An investigation into the effect of sodium content in plantain (*Plantago lanceolata*) on urine production in sheep

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Agricultural Science.

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The effect of the high sodium content of plantain on urine production in sheep was investigated. Two groups of sheep (n=10) were fed either perennial ryegrass or plantain for 3 days. Allocated feeds were balanced for water and sodium. Water intake, faecal water, and urine volume were measured daily to determine water balance. Plasma and urine samples were measured twice daily for sodium and osmolality. Creatinine, urea and free water clearance were calculated twice daily for each animal. After 10 days the trial was repeated for 2 days using two different groups of sheep. Plantain sheep had a higher urine volume (increase of about 50%) and a lower daily water balance (by about 600 ml) than the ryegrass sheep. Sodium excretion (g/day) in urine was higher in the plantain group. The day with the largest difference in water balance and smallest difference in sodium excretion was Day 3 of Week 1. There were positive relationships shown between the extra sodium ingested by the ryegrass group and the urine volume and sodium excreted in urine. These results confirm plantain causes an increase in dilute urine, without effecting total osmols excreted. They suggest that the higher sodium content in plantain accounts for some of the increase in urine production of the sheep, but the small differences in sodium excreted and large differences in urine volumes suggest sodium is not the only factor causing the diuresis.

Keywords: Plantain, diuresis, water balance, clearance, sodium ingestion, sodium excretion

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Chapter 1

Introduction

Narrow-leaved plantain (*Plantago lancelota*) occurs naturally in many pastures as a weed, however, it has only recently been researched as an alternative forage species to ryegrass in New Zealand pasture based farming systems. It is often used in a plant mixture, is rapid to establish, drought and heat tolerant, high in minerals and highly palatable to animals (Stewart, 1996). It also has potential medicinal properties (Deaker *et al*., 1994). Plantain has a higher concentration of some minerals compared with ryegrass, particularly sodium, cobalt, calcium, copper and zinc (Rumball *et al.,* 1997).

Previous studies have shown a diuresis occurs in animals fed plantain (Deaker *et al*., 1994; Wilman & Derrick, 1994; O'Connell *et al*., 2016), however, the cause of this effect is still unknown. O'Connell *et al*. (2016) found that plantain caused an increase in urine production, and the urine was more dilute but total solute excretion was unchanged. Lindsay (2016) was the first study to investigate the cause of the diuresis in sheep. It was found that there was very little difference in creatinine and urea clearance values of sheep fed plantain or grass, and it was concluded that plantain was not affecting glomerular or proximal tubule function (Lindsay, 2016).

Plantain causing a diuresis in animals may have positive environmental effects, therefore understanding the mechanisms will be beneficial to the agricultural industry. A diuretic effect of a plant results in less nitrogen loading on the soil, as the urine is more dilute (O'Connell *et* al., 2016) and therefore has a lower nitrogen concentration. Less nitrogen loading is beneficial as it will decrease leaching into waterways and therefore help to stop a decrease in water quality. Totty *et al*. (2013) found that plantain altered the nitrogen partitioning of animals, resulting in a reduction of 30-34% of urinary nitrogen concentration in cows grazing a diverse plant mixture (including plantain) compared with cows grazing ryegrass/white clover.

Total body fluid makes up about 60% of an animal's body weight (Physiology Web n.d.), water volume in the body is regulated with sodium. Osmosis is the flow of water from areas with high water potential to low water potential, areas of low water potential have a high concentration of sodium (Cunningham & Klein, 2007). In other words, water flows from areas with low sodium concentration to areas with high sodium concentration. This suggests that the higher sodium content in plantain could be resulting in more water being excreted in urine.

The aim of this project was to determine if the larger sodium content in plantain compared with ryegrass is the cause of the diuretic effect. It has been shown that plantain causes an increase in production of a more dilute urine in sheep (O'Connell *et al.,* 2016; Lindsay, 2016). It has also been shown that creatinine and urea clearance were unaltered by ingestion of plantain, suggesting that the effect of plantain is not on the glomerular and proximal tubule function (Lindsay, 2016). The cause of the diuretic effect of plantain therefore remains unknown. This present study was conducted to confirm the diuretic effect of plantain, and support the findings of Lindsay (2016) that the creatinine and clearance values after ingestion of plantain remain unchanged, and also to determine whether the higher sodium content in the plantain is causing the diuresis. This was achieved by balancing water and sodium input of the sheep and measuring sodium excretion in the urine and faeces. It was hypothesized that the higher sodium content will account for some of the diuretic effect, but not all of it.

Chapter 2

Literature Review

2.1 Plantain (*Plantago lanceolata*)

Plantain as a forage is a relatively new idea and the extent of the effects it has on an animal is still being researched. Plantain was originally a weed and can be found in many roadside pastures. Plantain is rapid to establish, drought and heat tolerant, high in minerals and highly palatable to grazing animals (Stewart, 1996). Plantain has similar breakdown characteristics to ryegrass (Stewart, 1996). On farm, plantain is often used in a plant mixture, as opposed to being fed on its own.

2.1.1 Productivity

An important factor to consider when trialling alternative forage crops is the pasture yield compared with the original forage, which in New Zealand is predominantly a ryegrass/white clover mixture. Plantain has shown to have similar average yields to this standard mix, tending to have higher yields in summer and lower in winter (Stewart, 1996; Woodward *et al*., 2013). Woodward *et al.* (2013) found in a three-year trial in the Waikato that a mixed pasture, containing plantain, produced 12% more dry matter than the ryegrass/white clover pasture over summer and autumn of year two of the trial, and 47% more in the summer and autumn of year three. This trial also found that plantain yielded 36% less in winter than the standard pasture. Over the three-year trial the ryegrass/white clover and mixed pasture produced similar dry matter yields, with mean annual yields of 15.3 t DM/ha and 14.7 t DM/ha respectively.

2.1.2 Animal Production

Plantain has the potential to affect rumen function and therefore can change milk production. Stewart (1996) stated that the antimicrobial compounds present in plantain can inhibit rumen fermentation and change the volatile fatty acid composition of the rumen contents. These changes have the potential to affect animal performance including decrease in the severity of bloat and increased milk production.

Totty *et al.* (2013) found that cows grazing the diverse pasture had a higher milk yield of 16.9 L/day compared with 15.2 L/day for the cows grazing the ryegrass pasture. However, there was no significant difference between the milk solid yields of the cows grazing the two pasture types.

Table 2-1: Milk production in cows grazing a diverse pasture mix compared with cows grazing a ryegrass pasture. (From Totty et al. 2013)

	DIVERSE	RYEGRASS
Milk yield (L/day)	16.9	15.2
Milk solids (kg/day)	1.47	1.55
Milk fat (%)	5	6.1
Milk protein	3.9	4.1

Animals grazing plantain have been shown to have similar live weight gains to animals grazing endophyte free perennial ryegrass. Animals grazing plantain as part of a pasture mix have been shown to have greater live weight gains than animals grazing perennial ryegrass (Stewart, 1996).

2.1.3 Mineral Content

Plantain, when compared with ryegrass and white clover, has a higher content of sodium, cobalt, calcium, copper and zinc (Rumball *et al*, 1997). Stewart (1996) stated that animals grazing plantain had a higher retention of calcium, magnesium and sodium than animals grazing perennial ryegrass: for calcium, it was four times higher. Lindsay (2016) reported that the sodium content of plantain was double that of ryegrass (0.757% DM and 0.343% DM respectively).

2.2 Kidneys

The kidneys filter plasma and plasma constituents from the blood and then selectively reabsorb water and other useful components from the filtrate, ultimately excreting excesses and waste products (Frandson *et al*, 2009). The urinary system is responsible for maintaining the relatively constant volume and composition of the body fluids. The kidneys regulate the body balance of water, electrolytes, acids and bases by adjusting the output of urine in response to changes in diet and metabolism. The kidneys also excrete metabolic waste products in the urine, including urea and creatinine (a by-product of skeletal muscle metabolism) (Frandson *et al*, 2009).

The kidneys are made up of thousands of microscopic functional units called nephrons. The nephrons consist of a spherical structure (Bowman's capsule) that contains an arteriolar complex termed the glomerulus and a single-long convoluted tubule. The single tubule is divided into three segments – proximal tubule, loop of Henle, and distal tubule (Reece, 2005). There are three processes involved in the formation of urine – filtration, reabsorption, and secretion. The blood is filtered at Bowman's capsule and then the filtrate flows through the tubules and collecting ducts where reabsorption and secretion occur. Reabsorption is the removal of substances from the filtered fluid, which are then returned to the blood. Secretion is the addition of substances to the filtered fluid (Frandson *et al*, 2009).

2.2.1 Glomerular filtration

The glomerular filtrate is the fluid and fluid constituents that pass from the blood plasma in the glomerulus into the urinary space of Bowman's capsule. The glomerular filtrate passes through a barrier which acts as a filter, allowing small molecules to go through but blocking passage of larger molecules. Blood cells and high molecular weight proteins are too large to pass through this barrier, but most other plasma constituents can. Glomerular filtration rate (GFR) is controlled by the pressure of blood in the glomerular vessels and the slightly opposing hydrostatic pressure of the filtrate within Bowman's capsule. In mammals GFR is largely controlled by arteriolar blood pressure and, thus, remains relatively stable. Lowered blood pressure leads to vasoconstriction of pre-glomerular vessels, causing a reduced GFR and, if prolonged and excessive can ultimately lead to renal failure (Frandson *et al*, 2009).

2.2.2 Tubular Function

As stated above tubular function involves two main processes: reabsorption and secretion. About 80% of the glomerular filtrate is reabsorbed in the proximal convoluted tubules, the remaining 20%, called the facultative reabsorption takes place in the distal tubular segments. This facultative process is under endocrine control from the posterior pituitary hormone; antidiuretic hormone (ADH, also called vasopressin) and from the adrenal cortex involving the mineralocorticoid aldosterone. ADH is primarily involved in maintaining osmotic concentration of the extracellular fluid (ECF), and aldosterone can increase the reabsorption of sodium ions from the urine in order to maintain sodium concentration and the volume of ECF. Tubular secretion is largely regulated by carrier processes that seem to be specific for individual waste products, toxins and foreign molecules, but the secretion of hydrogen and potassium ions occurs as a consequence of aldosterone-directed reabsorption of sodium (Frandson *et al*, 2009).

The process of water reabsorption alone – i.e. not water reabsorption that is associated with the reabsorption of sodium – is regulated by a mechanism involving the formation of a high osmotic concentration of interstitial fluid (ISF) in the renal medulla through action of tubular cells in the Loop of Henle plus the opening of water channels in the distal tubules and collecting ducts caused by ADH. This allows water to be passively reabsorbed down the osmotic gradient and is the process of water conservation that operates continuously in all mammals, except when there is a diuresis (Reece, 2005).

2.2.3 Osmosis

Osmosis is the movement of water across a semipermeable membrane in response to the difference in electrochemical potential of water on the two sides of the membrane (Cunningham & Klein, 2007).

Na^+ and Cl^- cannot move through a lipid bilayer to balance out solute concentration on either side of the membrane, instead water moves from the side with high water potential (low solute concentration) to the side with lower water potential (high solute concentration) to balance out the solute concentrations (Cunningham & Klein, 2007). Osmosis can therefore be described as water following sodium (and chloride) ions.

2.2.4 Clearance

Glomerular filtration rate is one of the most important parameters of renal function, and is largely determined by clearance. Clearance is the rate that plasma is cleared of a substance, it is the sum of the rates of filtration and secretion minus the rate of reabsorption of the substance (Cunningham & Klein, 2007). The most widely used measure of glomerular filtration in clinical situations is creatinine clearance. Creatinine is a waste product of muscle metabolism, and is only secreted in very small amounts in kidney tubules (Cunningham & Klein, 2007). Urea is a waste product created by protein metabolism, it is both secreted and reabsorbed by kidney tubules and therefore is less illustrative of glomerular filtration rate. Free water clearance is the volume of blood plasma that is cleared of solute-free water by the glomerulus per unit time (Vincent & Hall, 2012) and is a good indication of glomerular function.

2.2.5 Diuretics

A diuresis is an excessive production of urine, there are two main forms of diuresis; water and osmotic. Water diuresis occurs when an excessive amount of water is consumed. This results in the excess water, not needed for reabsorption, being secreted as urine. Osmotic diuresis is a result of substances in the kidney tubules that cannot be reabsorbed, for example glucose. This results in an increase in osmotic pressure in the kidney tubules, resulting in water flowing into the tubules and ultimately in an increase in urine production.

A diuretic promotes the formation of urine by the kidney. There are different forms of diuretics, that all work slightly differently. Ultimately the diuretic alters the ability of the kidney to reabsorb certain ions and molecules which causes them to be excreted as urine, this process attracts water and leads to an increase in urine volume (Rose, 1991). Diuretics are often used in medicine to help with treatment of different conditions such as high blood pressure.

The different types of diuretics include; loop diuretic, thiazide diuretics, potassium-sparing diuretics, and osmotic diuretics. Loop diuretics work by inhibiting sodium reabsorption from the loop of Henle. This increases the sodium ions excreted in urine, which in turn increases the water excreted in urine, and ultimately urine production (MedicineNet, 2016). Thiazide diuretics enhance the excretion of sodium and potassium ions by preventing sodium reabsorption from the distal convoluted tubule in the nephron (Rose, 1991). This causes an increase in water excretion and therefore urine volume. Potassium-sparing diuretics act by blocking the exchange of Na^+ for K^+, resulting in excretion of sodium, and therefore water (MedicineNet, 2016). Osmotic diuretics are substances that increase osmotic concentration (and osmotic pressure) within the nephron tubules, which decreases water reabsorption (Atherton *et al*., 1968).

2.3 Effects of plantain

Consumption of plantain has been shown to increase urine volume (Wilman & Derrick 1994; O'Connell *et al*., 2016; Lindsay, 2016). Wilman & Derrick (1994) found that plantain resulted in nearly double the weight of urine produced than that of a ryegrass diet (1.32 kg/sheep/day and 0.73 kg/sheep/day respectively). O'Connell *et al*. (2016) found that despite balancing water intake of feed, sheep eating plantain produced a higher urine volume than ryegrass sheep, suggesting a diuretic effect. The urine volume of the plantain sheep was 1.7 L greater than ryegrass sheep on Day 1 of the trial, and was 0.5 L greater for the remainder of the trial (O'Connell *et al*., 2016). The urine produced from the plantain sheep was more dilute than that of the ryegrass sheep, with a lower specific gravity and osmolality in the urine of the plantain group, however both groups had similar total osmols per day (O'Connell *et al*., 2016).

Lindsay (2016) was the first study that investigated the reason for the diuretic effect of plantain. It was confirmed urine volume was greater in sheep fed plantain compared with sheep fed ryegrass, despite balancing water intake. Urine production was 880 ml greater in plantain sheep, and daily water balance 660 ml less in plantain sheep compared with ryegrass sheep, confirming the diuretic effect (Lindsay, 2016). There was very little difference between creatinine and urea clearance values, which suggests plantain is not affecting glomerular and proximal tubule function (Lindsay, 2016).

2.4 Objectives of study

The role of plantain in agricultural systems is still a relatively new subject with research into the effects of plantain on animals still ongoing. In previous studies (O'Connell *et al*., 2016; Lindsay, 2016) it has been shown that plantain causes a diuretic effect in sheep. However, the exact cause of this diuresis still remains unknown. The findings of the study conducted by Lindsay (2016) show that the diuretic effect of plantain is not on glomerular and proximal tubule function.

With the knowledge that water 'follows' sodium ions in order to maintain homeostasis in the fluid, the higher sodium content of plantain could be the cause of the diuresis. The objectives of this study were to determine if this is true. To do this, the current study worked to balance both water and sodium intake of the two groups of sheep.

Chapter 3

Methods

3.1 Trial Design

3.1.1 Sheep and Feed

This study used 20 Romney cross five-month-old ewe hoggets owned by commercial seed company PGG Wrightson Seeds (Hornby, New Zealand). Leading up to the experiment they were grazed on predominantly ryegrass pasture near Lincoln, Canterbury, New Zealand (Kimihia Research Centre). On 1 May 2017, they were transported 5.2 km to the Johnstone Memorial Laboratory of Lincoln University (-43.644175 E, 172.451685 S) where they were held indoors for 5 days in individual metabolism crates with no access to drinking water. On that first day (1 May) all sheep were allocated perennial ryegrass (*Lolium perenne).* The following morning all feed refusals were discarded. On Day 0 of the trial the sheep were all allocated 4.7 kg fresh weight of perennial ryegrass. On the morning of 3 May (Day 1) the sheep were allocated to one of the two dietary treatments; perennial ryegrass (*Lolium perenne,* n = 10) or plantain (*Plantago lanceolata*, cultivar PG742, n = 10). The allocation of treatments was balanced for urine volumes recorded on Day 0.

The sheep were held in two separate rooms, one room contained six plantain and five grass treatments and the other contained four plantain and five grass treatments. The trial ended on the 6th May 2017.

All procedures conducted in this trial were under approval of the Lincoln University Animal Ethics Committee.

3.1.2 Liveweight

Non-fasted live weight of the sheep was obtained immediately prior to the start of the trial and immediately after its completion using electronic scales (XR5000, Tru-test, Auckland, New Zealand). Daily weight loss of sheep was calculated using the following equation:

(Initial weight – Final weight)/Number of days.

3.1.3 Feed Harvesting

Plantain and ryegrass monocultures were grown at the Kimihia Research Centre. Feed was harvested at about 1330 h each day using a motorised forage harvester (Wintersteiger Cibus F, Wintersteiger AG, 4910 Ried, Austria) which weighed out and collected the required pasture into a wool fadge. The feed was immediately transported to the Johnstone Memorial Laboratory where three samples of each feed were weighed into perforated bags and placed in a 90°C oven for 24 hours to dry. The samples were then reweighed to determine the dry matter percentage of the feed, using the following calculation; average dry weight/average fresh weight x 100. The remaining feed was kept in the wool fadges and placed in a refrigerator overnight. Harvesting the day before meant the feeds did not absorb moisture from overnight dew and rain.

The feed was fed at 0.75 kg DM/hd/day. The fresh weight was determined using the dry matter percentage of the feed from the previous day. Any refused feed was weighed and discarded, this weight was subtracted from the weight of the allocated feed to determine the intake of feed of each animal. The feed was allocated at about 1000 h each day.

Water was added to the grass feed using a spray bottle. The amount of water was determined by calculating the difference in water content of the two feeds using the dry matter percentages. Salt (NaCl) was dissolved in this water to balance sodium ingestion of the two feeds. The amount was determined by calculating the difference in sodium content of the two feeds and dividing this by 39.33% (percentage of sodium in sodium chloride).

3.1.4 Urine and Faeces

Urine and faeces were collected separately in trays and containers placed under the metabolism crates. To prevent mixing of faeces and urine, a net separator (shade cloth stretched over a frame and placed over the collection container) was used to collect the faeces and allow urine to flow through into the container.

At 0830 h each day the metabolism crates were cleaned and the faeces was collected for each animal. The faeces were weighed and a sample was taken and placed in a snap lock polythene bag and frozen (-20 °C) for analysis. Another two samples were taken and weighed individually. These samples were placed in perforated plastic bags and put in an oven then dried for 48 h at 90°C. Once dry the samples were reweighed and the dry weight was used to calculate the dry matter content of the faeces (dry weight/fresh weight x 100). The average of the two samples for each animal was used to determine the faecal water content.

At each urine collection (i.e. twice per day) a sample of freshly voided urine was collected – fresh urine. Following this, 125 ml of sulphuric acid (at 5% concentration) was placed in the urine containers prior to further collection of urine to prevent loss of nitrogen by volatilisation. Urine volume was measured twice daily; at 0830 h and 1700 h. The volume was determined by pouring urine from the collection container into a measuring cylinder and recording the total volume. A 50ml sample was taken from this acidic urine and placed in a freezer (-20 °C) for further analysis; the remainder of the urine was discarded. The urine collection containers were rinsed with tap water before being placed back under the metabolism crates.

Within 2 hours of collection, pH was determined for the fresh urine samples using a portable pH reader (Mettler Toledo Seven Easy, Auckland, New Zealand). Refractive index was determined using a portable refractometer (Atago R5000, Tokyo, Japan). The refractive index was used to calculate specific gravity and osmolality of the urine. Urine osmolality was calculated using the formula: (Specific gravity – 1) x 32000.

The remainder of the urine sample were kept in their collection containers and placed into a plastic snap-lock bag and placed in a freezer (-20°C) for storage.

Total urine volume for the day was calculated as; total volume of urine collected at two times during day + volume of the two fresh urine samples (2 x 50ml) – volume of acid added to each collection container (2 x 125ml).

3.1.5 Daily Water Balance

Daily water balance was calculated as total water consumed – (daily faecal water volume + daily urine volume).

For the sheep fed plantain total water consumed was calculated as (feed eaten) – (feed eaten x (DM% of feed/100)).

For sheep fed grass total water consumed was calculated as (feed eaten – (feed eaten x DM% of feed/100)) + (water allocated – water refusal).

3.1.6 Blood Samples

At approximately 0930 h and 1800 h each day a 9 ml blood sample was taken from each sheep. The blood sample was taken by jugular venepuncture while the sheep were manually restrained. To take the blood samples 0.9 x 25 mm needles were used (Becton Dickinson Vacutainer Systems, Precision Glide, Plymouth, United Kingdom) and evacuated plastic tubes containing lithium heparin as anticoagulant (Greiner Bio-One, Vacuette, Kremsmünster, Austria). Immediately after the blood sample was taken the blood tube was inverted several times to ensure dispersal of the anticoagulant.

The haematocrits (packed cell volume) of the blood samples was determined in duplicate using the first and last blood samples of the week. Microhaematocrit tubes were filled with blood and plugged with putty. The tubes were spun in a microhaematocrit centrifuge (Heraeus Sepatech Haemofuge, Hanau, Germany) for 5 minutes and the packed cell volume was determined using a microhaematocrit reader (Hawksley Micro-haematocrit Reader, London, England).

The blood samples in the evacuated plastic tubes were centrifuged at 2500 rpm for 20 minutes. Plasma was obtained by aspiration with disposable plastic Pasteur pipettes and transferred to labelled polycarbonate tubes, which were then frozen (-20 °C) for storage and further analysis.

3.1.7 Plasma and Urine Analysis

Plasma and urine samples were analysed at a commercial analytical laboratory (Gribbles Veterinary, Christchurch, New Zealand). Both plasma and urine were analysed for sodium, total protein, creatinine and urea.

3.1.8 Clearance calculations

Free water clearance was calculated as:

Urine osmolality x Urine flow rate / Plasma osmolality.

Creatinine and urea concentrations were used to calculate clearance values using the following formula:

Urine flow rate x Urine concentration / Plasma concentration.

3.2 Week Two

The trial was repeated for a second week (15^{th} – 19^{th} May), however a different group of sheep were used so the second week was considered a separate trial. The trial design remained the same for the second week, however, the trial ended one day earlier in the second week.

3.3 Statistical Analysis

Data for each week was kept and analysed separately, producing two sets of results. Data for each group was averaged on Microsoft Excel into groups of; total feed group, feed group daily, and feed group morning and afternoon. These groups were analysed using a one-way and two-way ANOVA on Minitab 17. For some measurements the plantain group was compared with the three ryegrass sheep that had the highest water intake. Relationships between the sodium ingested and other measures of the ryegrass sheep were determined using a scatter plot graph made in Microsoft Excel, a trend line was added to help determine the relationship.

Measurements taken on the first and last day of the trial were analysed by calculating the difference between these two values, and putting the averages of the differences into a one-way ANOVA.

All data was considered significant if $P < 0.05$.

Chapter 4

Results

4.1 Trial Results

The results of the two weeks of the trial were kept and analysed as two separate trials as they used different sheep and therefore Week 2 could not be considered a repeat of Week 1. In the second week, a sheep in the grass group was removed on Day 0, due to general poor health. The data from the blood samples and live weight of this sheep were not used for analysis.

4.2 Week one results

4.2.1 Sodium

There was no difference between overall plasma or urine sodium (mmol/L) between the two feeds (Table 4-1). The afternoon values of plasma sodium were higher than the morning concentrations (Table A.4). The difference in urine sodium concentration decreased over the three days (Table A.3). There was a significant interaction between feed and day, and feed and time, for sodium concentration in the urine (Table 4-1).

There was a significant difference in sodium excreted in urine (g/day) between the two feeds (Table 4-1), the plantain group had a higher sodium concentration (g/day) in the urine excreted than the ryegrass sheep (Table 4-1). The difference between the two groups decreased over the three days (Figure 4-1 & Table A.3). Both groups had a higher sodium excretion concentration in the morning than afternoon (Figure 4-1 & Table A.4). There was a significant interaction between feed and day, and between feed and time (Table 4-1).

Table 4-1: Mean sodium (Na^+) output (± S.E.M.) of sheep fed plantain (n=10) or ryegrass (n=10) for 3 days. Statistical significance values given as * is *P* < 0.05, ** is *P* < 0.01, * is *P* < 0.001 'very highly significant', ns is not significant.**

	Plantain (n=10)	Ryegrass (n=10)	Feed	Day	Time	Feed * Day	Feed * Time
Plasma (mmol/L)	147.8 ± 2.3	147.9 ± 2.5	ns	ns	***	ns	ns
Urine (mmol/L)	46.2 ± 0.4	46.1 ± 0.4	ns	***	ns	**	**
Urine (g/day)	3.05 ± 0.2	2.17 ± 0.2	***	***	***	*	**

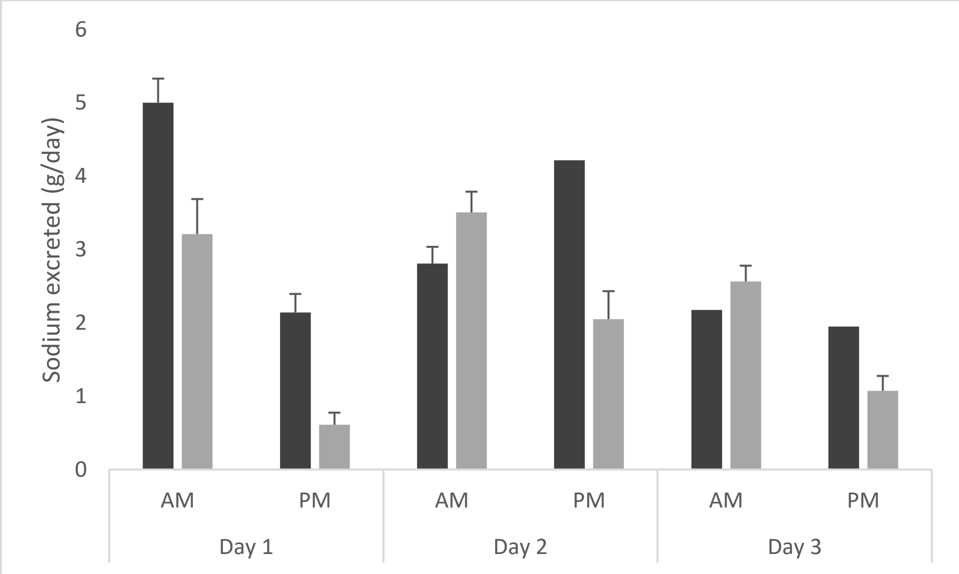

Figure 4-1: Mean sodium excretion in urine in sheep fed either plantain (n=10) (dark grey) or ryegrass (n=10) (light grey) for 3 days. Error bars show + S.E.M.

The plantain group consumed more sodium than the ryegrass group (Table 4-2), this was based on the sodium concentration values from Lindsay (2016).

Table 4-2: Sodium ingested (± S.E.M.) of sheep fed either plantain (n=10) or ryegrass (n=10) for 3 days. Statistical significance values given as * is *P* < 0.05, ** is *P* < 0.01, * is *P* < 0.001, ns is not significant.**

	Plantain (n=10)	Ryegrass (n=10)	Feed	Day	Feed * Day
Sodium ingested (g/day)	5.58 ± 0.06	4.47 ± 0.06	***	ns	ns

4.2.2 Clearance

There was a significant difference between the creatinine and urea clearances between the two groups, both were higher in the ryegrass sheep (Table 4-3). The urea clearance was higher in the afternoon for both groups (Table A.4). There was a significant interaction between feed and day for both creatinine and urea clearances (Table 4-3).

There was no significant difference between the overall mean free water clearances for the two groups (Table 4-3). However, in both groups the free water clearance decreased on the middle day and then increased again on the last day (Figure 4-2, Table A.3). The afternoon clearance was higher in both groups (Figure 4-2, Table A.4).

Table 4-3: Mean daily clearance values (± S.E.M.) of sheep fed plantain (n=10) or ryegrass (n=10) for 3 days. Statistical significance values given as * is *P* < 0.05, ** is *P* < 0.01, * is *P* < 0.001, ns is not significant.**

	Plantain (n=10)	Ryegrass (n=10)	Feed	Day	Time	Feed * Day	Feed * Time
Creatinine (ml/min)	83.96 ± 3.7	98.67 ± 5.5	*	ns	ns	***	ns
Urea (ml/min)	42.31 ± 2.5	50.4 ± 3.2	*	ns	***	***	ns
Free water (ml/min)	2.797 ± 0.2	2.574 ± 0.2	ns	***	**	ns	ns

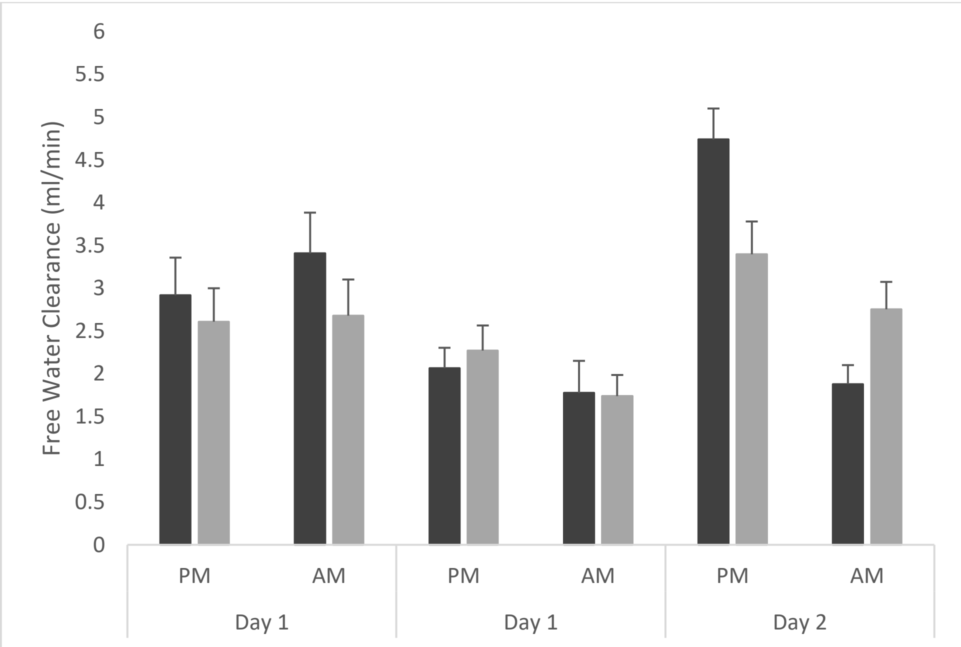

Figure 4-2: Mean free water clearance of sheep fed plantain (n=10) (dark grey) or ryegrass (n=10) (light grey) for 3 days. Vertical bars show + S.E.M.

4.2.3 Daily water balance

There was a significant difference between average daily water balances of the two feeds (Table A.2). Both groups had a positive mean daily water balance, the grass group had a higher water balance (Figure 4-3, Table A.2).

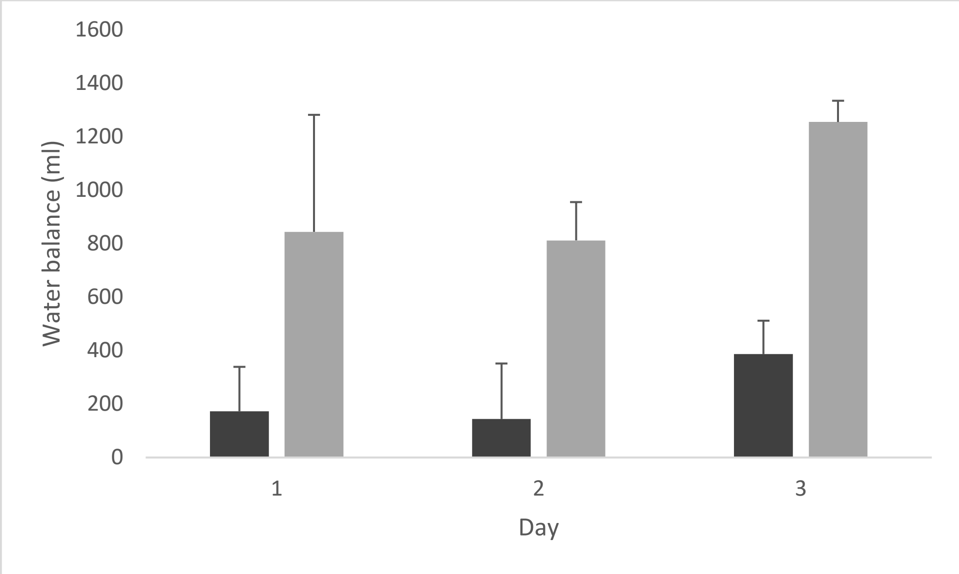

Figure 4-3: Mean daily water balance of sheep (n=10) fed plantain (dark grey) or ryegrass (light grey) for 3 days. Vertical bars show + S.E.M.

4.2.4 Other measurements

Both groups lost weight during the experiment, although not significant, the weight loss was greatest in the plantain group (Table 4-4).

Table 4-4: Mean (± S.E.M.) daily loss of live weight of sheep fed plantain or grass for 3 days.

	Plantain (n=10)	Grass (n=10)	Significance (P value)
Live weight loss (kg/day)	0.62 ± 0.093	0.4125 ± 0.115	0.177

Changes in packed cell volume (PCV) were not significant between the two groups (Table 4-5). Both groups had a larger packed cell volume on the last day compared with the first, this difference was larger in the ryegrass sheep (Table 4-5).

Table 4-5: Mean (± S.E.M.) packed cell volume in blood of sheep fed plantain (n=10) or ryegrass (n=10) for 3 days.

	Plantain (n=10)	Ryegrass (n=10)	Significance (P value)
Packed cell volume difference (%)	3.08 ± 0.9	3.88 ± 0.5	0.438
PCV Day 0 (%)	39.1 ± 1.1	39.4 ± 0.6	0.834
PCV Day 4 (%)	36.05 ± 1.2	35.53 ± 0.4	0.671

4.2.5 Extra comparisons

The three ryegrass sheep that drank the most of the extra water provided to them were compared as a separate group with the plantain group.

There was no difference between the sodium excreted (g/day) in urine between the plantain group and the three ryegrass sheep (Table 4-6).

Table 4-6: Mean sodium excretion of sheep fed plantain (n=10) or ryegrass (n=3) for 3 days. Statistical significance values given as * is $P < 0.05$, ** is $P < 0.01$, * is $P < 0.001$, ns is not significant.**

	Plantain (n=10)	Ryegrass (n=3)	Feed	Day	Time	Feed * Day	Feed * Time
Na^+ urine (g/day)	3.05 ± 0.2	2.53 ± 0.4	ns	*	***	ns	**

The daily water balance of sheep fed plantain was lower on all three days compared with the three ryegrass sheep with the highest water intake (Figure 4-4).

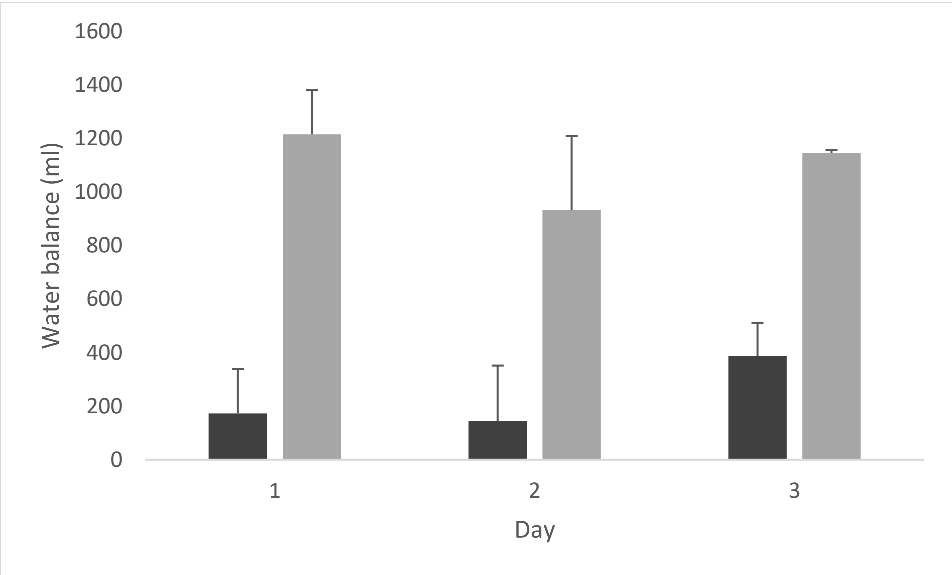

Figure 4-4: Mean daily water balance of sheep fed plantain (n=10) (dark grey) or ryegrass (n=3) (light grey) for 3 days. Vertical bars show + S.E.M.

4.2.6 Ryegrass sheep

The sodium intake of the sheep (added as sodium chloride) was compared with other measurements to test for relationships between the data.

There is some relation between the sodium ingested by the ryegrass sheep and the urine volume (Figure 4-5).

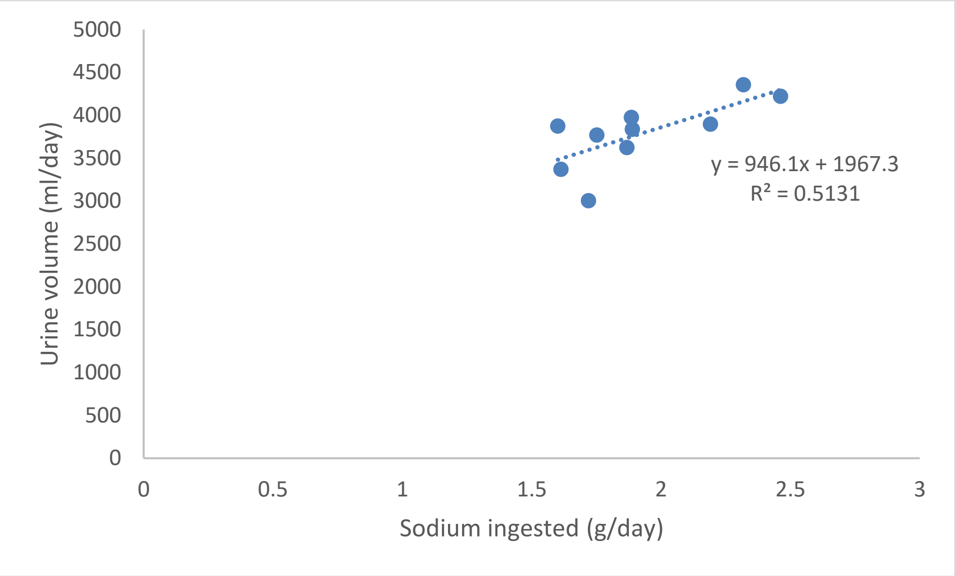

Figure 4-5: Urine volume (ml) compared with sodium ingested (g/day) of ryegrass sheep (n=10) over 3 days.

There is some relation between the Na^+ excreted and the Na^+ ingested by the ryegrass sheep (Figure 4-6).

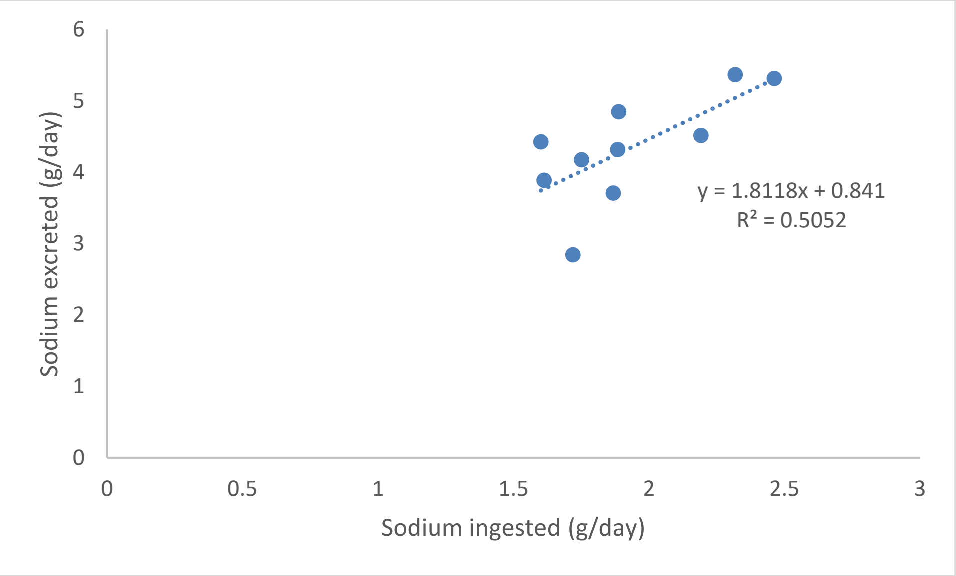

Figure 4-6: Na^+ excreted in urine (g/day) compared with sodium ingested (g/day) of ryegrass sheep (n=10) over 3 days.

There is a very weak relationship between the faeces water and Na^+ intake of the ryegrass sheep (Figure 4-7).

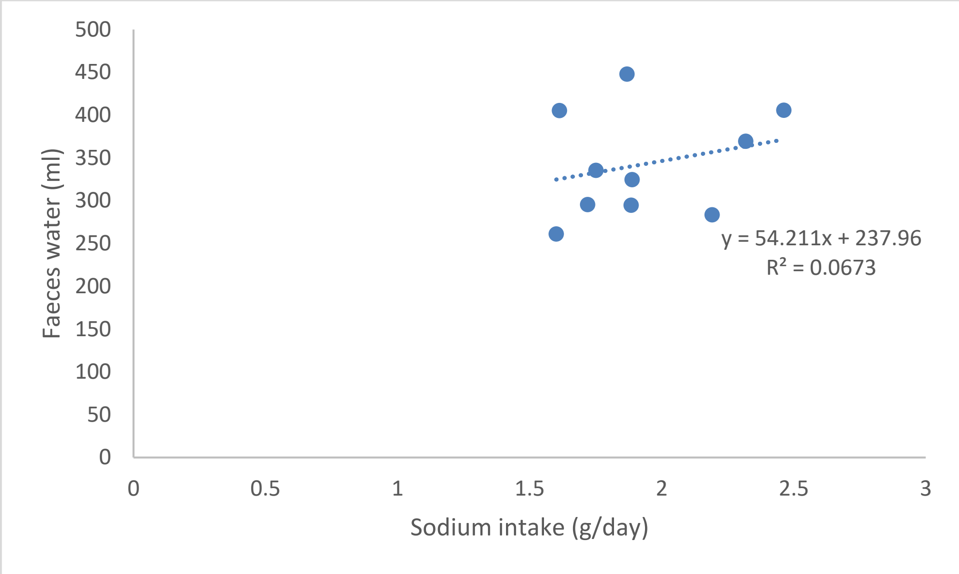

Figure 4-7: Faeces water (ml) compared with sodium intake (g/day) of ryegrass sheep (n=10) over 3 days.

4.3 Week two results

4.3.1 Sodium

There was no difference in overall plasma sodium concentration (mmol/L) between the two groups (Table 4-7). The concentrations were higher in the afternoon for both groups (Table B.4). The urine sodium concentration (mmol/L) was higher in the plantain sheep (Table 4-7). There was a significant interaction between feed and time in the urine sodium concentration (Table 4-7).

There was a significant difference in sodium excreted (g/day) in urine between the two groups (Table 4-7), which was higher in the plantain group (Table 4-7 & Figure 4-8). There was a significant interaction between feed and time in sodium excretion (Table 4-7).

Table 4-7: Mean sodium (Na^+) output (± S.E.M.) of sheep fed plantain (n=10) or ryegrass (n=9) for 2 days. Statistical significance values given as * is *P* < 0.05, ** is *P* < 0.01, * is *P* < 0.001, ns is not significant.**

	Plantain (n=10)	Ryegrass (n=9)	Feed	Day	Time	Feed * Day	Feed * Time
Plasma (mmol/L)	147 ± 0.4	147 ± 0.4	ns	ns	***	ns	ns
Urine (mmol/L)	51.7 ± 2.8	40.3 ± 2.5	**	ns	ns	ns	***
Urine (g/day)	2.96 ± 0.2	1.56 ± 0.1	***	ns	ns	ns	*

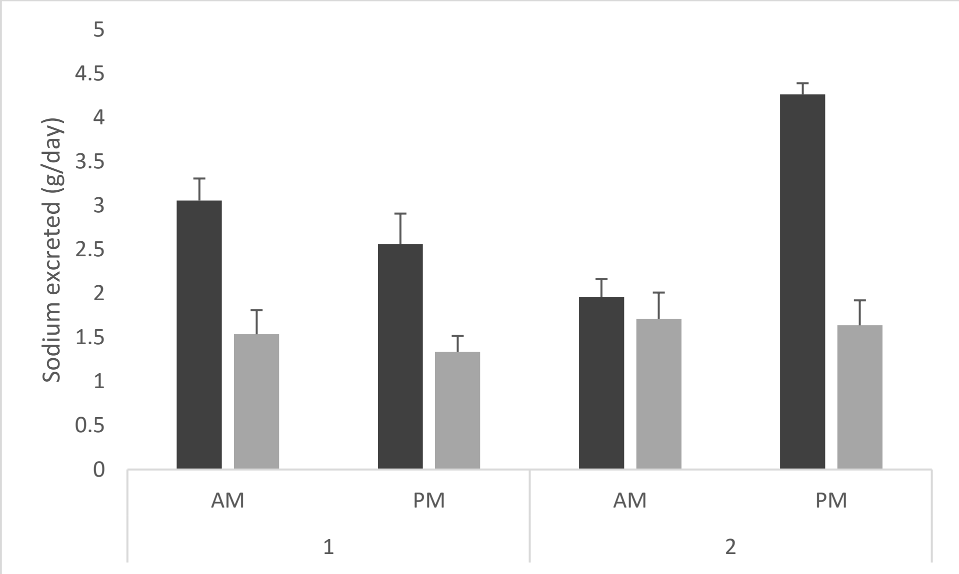

Figure 4-8: Mean sodium excretion in urine in sheep fed either plantain (n=10) (dark grey) or ryegrass (n=9) (light grey) for 2 days. Error bars show + S.E.M.

The plantain group consumed more sodium (g/day) than the ryegrass sheep, however this difference was not significant (Table 4-8). This was based on the sodium content of the feed from Lindsay (2016).

Table 4-8: Sodium ingested (± S.E.M.) of sheep fed either plantain (n=10) or ryegrass (n=9) for 2 days. Statistical significance values given as * is *P* < 0.05, ** is *P* < 0.01, * is *P* < 0.001, ns is not significant.**

	Plantain (n=10)	Ryegrass (n=9)	Feed	Day	Feed * Day
Sodium ingested (g/day)	5.67 ± 0.01	4.47 ± 0.04	***	ns	ns

4.3.2 Clearance

There was no difference in the mean creatinine clearance between the two groups (Table 4-9), the afternoon values were higher for both groups (Table B.4). There was a significant difference in urea clearance between the two groups with plantain having higher values (Table 4-9). In the plantain group urea clearance decreased, whereas in the grass group it increased over the two days (Table B.3).

There was a significant difference in mean free water clearance between the two groups (Table 4-9). Overall ryegrass was higher (Table 4-9), however the plantain group was higher in the afternoon of the second day (Figure 4-9). There was a significant interaction between feed and day (Table 4-5) and a significant interaction between feed and time (Table 4-9).

Table 4-9: Mean daily clearance values (± S.E.M.) of sheep fed plantain (n=10) or ryegrass (n=9) for 2 days. Statistical significance values given as * * is *P* < 0.05, ** is *P* < 0.01, * is *P* < 0.001, ns is not significant.**

	Plantain (n=10)	Ryegrass (n=9)	Feed	Day	Time	Feed * Day	Feed * Time
Creatinine (ml/min)	104 ± 5.5	89.7 ± 8.8	ns	ns	***	ns	ns
Urea (ml/min)	58.4 ± 5.8	50.5 ± 5.2	*	*	***	ns	ns
Free water (ml/min)	1.52 ± 0.2	2.07 ± 0.2	*	ns	ns	*	**

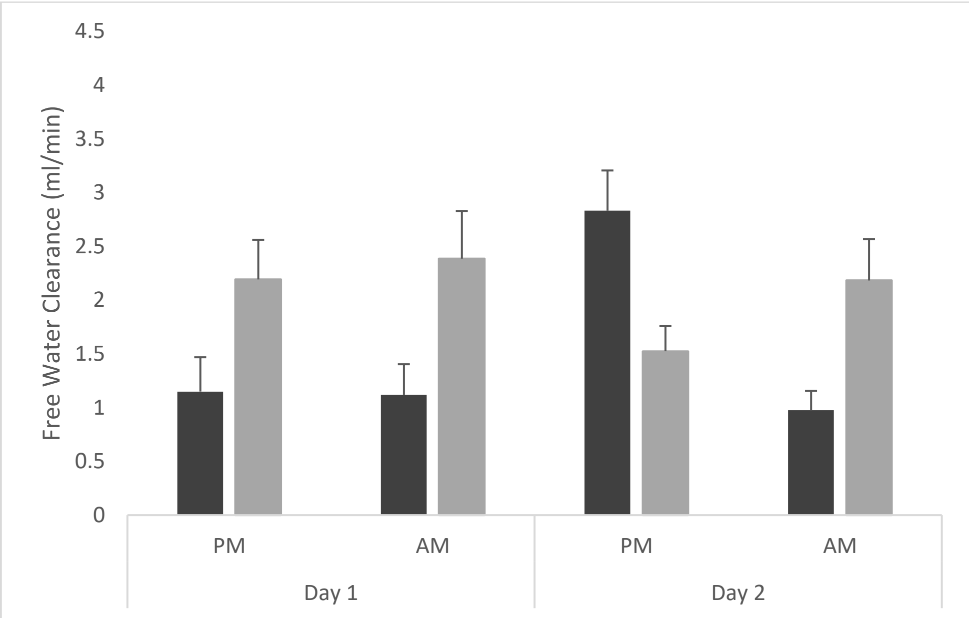

Figure 4-9: Mean free water clearance of sheep fed plantain (n=10) (dark grey) or ryegrass (n=9) (light grey) for 2 days. Vertical bars show + S.E.M.

4.3.3 Daily water balance

Both groups had a positive water balance throughout the trial (Figure 4-10). The water balance in the plantain group was consistently lower over the two days (Table A.2, Figure 4-10).

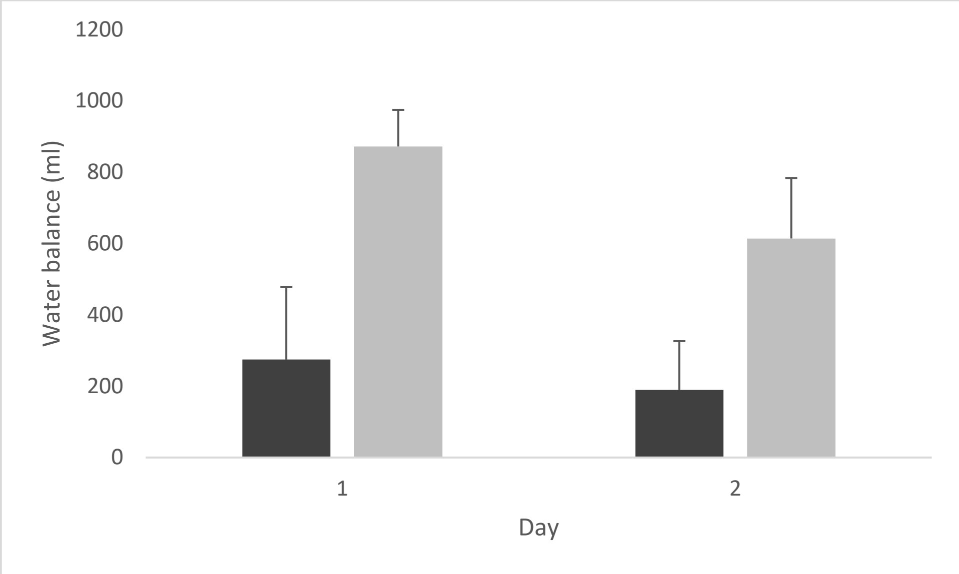

Figure 4-10: Mean daily water balance of sheep fed plantain (n=10) (dark grey) or ryegrass (n=9) (light grey) for 2 days. Vertical bars show + S.E.M.

4.3.4 Other measurements

Both groups of sheep lost weight throughout the trial, this was greatest in the plantain group (Table 4-10). This difference was significant, however, the amount of weight lost was small (Table 4-10).

Table 4-10: Mean (± S.E.M.) daily loss of live weight of sheep fed plantain (n=10) or ryegrass (n=9) for 2 days.

	Plantain (n=10)	Ryegrass (n=9)	Significance (P value)
Live weight loss (kg/day)	0.825 ± 0.11	0.46 ± 0.13	0.041

Both groups had a lower packed cell volume on the last day compared with the first, this difference was largest in the ryegrass sheep, neither difference was significant (Table 4-11).

Table 4-11: Mean (± S.E.M.) packed cell volume in blood of sheep fed plantain (n=10) or ryegrass (n=9) for 2 days.

	Plantain (n=10)	Ryegrass (n=9)	Significance (P value)
Packed cell volume difference (%)	-0.7 ± 0.77	-1.17 ± 0.66	0.657
PCV Day 0 (%)	36.5 ± 0.7	36.6 ± 0.8	0.937
PCV Day 3 (%)	37.2 ± 0.7	37.8 ± 0.9	0.62

4.3.5 Extra comparisons

The three ryegrass sheep that drank the most of the extra water provided to them were compared as a separate group with the plantain group.

There was a significant difference between Na^+ excreted in urine between the plantain and three ryegrass sheep that drank the most additional water (Table 4-12).

Table 4-12: Mean sodium excretion of sheep fed plantain (n=10) or ryegrass (n=3) for 2 days. Statistical significance values given as * is *P* < 0.05, ** is *P* < 0.01, * is *P* < 0.001, ns is not significant.**

	Plantain (n=10)	Ryegrass (n=3)	Feed	Day	Time	Feed * Day	Feed * Time
Na^+ urine (g/day)	2.96 ± 0.2	1.55 ± 0.3	***	ns	ns	ns	ns

Daily water balance was higher in the ryegrass sheep than in the plantain sheep each day (Figure 4-11). Daily water balance was positive on both days for both groups (Figure 4-11).

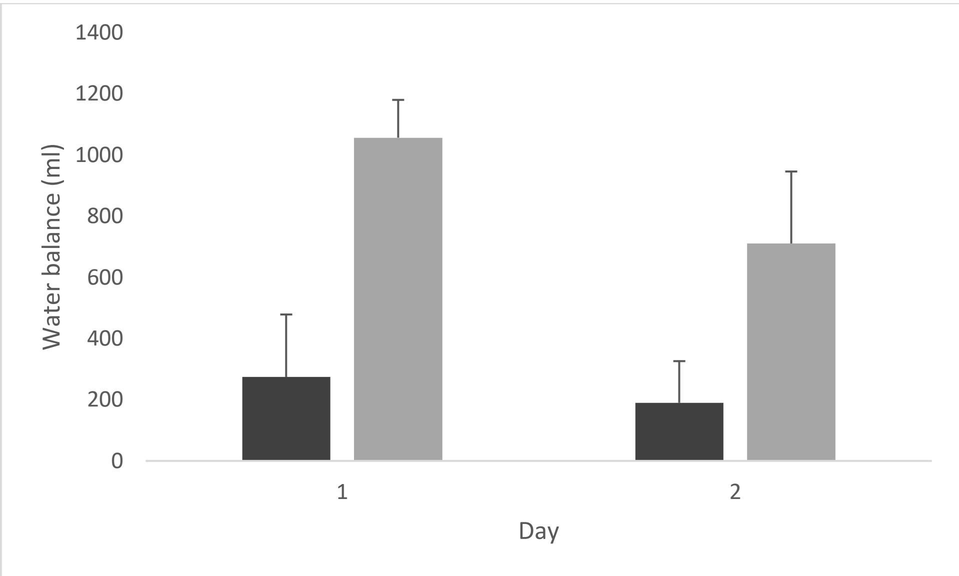

Figure 4-11: Mean daily water balance of sheep fed plantain (n=10) (dark grey) or ryegrass (n=3) (light grey) for 2 days. Vertical bars show + S.E.M.

4.3.6 Ryegrass sheep

The sodium intake of the sheep (added as sodium chloride) was compared with other measurements to test for relationships between the data.

There was very little relationship between urine volume and sodium intake (Figure 4-12).

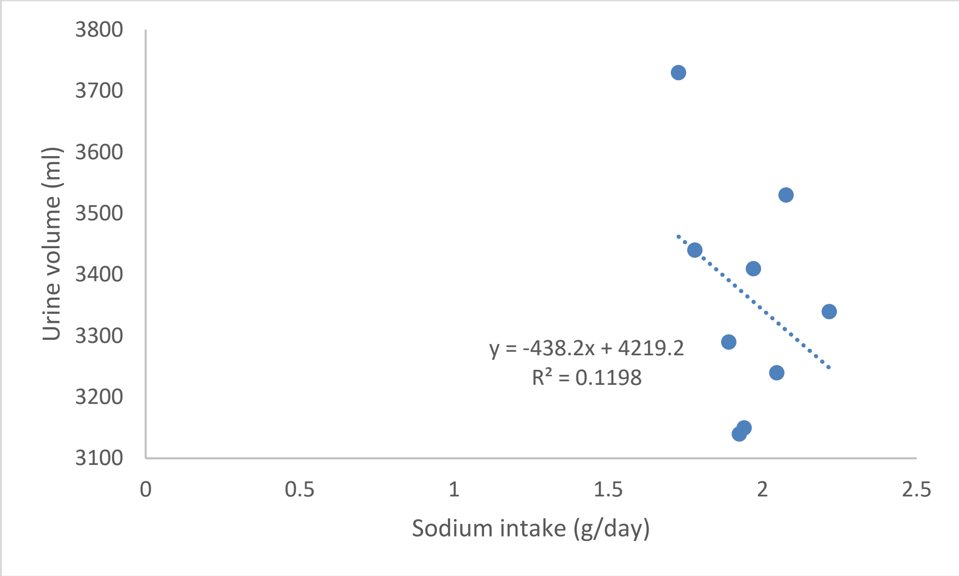

Figure 4-12: Urine volume (ml) compared with sodium ingested (g/day) of ryegrass sheep (n=9) over 2 days.

There is very little relationship between Na^+ excreted and Na^+ intake of ryegrass sheep (Figure 4-13).

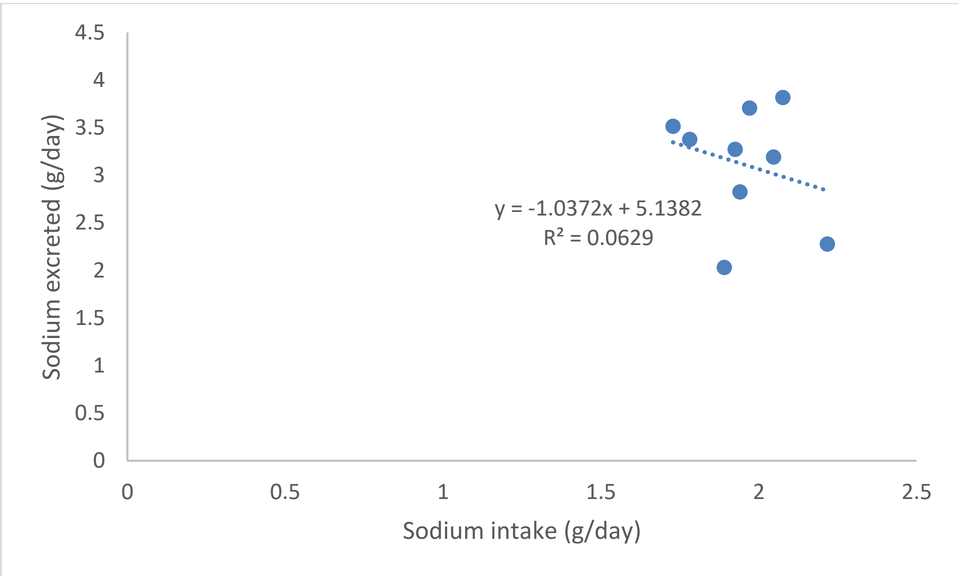

Figure 4-13: Na^+ excreted in urine (g/day) compared with sodium ingested (g/day) of ryegrass sheep (n=9) over 2 days.

There is very little relationship between faeces water and Na^+ intake in ryegrass sheep (Figure 4-14).

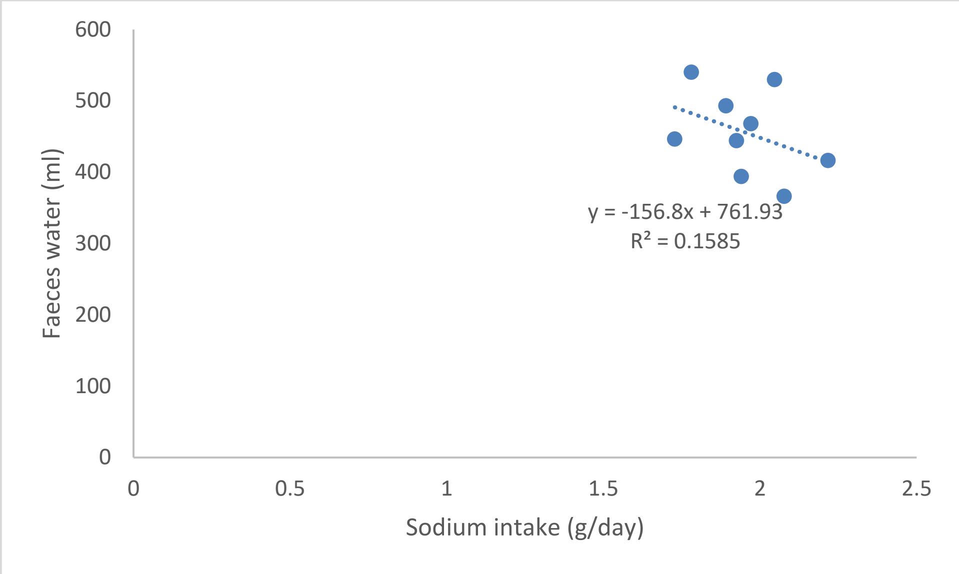

Figure 4-14: Faeces water (ml) compared with sodium intake (g/day) of ryegrass sheep (n=9) over 2 days.

Chapter 5

Discussion

These results have reinforced the findings of others, that sheep fed plantain have a higher urine volume than sheep fed ryegrass. However, the attempt to determine whether this diuresis can be attributed to the higher sodium content of plantain was not fully achieved, some of the results suggest that sodium is partially, but not fully responsible for the increase in urine volume. The urine produced by the plantain sheep had a similar total osmolality to the ryegrass sheep, despite having a higher sodium content.

5.1 Confirmed results

5.1.1 Diuresis

In both weeks of this trial the sheep fed plantain produced a higher volume of more dilute urine than sheep fed ryegrass. This is similar to results of other trials indicating a water diuresis has occurred. Sheep fed plantain here produced on average 50% more urine than those fed ryegrass in both weeks of the trial (1922 ml in Week 1, and 838 ml in Week 2). Wilman & Derrick (1994) recorded similar results with urine volumes being 53% greater in sheep fed plantain than ryegrass. O'Connell *et al*. (2016), and Lindsay (2016) both recorded larger volumes of more dilute urine following ingestion of plantain compared with ryegrass with no change in total osmol excretion, and concluded that a water diuresis had occurred. The day with the largest difference in water balance was Day 3 of Week 1. Therefore, some of the data for this day have been analysed separately to see of this effect can be related to some of the other measurements. In the present study, the plantain had a higher water content than usual (around 90%) hence it was difficult to achieve a balanced water intake for the two groups. Clearly some of the increased urine output of the plantain-fed sheep is simply a result of the extra water intake of these sheep. For this reason, it is more appropriate to compare water balance in the two groups. The higher water balance of ryegrass-fed sheep (Figure 4-3, Figure 4-10) shows that urine output of the plantain sheep exceeded that of the ryegrass sheep and provides additional support for the contention that plantain has a diuretic effect.

5.1.2 Clearance

The free water clearance in this trial did not differ between groups in the first week, but did overall in the second week. On the third day of the first week it was the same between the two groups. As free water clearance is the volume of blood plasma that is cleared of solute-free water by the glomerulus (Vincent & Hall, 2012), the similar values between the two groups suggest that plantain is not affecting glomerular function. The creatinine and urea clearance values were similar between feeds for the two weeks. However, on Day 3 of Week 1 there was a large difference between the mean clearance values of the two groups. When the three ryegrass sheep that drank the most water were compared with the plantain group for this day, there was no difference in creatinine or urea clearance values between the two groups. This suggests that it was not plantain itself affecting clearance values, but the difference in water consumption. Lindsay (2016) also found no difference in clearance values between the plantain and ryegrass groups. As creatinine clearance is a good indicator of glomerular filtration rate, the lack of difference between the clearance values of the two groups suggests that ingestion of plantain does not affect glomerular and proximal tubule function.

5.1.3 Urine pH

There was no difference in pH between the two feed groups. Lindsay (2016) found similar results using the same cultivar of plantain ('PG742'). O'Connell *et al.* (2016) found that feeding plantain (cultivar 'Ceres Tonic') reduced urinary pH. It is likely that the differences between these studies is due to the differences in plantain cultivars used.

5.2 Sodium

5.2.1 Input of sodium

Plantain has a higher sodium content than ryegrass (Rumball *et al*, 1997; Lindsay, 2016). However, it is not known how much of the sodium in plantain is available to the animal, in this experiment it was assumed it is all available. Salt (NaCl) was dissolved in the water added to the grass feed in an attempt to balance out the sodium ingested by the two groups. As the water was consumed on a voluntary basis, and there was a large amount of water added, each ryegrass sheep consumed different amounts, and therefore different amounts of sodium. However, to account for this difference, comparisons for sodium excretion between the three sheep that consumed the most water supplied to them, and the plantain sheep were made. These comparisons were the best representation of the aim of this trial.

5.2.2 Urine excretion of sodium

The overall excretion of sodium in the urine was higher in the plantain group than the ryegrass group in both weeks of the trial, Lindsay (2016) found without balancing sodium intake, urine sodium concentration to be higher in the plantain sheep than the ryegrass sheep (76.5 mmol/L and 41.3 mmol/L respectively). In Week 1 of the current trial, when the sodium excretion of the three ryegrass sheep that drank the most were compared with the plantain group, there was no difference between the two groups ($P>0.05$). Looking at the days individually in the first week, the day with the smallest difference in Na^+ excreted in urine was on Day 3 (plantain: 2.06 g/day, and grass: 1.8 g/day), which was also the day with the largest difference in water balance between the two feeds (plantain: 387 ml and grass: 1254 ml). Bannink *et al*. (1999) found a close relationship between sodium excreted in urine and urine volume of lactating dairy cows. This relationship can be explained by osmosis, movement of sodium and other solutes in the body is limited, and therefore water moves from areas of high water potential (low concentration of solute) to areas of low water potential (high concentration of solute) (Cunningham & Klein, 2007). This means that when there is excess sodium excreted in the urine by the kidneys, the water moves to lower the water potential, therefore increasing the volume of the urine.

These results show that despite there being more sodium excreted in the urine of the plantain sheep each day, when looking at the sheep from the two groups with the closest sodium intake there was no difference in sodium excretion. The day with the largest difference in water balance has the smallest difference in sodium excreted, which suggests that sodium is not the only factor in the excess urine production.

5.2.3 Sodium relationships in ryegrass sheep

The additional sodium consumed as salt by the ryegrass sheep was compared with different measures to determine if there was a relationship between them. In the second week of the trial there appeared to only be weak relationships. However, in the first week there were stronger relationships. These stronger relationships in Week 1 are likely due to the effects of Day 3 of the trial which appeared to be the show the most dramatic differences between the two feeds.

The relationship between the sodium ingested and the urine volume of the ryegrass sheep suggest that sodium is having an effect on the urine production in the sheep. This can be explained by osmosis (Cunningham & Klein, 2007), when the sodium concentration of the urine increases the water moves to lower the water potential and therefore increases the volume of the urine. Spek *et al*. (2012) found a positive linear relationship between sodium intake of cows and urine production. They also found there was a positive linear relationship between the sodium intake and water consumption, meaning the more sodium the cows were fed, the more water they drank, leading to an increase in urine production.

There was a very weak relationship between Na^+ consumed and faeces water, suggesting that Na^+ intake does not have a large effect on faeces production.

The increase in urine production in this study cannot be fully explained in the same way. While the sheep fed plantain had a higher sodium and water intake than that of the ryegrass sheep, the daily water balance allows for the difference in water intake, and was still significantly higher in the plantain sheep. The daily water balance of the three ryegrass sheep that consumed the most of the water provided was compared with the plantain group and there was a significant difference between the two groups. This suggests that the water in the urine produced by the plantain sheep is coming from within the body as the increase in water intake by the ryegrass sheep is still resulting in a lower urine production. The results of Lindsay (2016) and O'Connell *et al*. (2016) also showed a lower water balance in sheep consuming plantain than sheep consuming ryegrass, despite balancing the water intake.

These results show that there is a relationship between Na^+ consumed and urine production, and therefore some of the increase in urine production of the plantain sheep can be explained by the larger sodium content in the feed.

The relationship between Na^+ consumed and Na^+ excreted shows that the more sodium the animals consumed the more Na^+ excreted in the urine. This relationship was expected as an increase in the consumption of salt leads to the kidneys excreting excess sodium in the urine (Cunningham & Klein, 2007).

5.2.4 Osmolality

The total osmols excreted in the urine were similar between the two feeds on Day 3 of Week 1. The total osmols excreted in the urine on Day 3 of Week 1 were 1182.5 mOsm for plantain and 1239.8 mOsm for ryegrass. While the plantain sheep have excreted more sodium on this day, the ryegrass sheep have excreted more of other elements to make up the solute output. Lindsay (2016) and O'Connell *et al*. (2016) reported similar results of total osmols excreted being unaffected by diet.

5.3 Water balance

5.3.1 Water ingested

Despite efforts to balance water intake of the two groups there was a large difference in water ingested by sheep in both weeks of the trial. The feed was harvested the day before being fed so that the feed didn't absorb moisture from overnight rain and dew. Despite this, there were still large differences in dry matter between the two feed types, which meant large amounts of water (up to 2500ml) were added to the grass feed in an attempt to balance this. The water was consumed on a voluntary basis, thus due to the large quantities of water, the sheep did not drink all of the water added to the feed in either week of the trial. This meant the water intake of the sheep was not balanced as successfully as intended, and therefore urine volume alone is not the best indicator of diuresis. One option that was considered to remove the variation on water ingestion due to voluntary consumption was to give the water to the sheep through a drench gun. However, this idea was dismissed as it would not give an accurate representation of normal water intake of sheep.

5.3.2 Daily water balance

Calculating daily water balance removes the effects of difference in water intake of the sheep by comparing the difference in water input and output of the sheep. The output included both urine and faecal water. Daily water balance of the plantain sheep was lower than that of the ryegrass sheep for each day, both weeks of the trial. Despite the plantain sheep ingesting 22% and 28% more water than ryegrass sheep in Week 1 and two respectively, they produced 47% and 45% more water in urine and faeces. Lindsay (2016) reported similar results with lower daily water balance values in plantain than in ryegrass each day of the trial. The lower water balance in the plantain group shows that the larger urine volume of these sheep was not due to the larger water intake.

5.3.3 Other water losses

Animals lose water from evaporation from the skin and in breath. However, it was assumed that the amount of water being lost in this way was insignificant to the trial, as no animal was observed to have heavy breathing or any other sign of heat stress. It was therefore also assumed that all the animals were losing the same amount of water in these ways, as they were all kept in the same environment.

There were no signs of dehydration in the animals. The grass sheep not ingesting all the water available to them is a good indication that they were not dehydrated. The packed cell volume showed no significant change throughout the trial in both groups each week, it also never went outside of the normal range (27-45%) (Fielder, n.d.). The plasma sodium was unaltered by diet, which also indicates the animals were not under water stress.

5.3.4 Weight loss

In both weeks of the experiment, both groups of sheep lost weight. In the first week the plantain group lost 0.62 kg and the ryegrass group lost 0.4 kg, in the second week the plantain group lost 0.8 kg and the ryegrass group 0.46 kg. Loss of water accounts for up to 45% of body weight losses in sheep (Macfarlane *et al*., 1963), therefore it is likely the weight loss in this experiment was due to a loss in water. Alternatively, weight loss may also be due to the mobilisation of body tissues. When there is an increase in physiological demand, body fat, and some muscle, is mobilised to meet the greater energy demand (Geenty, 1983). The use of radiolabelled chemicals such as sodium thiosulphate, which can be used to detect changes in extracellular water, and body composition (MacFarlane *et al*., 1975). Changes in concentration of this chemical following ingestion of plantain would show whether water was lost from the rumen and gut or from extracellular areas. To determine changes in body composition, the animals could be slaughtered after the trial. There are also less invasive methods such as analysing subcutaneous fat samples for adipocyte size and depth of tissue, and blood tests for non-esterified fatty acids (NEFA), which are released during fat metabolism (Reid *et al*., 1986; Rodrigues *et al*., 2010), or body condition scoring. Changes in body tissues would influence water balance of the sheep, and therefore, could be an important consideration in future research.

5.4 Differences between two weeks

There were some differences in the overall averages of different measurements between the two weeks. Most of these can be explained by the difference in values on the Day 3 of Week 1, which had the largest difference in daily water balance between the two feeds.

5.4.1 Differences between morning and night

There were some differences between the data collected in the morning and night. Urine volume was larger in the morning collections, this was due to the longer interval between evening and morning collections, compared with the interval between morning and evening. The feed was allocated to the sheep after the morning collection of urine and blood, it was observed that most sheep had eaten the majority of the feed before the evening collection of samples. Therefore, the differences in morning and evening values may be due to the rate of consumption of the feed. This theory cannot be proven as the feed was not weighed in the evenings, it was based purely on observation.

5.5 Further study

Investigation into the availability of sodium in plantain would help to support the findings of this study. Further research into determining factors, other than the higher sodium levels, that are contributing to the increased urine production would help to understand the mechanisms causing the diuresis.

This study, along with those conducted by O'Connell *et al*. (2016) and Lindsay (2016), looked at the short-term effects of plantain on sheep. The long-term effects should be investigated including health implications involved with diuresis. Deaker *et al*. (1994) found lambs grazing plantain had larger kidneys than those graze ryegrass, they suggested this was due to increased metabolic loading in sheep fed plantain. A trial looking at kidney size and function would involve slaughter of the animals post-trial and would need to be a long-term trial.

This trial, along with similar trials (O'Connell *et al*., 2016; Lindsay, 2016) looked at the diuretic effects of plantain when fed as the sole dietary component. As plantain is typically used in pasture mixes rather than as a sole feed (DairyNZ, n.d.), future research could look at the amount of plantain in the diet that is required to see the diuretic effect. This research could also be used to look at the health impacts on the kidney caused by plantain at different feed amounts, to determine if there is an optimum amount of plantain to get the diuretic benefits without having negative impacts on the kidneys.

5.6 Conclusions

The results from the comparisons between the ryegrass sheep that had the highest sodium intake and the plantain group showed there was very little difference in the amount of sodium excreted in the urine, and there was a large difference in daily water balance (and urine volume). These comparisons are the best representation of the aim of this trial, and show that sodium is not fully responsible for the increased urine volume of the plantain sheep.

- Ingestion of plantain causes an increased production of dilute urine without altering total solute excretion.
- Creatinine and urea clearance values were unaltered by feed type suggesting that plantain is not affecting glomerular and proximal tubule function.
- There is a relationship between Na^+ ingested and urine production, which can be explained by osmosis. Some of the increase in urine production can be explained by the higher sodium content. However, the small differences in Na^+ excreted in the urine and the large differences in urine volume, suggest that Na^+ is not the only factor in the increase in urine production of the sheep.

Appendix A

Week one results

Table A.1: Overall means (± S.E.M.) for daily/twice daily measurements of sheep fed plantain (n=10) or ryegrass (n=10) for 3 days.

	Plantain (n=10)	Ryegrass (n=10)	Significance (*P* value)
Urine			
Volume (ml)	5715.7 ± 99.3	3793.3 ± 111.2	0.000
pH	8.2 ± 0.04	8.3 ± 0.08	0.177
Total Protein (g/L)	0.05 ± 0.006	0.09 ± 0.009	0.000
Urea (mmol/L)	59.6 ± 2.7	1204.7 ± 5.3	0.000
Creatinine (mmol/L)	97.7 ± 50.6	2095.7 ± 74.06	0.000
Osmolality (mosmol/kg)	267.3 ± 26	333.2 ± 21	0.021
Plasma			
Total Protein (g/L)	74.7 ± 0.5	71.4 ± 0.6	0.000
Urea (mmol/L)	5.46 ± 0.2	5.29 ± 0.1	0.449
Creatinine (mmol/L)	52.88 ± 0.6	58.33 ± 0.9	0.000
Osmolality (mmol/kg)	287.8 ± 1.78	277.2 ± 1.75	0.724
Faecal water (ml)	436 ± 21	389 ± 51	0384

Table A.2: Significance for measurements of sheep fed plantain (n=10) or ryegrass (n=10) for 3 days. Statistical significance * is *P* < 0.05, ** is *P* < 0.01, * is *P* < 0.001, ns is not significant, and na is not applicable.**

	Feed	Day	Time	Feed*Day	Feed*Time
Urine					
Volume	***	***	***	ns	**
pH	ns	ns	***	ns	*
Total Protein	***	*	ns	ns	ns
Creatinine	***	***	ns	ns	***
Urea	***	***	***	***	***
Osmolality	*	***	ns	**	ns
Plasma					
Urea	ns	ns	***	***	ns
Creatinine	***	ns	***	ns	ns
Total Protein	***	ns	**	ns	ns
Osmolality	ns	ns	ns	ns	ns
Water					
Ingested	***	***	na	**	na
Balance	***	ns	na	ns	na
Faeces	ns	*	na	ns	na

Table A.3 Mean daily values (± S.E.M.) for sheep feed plantain (n=10) or ryegrass (n=10) for 3 days

	Plantain (n=10)			Ryegrass (n=10)		
Day	1	2	3	1	2	3
Urine						
Volume (ml)	5139 ± 216.4	6322 ± 130.7	5686 ± 137.3	3150 ± 209.8	4190 ± 154.6	4040 ± 197.6
pH	8.175 ± 0.07	8.251 ± 0.07	8.061 ± 0.09	8.099 ± 0.17	8.368 ± 0.14	8.407 ± 0.09
Sodium (mmol/L)	58.6 ± 3.5	48.3 ± 3.4	31.75 ± 2.4	44.8 ± 5.7	55.65 ± 2.8	37.9 ± 2.7
Urea (mmol/L)	60.7 ± 4.6	51.1 ± 4.8	66.95 ± 3.9	125.8 ± 10.1	79.95 ± 4.7	87.25 ± 6.4
Creatinine (mmol/L)	1337.5 ± 78.8	1091.95 ± 98.4	1184.55 ± 79.9	2439.8 ± 128.9	1788.7 ± 96.2	2058.5 ± 118.1
Protein (g/L)	0.07 ± 0.02	0.04 ± 0.002	0.03 ± 0.002	0.10 ± 0.02	0.08 ± 0.0097	0.08 ± 0.008
Osmolality (mOsm/kg)	461.4 ± 53	152.32 ± 17	188.2 ± 14	398.5 ± 45	304.64 ± 36	295.7 ± 23
Sodium (g/day)	3.6 ± 0.4	3.5 ± 0.3	2.06 ± 0.2	1.9 ± 0.4	2.78 ± 0.3	1.8 ± 0.2
Plasma						
Protein (g/L)	73.95 ± 0.9	75.1 ± 0.95	74.93 ± 0.8	70.95 ± 0.98	72.35 ± 1.14	70.95 ± 0.97
Creatinine (mmol/L)	52.75 ± 1.04	52.35 ± 1.05	53.53 ± 1.05	60.3 ± 1.54	57.1 ± 1.4	57.6 ± 1.6
Urea (mmol/L)	4.54 ± 0.2	5.5 ± 0.3	6.34 ± 0.2	5.74 ± 0.2	5.25 ± 0.1	4.88 ± 0.1
Sodium (mmol/L)	148.1 ± 0.7	147.6 ± 0.8	147.6 ± 0.7	148.4 ± 0.5	147.7 ± 0.5	147.6 ± 0.7
Osmolality (mmol/kg)	273.8 ± 4.2	278.3 ± 2.5	282.2 ± 1.9	274.6 ± 4.3	278.1 ± 2.6	279 ± 1.6
Clearance						
Creatinine (ml/min)	90.2 ± 7.05	90.56 ± 4.8	71.12 ± 6.4	79.62 ± 4.8	90.56 ± 4.9	125.8 ± 13
Urea (ml/min)	48.65 ± 5.2	43.02 ± 3.8	35.26 ± 3.4	42.2 ± 2.9	45.27 ± 3.6	63.67 ± 7.9
Free Water (ml/min)	3.16 ± 0.32	1.92 ± 0.22	3.31 ± 0.4	2.64 ± 0.28	2.004 ± 0.2	3.07 ± 0.26
Water						
Ingested (ml)	5823.8 ± 11.2	6901.62 ± 154	6425.92 ± 180	4802.9 ± 116	5324.95 ± 96	5627.4 ± 74
Balance (ml)	172.52 ± 166	143.74 ± 208	386.56 ± 125	843.59 ± 437	811.6 ± 143	1254.32 ± 80
Faeces (ml)	512 ± 40	441 ± 33	353 ± 13	509 ± 148	323 ± 24	333 ± 24

Table A.4: Mean values (± S.E.M.) taken twice a day of sheep fed plantain (n=10) or ryegrass (n=10) for 3 days

	Plantain (n=10)		Ryegrass (n=10)	
Time	AM	PM	AM	PM
Urine				
Volume (ml)	3288 ± 100	2426 ± 132	2589.67 ± 132	1203.67 ± 90
pH	8.25 ± 0.05	8.08 ± 0.07	8.58 ± 0.09	8 ± 0.11
Sodium (mmol/L)	43.1 ± 3.3	49.33 ± 3.1	51.2 ± 2.4	41.07 ± 4.1
Urea (mmol/L)	63.3 ± 3.5	55.9 ± 4	75.6 ± 3.7	119.73 ± 7.3
Creatinine (mmol/L)	1390.7 ± 65	1018.7 ± 62	1892.3 ± 76	2299 ± 117
Protein (g/L)	0.05 ± 0.01	0.04 ± 0.002	0.10 ± 0.02	0.07 ± 0.004
Osmolality (mOsm/kg)	256.9 ± 39.5	277.8 ± 34.3	298.2 ± 26.7	364.4 ± 31.5
Sodium (g/day)	3.33 ± 0.3	2.77 ± 0.2	3.1 ± 0.2	1.2 ± 0.2
Plasma				
Protein (g/L)	75.83 ± 0.6	73.5 ± 0.7	72.7 ± 0.8	70.13 ± 0.8
Creatinine (mmol/L)	55.8 ± 0.6	50 ± 0.7	59.6 ± 1.2	57.07 ± 1.3
Urea (mmol/L)	5.88 ± 0.2	5.03 ± 0.2	5.50 ± 0.1	3.81 ± 0.2
Sodium (mmol/L)	145.17 ± 0.3	150.33 ± 0.3	145.33 ± 0.2	150.4 ± 0.2
Osmolality (mmol/kg)	276.7 ± 2.8	279.47 ± 2.2	275.8 ± 2.7	278.63 ± 2.3
Clearance				
Creatinine (ml/min)	83.43 ± 4	84.49 ± 6.3	91.95 ± 3.7	105.4 ± 10.2
Urea (ml/min)	36.41 ± 1.9	48.21 ± 4.4	39.32 ± 1.7	61.44 ± 5.6
Free water (ml/min)	2.35 ± 0.25	3.24 ± 0.3	2.38 ± 0.2	2.76 ± 0.22

Table A.5: Dry matter percentage of plantain and ryegrass each day.

	Day 1	Day 2	Day 3
Plantain	11.4%	9.6%	10.2%
Grass	18.4%	16.6%	15.2%

Appendix B

Week two results

Table B.1: Overall means (± S.E.M.) for daily/twice daily measurements of sheep fed plantain (n=10) or ryegrass (n=9) for 2 days.

	Plantain (n=10)	Ryegrass (n=9)	Significance (*P* value)
Urine			
Volume (ml)	2520 ± 93	1681.7 ± 68	0.000
pH	8.09 ± 0.07	8.24 ± 0.09	0.101
Total Protein (g/L)	0.09 ± 0.01	0.09 ± 0.008	0.823
Urea (mmol/L)	60.08 ± 3.2	90.05 ± 6	0.000
Creatinine (mmol/L)	1483.6 ± 72	1936.2 ± 127	0.002
Osmolality (mosmol/kg)	114.24 ± 11.8	277.29 ± 176	0.000
Plasma			
Total Protein (g/L)	76.2 ± 0.5	76.2 ± 0.9	0.972
Urea (mmol/L)	27.6 ± 3.9	30.3 ± 4.3	0.012
Creatinine (mmol/L)	27.9 ± 23.5	30.72 ± 4.4	0.008
Osmolality (mmol/kg)	287.6 ± 0.8	289.42 ± 1.1	0.123
Faecal water (ml)	558 ± 40	456 ± 21	0.005

Table B.2: Significance for measurements of sheep fed plantain (n=10) or ryegrass (n=9) for 2 days. Statistical significance * is *P* < 0.05, ** is *P* < 0.01, * is *P* < 0.001, ns is not significant, and na is not applicable.**

	Feed	Day	Time	Feed*Day	Feed*Time
Urine					
Volume	***	ns	*	ns	ns
pH	ns	ns	***	ns	ns
Total Protein	ns	ns	ns	ns	ns
Creatinine	**	ns	ns	ns	ns
Urea	***	ns	**	ns	*
Osmolality	***	ns	**	*	**
Plasma					
Urea	*	***	**	ns	ns
Creatinine	**	***	**	*	ns
Total Protein	ns	ns	*	ns	ns
Osmolality	ns	**	***	ns	ns
Water					
Ingested	***	*	na	***	na
Balance	**	ns	na	ns	na
Faeces	**	***	na	**	na

Table B.3: Mean daily values (± S.E.M.) for sheep feed plantain (n=10) or ryegrass (n=9) for 2 days

	Plantain (n=10)		Ryegrass (n=9)	
Day	1	2	1	2
Urine				
Volume (ml)	2542 ± 154	2498 ± 109	1532.8 ± 71.5	1830.6 ± 106
pH	8.2 ± 0.1	8.02 ± 0.1	8.164 ± 0.2	8.317 ± 0.1
Sodium (mmol/L)	49.9 ± 3.9	53.5 ± 4.2	37.7 ± 3.5	42.9 ± 3.4
Urea (mmol/L)	58.3 ± 5.1	61.85 ± 3.9	99.4 ± 9.3	80.7 ± 7
Creatinine (mmol/L)	1480.8 ± 74.7	1486.35 ± 126	2072.9 ± 208	1799.5 ± 144
Protein (g/L)	0.11 ± 0.02	0.07 ± 0.009	0.1 ± 0.01	0.09 ± 0.01
Osmolality (mosmol/kg)	94.08 ± 17.8	134.4 ± 14.5	313.6 ± 44.8	230 ± 41.4
Sodium (g/day)	2.8 ± 0.2	3.11 ± 0.29	1.44 ± 0.16	1.68 ± 0.2
Plasma				
Protein (g/L)	75.5 ± 0.81	76.9 ± 0.64	75.72 ± 1.25	76.59 ± 1.3
Creatinine (mmol/L)	50.75 ± 1.2	5.03 ± 0.33	56.11 ± 1.8	5.32 ± 0.2
Urea (mmol/L)	3.91 ± 0.3	51.25 ± 1.4	5.31 ± 0.3	55.19 ± 1.8
Sodium (mmol/L)	147.35 ± 0.7	146.6 ± 0.5	147.11 ± 0.6	146.94 ± 0.5
Osmolality (mmol/kg)	288.8 ± 1.1	286.4 ± 1.2	291.8 ± 1.7	287.06 ± 1.2
Clearance				
Creatinine (ml/min)	107.7 ± 8.5	100.33 ± 7.1	83.59 ± 10.3	96.185 ± 15
Urea (ml/min)	66.85 ± 10.1	49.955 ± 7.5	49.52 ± 7.4	51.6 ± 7.5
Free water (ml/min)	1.13 ± 0.21	1.9 ± 0.3	2.28 ± 0.3	1.83 ± 0.23
Water				
Ingested (ml)	6046.42 ± 25.7	5613.94 ± 0.9	4420.27 ± 39	4703.83 ± 54.4
Balance (ml)	274.37 ± 204	189.7 ± 136	872.08 ± 103	614.02 ± 170
Faeces (ml)	688 ± 42	428 ± 34	483 ± 34	429 ± 21

Table B.4: Mean values (± S.E.M.) taken twice a day of sheep fed plantain (n=10) or ryegrass (n=9) for 2 days

	Plantain (n=10)		Ryegrass (n=9)	
Time	AM	PM	AM	PM
Urine				
Volume (ml)	2650 ± 141	2390 ± 118	1791.67 ± 93	1571.67 ± 94.5
pH	8.273 ± 0.09	7.899 ± 0.1	8.584 ± 0.04	7.928 ± 0.1
Sodium (mmol/L)	41.6 ± 2.9	61.75 ± 3.7	43.706 ± 3.5	36.556 ± 3.3
Urea (mmol/L)	56.9 ± 4.9	63.25 ± 4	72.353 ± 7.6	108.22 ± 7.1
Creatinine (mmol/L)	1640.5 ± 107	1326.7 ± 86	1900.9 ± 185.4	1985.4 ± 178.7
Protein (g/L)	0.0873 ± 0.02	0.0887 ± 0.02	0.1027 ± 0.01	0.0809 ± 0.007
Osmolality (mosmol/kg)	112 ± 18.2	116.5 ± 15.4	367.4 ± 50.8	192.4 ± 25
Sodium (g/day)	2.51 ± 0.2	3.41 ± 0.26	1.62 ± 0.2	1.49 ± 0.17
Plasma				
Protein (g/L)	0.7112 ± 0.7	0.6955 ± 0.7	77.471 ± 1.3	74.833 ± 1.1
Creatinine (mmol/L)	29.63 ± 5.4	26.145 ± 5.2	33.712 ± 6.5	29.261 ± 6.1
Urea (mmol/L)	30.04 ± 5.8	25.115 ± 5.2	29.918 ± 6.2	29.106 ± 6.1
Sodium (mmol/L)	144.75 ± 0.3	149.2 ± 0.4	145 ± 0.2	149.06 ± 0.3
Osmolality (mmol/kg)	284.55 ± 0.9	290.65 ± 1	287.56 ± 1.7	291.28 ± 1.3
Clearance				
Creatinine (ml/min)	82.45 ± 4	125.54 ± 8	64.5 ± 7.7	113.52 ± 13.7
Urea (ml/min)	27.7 ± 1.8	89.12 ± 6	23.7 ± 3	74.31 ± 5.3
Free Water (ml/min)	1.05 ± 0.16	1.99 ± 0.3	2.29 ± 0.3	1.88 ± 0.23

Table B.5: Dry matter percentage of plantain and ryegrass each day.

	Day 1	Day 2
Plantain	11%	11.8%
Ryegrass	18.9%	17.8%

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