Charles Sturt University

The effects of an oral rehydration solution on rumen pH and blood glucose in feed restricted ewes

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Table of Contents

List of Tables

List of Figures

Declaration

I, Teagan Colless

Hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgement is made in the dissertation. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere is fully acknowledged.

The research presented and reported in this dissertation was conducted in compliance with the National Health and Medical Research Council Australian Code for the Care and Use of animals for scientific purposes 8th edition (2013). The research study received animal ethics approval from the Charles Sturt University Animal Care and Ethics Committee, Approval Number *A25049*.

I confirm that my Honours Supervisor agrees that this dissertation is ready for examination.

Teagan Colless_____ ___10May2026____

Signature Date

Allan Gunn DACT, FRCVS___ ___11May2026____

Supervisor's signature Date

Abstract

Background

Pregnancy toxaemia is a metabolic disorder of late gestation in ewes characterised by negative energy status, hypoglycaemia and hyperketonaemia. Treatment includes glucose containing oral rehydration solutions, but the effects on rumen pH and risk of sub-acute ruminal acidosis and acidosis remain unclear. This study evaluated the effects of an oral rehydration solution on rumen pH and metabolic variables in feed-restricted ewes.

Methods

Twelve non-pregnant Merino ewes were randomly allocated to Treatment (oral rehydration solution) or Control (water) groups (n = 6) following 24 h feed restriction. Rumen pH, blood glucose, insulin and electrolytes concentrations and blood gases were measured prior to, and post-treatment. Data was analysed using mixed-effects models and Welch two-sample t-tests.

Results

Rumen pH decreased over time in both groups, with a greater reduction in the Treatment groups; however, values remained within the physiological range, and no acidosis occurred. Oral rehydration solution administration increased blood glucose and insulin concentrations (Δ glucose $p = 0.002$; Δ insulin $p = 0.040$). Blood sodium and chloride concentrations increased ($p < 0.001$), while other variables did not ($p > 0.05$).

Limitations

Limitations included the small sample size (n = 12), and use of healthy, non-pregnant ewes, which did not replicate changes in late gestation, nor the metabolic alterations associated with clinical pregnancy toxaemia.

Conclusion

The oral rehydration solution increased blood glucose, insulin and electrolyte concentrations without inducing ruminal acidosis despite reducing rumen pH. These findings supported the use of oral rehydration solutions as a therapy for hypoglycaemia, although, further research in pregnant and clinically affected ewes is required.

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Glossary of Acronyms

ADH	Anti-diuretic hormone
ANOVA	Analysis of variance
BCS	Body condition score
bHB	Beta-hydroxybutyrate
CNS	Central nervous system
CI	Confidence interval
IL-1β	Interleukin-1beta
IV	Intravenous
MCP-1	Monocyte chemoattractant protein-1
ME	Metabolisable energy
NEFA	Non-esterified fatty acid(s)
NSAID	Non-steroidal anti-inflammatory drug(s)
ORS	Oral rehydration solution
PT	Pregnancy toxaemia
SARA	Subacute ruminal acidosis
SGLT-1	Sodium-glucose co-transporter 1
TNF-α	Tumour necrosis factor alpha
VFA	Volatile fatty acid(s)
VLDL	Very low-density lipoproteins
Vytrate	Vytrate™ liquid concentrate, Jurox

Chapter 1: Literature Review

1.1 Introduction

Metabolic disorders in periparturient ewes are a significant cause of mortality in sheep production systems (Kirk et al., 2025). One of these metabolic diseases is pregnancy toxaemia (PT), which is also referred to as twin lamb disease, gestational toxaemia, ketoacidaemia, or ovine ketosis. It is a relatively common disorder of late pregnant ewes, typically in the last 5-6 weeks of gestation (Van Saun, 2000), especially in those ewes with more than one fetus. Pregnancy toxaemia is associated with severe metabolic and pathological disturbances, and a poor prognosis for survival of both the dam and the fetuses, resulting in welfare compromise and production and economic losses (Souto et al., 2019; Scott et al., 1995b). Sandoval et al. (2026) described seven outbreaks of pregnancy toxaemia in sheep and goats in Argentina, reporting morbidity rates ranging from 3–24% and mortality rates of up to 100% among affected animals. Most sheep in Australasia are extensively grazed resulting in limited information on the incidence of PT. Field studies of peri-parturient ewes in Australia reported an overall mortality risk of at least 2–2.5% during late pregnancy and lambing, with metabolic diseases, including pregnancy toxaemia, contributing substantially to these losses under commercial conditions (Kirk et al., 2025).

Pregnancy toxaemia is a poorly understood metabolic disorder of aberrant glucose homeostasis, with a poor prognosis regardless of treatment in clinical cases (Henze et al., 1998). Monitoring of animals in extensive flocks is often infrequent, typically resulting in a hopeless prognosis once the condition is detected clinically. Clinical signs include, but are not limited to, separation from the flock, anorexia and dull mentation, central blindness, bruxism, ptyalism, head pressing, star gazing, facial tremors, hyperaesthesia, and incoordination leading to recumbency (Khames Mustafa et al., 2023; Simões et al., 2020). Treatment regimens include the enteral administration of 160 mL of a proprietary oral rehydration solution (ORS) such as Vytrate™ liquid concentrate, Jurox (Vytrate), aimed at improving blood glucose concentrations (Buswell et al., 1986). The use of oral glucose in feed-restricted ruminants is controversial due to concerns about rumen microbiome disruption and induction of rumen acidosis. The focus of this was the pathophysiology of PT and treatment regimens associated with the condition.

1.2 Pathophysiology of pregnancy toxaemia

1.2.1 Negative energy status and glucose dysregulation

Pregnancy toxaemia occurs when the escalating feto-placental energy and glucose demands of late gestation, and lactogenesis/colostrogenesis, result in disturbances of maternal glucose and lipid metabolism. The energy imbalance typically occurs in multitocous pregnancies and is exacerbated by

the relative decrease in the size of the gastrointestinal tract and rumen due to the increased size of the gravid uterus. Fetal growth in sheep is non-linear, with approximately 90% of fetal mass accumulated during the last third of gestation (Makela et al., 2022). Rapid fetal growth and increased feto-placental glucose utilisation near term substantially increase maternal energy requirements (Leury et al., 1990; Wallace et al., 2002). Concurrently, progressive uterine enlargement restricts rumen capacity and voluntary feed intake, limiting metabolisable energy (ME) availability resulting in a negative energy status and hypoglycaemia. Homeostatic mechanisms become aberrant, resulting in homeorhesis.

Pregnancy toxaemia is often described in the literature as occurring in four forms: primary, resulting from decreased energy intake; obesity-related PT, affecting over-conditioned ewes with a body condition score (BCS) > 3.5/5; starvation PT occurring in under-conditioned ewes with prolonged inadequate nutrition; and secondary PT arising due to concurrent disease processes (Andrews, 1997; Crilly et al., 2021). These classifications are based on nutritional status and underlying risk factors. However, this framework is largely descriptive and is not formally defined in primary research studies.

Insufficient ME intake during late gestation limits the ability of ewes to meet feto-placental energy requirements. Ewes, particularly those that are over conditioned, mobilise adipose tissue through lipolysis, whereby triglycerides are hydrolysed into glycerol and non-esterified fatty acid (NEFA). The NEFA are transported to the liver, where they undergo β-oxidation to form acetyl-CoA, which can then be converted into ketone bodies, beta-hydroxybutyrate (bHB), acetoacetate and acetone which serve as alternative maternal energy substrates (Figure 1.1) (Souto et al., 2019). The mobilisation of adipose reserves and production of ketone bodies occurs faster than they can be metabolised resulting in hyperketonaemia (Vasava et al., 2016). Excessive NEFA mobilisation overwhelms hepatic capacity, and triglycerides accumulate in the liver because ruminants have a limited ability to export them as very low-density lipoproteins (VLDL), leading to hepatic lipidosis which is a common postmortem finding of PT (Gross et al., 2013). Recent experimental evidence indicated that in ewes with PT, negative energy status disrupts hepatic lipid metabolism by altering the expression of genes involved in fatty acid oxidation, acetyl-CoA metabolism, and triglyceride synthesis, leading to fat accumulation, hepatocyte structural damage, and exacerbation of the disease. This results in an enlarged liver with rounded edges, and a deep yellow colour, at post-mortem examination (Xue et al., 2019).

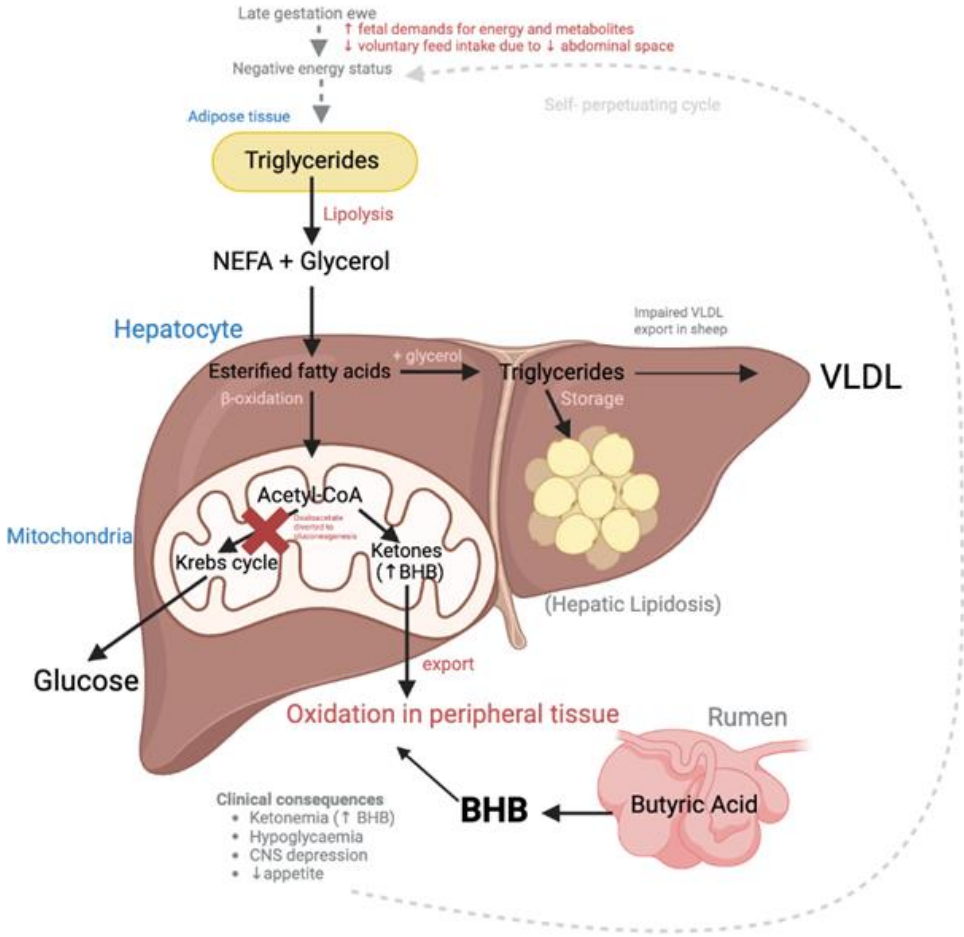

Figure 1.1 Disrupted glucose homeostasis and ketogenesis in ovine pregnancy toxaemia (created with BioRender.com)

Circulating ketone bodies are acidic and contribute to the metabolic derangements observed in PT, notably metabolic acidosis (Gomez et al., 2015). This creates a negative spiral, as metabolic disturbances lead to central nervous system (CNS) aberrations and overt clinical signs of PT (Schlumbohm & Harmeyer, 2004), which in turn exacerbate inappetence and further worsen the ewe's energy and metabolic status.

1.2.2 Risk factors

Late gestation, particularly in twin-bearing, ewes have impaired energy and glucose metabolism. There is a direct antilipolytic action of D-bHB on adipose tissue, thereby promoting high concentrations of circulating ketone bodies (hyperketonaemia) and increasing susceptibility to PT (Harmeyer & Schlumbohm, 2006). In mammals, D-bHB is the biologically active form of bHB which is produced via ketogenesis and can be oxidised by extrahepatic tissues as an energy substrate, whereas L-bHB is a racemic by-product which is unable to be metabolised for energy utilisation (Van Rijt et al., 2021).

Normal blood D-bHB concentration in ewes is < 0.8 mmol/L (Öztürk, 2023). Ewes that have poor energy status (severely underfed or have low-quality diets) are at a high risk of developing PT. Concurrent diseases such as Ovine Johne's Disease, or *Fasciola hepatica* further exacerbate this risk by reducing nutrient absorption and increasing metabolic demand through chronic inflammation, thereby lowering the ewe's ability to meet maternal and feto-placental energy requirements (McGregor et al., 2015**)**. Affected ewes may display clinical signs of PT when bHB concentrations exceed 3 mmol/L (Simões et al., 2020). Ewes with an ideal BCS of 2.5–3.5 had a 19.7% lower risk of elevated bHB concentrations compared with ewes with BCS of ≤2.0 or ≥4.0. In addition, ewes possessing gastrointestinal helminth eggs in their faeces (detected by faecal flotation) exhibited 12.3% higher bHB concentrations (Ratanapob et al., 2018). The presence of gastrointestinal helminth eggs, although not directly pathological, serves as a proxy for established parasitic burden and associated metabolic and inflammatory stress. This stress exacerbates the negative energy status and increases susceptibility to ketonaemia, and PT. Elevated faecal egg counts were correlated with raised bHB concentrations and negatively associated with maternal blood glucose concentrations and lamb birthweight in affected ewes (Barbagianni et al., 2015a).

1.2.3 Clinical manifestations of pregnancy toxaemia

Clinical signs of PT typically appear once the metabolic derangements are advanced. Neurological abnormalities arise from cerebral hypoglycaemia, reflected by low cerebrospinal fluid glucose. This impairs brain function resulting in observed incoordination, obtundation and other CNS manifestations (Scott et al., 1995a).

Yarim et al. (2007) demonstrated that ewes with PT exhibit progressively higher plasma concentrations of proinflammatory cytokines interleukin-1 beta (IL-1β), tumour necrosis factor alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1) in conjunction with worsening clinical and biochemical indicators, suggesting that these cytokines contribute to the pathogenesis of the disorder, and may serve as prognostic biomarkers. The marked elevation of these cytokines may reflect an energetically costly inflammatory response in which immune-driven hypermetabolism, glucose diversion, hypophagia and associated mineral disturbances further exacerbate the negative energy status. This creates a self-perpetuating cycle in which energy deficit, hyperketonaemia, and CNS derangements impair appetite and function, driving progressively worsening metabolic and clinical presentation.

Another biomarker recently proposed for subclinical and clinical PT is fructosamine (Iqbal et al., 2022). Prolonged negative energy status and hypoglycaemia will result in decreased blood concentrations of fructosamine due to decreased non-enzymatic glycation of serum proteins over time (Hasanabadi et al., 2019). Iqbal et al. (2022) identified fructosamine as the most robust diagnostic

biomarker for clinical and subclinical PT, outperforming NEFA. Blood creatinine, potassium, and lactate dehydrogenase were also shown to have strong prognostic value for the disease outcome (Iqbal et al., 2022). Non-esterified fatty acids are a biomarker of PT as elevated blood concentrations reflect excessive mobilisation of adipose tissue in response to the negative energy status (Mohammadi et al., 2016). Creatinine concentration is increased due to reduced renal perfusion and increased muscle catabolism associated with severe metabolic stress, while blood potassium concentration decreases with reduced feed intake and acid-base disturbances. Lactate dehydrogenase is increased due to tissue damage and cellular hypoxia to the liver, heart and skeletal muscle associated with disease severity (Iqbal et al., 2022).

Clinically, PT can resemble hypocalcaemia or hypermagnesemia, and affected ewes are often afflicted by multiple concurrent metabolic disturbances. Measuring serum calcium, magnesium and bHB concentrations, as well as response to appropriate treatment can aid in the diagnosis of an obtunded, recumbent periparturient ewe, however, the syndrome is often linked to various metabolic aberrations (Abbott, 2024). This underscores the need to define PT as a metabolic syndrome characterised by hyperketonaemia and negative energy status, rather than a diagnosis due to a single biochemical aberration. Doing so allows PT to be distinguished from concurrent conditions such as hypocalcaemia or sepsis, a systemic inflammatory response caused by severe infection (Obonyo et al., 2024; Passmore et al., 2018).

Hypocalcaemia may occur in ewes with PT, with approximately 20% of affected animals reported to have concurrent hypocalcaemia (Aitken, 2008), although its clinical significance remains unclear. In late gestation, increased fetal calcium demand driven by skeletal mineralisation coincides with reduced feed intake and mobilisation of body reserves, which often occurs concurrently with negative energy status and ketone body accumulation (Abdelrahman, 2008). Hypocalcaemia alone does not induce PT, but experimental evidence demonstrated that when combined with hyperketonaemia it markedly suppresses endogenous glucose production, thereby exacerbating hypoglycaemia and promoting disease progression (Schlumbohm & Harmeyer, 2003). Understanding these biomarkers and clinical signs is crucial for early disease detection and facilitates both preventative strategies and therapeutic interventions for PT.

1.2.4 Alternative methods for detecting pregnancy toxaemia

A range of clinical, biochemical, and post-mortem methods are available to aid in the detection of PT in ewes, particularly where conventional laboratory testing is impractical. Alternative methods complement blood biochemistry and are especially valuable in field settings with limited easily available diagnostic infrastructure. A preliminary diagnosis is often based on clinical presentation,

with affected ewes typically in late gestation showing reduced appetite or anorexia, poor mobility or recumbency, and dull mentation, particularly in multitocous animals.

Blood bHB concentrations remain the most widely used diagnostic measure and can be readily assessed in the field using handheld ketometers, where values >1.1 mmol/L are considered abnormal (Panousis et al., 2012; Pichler et al., 2014). Ketone bodies can also be detected in urine (ketonuria). Tests for ketonuria mainly detect acetoacetate, which is excreted more slowly and variably, making it less reliable for early or accurate diagnosis (Glengarry et al., 2025). When ketone bodies are elevated, and plasma bHB is elevated above 0.7 mmol/L, acetoacetate can be detected in the urine using nitroprusside-based dip stick (Lynch & Jackson, 1983). While urine dipsticks provide an alternative diagnostic tool, bHB predominates in blood and therefore provides a more accurate and reliable indicator of current metabolic status and disease severity.

Post-mortem examination findings provide evidence of PT in fatal cases, with characteristic lesions including hepatic lipidosis, reduced adipose reserves, pale, enlarged and friable liver, the presence of multiple fetuses and in some cases enlarged adrenal cortex due to increased production of cortisol (Hill et al., 1984). More recent studies have demonstrated that pregnancy toxaemia is associated with multi-organ pathology, including hepatic lipidosis with hepatocellular vacuolation, renal tubular degeneration, and systemic congestion affecting organs such as the lungs, spleen, and brain, reflecting the widespread metabolic and oxidative disturbances characteristic of the disease (Adam et al., 2024; Tharwat et al., 2024). Measurement of bHB concentrations in aqueous humour is a reliable post-mortem diagnostic method, with concentrations >2 mmol/L considered diagnostic and >0.8 mmol/L supportive of PT (Scott et al., 1995b). Despite the utility of blood and urine ketone testing for early detection, their routine use remains limited due to impracticalities in extensive grazing systems such as those common in Australia, increasing reliance on clinical assessment and post-mortem evaluation.

1.2.5 Sequelae to pregnancy toxaemia

Pregnancy toxaemia has significant consequences for both ewes and fetuses, affecting metabolic function, reproductive performance, and neonatal survival (Barbagianni et al., 2015b; Turgut et al., 2025). In healthy ruminants, glucose homeostasis is predominantly maintained through the utilisation of rumen-derived propionic acid as a substrate for hepatic gluconeogenesis (Lemosquet et al., 2009). In fasted or feed-restricted states, such as in late gestation, aberrant availability of gluconeogenic precursors leads to an energy deficit that hepatic gluconeogenesis cannot overcome. The inability to maintain maternal blood glucose concentrations results in hypoglycaemia, compromising both maternal and fetal viability. This reflects the combined effects of decreased ruminal propionic acid production secondary to reduced feed intake, and escalating fetal glucose demands, mechanisms that are examined in detail in the aetiopathogenesis section of this review.

Hyperketonaemic ewes have an increased risk of dystocia, retained fetal membranes, metritis, mastitis, and reduced subsequent milk yield. Subclinical hyperketonaemia is associated with impaired immune function, hepatic triglyceride accumulation and fibrosis, altered acute-phase protein profiles, and diminished mammary lymphoid tissue (Barbagianni et al., 2015b; Barbagianni et al., 2015c). Dysregulation of angiogenic and hypoxia-related genes in ewes affects placental vascular development and transplacental nutrient exchange; however, surviving ewes typically show no long-term reproductive deficits (Kasimanickam, 2016). This sequence of metabolic disruptions results in the clinical presentation of PT and illustrates the need for early identification and intervention to restore energy balance to prevent mortality of both the ewe and fetuses.

Fetal consequences include reduced fetoplacental unit oxygen perfusion and glucose supply from the ewe, fetal hypoxia and acidosis, lower birthweights, and increased perinatal mortality (Gomez et al., 2015). Clinical or subclinical ketonaemia can delay lactogenesis, reduce colostrum quantity and quality, increase lamb hypothermia risk due to greater brown fat utilisation, collectively compromising neonatal colostrum intake and survival (Kelly et al., 2025). These fetal and neonatal consequences illustrate the critical impact of maternal metabolic imbalance on lamb survival, highlighting the need for early detection and intervention to improve both ewe and offspring outcomes.

1.3 Rumen function and glucose metabolism in pregnancy

The reticulo-rumen are part of the forestomachs containing a diverse microbiome that metabolise dietary substrates via fermentation into volatile fatty acids (VFA), primarily acetic acid, propionic acid, and butyric acids (Xie et al., 2025). Propionic acid, produced from dietary non-structural carbohydrates, serve as an energy source via hepatic gluconeogenesis compared to monogastric species which utilise dietary glucose directly via intestinal absorption (Millen et al., 2016).

Normal rumen pH ranges from 6.4 to 6.8 (Jasmin et al., 2011). Typically, ruminal acidosis occurs due to the intake of rapidly fermentable carbohydrates such as glucose or starch, and a low fibre diet (Jasmin et al., 2011; Tufani et al., 2013). Sub-acute ruminal acidosis (SARA) is a digestive disorder in ruminants characterised by prolonged periods of moderately low rumen pH. Various definitions have been proposed including pH values ranging from 5.0-5.5 for SARA whereas ruminal acidosis is typically defined as a mean pH < 5 which is associated with elevated lactic acid concentrations (Dagnaw Fenta et al., 2023; Lettat et al., 2010; Oetzel, 2000).

Ruminants with grain-induced acidosis and SARA exhibit reduced feed intake, decreased rumen motility, increased rumination time, and marked rumen microbial dysbiosis as well as systemic effects of acidaemia and likely bacteraemia due to reduced rumen wall integrity. This dysbiosis exacerbates

inappetence and hypoglycaemia, as well as the systemic effects of acidaemia and likely bacteraemia due to reduced rumen wall integrity increasing the probability of ewe mortality (Minami et al., 2020). Lactic acidosis causes rapid reductions in feed intake, rumen motility, and overall activity, accompanied by altered biochemical parameters and rumen pH (Zein-Eldin et al., 2014). Interventions such as ruminal fluid transfaunation can restore microbial diversity, VFA production, and reduce lactate accumulation. This highlights the role of dysbiosis in exacerbating negative energy status (Liu et al., 2019). In ewes with PT, comparable reductions in rumen microbial richness, including key fermentative bacteria such as *Prevotella, Butyrivibrio, Ruminococcus, Lachnospiraceae,* and *Oribacterium*, have been associated with decreased VFA production and glucose availability, further exacerbating hypoglycaemia, elevated bHB concentrations, oxidative stress, and the risk of mortality (Chen et al., 2024). Collectively, these processes highlight that disturbances in rumen pH and microbial function can exacerbate negative energy status and metabolic instability in ewes with PT, underscoring the need for targeted research evaluating how ORS influence rumen pH and microbiota to ensure their therapeutic use does not inadvertently worsen ruminal dysfunction.

1.3.1 The role of the rumen in nutrition

Rumen microbial fermentation rapidly converts most dietary glucose to VFA, however, there has been some evidence that sheep are capable of directly absorbing glucose across the ruminal epithelium into the bloodstream via the sodium-glucose co-transporter 1 (SGLT-1) in (ovine) rumen epithelium. Isolated ruminal tissues actively transported glucose analogues in a sodium-dependent manner, indicating epithelial uptake rather than microbial alteration (Aschenbach et al., 2000a). Results from *in vivo* reticulorumen experiments indicated that a measurable proportion of glucose could be absorbed from ruminal contents in a Na^+ dependent process, indicating active transport from the forestomach (Aschenbach et al., 2000b). Additional research showed ruminal SGLT-1 activity could be modulated by physiological signals such as β_2-adrenoceptor stimulation thus ameliorating consequences of lactic acidosis (Aschenbach et al., 2002). Direct ruminal glucose absorption is generally a minor contributor to systemic glucose supply and overall energy metabolism, compared with VFA uptake and post-ruminal intestinal absorption (Liu et al., 2020). These findings suggested that glucose could cross the rumen wall and enter the hepatic portal circulation in sheep, challenging the traditional assumption that ruminal glucose absorption is negligible. The importance subscribed to direct rumen glucose absorption is minimal.

1.3.2 Endocrine adaptations in late gestation

During late gestation, normal endocrine adaptations in ewes include interactions with pregnancy-associated hormones and reduced maternal insulin concentrations, which decrease pancreatic responsiveness to insulinotrophic signals and decrease lipid synthesis (Lomax et al., 1979; Regnault et

al., 2004). This promotes a shift towards lipolysis, leading to increased production of NEFA, which cannot be utilised by the gravid uterus (Pethick et al., 1983). Consequently, maternal energy metabolism becomes increasingly reliant on gluconeogenesis to meet the rising energy demands of the feto-placental unit. These metabolic adaptations are accompanied by altered pancreatic insulin secretion and increased peripheral tissue and pancreatic β-cell resistance to insulin, further exacerbating the negative energy status. Duehlmeier et al. (2013) reported a breed-associated insulin resistance in German Blackheaded Mutton ewes that led to an increased risk of developing PT. Moallem et al. (2012) demonstrated that as the number of fetuses increased, ewes developed progressively greater metabolic disruption, characterised by rising NEFA and bHB concentrations, and markedly reduced triglycerides, cholesterol, and insulin concentrations. This insulin suppression was identified as a key homeorhetic adaptation to redirect glucose to the brain and fetoplacental units; however, alongside increased NEFA mobilisation, it promoted ketogenesis and increased susceptibility to gestational ketonaemia and PT in ewes carrying multiple fetuses. These endocrine adaptations illustrate that progressive reductions in insulin concentration and sensitivity during late gestation impair glucose homeostasis. Diminished insulin receptor responsiveness limits peripheral glucose utilisation and heightens reliance on lipolysis and ketogenesis, thereby increasing the risk of PT, particularly in ewes carrying multiple fetuses.

1.4 Current treatment strategies

Therapeutic intervention for the treatment of PT should aim to address dehydration, metabolic acidosis and electrolyte imbalances while supporting the re-establishment of glucose homeostatic mechanisms and VFA production and reducing the production of NEFA and ketone bodies.

1.4.1 Enteral treatment options

A variety of treatment regimens have been recommended, including feeding energy-dense rations to non-anorexic sheep, or oral drenches with glucose and glucogenic precursors such as sodium propionate, sodium lactate, glycerol, and propylene glycol (Cal-Pereyra et al., 2015). Increasing ME density in the management of PT is typically achieved by increasing the supply of rapidly fermentable or soluble carbohydrates, thereby, enhancing ruminal propionic acid production and hepatic gluconeogenesis. The use of dietary fat is limited in sheep due to its potential to impair rumen function and fibre digestion which is already compromised in sheep with PT (Kowalczyk et al., 1977).

Enteral treatment options include electrolyte solutions with a combination of glucose and electrolyte components. Buswell et al. (1986) reported that a concentrated ORS (Vytrate) containing 44.6 g glucose, 8.55 g sodium chloride, 6.17 g glycine, 4.08 g potassium dihydrogen phosphate, 0.12 g potassium citrate, and 0.525 g citric acid in 160 mL produced a significantly faster and greater rise

in plasma glucose concentrations in ewes than an equivalent glucose dose delivered in water. This suggested the formulation may better correct the metabolic derangements of PT by rapidly increasing blood glucose concentration while providing electrolytes and energy substrates. Although glycerol induced a similar but more prolonged glycaemic response, and propylene glycol had minimal effect, the ORS appeared to offer the most rapid correction of hypoglycaemia. A follow-up field study using the ORS drench every 4-8 h reported recovery rates of 90% in mild and 55% in severe naturally occurring PT cases (overall 68%) (Buswell et al., 1986). By contrast, when 160 mL of Vytrate three times daily in conjunction with administered with 160 mg of recombinant bovine somatotropin to treat naturally occurring ovine PT in multi-gravid ewes it resulted in markedly lower survival rates of 34.8% for ewes (Buswell et al., 1986). These contrasting outcomes highlight variability in treatment efficacy and underscore the limited contemporary evidence evaluating this ORS formulation in the management of PT, representing a clear gap in the literature.

Treatment strategies should aim to restore depleted blood glucose concentrations and reduce ketone body synthesis thereby mitigating the development of hyperketonaemia. Oral glucose administration is easier than intravenous (IV) delivery; however, its effectiveness is uncertain because ruminal fermentation is likely to metabolise most glucose, providing little direct replenishment of blood glucose due to minimal direct rumen absorption (Martín-Alonso et al., 2019a). Nevertheless, non-hypertonic ORS may still benefit ewes with dehydration, uraemia, or metabolic acidosis. Oral glucose administration has raised concerns about potential side effects, including altering rumen motility and pH, especially in feed-restricted or inappetent ewes such as ewes with PT, where microbial fermentation patterns may be altered. This uncertainty regarding the metabolic effects of glucose-based ORS underscores the need for evidence-based treatment strategies for PT.

Brozos et al. (2011) recommended using two oral doses of 150-200 mL of propylene glycol on day one, then 60 mL per dose for up to 6 d. Alternatively, 60 mL oral glycerol twice daily for 3-6 d could be used instead of propylene glycol. Other highly soluble carbohydrate sources such as liquid molasses may be used as an energy source but act more slowly and can risk ruminal disruption and predisposition to ruminal acidosis. Supplementation with 30% molasses over three weeks was associated with improved rumen fermentation parameters, without significant alterations in electrolyte balance or acid-base homeostasis (Osman et al., 2020). Wang et al. (2024) demonstrated that negative energy status was associated with reduced concentrations of acetic and butyric acids, shortened rumen papillae, and alterations in rumen bacterial structure and diversity, indicating that compromised rumen function may further limit the effectiveness and safety of oral energy supplementation. These findings illustrate both the potential use, and limitations of current enteral therapies for PT, highlighting the need to explore alternative strategies that restore glucose homeostasis to the pregnant ewe while minimising adverse effects on rumen function and overall ewe health.

1.4.2 Alternative treatment options

There is limited evidence to support the IV administration of propylene glycol, glycerol, or dextrose in comparison with enteral treatment options, and insulin has only been shown to be effective as an adjunct to other treatment options (Cal-Pereyra et al., 2015). Kalyesubula et al. (2019) reported that IV administration of 170 mL isotonic saline with 25.5 mL of glycerol reduced hypoglycaemia, hyperketonaemia and adipose lipolysis by directly supplying glycerol as a gluconeogenic substrate to the liver, thereby, bypassing rumen metabolism.

El-Hamamsy et al. (1990) demonstrated that inducing oesophageal groove closure in adult ewes using lysine vasopressin (0.1 IU/kg) followed by oral glucose markedly improved recovery from PT. This resulted in sustained increases in plasma glucose concentrations and significant reductions in NEFA and ketone body concentrations compared with standard IV glucose therapy, which showed minimal clinical or biochemical benefit.

The use of non-steroidal anti-inflammatory drugs (NSAID), such as flunixin meglumine, is beneficial as an analgesic, and to prevent inflammatory cascades associated with PT, however, the nephrotoxic side effects need to be considered in such cases (Zamir et al., 2009).

If fetal viability or dam prognosis is poor, termination of pregnancy will reduce metabolic demand and mitigate progression of PT. This can be achieved via induction of parturition using corticosteroids, or caesarean to eliminate the metabolic demand for energy from the fetoplacental unit (Andrews, 1997; Ingoldby & Jackson, 2001). It has been reported that only 33% of clinically affected ewes recovered with this sort of intervention (Aitken, 2008; Vijayanand et al., 2022). Producers should aim to focus on preventative rather than curative measures.

1.5 Oesophageal groove influence on treatment

The oesophageal groove presents a promising mechanism to enhance enteral therapies in PT, as it might allow orally administered glucose and electrolytes to bypass ruminal degradation, reach the abomasum and small intestine rapidly, and potentially improve energy homeostasis in affected ewes.

1.5.1 Anatomy and physiology of the oesophageal groove

The oesophageal groove is a muscular structure connecting the oesophageal cardia to the reticulo-omasal orifice, forming a channel that allows ingested liquid to bypass the rumen and pass directly to the abomasum, thereby enabling the young ruminant to function in a monogastric-like manner (Martín-Alonso et al., 2019b). It directs fluids past the rumen and reticulum directly into the abomasum (Ørskov & Benzie, 1969). Although primarily functional in neonates, understanding the

developmental physiology informs strategies to stimulate groove closure in adult ewes for therapeutic purposes. Embryologically, the reticular groove differentiates early in sheep and goats, while postnatal rumen development progresses through three stages: non-ruminant reliance on milk (birth-3 weeks), transitional solid feed intake with increasing VFA production (3-8 weeks), and full ruminant function by 8 weeks. Exclusive milk diets in lambs may delay rumen development and maturation with decreased rumen weight and papillae length as well as reduced abundance of carbohydrate degrading bacteria (Huang et al., 2023).

Activation of the oesophageal groove involves both the vagal nerve (CN X) and the local myenteric plexus. Afferent trigeminal stimulation by milk components (albumin, glucose, copper in ovine; sodium in bovine), reinforced by cortical and sucking inputs, drives an efferent response that contracts the groove musculature while inhibiting rumino-reticular motility (Comline & Titchen, 1951; Denac et al., 1990; Newhook & Titchen, 1976). Consequently, central or peripheral stimuli may trigger closure of the oesophageal groove in adult sheep, permitting orally administered fluids to bypass the rumen. This reflex can be conditioned in young animals using visual and auditory stimuli and when fluid is deposited into the back of the mouth (Comline & Titchen, 1951).

In adult sheep, the oesophageal groove is typically inactive, but closure can be triggered, particularly by voluntary swallowing of fluids and in fasted animals, which allows bypassing of the forestomachs. Many orally administered substances, including glucose, electrolytes, NSAID, antibiotics, and anthelmintics, are normally degraded by rumen microbes; however, if the groove closes, these fluids can reach the abomasum directly, allowing rapid absorption within the gastrointestinal tract (Elbadawy et al., 2016; González et al., 2001; Marini et al., 2016). In PT, triggering oesophageal groove closure would allow orally administered glucose to bypass ruminal fermentation, facilitating rapid intestinal absorption, correcting energy deficits, and mitigating negative energy status without altering rumen pH (Martín-Alonso et al., 2019a).

1.5.2 Agents that induce closure of the oesophageal groove

Many compounds have been shown to induce closure of the oesophageal groove in sheep with enteral administration, such as copper sulphate, copper acetate, copper chloride (Monnig, 1935; Tsiamitas & Brikas, 1981; Watson, 1941), zinc acetate, zinc sulphate (Smith et al., 1977), cobalt sulphate (Sargison et al., 1999), sodium sulphate, bicarbonate, chloride, acetate, and salicylate (González-Montaña et al., 2014). Physiologically, anti-diuretic hormone (ADH) is continuously synthesised and secreted by the posterior pituitary to maintain water and electrolyte homeostasis. Under conditions of increased plasma osmolality or dehydration, ADH secretion is further stimulated. In addition to its role in renal water conservation, ADH (vasopressin) has been shown to influence oesophageal groove function, with increased circulating concentrations associated with groove closure. This results in

liquids bypassing the rumen and passing directly to the abomasum and small intestine for rapid absorption (Mikhail et al., 1988). Although oesophageal groove closure has been investigated in adult ruminants for decades, responses to proposed stimulatory agents remain inconsistent and unpredictable. Consequently, vasopressin has been explored as a pharmacological method of inducing groove closure to improve oral treatment strategies for conditions including primary ketosis, non-specific diarrhoea and PT. González-Montaña et al. (2014) demonstrated that IV administration of vasopressin at a dose of 0.08 IU/kg body weight resulted in complete oesophageal groove closure in adult sheep without inducing adverse effects.

Glucose-containing fluids deposited into the oral cavity can stimulate oropharyngeal and lingual receptors, which, via vagally mediated reflex pathways, induce oesophageal groove closure. The precise mechanisms underlying this response remain incompletely understood. This rumen bypass effect has been observed in 42% of grazing sheep following oral administration of anthelmintics combined with glucose (Prichard & Hennessy, 1981). The importance of this in relation to PT was first noted by El-Hamamsy et al. (1990) where oral treatment of a local vasopressin preparation IV at 0.1 IU/kg followed by 50 g glucose in 100 mL water orally was successful in inducing oesophageal groove closure resulting in increasing plasma glucose concentrations and reducing NEFA and ketone body concentrations in ewes displaying clinical signs of PT. More contemporary studies have shown that administering 0.08 IU/kg body weight IV lysine-vasopressin and a 50 g oral glucose solution in fasted ewes induced oesophageal groove closure. This enabled glucose to bypass the rumen, and be absorbed in the intestines, increasing blood glucose concentrations for up to 6 h without adverse effects (González-Montaña et al., 2014; Martín-Alonso et al., 2019a). These findings support the premise that reliable oesophageal groove closure could allow oral administration of glucose and electrolytes to bypass the rumen fermentation, enhancing systemic glucose availability without disrupting rumen pH, representing a physiologically sound adjunctive therapy for PT.

The use of oral glucose in ruminants is controversial, as most dietary glucose is rapidly fermented within the rumen rather than being rapidly absorbed into the blood, limiting its contribution to systemic glucose availability (Aschenbach et al., 2000a; Aschenbach et al., 2000b). Administration of rapidly fermentable carbohydrates may disrupt rumen microbial ecology and lower rumen pH, increasing the risk of subacute or ruminal acidosis, particularly in feed-restricted or anorexic ewes (Dagnaw Fenta et al., 2023; Jasmin et al., 2011; Lettat et al., 2010; Tufani et al., 2013). Recent evidence indicated ewes with PT already exhibited rumen microbial dysbiosis and reduced VFA production, potentially increasing susceptibility to ruminal dysfunction following oral glucose administration (Chen et al., 2024).

1.6 Research objective

Despite widespread on-farm use, there is a paucity of empirical data evaluating the effects of glucose-based ORS on rumen pH and systemic glucose concentrations in feed-restricted ewes. Determining whether an ORS administration adversely alters the rumen environment is critical to ensuring that commonly used therapeutic interventions do not inadvertently exacerbate metabolic instability. The objective of this study was, therefore, to investigate the effects of a proprietary ORS on rumen pH and blood glucose concentrations in feed-restricted ewes.

Chapter 2: Materials and Methods

Ethics approval was received for this work by the Charles Sturt University Animal Care and Ethics Committee on 24 March 2025, Approval Number *A25049.* This study was conducted at Charles Sturt University utilising ewes from the University's commercial flock located on the university campus at Wagga Wagga, New South Wales. Prior to recruitment into the trial a clinical examination was conducted on each ewe and no abnormalities were detected.

2.1 Experimental design and animal preparation

The study was conducted over three consecutive days. Twelve non-pregnant, two-year-old Merino ewes (bodyweight 57–67 kg) were randomly allocated to either a Control group (n = 6) or a Treatment group (n = 6). Within each group, ewes (n = 2 for each Control and Treatment group per day) were randomly assigned to one of three trial days. The Treatment group received 160 mL proprietary ORS Vytrate liquid concentrate (glucose 278.75 g, sodium chloride 53.44 g, glycine 38.56 g, potassium phosphate monobasic 25.53 g, potassium citrate 0.75 g, citric acid monohydrate 3.28 g) three times at 4 h intervals as per manufacturer recommendations. The Control group received 160 mL aliquots of tap water orally at the same times as the Treatment group was administered the ORS.

Before the trial, sheep were housed in a shaded yard (6 m × 5.85 m) with *ad libitum* access to reticulated fresh water and lucerne hay. Ewes were subsequently moved into indoor pens (1.9 m x 1.93 m per two ewes) and fasted for 24 h before the study, while maintaining access to water. Water was withheld 30 min prior to the commencement of the trial and for the 10 h duration of the experimental period.

2.2 Treatment administration

At the start of the trial, time zero (trt1), and again at 4 h (trt2), and 8 h (trt3), ewes received 160 mL of either water (Control) or ORS (Treatment). Administration was performed using a 60 mL catheter-tip syringe, slowly dispensing the solution into the cheek pouch to stimulate voluntary swallowing and ensure complete ingestion of the liquid.

2.3 Blood collection and analysis

Venous blood was collected via jugular venipuncture, for insulin and glucose assay, as well as for blood gas and electrolyte analysis at 30 min prior to treatment administration (t0) and again 2 h after treatment (t2).

2.3.1 Insulin assay

Blood was drawn using a 19G needle into a plain red-top tube (BD Vacutainer 4 mL tube 13 mm x 75 mm). After clotting, the sample was centrifuged for 10 min (Eppendorf Centrifuge 5702, 13.5 cm arm length at 4.4 x1000 rpm). Serum was removed manually via pipette into a 5 mL TechnoPlas specimen container and stored frozen at -4°C for subsequent insulin analysis using a solid-phase, enzyme-labelled chemiluminescent immunometric assay (IMMULITE® 2000 Insulin system; Siemens Healthcare Diagnostics, UK).

2.3.2 Glucose assay

Using the same venipuncture blood draw as for the insulin assay, a fluoride oxalate grey-top tube (BD Vacutainer 4 mL tube 13 mm x 75 mm) was filled and immediately inverted to ensure thorough mixing with the anticoagulant. The tube was then centrifuged at 3500 rpm for 10 min (Thermoscientific multifuge X pro). The serum was decanted into smaller aliquot tubes and refrigerated at ~4°C (less than 24 h). Serum glucose concentrations were quantified using an enzymatic UV (hexokinase) method on an automated clinical chemistry analyser (Beckman Coulter AU480; Beckman Coulter Inc., Brea, CA, USA), according to the manufacturer's instructions.

2.3.3 Blood gas analysis

Blood was collected from the same venipuncture into a heparinised, air-free syringe (BD A-line blood gas syringe 3.0 mL). The air was immediately removed, and the syringe gently inverted. Blood gas, electrolyte and metabolite parameters were measured within 10 min of collection using a whole blood analyser (GEM® Premier™ 5000; Werfen, Bedford, MA, USA), according to the manufacturer's instructions.

2.4 Ruminal fluid sampling and analysis

Relative to treatment delivery (water or ORS), ruminal fluid samples were collected via oral oesophageal intubation at the following 11 time points: -0.5 h, 0.5 h, 1 h, 2 h, 4.5 h, 5 h, 6 h, 7 h, 8.5 h, 9 h, and 10 h. A roll of elastoplast (Tensoplast Vet) served as a mouth gag to facilitate the passage of the tubing through the oral cavity. For ewes (numbered) 1 to 4, samples were collected from -0.5 h to 2 h using a suction-based tubing connected to a manual pump with a weighted filter (20 mm internal diameter, 22 mm external). However, for samples collected from 4 h to 10 h a smaller-diameter tubing was used (foal stomach tube 9.5 mm OD x 213 cm), with a plastic internal stylet using with suction from a 60 mL catheter tipped syringe. For ewes numbered 5 to 12, all samples were collected using 2 m length tubing (10 mm external diameter, 6 mm internal diameter) and a 60 mL catheter tip syringe

(Terumo), to apply suction to aid in siphoning out the ruminal fluid. All samples were collected into a clean, dry TechnoPlas 70 mL specimen container.

Control ewes (n = 6) were sampled at all 11 time points between 0 and 10 h. Treatment ewes (n = 6) were assessed in a staggered design across four treatment phases/periods to produce a cumulative effect. Treatment phase 0 (trt0, red), represented baseline measurements collected prior to any treatment administration at -0.5 h; Treatment phase 1 (trt1, green), representing measurements collected at 0.5 h, 1 h and 2 h following the first treatment administered at 0 h; Treatment phase 2 (trt2, blue), represented measurements collected at 4.5 h, 5 h, 6 h and 7 h following the second treatment administration at 4 h; and Treatment phase 4 (trt3, aqua) represented measurements collected at 8.5 h, 9 h and 10 h following the third treatment administration at 8 h. The study design and sampling frequency are summarised in Table 2.1.

Table 2.1 Study design and timing of rumen pH measurements for the Control and Treatment groups.

Time (h)	Control	Treatment phase 0	Treatment phase 1	Treatment phase 2	Treatment phase 3
-0.5	6	6	0	0	0
0.5	6	0	6	0	0
1	6	0	6	0	0
2	6	0	6	0	0
4.5	6	0	0	6	0
5	6	0	0	6	0
6	6	0	0	6	0
7	6	0	0	6	0
8.5	6	0	0	0	6
9	6	0	0	0	6
10	6	0	0	0	6

Note: The numbers in the table indicate the number of sheep sampled at a given timepoint.

2.4.1 Ruminal fluid pH

Measurement of ruminal fluid pH was taken within 5 min post sampling to avoid shifts in pH due to changes in temperature or ion activity. The ruminal fluid was gently agitated, the pH and temperature of the sample was first measured and recorded using the calibrated “S20 SevenEasy pH” meter (Mettler-Toledo), followed immediately by the “Basic pH Meter” (Denver Instrument). Once a stable reading was obtained on both devices, readings were recorded and averaged for analysis, the sample was then discarded. Electrodes were rinsed with distilled water and blotted dry between samples. Specimen containers were cleaned, and rinsed twice, and dried by wiping with paper towel.

2.4.1.1 pH Meter calibration

The “S20 SevenEasy pH” meter (Mettler-Toledo) and a “Basic pH Meter” (Denver Instrument) and their electrodes were calibrated at the beginning of each trial day, to ensure accuracy and precision of pH measurements. Calibration was performed using three standard buffer solutions with pH values of 4.0, 7.0, and 10.0. Electrodes were first rinsed with distilled water and gently blotted dry with paper towel between each buffer.

Calibration was performed according to manufacturer instructions using a two- or three-point standard curve, with electrode slope (%) monitored to ensure accuracy. Calibration was considered acceptable when the slope was within 90–105%, a criterion that was consistently met throughout the trial, with a “good electrode” status displayed at each calibration. When not in use, electrodes were stored in a pH 4.0 storage solution and routinely inspected for damage, dryness, or residue accumulation to maintain measurement integrity.

2.5 Post-trial animal care

At the conclusion of the trial, all sheep were clinically examined, subcutaneously administered meloxicam at 1 mg/kg body weight for post procedural analgesia, and provided *ad libitum* access to fresh water and lucerne hay. They returned to the paddock with their cohorts with no adverse effects observed.

2.6 Statistical analysis

Statistical analysis was performed using R statistical software (R Core Team, 2025). Mixed-effects regression models were used to account for repeated measures within animals, with animal ID included as a random effect. Fixed effects included treatment group and time. Where no significant interaction between treatment and time was identified ($p = 0.875$), models were simplified to include main effect terms only, and post-hoc pairwise comparisons were performed based on Tukey’s honest significance differences (TukeyHSD) test in R (Crawley, 2013). Estimated marginal means and 95% confidence intervals (CI) were derived from the models to assess differences between treatment timepoints. For blood variables measured at baseline -0.5 h (t0) and 2 h (t2) post treatment (trt1), within-animal changes ($\Delta = t2 - t0$) were calculated and compared between groups using Welch two-sample t-tests, with between-group differences expressed as Control minus Treatment. Statistical significance was set at $p < 0.05$.

Chapter 3: Results

3.1 Rumen pH

Rumen pH decreased over time in both the Treatment and Control ewes, with a greater reduction observed in Treatment ($p = 0.002$) compared to the Control ewes (Figures 3.1 and 3.2). Median rumen pH in Treatment ewes declined from 7.92 at baseline (trt0) to 7.06 at the final treatment period (trt3), whereas median rumen pH in Control ewes remained relatively stable over time (7.62). Variability in rumen pH measurements increased during later treatment periods. Despite these rumen pH decreases, rumen pH remained within the physiological range in all animals, and no cases of ruminal acidosis (pH <5.2) were observed. Descriptive boxplot representations (Figures 3.1 and 3.2) were consistent with inferential analyses derived from the mixed-effects regression model, both demonstrating a progressive reduction in rumen pH following repeated ORS administration. For Treatment ewes, trt0 represented the baseline pre-treatment measurement collected at -0.5 h and therefore served as the within-animal control for subsequent treatment periods. Control measurements represented pooled rumen pH values collected from untreated ewes across all 11 study time points. There was no significant difference between Control and Treatment ewes at the start of the study (Figure 3.1), confirming the effect of the randomisation of the ewes.

A mixed-effects regression model with both main effect terms and the interaction term was initially fitted to the data, and no interaction between treatment and time ($p = 0.875$) was identified; therefore, the final model included main effect terms only (Figure 3.3).

Estimated marginal means were calculated for each treatment period with animal identification (number) included as a random effect to account for repeated measures. Control represented pooled measurements from Control ewes collected across all study time points, while trt0 represented baseline pre-treatment measurements from Treatment ewes. A progressive decline in modelled rumen pH was observed across treatment periods.

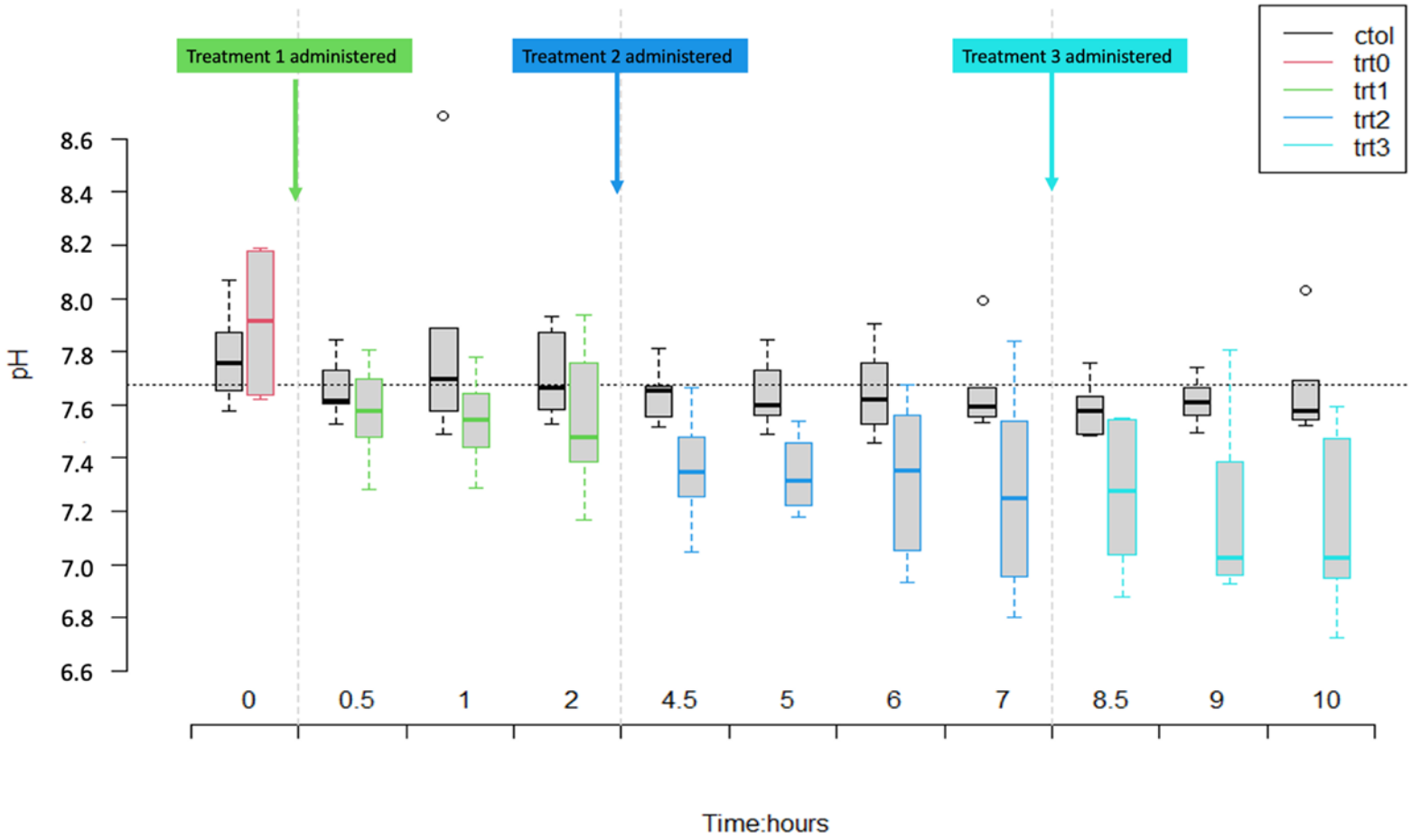

Figure 3.1 Distribution of rumen pH over time by treatment group.

Note: Box-and-whisker plots display median (horizontal line), interquartile range (box; 25th-75th percentile), and range (whiskers). Control animals (ctol) are shown in black. Treatment timepoints (trt1-trt3) are represented sequentially with trt1 in green; trt2 in blue; and trt3 in aqua. The black horizontal dotted line represents the average of the ctol group pH level over time. The vertical dotted lines represent when the treatment (160 mL ORS for Treatment group or 160 mL water for the ctol group).

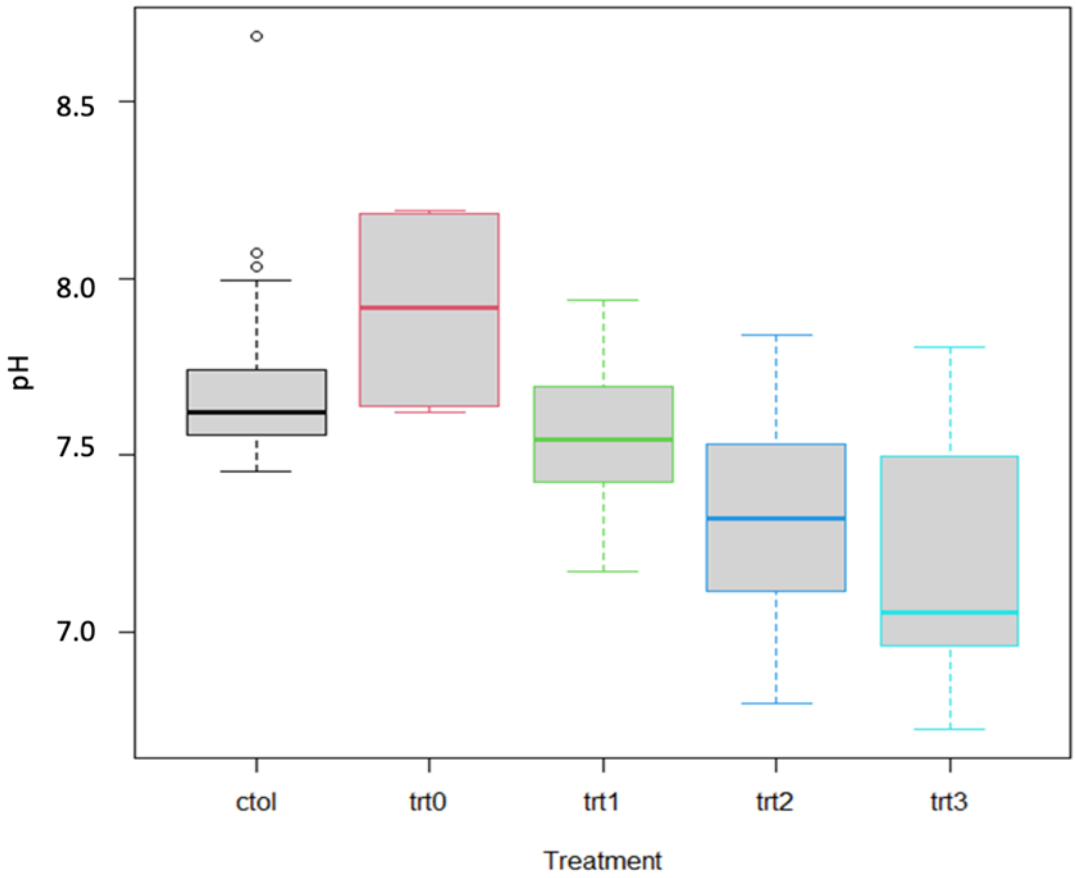

Figure 3.2 Distribution of rumen pH by treatment group.

Note: Box-and-whisker plots display median (horizontal line), interquartile range (box; 25th–75th percentile), and range (whiskers). Control animals are shown in black and treatment periods are shown sequentially as trt0 (red), trt1 (green), trt2 (blue), and trt3 (aqua).

Post-hoc pairwise comparisons, based on the main effects of treatment and time, demonstrated reductions in rumen pH between baseline measurements (trt0) and all subsequent treatment periods (trt1-trt3). This indicated a progressive decline in rumen pH following repeated ORS administration. Differences were also identified between trt1 and both trt2 and trt3, supporting a continued reduction in rumen pH over time. In contrast, no difference was detected between trt2 and trt3, suggesting that rumen pH stabilised following the second treatment period. The greatest estimated mean differences relative to baseline were observed at trt2 and trt3 (Figure 3.4).

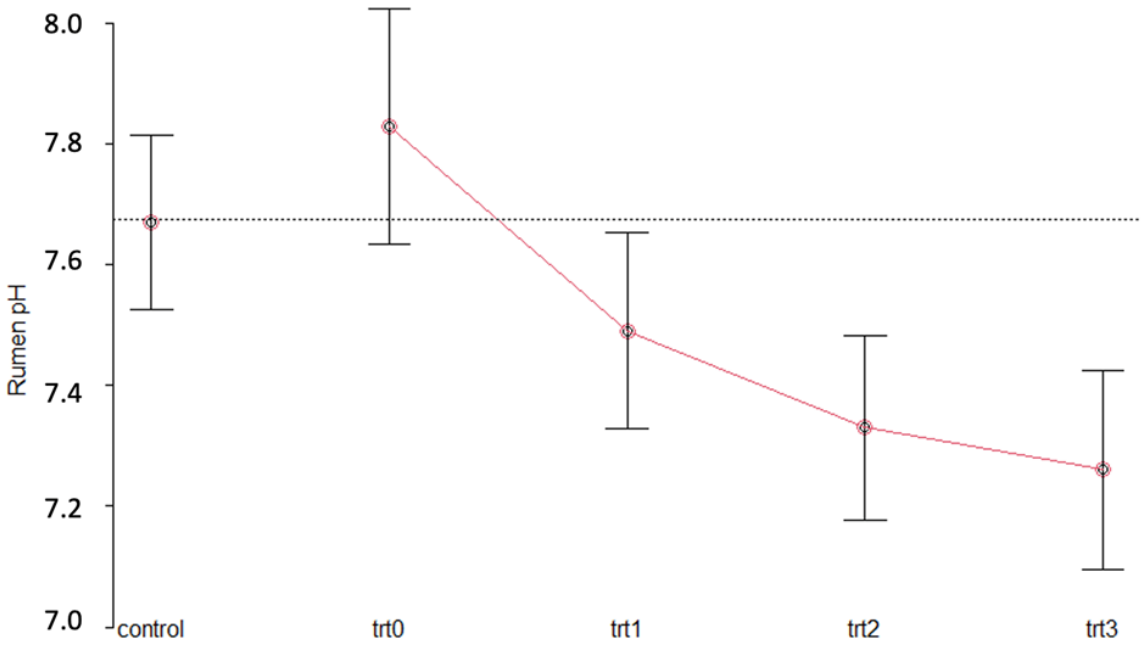

Figure 3.3 Model rumen pH for the Control and Treatment groups based on mixed-effects regression analysis. Note: Points represent estimated marginal means, and error bars indicate 95% confidence intervals.

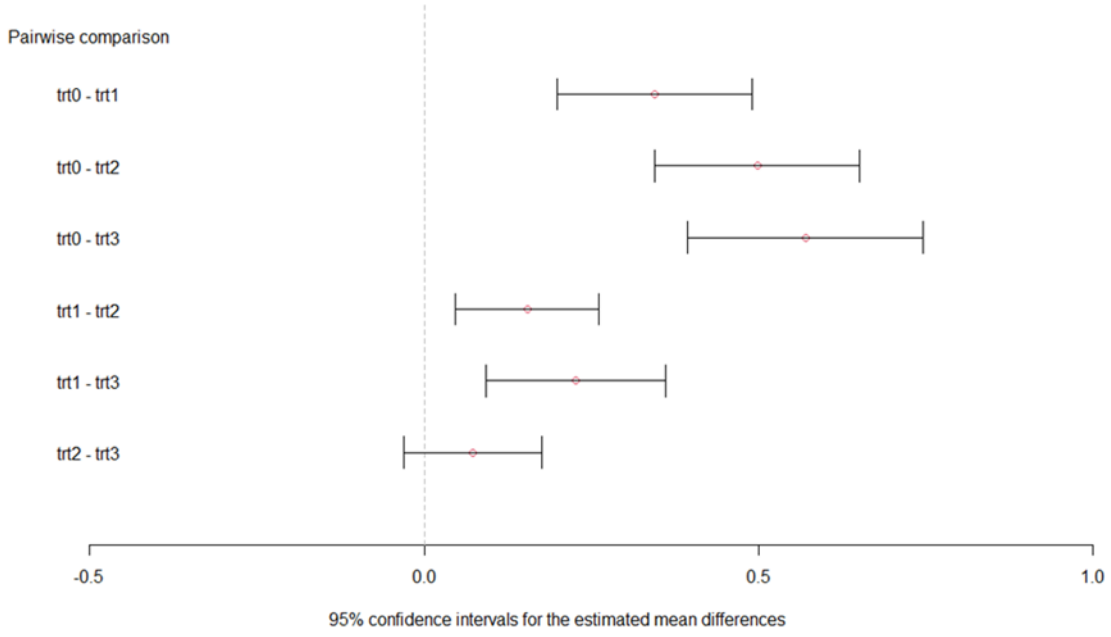

Figure 3.4 Post-hoc pairwise comparisons of model-estimated rumen pH between treatment periods.

Note: Points represent estimated mean differences, and error bars indicate 95% confidence intervals derived from the mixed-effects model. Confidence intervals crossing zero indicated no significant difference. Comparison was made of the Treatment group (n = 6) not the Control group.

3.2 Blood parameters

3.2.1 Glucose concentrations

Blood glucose concentrations increased following ORS administration and were higher in the Treatment group at 2 h compared to Control ewes, as shown in Figure 3.5 and Table 3.1. The change in blood glucose concentration from baseline to 2 h (Δ glucose) was also greater in the Treatment group compared to Control (mean difference −3.23 mmol/L, 95% CI −4.96 to −1.51; $p = 0.002$).

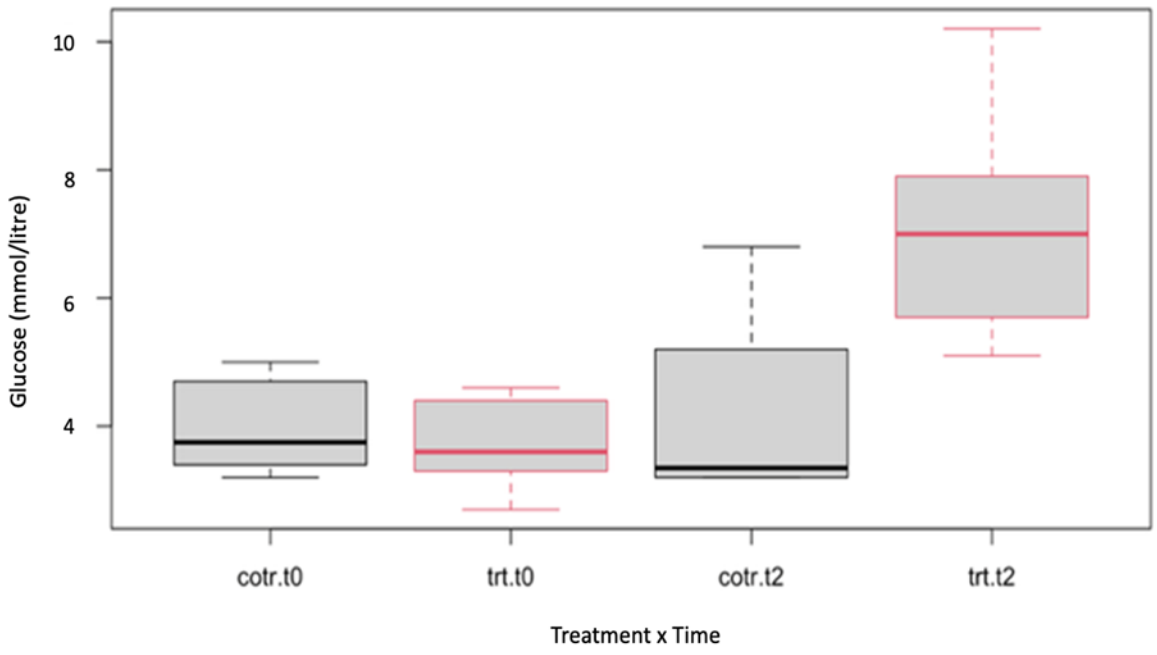

Figure 3.5 Blood glucose concentrations by treatment group at baseline (t0) and 2 h post-administration (t2).

Note: Box-and-whisker plots display median (horizontal line), interquartile range (box; 25th–75th percentile), and range (whiskers). Black represents the Control group and red represents the Treatment group. The left panel (t0) represents baseline measurements prior to ORS administration, while the right panel (t2) represents measurements collected 2 h following ORS administration.

Table 3.1 Mean glucose (mmol/L) and insulin (uM/L) concentrations of ewes at baseline (t0) and 2 h post administration of either water (Control) or oral rehydration solution (Treatment).

Parameter	Control	Treatment	Between-group difference (95% CI)	*p*-value
Glucose at t0 (mmol/L)	3.97	3.70	0.27 (-0.66 to 1.19)	0.534
Glucose at t2 (mmol/L)	4.18	7.15	-2.97 (-5.14 to -0.80)	0.013
Δ glucose (t2 - t0)	0.22	3.45	-3.23 (-4.96 to -1.51)	0.002
Insulin at t0 (uM/L)	8.93	6.75	2.18 (-11.10 to 15.47)	0.719
Insulin at t2 (uM/L)	7.50	19.15	-11.65 (-35.85 to 12.55)	0.286
Δ insulin (t2 - t0)	-1.43	12.40	-13.83 (-26.87 to -0.80)	0.040

3.2.2 Insulin concentrations

Serum insulin concentrations increased over time in the Treatment group following administration of trt1, whereas concentrations remained stable or slightly decreased in the Control group (Figure 3.6.). The change in insulin concentration (Δ insulin; t2 − t0) was greater in the Treatment group compared with the Control (−13.83, 95% CI −26.87 to −0.80; $p = 0.040$). No differences in absolute insulin concentrations were observed between groups at baseline ($p = 0.719$) or at 2 h post-treatment ($p = 0.286$), as shown in Table 3.1. However, there was a greater ($p = 0.040$) change in insulin concentrations between baseline and 2 h post-treatment ($p = 0.040$) for the Treatment ewes compared with the Control ewes.

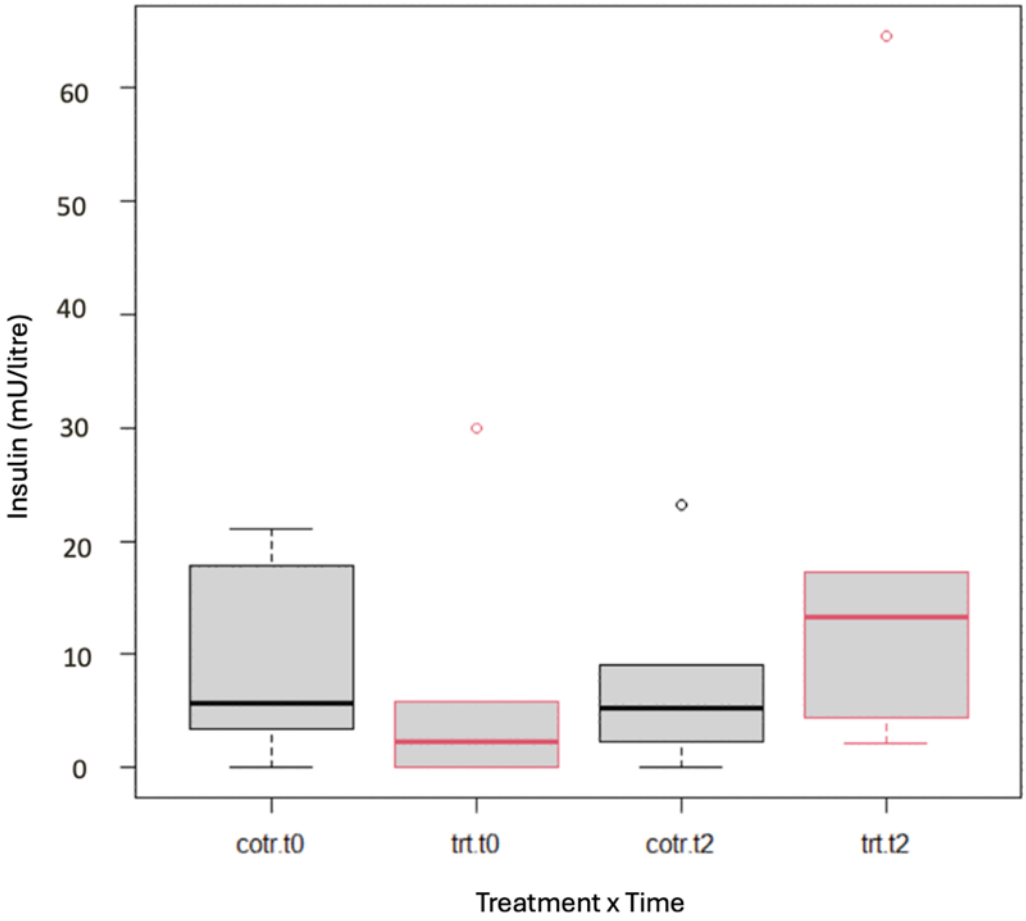

Figure 3.6 Serum insulin concentrations by treatment group at baseline (t0) and 2 h post-administration (t2).

Note: Box-and-whisker plots display median (horizontal line), interquartile range (box; 25th–75th percentile), and range (whiskers). Values are presented for Control (black) and Treatment groups (red) after one administration of the oral rehydration solution (trt1).

3.2.3 Blood electrolytes

Sodium and chloride concentrations increased following treatment (Table 3.3). The change in sodium (Δ Na^+) and chloride (Δ Cl^-) concentrations was significantly greater in the treatment group compared to control (both $p < 0.001$). At 2 h both sodium (Figure 3.7) and chloride (Figure 3.8) concentrations remained higher in the Treatment group (Na^+ $p < 0.001$; Cl^- $p = 0.0166$).

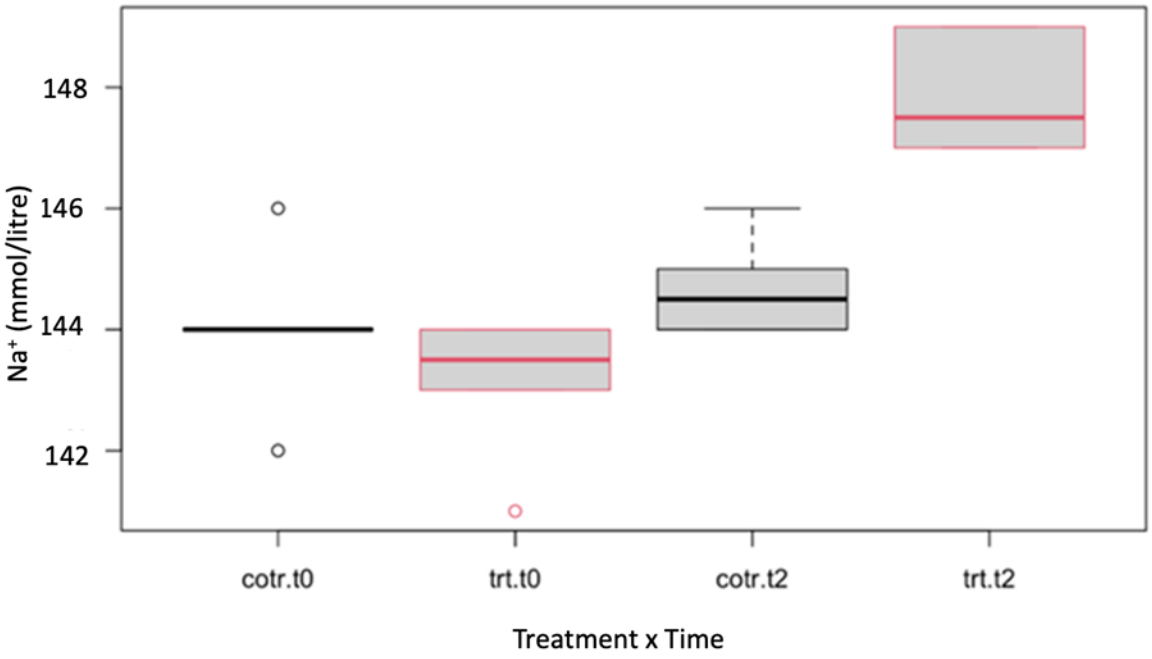

Figure 3.7. Mean blood sodium (Na^+) concentrations (mmol/L) of ewes at baseline (t0) and 2 h post administration of either water (Control) or oral rehydration solution (Treatment).

Note: Box-and-whisker plots display median (horizontal line), interquartile range (box; 25th–75th percentile), and range (whiskers). Values are presented for Control (black) and Treatment groups (red) after one administration of the ORS (trt1), demonstrating higher post-treatment values in the treatment group.

* Laboratory Na^+ reference interval: 141–149 mmol/L

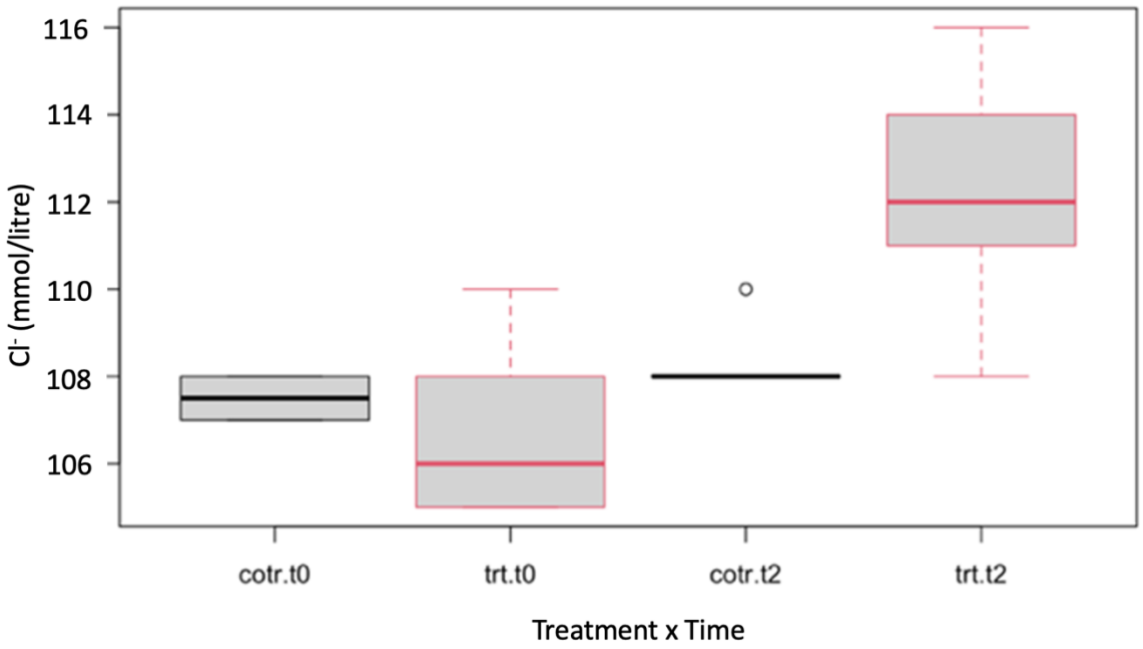

Figure 3.7. Mean blood chloride (Cl^-) concentrations (mmol/L) of ewes at baseline (t0) and 2 h post administration of either water (Control) or oral rehydration solution (Treatment).

Note: Box-and-whisker plots display median (horizontal line), interquartile range (box; 25th–75th percentile), and range (whiskers). Values are presented for control (black) and treatment groups (red) after one administration of the ORS (trt1), demonstrating higher post-treatment values in the treatment group.

* Laboratory Cl^- reference interval: 102–110 mmol/L

3.4 Other variables

All pre-trial clinical examination parameters, including general appearance, body condition, respiratory rate, and heart rate were within normal limits. As shown in Table 3.3, no differences ($p > 0.05$) were observed between groups for all measured variables at the start of the study, including glucose, insulin, blood gas (pH, PCO_2, PO_2), electrolyte (sodium, potassium, chloride, calcium), haematocrit and lactate. Haematocrit decreased over time in both groups, from 33.50% to 31.50% in the control group and from 33.33% to 30.17% in the treatment group, with a greater numerical reduction observed in the treatment group (Δ −3.17 vs −2.00; $p = 0.128$).

Similarly, lactate concentrations decreased in both groups, from 3.38 mmol/L to 1.17 mmol/L in the control group and from 2.27 mmol/L to 1.03 mmol/L in the treatment group, with a numerically greater reduction in the control group (Δ −2.22 vs −1.23; $p = 0.0879$), although this did not reach statistical significance.

Table 3.3 Mean blood biochemistry parameters of ewes at times baseline (t0) and 2 h post administration of either water (Control) or oral rehydration solution (Treatment).

Parameter	Control	Treatment	Between-group difference (95% CI)	*p*-value
Blood pH at t0	7.435	7.450	-0.015 (-0.047 to 0.017)	0.327
Blood pH at t2	7.465	7.465	0.000 (-0.031 to 0.031)	1.000
Δ Blood pH (t2 - t0)	0.030	0.015	0.015 (-0.012 to 0.042)	0.241
PCO_2 at t0 (mmHg)	37.67	39.33	-1.67 (-5.04 to 1.71)	0.297
PCO_2 at t2 (mmHg)	39.00	39.00	0.00 (-2.43 to 2.43)	1.000
Δ PCO_2 (t2 - t0)	1.33	-0.33	1.67 (-0.19 to 3.53)	0.072
PO_2 at t0 (mmHg)	40.67	39.33	1.33 (-3.92 to 6.59)	0.560
PO_2 at t2 (mmHg)	40.33	42.17	-1.83 (-6.00 to 2.33)	0.340
Δ PO_2 (t2 - t0)	-0.33	2.83	-3.17 (-8.28 to 1.94)	0.188
Na^+ at t0 (mmol/L)	144.00	143.17	0.83 (-0.73 to 2.40)	0.264
Na^+ at t2 (mmol/L)	144.67	147.83	-3.17 (-4.33 to -2.00)	<0.001
Δ Na^+ (t2 - t0)	0.67	4.67	-4.00 (-5.35 to -2.65)	<0.001
K^+ at t0 (mmol/L)	4.30	4.38	-0.08 (-0.48 to 0.32)	0.653
K^+ at t2 (mmol/L)	4.15	4.12	0.03 (-0.31 to 0.38)	0.833
Δ K^+ (t2 - t0)	-0.15	-0.27	0.12 (-0.22 to 0.46)	0.451
Cl^- at t0 (mmol/L)	107.50	106.67	0.83 (-1.23 to 2.89)	0.357
Cl^- at t2 (mmol/L)	108.33	112.17	-3.83 (-6.68 to -0.99)	0.0166
Δ Cl^- (t2 - t0)	0.83	5.50	-4.67 (-6.32 to -3.02)	<0.001
Ca^{2+} at t0 (mmol/L)	1.1483	1.1817	-0.0333 (-0.1567 to 0.0900)	0.559
Ca^{2+} at t2 (mmol/L)	1.1600	1.1667	-0.0067 (-0.1185 to 0.1052)	0.895
Δ Ca^{2+} (t2 - t0)	0.0117	-0.0150	0.0267 (-0.0208 to 0.0742)	0.239
Hct at t0 (%)	33.50	33.33	0.17 (-3.54 to 3.87)	0.922
Hct at t2 (%)	31.50	30.17	1.33 (-2.33 to 4.99)	0.435
Δ Hct (t2 - t0)	-2.00	-3.17	1.17 (-0.40 to 2.73)	0.128
Lactate at t0 (mmol/L)	3.383	2.267	1.117 (-0.51 to 2.744)	0.1536
Lactate at t2 (mmol/L)	1.167	1.033	-0.133 (-0.495 to 0.761)	0.6454
Δ Lactate (t2 - t0)	-2.217	-1.233	-0.983 (-2.153 to 0.187)	0.0879

Chapter 4: Discussion

Administration of a propietary ORS (Vytrate) increased blood glucose, sodium and chloride and serum insulin concentrations, and reduced rumen pH in healthy, feed-restricted ewes. Although rumen pH declined following administration of the ORS, it did not reach the thresholds associated with SARA (pH 5-5.5) or ruminal acidosis (pH<5) (Dagnaw Fenta et al., 2023; Lettat et al., 2010; Oetzel, 2000). These findings suggested glucose and electrolyte-containing oral formulations may improve systemic energy status without causing clinically significant disruption to rumen homeostasis under the conditions of this study. This is clinically relevant to the management of PT, as the use of oral glucose remains controversial due to concerns that rapid ruminal fermentation may exacerbate metabolic instability (Crilly et al., 2021; Mongini & Van Saun, 2023).

Whilst the administration of oral glucose has been shown to reduce rumen pH in sheep (Gregory et al., 2009), several buffering mechanisms and physiological factors may have mitigated this effect in the present study. These include salivary buffering and potential sample contamination with saliva, VFA absorption with associated bicarbonate exchange, and possible oesophageal groove activation allowing glucose to bypass the rumen into the abomasum. Other pathways, such as SGLT-1 could facilitate glucose absorption directly from the rumen (Aschenbach et al., 2000). These would not necessarily directly buffer the rumen pH but could decrease rumen glucose available for fermentation.

Ruminant saliva contains bicarbonate and phosphate ions that contribute to ruminal buffering by neutralising hydrogen ions produced during microbial fermentation and production of VFA, resulting in prevention of subsequent SARA or acidosis (Castillo-Lopez et al., 2021). Different salivary components have been linked to rumen microbial fermentation (Palma-Hidalgo et al., 2021). The composition of saliva, and hence buffering capacity, can be altered in sheep when under physiological stress, which may be relevant in ewes with PT (Contreras-Aguilar et al., 2019). Additionally, ruminal fluid samples were taken via oesophageal intubation, and contamination of the tube with saliva may have contributed to the measured rumen pH remaining above the normal pH range of 6.4-6.8 (Jasmin et al., 2011). Rumen pH declined progressively following treatment with the ORS, from a median of approximately 7.9 at baseline to around 7.1 2 h after the final administration. Rumen pH of the Control ewes remained relatively stable at approximately pH 7.6 throughout the study. Variability in rumen pH measurements increased during the later treatment periods, which may have reflected inter-animal differences in ruminal fermentation dynamics, buffering capacity, oesophageal groove activation, or direct ruminal glucose absorption as cumulative oral glucose administrations progressed.

In addition to salivary buffering, absorption of VFA across the ruminal epithelium is a major pathway for proton removal and is closely coupled with bicarbonate secretion via VFA^-/HCO_3^- exchange,

which reduces ruminal acid load and enhances buffering capacity (Aschenbach et al., 2011). This coordinated physiological response could explain the observed decrease in ruminal pH following oral glucose administration without progression to SARA or ruminal acidosis, as increased acid production was offset by epithelial absorption and bicarbonate-mediated buffering.

Functional studies have demonstrated the presence of SGLT-1 within the ovine ruminal epithelium, allowing direct absorption of glucose across the rumen wall prior to microbial fermentation (Aschenbach et al., 2000). It is therefore possible that the relatively small glucose load administered in the present study was partially absorbed directly from the rumen, limiting fermentable substrate availability and consequently reducing the extent of VFA and ruminal pH decline.

Alternatively, a degree of oesophageal groove activation may have occurred. Thus, directing the administered fluid directly into the abomasum, facilitating rapid intestinal absorption of glucose and contributing to the observed increase in blood glucose concentrations. This mechanism has been demonstrated in ewes, where glucose-containing solutions can stimulate oesophageal groove closure in some animals (42%) and result in increased systemic glucose availability (Prichard & Hennessy, 1981). This mechanism has also been demonstrated fluoroscopically in sheep, where pharyngeal stimulation with copper or cobalt sulphate solutions induced oesophageal groove closure and direct abomasal delivery of orally administered material in a proportion of animals (Sargison et al., 1999). The relatively high glucose content of the ORS (44.6 g in 160 mL) supported the hypothesis that limited ruminal exposure, due to oesophageal closure and glucose diversion to the abomasum, may have attenuated fermentation-associated declines in ruminal pH (Martín-Alonso et al., 2019b).

In ruminants, elevations in blood glucose concentrations following oral glucose administration stimulate pancreatic β-cell insulin secretion via glucose metabolism-dependent pathways, promoting peripheral glucose utilisation and maintaining glycaemic homeostasis (Guo et al., 2021). Insulin concentrations were measured in this study as blood glucose concentrations were not expected to increase. It was expected the glucose would enter the rumen, undergo rumen microbial fermentation and be synthesised into VFA. However, the observed increase in blood insulin concentration in the present study supported the interpretation that the rise in blood glucose concentrations was biologically meaningful rather than transient at a single timepoint.

There is limited evidence regarding peak insulin concentrations following orally administered glucose in sheep. Experimental studies in ewes have demonstrated that plasma insulin concentrations increased rapidly following IV glucose administration, with peak responses typically occurring within minutes, (Lunesu et al., 2023). Given insulin concentrations were likely to have already peaked prior to 2 h (t0 = 6.75, t2= 19.15, p = 0.040), this indicated rapid glucose absorption, supporting glucose

going directly to the abomasum and small intestine rather than via rumen fermentation, or possibly but unlikely, via SGLT-1 rumen absorption mechanisms.

Pregnant ewes were not used in this study, which was an important limitation when interpreting the clinical applicability of these findings to PT. During late gestation, ewes develop reduced insulin sensitivity and lower circulating insulin concentrations as part of normal metabolic adaptation to support fetal glucose supply (Regnault et al., 2004; Shorten et al., 2016). Consequently, although glucose administration can stimulate insulin secretion, the metabolic response in pregnant or pregnancy toxaemic ewes may be diminished compared to the healthy non-pregnant ewes used in the present study. This insulin resistance may reduce the effectiveness of glucose supplementation alone in clinically affected animals, highlighting the importance of multimodal treatment approaches incorporating glucogenic precursors, fluid and electrolyte therapy, insulin administration, and, in severe cases, induction of parturition or caesarean section (Crilly et al., 2021; Souto et al., 2019).

Buswell et al. (1986) demonstrated a peak increase in blood glucose concentrations 2 h following oral administration of an ORS (Vytrate) in non-pregnant ewes; however, there have not been further experimental studies supporting its application as a treatment regimen for PT. This is likely due to the concerns associated with rumen fermentation of glucose (Crilly et al., 2021). Pregnancy toxaemia is associated with altered rumen fermentation and microbial dysbiosis, characterised by reduced concentrations of key VFA and shifts in rumen microbial composition, which impair nutrient utilisation and further exacerbate negative energy status (Chen et al., 2024). Adding glucose to the rumen would likely enhance this dysbiosis and further disrupt ruminal fermentation, potentially exacerbating negative energy status and increasing the risk of ruminal acidosis.

Blood glucose concentrations vary throughout the course of PT, with ewes typically hypoglycaemic early in the disease process and become hyperglycaemic in later stages (Souto et al., 2019). This is why blood glucose is not considered a reliable prognostic indicator for PT (Iqbal et al., 2022; Sargison, 2007). Nevertheless, assessment of blood glucose concentrations prior to treatment remains an important component of the clinical evaluation and therapeutic management of PT. Early cases of PT can be managed with glucogenic precursors such as propylene glycol or glycerol (Bayne, 2023; Brozos et al., 2011) or with glucose supplementation (Buswell et al., 1986), to restore glycaemic homeostasis, increase energy availability, reduce lipolysis, and reduce mobilisation of NEFA and ketone body production. Glucogenic precursors such as propylene glycol undergo substantial ruminal microbial metabolism, generating propionic acid and other gluconeogenic substrates that may provide a more sustained energy source to the ewe and feto-placental unit (Ferraro et al., 2016; Kristensen & Raun, 2007). In contrast, oesophageal groove closure following oral glucose administration may facilitate more rapid systemic glucose absorption through partial bypass of the rumen, potentially

improving short-term glycaemic support and electrolyte delivery, but possibly reducing exposure of the solution to ruminal fermentation processes (Ørskov & Benzie, 1969).

Administration of the ORS resulted in a significant change in blood glucose concentrations at 2 h (t0= 3.70 mmol/L, t2= 7.15 mmol/L) compared with the Control group. This increase was likely to be clinically meaningful in the context of PT, as it suggested oral glucose delivery may be sufficient to partially correct hypoglycaemia and limit progression of ketogenesis (Martín-Alonso et al., 2019). Assessment of blood glucose concentrations prior to treatment may therefore help guide appropriate therapeutic intervention and identify animals most likely to benefit from glucose supplementation.

Several physiological mechanisms may account for the observed glycaemic response, despite expected ruminal fermentation, although their relative contributions cannot be determined in the present study. Liquid-phase digesta is cleared from the rumen more rapidly than solid material because it consists of fluid and small particles that are not retained within the rumen mat, allowing faster mixing and passage through the reticulo-omasal orifice (De Vega et al., 2025). This reduces ruminal retention time and limits exposure to microbial fermentation, increasing the likelihood that glucose reaches the small intestine for absorption. Although most dietary glucose is typically fermented into VFA in the rumen, a small proportion may escape fermentation (Liu et al., 2020). Glucose can also be directly absorbed across the ruminal epithelium via SGLT-1 mediated, sodium-dependent transport, although this contribution is likely minimal (Aschenbach et al., 2000a; 2000b; 2002). In addition, short-term fasting alters rumen microbial composition and fermentation dynamics, including reductions in key fermenting microbial species (Kim et al., 2019). This may reduce microbial glucose utilisation and increase the likelihood of glucose escaping ruminal fermentation, contributing to increased systemic availability. Rumen activity and coordinated motility could be affected by the PT and derangements associated with the disease complex.

The ORS was administered slowly into the cheek pouch via a catheter-tip syringe, relying on voluntary swallowing of the administered liquid by the ewe. This method may have stimulated the oesophageal groove closure reflex (Comline & Titchen, 1951; Martín-Alonso et al., 2019b; Ørskov & Benzin, 1969; Ruckebusch, 1989) and facilitated at least partial rumen bypass, thereby reducing ruminal exposure and fermentation of glucose. Whilst the observed increase in blood glucose concentration was consistent with this mechanism, the present study could not confirm this hypothesis, and further investigation is required.

There were significant increases in blood sodium and chloride concentrations in the Treatment group compared to the Control group. Addressing concurrent electrolyte and acid–base derangements, as well as dehydration, is an important part in the treatment of PT for both the dam and fetus, regardless of the therapeutic intervention selected (Bayne, 2023). The ORS contained electrolytes which likely

contributed to the observed increases in blood sodium and chloride concentrations. All sodium values were within laboratory reference ranges (141–149 mmol/L).

Chloride concentrations increased up to 112.17 mmol/L which was slightly above the upper reference range (laboratory reference interval 102–110 mmol/L), in the Treatment group, consistent with the development of mild hyperchloremia following ORS administration. From a strong ion perspective, chloride acts as a principal strong anion, and increases in its concentration reduce the strong ion difference, thereby promoting a shift toward metabolic acidosis (Funes & De Morais, 2017; Las et al., 2007). Despite this, systemic acid–base status remained stable in the Treatment group (pH 7.45 to 7.47), indicating that the degree of hyperchloremia induced was not sufficient to cause clinically relevant acidaemia in healthy, feed-restricted ewes, likely due to intact physiological buffering and renal compensatory mechanisms (Jaramillo-López et al., 2017). However, this finding should be interpreted cautiously in the context of clinical disease. In ewes with PT, where ketoacidosis is already present, the physiological capacity to compensate for additional acidification influences is likely reduced. Under such conditions, further increases in chloride may decrease the strong ion difference to a greater extent, predisposing to hyperchloraemic metabolic acidosis and exacerbating systemic acid–base derangement. This has important clinical implications, as treatment of PT is directed toward correcting negative energy status, dehydration, and electrolyte abnormalities while avoiding further deterioration in acid–base status (Ji et al., 2023). Therefore, the use of chloride-rich formulations may not be optimal in compromised animals, and alternative alkalinising sodium salts, such as sodium bicarbonate, acetate, or propionate, may provide a more physiologically appropriate approach by supporting acid–base balance while maintaining electrolyte and energy supplementation.

A decrease in haematocrit was observed in both the Control (t0 = 33.50%, t2 = 31.50%) and Treatment groups (t0 = 33.33%, t2 = 30.17%). As the ewes were water restricted during the trial, this may have reflected haemodilution and improved hydration status following oral fluid administration rather than a direct effect of electrolyte supplementation (Costa et al., 2025). At later sampling timepoints the ruminal fluid samples were more difficult to obtain as the fibre mat appeared to be drier, which could indicate that water absorption was occurring from the gastrointestinal tract, particularly the rumen in this case, to maintain blood volume.

A mild reduction in blood lactate concentrations was observed in both groups, with a slightly greater decrease in the Treatment group (2.27 mmol/L to 1.03 mmol/L) compared to Control (3.38 mmol/L to 1.17 mmol/L). Concurrently, PO_2 increased modestly in the Treatment group (39.33 mmHg to 42.17 mmHg) but remained stable in the Control ewes (40.67 mmHg to 40.33 mmHg). As lactate concentration reflects anaerobic metabolism and tissue perfusion, its reduction, alongside increased PO_2, suggested improved oxygenation and a shift toward aerobic metabolism following treatment

(Elmeligy et al., 2025; Kichmann et al., 2026). This interpretation was further supported by a reduction in haematocrit in both groups, which may indicate improved hydration status following oral fluid administration (Casamassima et al., 2016). However, given the use of healthy, non-pregnant ewes, these findings likely reflect normal physiological responses and may not translate to clinically compromised animals.

2.1 Limitations and future research

Several limitations must be considered when interpreting the findings of this study. The sample size, while determined to be appropriate based on a repeated-measures ANOVA power calculation (α = 0.05, power = 0.8), consisted of 12 animals (six per group). The use of healthy, non-pregnant ewes limited applicability to PT, as these animals did not reflect the metabolic demands of late gestation (Moallem et al. 2012), including fetal glucose requirements and altered insulin dynamics (Lomax et al., 1979; Regnault et al., 2004), and findings may not fully translate to clinical cases. The short study duration and fixed sampling timepoints (-0.5 h and 2 h) may not have captured peak or sustained responses, and the duration of the glycaemic effect remains unknown. Additionally, the absence of blood ketone and rumen VFA measurements limited interpretation of metabolic and fermentative changes. Collection of ruminal fluid samples via oesophageal intubation may have introduced saliva contamination and sampling variability, potentially affecting pH accuracy, although the methodology was consistent across animals.

Future studies should investigate effects in pregnant or clinically affected ewes and include measurement of blood ketone and rumen VFA (acetic acid, propionic acid, and butyric acid) concentrations. Techniques such as markers or imaging could also be utilised to confirm oesophageal groove activation and rumen bypass, alongside evaluation of different administration methods, such as direct drenching rather than slow delivery via the cheek pouch.

This study contributed to the veterinary literature by providing insight into both systemic and ruminal responses to Vytrate liquid concentrate in non-pregnant ewes, incorporating rumen pH data and mechanistic explanations for glucose utilisation. Previous work, such as Buswell (1986), demonstrated rapid increases in blood glucose concentrations but focused primarily on clinical outcomes with limited evaluation of the rumen environment.

2.2 Conclusion

Administration of a proprietary ORS significantly increased blood glucose concentrations 2 h following the first treatment compared with Control ewes. Although rumen pH progressively declined following repeated oral glucose administration, values remained above thresholds associated with SARA and overt ruminal acidosis. Blood sodium and chloride concentrations increased following treatment but generally remained within laboratory reference intervals.

These findings suggested slow oral administration of a proprietary ORS via the cheek pouch may represent a viable supportive treatment strategy for hypoglycaemic ewes. Potential oesophageal groove activation may have contributed to the observed changes in blood variables while limiting ruminal fermentation and acidosis. Further studies in pregnant and clinically affected ewes are required to determine the clinical applicability of this approach in PT.

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