not show a difference from low level inclusion of willow as a supplement. In this experiment, because the experiment time was much shorter than the duration of the above experiment (81 days) and the recommended feeding time (120 days), there was no significant difference in weight loss between the experimental group and the control group. Most past studies have shown that willow supplementation can reduce LW loss in lambs during the summer when they are free to feed, which was not statistically relevant in the present study (Moore et al., 2003; McWilliam et al., 2005; Pitta, 2007). In the summer grazing experiment, there was no significant difference in the weight change of lambs on willow feed blocks and control pastures, while the weight gain of lambs on willow feed blocks in late autumn decreased (Ramirez-Restrepo et al., 2009). In the autumn, nutrients are transferred from the leaves to the roots of the willow, so the nutrient level of the willow may be lower than that of the pasture in the autumn.

Willow contains secondary compounds that require detoxification in ruminants through processes such as oxidation or hydrolysis, which results in energy loss (Foley and McArthur, 1994; Illius and Jessop, 1995). In addition, the energy cost of detoxification can be measured in terms of heat increment of feeding, and an increase in the proportion of willow leaves increases the heat increment of feeding (White and Lawler, 2002). Although detoxification costs can be compensated by reduced CH_4 production, the energy expended for detoxification is higher than the energy saved by reduced CH_4 production (White and Lawler, 2002). Future studies should focus on measuring the secondary compounds ingested by the lambs as well as understanding the trade-offs of the detoxification process.

5.4.1. Methane

Dietary gross energy, DE, UE and FE of each treatment mean were determined, and ME was estimated. The linear relationship between ME and DE can be used to predict CH_4 energy loss, and this conversion method is suitable for pasture-based diets, so it matches the present experiment (Hales, 2019). In this experiment, the ME to DE ratio of the experimental group was greater than that of the control group, so the ratio of energy used for CH_4 emission was reduced by the willow supplement, although the difference was not significant.

The more scientifically based prediction models mainly predict CH_4 emissions based on DMI and ME (Benaouda et al., 2019). Lambs with low CH_4 emissions also had lower dietary digestibility (Pinares-Patino et al., 2011). However, in this experiment, because DMI and ME intake of the experimental group were higher than those of the control group, CH_4 emission of the experimental group was estimated to be higher than that of the control group. This needs further investigation because predicting CH_4 through ME and DMI models may overlook the effects of certain chemical components of different plant feeds on CH_4 emissions, such as secondary metabolites in willow. Therefore, to further explore the effect of willows on CH_4 emission reduction in ruminants, CH_4 emissions should be determined by other CH_4 emission measurement methods (eg. sulphur hexafluoride tracer method). As the nutritional value of willow is generally higher than that of pasture in the summer, this can lead to higher estimates of CH_4 emissions from the prediction models and thus lower prediction accuracy.

5.4.2. Faecal energy

The ratio of DE to GE under different treatments was similar but slightly higher in the experimental group than in the control group. However, there may be some error in this result because no collection bag attached to the lamb was used to collect faeces. Some pasture fell into the bottom faeces collector during the feeding of the lambs, which may lead to a high FE and a low DE result. On the other hand, during the process of collecting faeces, some faeces remained in the metabolic crate and the ground, which may lead to the reduction of FE loss in some lambs. The above reasons may lead to the results of the experiment that the LW loss of lambs in the experimental group is slightly higher than that in the control group, but the energy balance of lambs in the experimental group is higher than that in the control group. Consequently, there are some errors in the nutrient composition and energy of faeces in this study. In addition, higher pasture maturity results in lower ME intakes. In this study, the NDF content of pasture accounted for 56.3% of DM content. The decrease of DE to GE ratio was associated with the increase of FE loss caused by the decrease of diet quality (Ramirez-Restrepo et al., 2019).

5.4.3. Urine energy

Gross energy of urine samples from the experimental group was higher than that of the control group. Urine energy is not strongly related to DMI but is positively proportional to CP concentration (Hemphill et al., 2018). Urine contains 3% to 5% of GE intake due to energy loss (Johnson and Johnson, 1995). In this experiment, the UE loss of the experimental group and the control group accounted for 11.6% and 12.5% of the GE intake, respectively. Low GE due to insufficient dietary supply may be a reason. The negative N balance of lambs in the control group indicates that more N is not utilized, resulting in high UE. In the experimental group, although the N balance was positive, UE loss was similar. Lambs supplemented with willow may have higher concentrations of non-nitrogen organic compounds such as VFA and NSC in their urine, resulting in a lower urinary N content but a similar higher UE than control lambs. In other experiments, UE loss was inversely proportional to pasture maturity (Ramirez-Restrepo et al., 2019). However, the pasture in this experiment was relatively mature, so the urine N in this experiment may be partially derived from lamb itself.

6. Limitations and future prospects

There are some limiting factors in this study which lead to some errors in the experimental results. The refusal of lambs to feed while housed in individual indoor pens was primarily due to their reluctance to eat from the feeders. During feeding, some of the offered feed fell to the ground and mixed with faeces, making the faeces collection more labourious. Therefore, subsequent studies should use collection bags attached to lambs to collect faeces, which would improve the accuracy of experimental results.

This experiment did not measure the CH_4 emissions of lambs, and the prediction model may result in the erroneous CH_4 emissions due to the neglect of the chemical composition of the willow itself. This study did not include an analysis of secondary compounds in willow, due to its cost. This likely affected the estimation of energy lost as CH_4, leading to inaccuracies in the calculation of ME. Future research should prioritise direct measurement of CH_4 emissions of lambs fed willow as well as consider incorporating data on secondary metabolites, to further investigate the effects of willow on energy and N metabolism in lambs.

It would also be valuable to evaluate lamb performance and CH_4 emissions under grazing conditions; however, this is currently limited by the difficulty of accurately measuring intake. The performance results reported in this study have limited relevance, as the study was not powered to detect performance differences, even if they exist. Nevertheless, willow shows promise as a summer supplement for lambs, as they appear to readily consume it. In addition, estimating the carbon sink potential of willow within a lamb production system could offer insights into its role in offsetting emissions. Such information would help clarify potential pathways to adoption, particularly if willow contributes to both environmental and production goals.

7. Conclusion

Willow has important potential to promote energy and N utilization in ruminants. The results of this study showed that willow improved the apparent digestibility, ME intake and N balance of lamb, and significantly reduced urinary N concentration. Although the experimental results did not show that willow supports the growth of lambs and reduces the emission of CH_4, willow reduces the proportion of energy loss to CH_4 in GE. In addition, the nutritional value of *Salix alba* was comparable to that of the New Zealand hybrid willow used in other studies. When ruminants adapt to willow, *Salix alba* can be selected as a summer supplementary feed.

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