CARCASS AND EATING QUALITY OF SHEEP GRAZING
SALTBUsh BASED SALINE PASTURE SYSTEMS

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DECLARATION

I declare that this is my own account of my research and contains its main content work which has not been submitted for a degree at any tertiary educational institution.

......................................................

Kelly Lynne Pearce
Abstract

Forage halophytes such as saltbush (*Atriplex* spp) are being widely used to revegetate Australian saline land and can also provide a medium quality fodder source. There is widespread anecdotal evidence that sheep grazing on saltbush are leaner, tastier and juicier. This thesis investigated the potential to produce a high quality carcass with improved eating quality from grazing sheep on saltbush on saline land.

The first experimental chapter in this thesis details an animal house experiment investigating the carcass, eating and wool quality and physiological responses of sheep ingesting a 60:40 dried saltbush (*Atriplex nummularia*):barley grain (S+B), ration verses a 33:25:42 lupin grain:barley grain:oaten hay ration (Control, C) for 10 weeks prior to commercial slaughter (Chapter 4). Subsequently, two field experiments were conducted to examine the effects of grazing saltbush on saline land compared to dry senesced pasture on carcass and eating quality of lambs (Goomalling 2003) and hoggets (Wickepin 2004) (both chapter 6) for 14 weeks. Both chapters demonstrated that the ingestion of saltbush resulted in significantly less fat and in the 2 field experiments the ingestion of saltbush resulted in more lean on the carcass compared to sheep grazing a stubble/pasture (control) ration. These are positive findings for processors as costs of fat denudation are high so the lower the fat content and for farmers because less fat is deposited on the carcass per unit of liveweight gain. The decreased deposition of fat was attributed to the higher protein:energy ratio available for production, secondary compounds in saltbush and lower circulating insulin and higher growth hormone of the S+B fed sheep compared to the control fed sheep. Further work is needed to determine if these beneficial improvements in carcass composition can be achieved without compromising animal production. The long term grazing of saltbush did not result in commercially desirable hot carcass weights unless the sheep were
supplemented with a high energy source such as barley. The low growth rates are attributed to a decreased availability of energy substrates, low feed intake and increased energy output of sheep fed high salt diets. The low energy intake of the S+B fed sheep also resulted in an a significantly lower percentage of unsaturated fat and unchanged levels of saturated fat in the fat depots compared to the C treatment.

Consumer taste tests conducted on meat from experiments in both chapter 4 and 6 indicated no difference between the treatments for any of the eating quality traits assessed. This can be considered a positive result as sheep can be finished on saltbush without any detriment to eating quality. High vitamin E levels in the meat may have also prevented the development of rancid flavours and aroma. It can be speculated that saltbush does not impart beneficial flavour and aroma volatiles as previously thought; instead the high vitamin E levels inhibit off-flavour and aroma development compared to meat from sheep grazed on dry pasture.

The long term ingestion of saltbush also resulted in significantly lower urine specific gravity (USG), muscle dry matter and higher urine weights suggesting that the saltbush fed sheep had a better hydration status compared to control fed sheep. However, this finding did not correspond with higher hot carcass weight or dressing percentages. The increases in muscle fluid content of the saltbush fed sheep were attributed to changes in body composition. The saltbush fed sheep had a higher lean and lower fat content which corresponded with a greater body fluid content as found in the animal house study.

Under conditions where the body composition of sheep remains the same, the use of short term strategic feeding of components of saltbush was investigated (mimicked in the form of salt and betaine) to reduce dehydration and subsequent reductions in carcass weight and dressing
percentages (Chapter 7). Salt and or betaine were fed for 1 week either prior to a 48 h period of water deprivation or prior to 48 h commercial slaughter process where water was available in lairage from 24-48 h. Under both scenarios the diets did not result in improved dressing percentages, hot carcass weights, muscle dry matter or muscle weights. The ingestion of high salt diet prior to slaughter, did increase fluid retention in the extracellular spaces prior to slaughter however by 48 h both groups were at a similar physiological and therefore similar hydration status. Therefore similar levels of fluid were present in the muscles and no difference in carcass weight or dressing percentage could be expected. An important observation from the second experiment was that the high salt group drunk more water than the low salt fed sheep but the low salt group consumed fluid in lairage also. The low salt fed sheep may have been encouraged to drink water after observing the frequent drinking patterns of the high salt group.

This thesis has also shown that saltbush contains high levels of vitamin E ($\alpha$-tocopherol) (193 mg/kg dry matter). As a result the concentration of $\alpha$-tocopherol in plasma, liver and muscle of the saltbush fed sheep was elevated compared to those grazing dry pasture. The high muscle concentrations of vitamin E in the saltbush-grazed sheep resulted in improved meat colour stability. The high vitamin E levels did not influence the drip and cooking loss of the meat despite a decrease in the muscle dry matter of the meat. The browning of meat and increased drip loss results in large losses to the meat industry due to value deterioration at the supermarket. There is also great potential for the high vitamin E content in saltbush to be used for the prevention of nutritional myopathy instead of using expensive and labour intensive synthetic supplements.

In conclusion, this thesis has provided an insight into the carcass and eating quality of sheep grazed on saltbush based saline pasture systems. The most significant findings were that
ingesting saltbush can reduce the carcass fat content, improve meat colour stability and not result in any detriment to eating quality. A potentially useful way to incorporate these results into an Australian farming system may be to use saltbush on a short term basis, not for the length of period grazed in this thesis. The short term use of saltbush should provide sufficient grazing time for an elevation of vitamin E levels in the muscle to improve meat colour stability, increase the amount of lean and decrease fat levels of a carcass all without changing eating quality and decreasing liveweight. Further work is needed to ensure that these benefits can be achieved without compromising animal production. The opportunity to utilise saltbush to produce leaner carcasses with better colour stability may encourage farmers to consider previously unproductive land planted to saltbush to be a highly useful enterprise.
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Published and Submitted Papers

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And finally, who can forget the 400 sheep that died for the sake of this thesis….
# List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWG</td>
<td>Clean wool growth</td>
<td>SM</td>
<td>musculus semimembranosus&lt;br&gt;(Semimembranosus muscle)</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual x-ray absorptiometry</td>
<td>ST</td>
<td>musculus semitendinosus&lt;br&gt;(Semitendinosus muscle)</td>
</tr>
<tr>
<td>FER</td>
<td>Fractional excretion rate</td>
<td>USG</td>
<td>Urine specific gravity</td>
</tr>
<tr>
<td>GH</td>
<td>Growth hormone</td>
<td>VFA</td>
<td>Volatile fatty acids</td>
</tr>
<tr>
<td>IGF</td>
<td>Insulin-like growth factor.</td>
<td>PUFA</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>LL</td>
<td><em>musculus longissimus thoracis et. lumborum</em> (also referred to as the Loin)</td>
<td>NSW</td>
<td>New South Wales</td>
</tr>
<tr>
<td>ME</td>
<td>Metabolisable energy</td>
<td>SA</td>
<td>South Australia</td>
</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
<td>CP</td>
<td>Crude protein</td>
</tr>
</tbody>
</table>
Chapter 1

General Introduction

Soil and water salinisation in Australian dryland farming systems is caused by the accumulation of soluble salts in the upper soil profile due to the removal of native vegetation and extensive use of shallow rooted annual plant species. These shallow rooted plants allow 20-50 mm of additional water to percolate annually beyond the root zone compared with native vegetation causing the groundwater to rise (Nulsen et al. 1982). The salts in the lower parts of the soil profile dissolve into this increased volume of groundwater causing it to become saline. As the groundwater rises to the surface, the water evaporates leaving the salt on the soil surface. The increased accumulation of salts on the soil surface reduces the potential for non-salt tolerant crops and pastures to survive hence reducing productivity.

Soil salinisation is a major environmental problem in Australia. Almost six million hectares of Australia’s agricultural and pastoral land is at risk of becoming saline, including 1.8 million hectares in Western Australia. If the current trend continues in Western Australia up to 3 million hectares will be affected by 2020 and 6 million hectares will be affected by 2050, resulting in a loss of agricultural production equivalent to $400 million per year (National Land and Water Resources Audit 2001). This does not include the costs incurred for repairs and maintenance of buildings/infrastructure, dams, establishment of deep drains to counter rising water tables, desalinisation of water resources and restoration of natural environments. It is clear that
significant changes to land use practices are required to combat the problem (Masters et al. 2001).

There are hydrological and engineering techniques available to reduce the impact and growth of soil salinisation. These include contour banks and deep drainage to divert water, forestry plantations and other perennial plants to increase water usage as well as the desalinisation of water. However for most saline areas these reclamation options are not realistic in the short term and the challenge is to find a use for the land which will provide economic return as well as meet the environmental requirements of the broader community (Masters et al. 2001). One approach is to plant halophytic plants such as saltbush (*Atriplex spp*) with other salt tolerant pastures and grasses. Saltbush acts to lower the watertable and prevent further increases in salinity in the topsoil (Barrett-Lennard et al. 1996). Saltbush establishment is considered less expensive than improvements through hydrological and engineering techniques and thus more financially attractive.

The benefits of saltbush and saltland pastures are that they can produce biomass despite the high concentrations of salt in their root zones (Barrett-Lennard et al. 1996). They can provide a fodder source for livestock that allows for an increase in the carrying capacity of the farm and potential out-of-season production. Producers are particularly interested in the animal productivity likely from the plants given the investment in establishment.

There is widespread anecdotal evidence that grazing sheep fed saltbush grown on marginal and pastoral land will result in a superior meat product. With increasing importance being placed on the use of saltbush for saline land revegetation there is a unique opportunity to utilise these areas for meat production and increased profit. If grazing sheep on saltbush does result in superior
meat quality the utilisation of saline areas to produce a saltbush meat product may be a novel way to increase the profitability of these areas. An additional benefit is that the farming community may consider previously unproductive land planted to saltbush a more useful option. There is however, limited objective information on the effects of grazing saltbush dominated saline land systems on carcass and eating quality. Therefore to establish the economic viability of utilising saltbush based pasture systems for meat production it is important to identify the benefits and any possible deleterious effects on the carcass and eating quality of lambs grazed under such systems.

The following review will firstly discuss the role of saltbush for animal production. Secondly, this review will investigate the potential for saltbush based saline pastures to be used for meat production. The following experiments will investigate how ingesting saltbush affects the carcass and eating quality of sheep. The experimental approach was a combination of animal house and field experiments.
Chapter 2

Review of the Literature

2.1. Saltbush and saltland pastures for salinity management and animal production

The revegetation of saltland using halophytes such as saltbush has become more prevalent in recent years in the Western Australian farming community. The most commonly grown halophytes are Atriplex and Maireana spp. Salt tolerant pastures and grasses can also be grown between the rows of saltbush (referred to herein as interrow species). These often include barley grass (Hordeum leporinum), puccinellia (Puccinellia ciliata), balansa clover (Trifolium michelianum), annual ryegrass (Lolium multiforum), mintweed (Salvia reflexa), rumex (Rumex acetosella) and silvergrass (Vulpia bromoides) although many other options are possible (Masters et al. 2001). Over time the presence of saltbush may slow or reverse salinity at the surface and allow less salt tolerant pastures and grasses to volunteer or be cultivated.

2.1.1. Characteristics of Saltbush

Saltbush (Genus: Atriplex; Family: Chenopodiaceae) and bluebush (Genus: Maireana; Family: Chenopodiaceae) are halophytes indigenous to large areas of semi-arid and arid Australia. They are capable of surviving and growing in highly saline soils (100-200mM of sodium) by a process of salt accumulation in the bladders underneath the leaf. The establishment of saltbush and bluebush can help prevent further increases in salinity by lowering the water table. To achieve this the deep roots of the saltbush increase the water usage of the plant by around 50mm of groundwater per year in a 330mm rainfall district (Barrett-Lennard 2002). An added
The advantage of saltbush and bluebush for use on saline land is that they can provide about three times as much dry matter per unit of water transpired as conventional pastures because they are C4 plants and thus resistant to photorespiration (respiration without energy production) (Atiq-ur-Rehman et al. 1999). Saltbushes are the most commonly planted species and this thesis focuses on saltbush dominated saline pasture systems (Barrett-Lennard 2002). Old man saltbush is the most commonly grown species but other species grown in Australia include *Atriplex undulata* (wavy leaf saltbush), *Atriplex amnicola* (river saltbush), *Atriplex vesicaria* (bladder saltbush) and *Atriplex halimus* (Mediterranean saltbush or Sea orache).

### 2.1.2. The value of saltbush for animal production

The south western agricultural region of Western Australia is characterised by a Mediterranean-type climate, with hot dry summers and cool, wet winters. The annual rainfall ranges from 200-1000mm and most falls in the winter and spring. Under such conditions, livestock receive better quality feed in winter and spring when pasture growth rates are high (Atiq-ur-Rehman et al. 1999). During summer and autumn livestock graze on senesced pastures and crop residues that have a low energy and protein content. The liveweights of sheep during the summer and autumn tend to decline and this period is commonly referred to as the ‘autumn feed gap’. To reduce the effects of the feed gap it is routine for farmers to supplementary feed their animals with grain and/or hay but this can be an expensive option. An alternative is to grow perennials shrubs such as saltbush to provide biomass for livestock production all year round. Saltbushes are summer active so their nutritional value deteriorates little during the dry summer/autumn period (Wilson 1966b). Maximum benefits could be achieved by strategic grazing of these shrubs at this time as an alternative or in combination with grain supplements (Casson et al. 1994; Warren et al. 1996; Warren et al. 1995).
A consistent observation of sheep grazing saltbush is that they do not perform as well as expected. Sheep often lose up to 100g/day when grazing saltbush during summer (Morcombe et al. 1996; Warren et al. 1992; Warren et al. 1995). This has been attributed to the high salt and low metabolisable energy content of the plants (Masters et al. 2001).

Saltbush contains 30-150g/kg dry matter (DM) (3-15%) of sodium (Squires 1993; Wilson 1966a). The presence of high levels of sodium to the diet has been shown to significantly decrease feed intake and organic matter digestibility and subsequently the growth rates of the sheep (Barret-Lennard et al. 2005; Goodwin et al. 1986; Hemsley 1975; Nelson et al. 1955; Thomson et al. 1978). Masters et al. (2005) demonstrated a reduction in feed intake from 1.35kg/d to 0.67kg/d in sheep fed diets containing 0 and 80 gNa/kg DM sodium respectively.

Hemsley et al. (1975) also observed a 24% reduction in digestion of organic matter in the rumen when sheep consumed 150 g/head.d of sodium chloride (NaCl) compared to those on low sodium diet. The depressed organic matter digestibility can be attributed to the high water intake by sheep ingesting high quantities of sodium. The ingestion of high levels of sodium increases the extracellular fluid concentration (Guyton et al. 2000). To maintain homeostatic osmolarity of the extracellular fluid the thirst mechanism is triggered and water consumption increases (Macfarlane et al. 1967; Warren et al. 1996; Whittow 1968; Wilson 1994). The high water consumption tends to increase the rate of passage of feed through the rumen highlighted in the study by Hemsley et al. (1975) where the residence time of feed in the rumen dropped from 20 hours to 12 hours in sheep fed the 150g/head.d of NaCl were compared to those fed a low salt diet. This provides less time for the rumen microbes to digest organic matter. In addition, saltbush has been shown to impair the absorption of volatile fatty acids from the rumen (Weston
et al. 1970). Volatile fatty acids (VFAs) are formed by the fermentation of plant materials such as cellulosics, starches and sugars. For the ruminant animal, VFAs are the major source of energy. VFAs are believed to contribute approximately 70% to the caloric requirement of ruminants (Bergman 1990). With the depression in feed intake, digestibility of the feed and VFA absorption it is unlikely that the sheep can consume sufficient quantities of digestible organic matter to grow. Saltbush also contains roughly 7MJ/kg DM of metabolisable energy (Wilson 1994). The maintenance level required by a 30kg sheep is 7MJ/d (McDonald et al. 1995). If the sheep is not able to eat 1kg DM/d needed to meet its energy requirements then the animal will lose condition. Under these conditions, the poor performance of sheep fed saltbush is not surprising.

A positive feature of saltbush is that it contains 2-3% nitrogen (N) (Arieli et al. 1989; Weston et al. 1970; Wilson 1966b), similar to lucerne hay (Chriyaa et al. 1997). Crude protein (CP) is derived mathematically from this N content (CP = N x 6.25) indicating that saltbush contains 12.5-19% protein. Growing sheep require a diet of at least 12% protein for liveweight gain (McDonald et al. 1995). However, CP does not give a true indication of protein in saltbush as up to 50% of the N in saltbush is non-protein nitrogen (NPN) including betaines (up to 6gN/kg DM or equivalent to 4% crude protein (Storey et al. 1977) and nitrates (3-5gN/kg DM or 2-3% CP (Masters et al. 2001)). While rumen microbes do have the ability to manufacture protein from soluble N compounds, this process is dependant on a good supply of digestible energy. Nevertheless, saltbush plantations can serve as crude protein supplements during summer-autumn when other feed is dry and deficient in protein and minerals (Chriyaa et al. 1997; Jones et al. 1987) particularly if the sheep are provided with an energy supplement at the same time.
2.2. The role saltbush in wool and meat production

The Australian farming system can incorporate both wool and meat production. The economic viability of grazing saltbush must take into account both aspects of production. There have been a few studies conducted on the role of saltbush for wool production and results to date are positive. There is very little information however on the role of saltbush for meat production.

2.2.1. Saltbush and wool production

Since the introduction of wool sheep to the pastoral regions, pastoralists have been advocating the benefits of saltbush for wool growth. The pastoralists have associated the high wool clips and lamb marking percentages with grazing saltbush (Warren et al. 1996).

The literature to date also suggests that saltbush is suitable for wool production. Hemsley et al. (1975) and Masters et al. (2005) have observed high wool growth efficiency with high salt diets. Masters et al. (2005) reported the efficiency of wool growth went from 0.89 mg/cm².kg organic matter intake to 1.57 mg/cm².kg organic matter intake in sheep fed 0 and 80 gNa/kg DM sodium respectively. From the point of view of a specialist wool growing enterprise this result is highly significant. It indicates that including plant material with a high salt concentration can increase total wool production per kg of available organic matter. The individual feed intakes may be reduced and the sheep would probably not gain weight but wool would be finer and the amount of produced per unit of available feed would increase.

Wool growth is highly responsive to protein available for absorption in the small intestine (Hemsley 1975). High salt diets increase water intake and the rate of passage of nutrients in sheep. Increased rate of passage will mean lower degradation of protein by micro-organisms in
the rumen. The result will be more undegraded protein available for absorption in the small intestine. Therefore it is possible that the consumption of saltbush could increase wool growth however the lower amount of available metabolisable energy in saltbush may suppress this potential. In addition, saltbush contains twice as much sulphur as green pastures and four times as much as dried annual pastures (Morecombe et al. 1991). This, coupled with the high N levels may facilitate the synthesis of the sulphur containing amino acids in the rumen.

2.2.2. Saltbush and meat production

There is widespread anecdotal evidence suggesting that meat from saltbush grazed sheep is leaner, tastier and juicier. These claims have resulted in the establishment of two niche markets in Eastern Australia.

Saltbush Dija is a product from South Australia. The meat is from sheep grazed on natural (non-saline) saltbush pastures in pastoral country. It is claimed that the meat has a unique, intense, game-like flavour. A strict quality assurance program specifies that sheep must be 100% merino, 18 to 30 months of age, have spent at least 400 days grazing salt/blue bush pasture and upon slaughter have a carcass weight of 22-29kg and a fat depth of 5-20mm. The product is also aged for a minimum of 3 weeks prior to consumption. Since the product was launched, sales have been between 100-200 sheep per day. The product is sold to 5 star hotels, boutique restaurants and delicatessens.

A second product from New South Wales is called Saltbush Mutton. Sheep (18+ months of age) are finished on old man saltbush grown on marginal non-saline land. The producers used their saltbush stands in conjunction with other pasture before sending the animals to a central
property for finishing with grain supplementation. They claim that the meat is lean, clean, crisp finish and lasting flavour and superior in terms of taste and texture. It was also said to lack the heavy, fatty after taste of pasture fed animals. This system is not currently in production. (Andrew Sippell, Pers comm.)

Based on these claims it may be possible that sheep grazing saltbush on saline land will also have a superior meat quality. If so, utilising saline areas to produce a saltbush meat product may be a novel way to increase profitability of these areas. An additional benefit is that the farming community may consider previously unproductive land planted to saltbush a more useful option. There is however, no objective information on the effects of grazing saltbush grown on saline land on carcass and eating quality of sheepmeat. There is also little information on the effects of grazing animals on saltbush grown on pastoral or non-saline land despite the development of branded “saltbush sheepmeat” niche products from these areas. Therefore to establish the economic viability of alternative systems for utilising saltbush based pasture systems it is important to identify the benefits and any possible deleterious effects on the carcass and eating quality of lambs grazed under such systems. The following sections of this review will detail the potential advantages and disadvantages of grazing saltbush on the carcass and eating quality of sheepmeat. (Andrew Sippell, Pers comm.)

2.3. Saltbush and carcass quality

Carcass quality is a term used to describe the carcass weight and carcass composition, meat appearance and muscle biochemistry of a carcass. To date there has only been one comprehensive study conducted on the carcass quality of sheep grazing saltbush by Hopkins et al. (1999). In this study, 120 Suffolk x Merino lambs were grazed on either saltbush
supplemented with oats, saltbush supplemented with lucerne hay or green lucerne pasture alone for 68 days. Carcass quality attributes evaluated included hot carcass weight, GR fat depth, ultimate pH and meat and fat colour. Notably, this study was conducted on saltbush on non-saline marginal land and its relevance to a saline land system is not known.

2.3.1. Carcass weight

Australians meat processors are increasingly demanding higher carcass weights. The higher the carcass weight the greater the quantity of saleable product and thus the higher the profit for the processor. Recent studies have also indicated a strong consumer preference in Australia for retail cuts from heavier carcasses as tenderness and juiciness appear to be positively correlated to carcass weight (Hopkins et al. 1985).

2.3.1.1. Carcass weight and liveweight

Carcass weight is dependant on the liveweight at slaughter, therefore the capacity of the sheep to gain weight when fed saltbush will determine carcass weight. The literature suggests that saltbush alone will only support maintenance of liveweight (Casson et al. 1994; Squires 1993; Warren et al. 1990; Warren et al. 1992; Warren et al. 1996; Warren et al. 1995). As mentioned previously, saltbush is a valuable protein supply but it is low in metabolisable energy as a proportion of DM. Therefore if an animal is to gain weight whilst grazing saltbush it needs a ration balanced for both protein and energy (Casson et al. 1994; Squires 1993; Warren et al. 1990; Warren et al. 1992; Warren et al. 1996; Warren et al. 1995).

Supplements including grains and hay and the interrow species (plants growing between the rows of saltbush) such as grasses and clovers may increase the metabolisable energy for an
animal grazing saltbush (Atiq-ur-Rehman et al. 1999). Warren and Casson (1994) and Roberts (2001) both reported a complementary effect of feeding a mixed diet of saltbush and chaff or pasture, with dry matter intake (DMI) doubling over feeding saltbush or chaff alone. The higher intake of the mixed diet may be attributed to the higher energy and protein and lower salt of the mixed ration. In both studies, significantly higher liveweight gains were achieved in the group fed a mixed ration compared to the group fed saltbush or chaff alone.

The choice of supplement used when finishing a sheep for slaughter must be considered carefully. The use of interrow pastures species alone to supplement saltbush is unreliable as low rainfall years may result in reduced growth and nutritive value of these plants. Furthermore, these interrow species are also of low nutritive value during summer and autumn (Atiq-ur-Rehman et al. 1999). In these situations, the sheep will need to be supplemented with hay and or grain in addition to the interrow pasture. However, the use of hay may not be good enough in a finishing system. The extra gut fill associated with the consumption of hay may confound liveweight measurements. Hay may also not provide enough digestible nutrients if fed alone. The use of hay was a problem encountered by Hopkins et al. (1999) who observed significantly lower hot carcass weights for sheep fed saltbush and lucerne hay treatment compared to those fed green lucerne pasture. This occurred despite there being no significant difference in final liveweight between the groups. The use of cereal grain alone may be a more dependable and efficient option when finishing animals. It is a source of rapidly fermentable carbohydrates (mainly starch) that will aid the microbial utilisation of nitrogen from saltbush and keep animals in a positive energy balance (Fontenot et al. 1955; Hemsley et al. 1975). Chriyaa et al. (1997) found that for each kilogram of saltbush fed, voluntary barley intake increased by 340-630g, inferring complementarity. Hassan and Abdel-Aziz (1979) reported that 150g/head.d of barley was the minimum required to ensure sheep fed saltbush maintained bodyweight and at this
point, nitrogen retention was significantly greater than rations with less grain. Franklin-McEvoy (2002) demonstrated that supplementing with 250g/head.d of barley permitted liveweight gains of approximately 63g/d compared to 5g/d in the unsupplemented group. Therefore, the use of saltbush based pasture systems in combination with a grain supplement to finish animals for slaughter may provide a potential strategy to produce acceptable carcass weights from saline pastures.

2.3.1.2. Carcass weight and water retention

The process of transportation and lairage to the abattoir may also affect the final carcass weights. Jacob et al. (2005b) demonstrated that after a 24 and 48 hour transport and lairage period there was a carcass weight decrease of 2.4 and 2.8% respectively compared with sheep slaughtered without undergoing transportation and lairage. This carcass weight loss is detrimental to both the farmers and the processors. Particularly to farmers who receive payment from the abattoir for their sheep based on the carcass weight and grade specifications. This decrease in carcass weight has been attributed to the level of hydration at the time of slaughter (Carragher et al. 1996; Thompson et al. 1987). Although water is available in lairage prior to slaughter sheep may not drink due to the unfamiliar surroundings and high sheep density per pen. This water deprivation can cause dehydration of body tissues and the loss of carcass weight (Gortel et al. 1992; Horton et al. 1996; Wythes et al. 1985). There is also the possibility that dehydration may cause some sheep to become stressed and have increased muscle pH post-mortem. This in turn impairs meat quality. Dehydration can be averted if water is made easily available in lairage and the sheep drink it.
Grazing saltbush may promote the consumption of water in lairage as the high salt content of the saltbush increases the sheep’s thirst. In addition the consumption of high levels of saltbush may also increase the retention of body water during grazing. The changes in water consumption and increased retention may influence carcass weight changes resulting from transport and lairage. The interaction between grazing saltbush, transportation and lairage on carcass quality requires further investigation under commercial conditions.

**2.3.2. Carcass composition**

Carcass composition is the proportion of lean (protein) and fat in a carcass. Optimal composition desired by processors is a high protein and low fat content. The costs of fat denudation are high so the lower the fat content, the higher the profit per animal. In recent times, carcass video imaging technologies (called Viascan©) have been introduced into Australia to enable the prediction of lean meat yield (Hopkins et al. 2004). It is envisaged that premiums will be paid to farmers who produce leaner carcasses. Further benefits to farmers of producing leaner carcasses are that less fat is deposited per unit of liveweight gain (Warriss 2000). Consumers also prefer to purchase meat with less visual fat despite a positive correlation between eating quality and fat content (Hopkins et al. 1985).

There is some anecdotal evidence that saltbush fed sheep are leaner than similarly sized sheep fed low salt feed. Despite these claims, the effect of grazing saltbush on the carcass composition of sheepmeat has not been previously investigated. There is some indication in the literature that ingesting a high salt load or compounds such as betaine and chromium, all present in saltbush, may affect the rate of fat and protein deposition. The following sections review the potential for similar results to be obtained from grazing saltbush.
2.3.2.1. **Impact of ingesting a high salt load on fat and protein deposition**

Kraidees *et al.* (1998) observed a change in carcass composition from feeding *Salicornia bigelovii* Torr. *Salicornia bigelovii* Torr is a high yielding halophyte that is used by the oil industry in the Middle East. It has been suggested that the by-products of *Salicornia* can be used for animal production but, like *Atriplex*, it contains high concentration of NaCl, up to 30% DM. In this experiment, Najdi ram lambs were fed a ration containing either 0, 10, 20 or 30% *Salicornia* by-products. The sodium concentrations of the final diets were 4.5, 23, 35.9 and 47.9 g/kg DM. The chemical composition of the carcasses was examined (Table 2.1) and as the inclusion rate of *Salicornia* increased there was a corresponding decrease in ether extract (fat), increase in moisture and non significant increase in protein content. There was an 11-22% decrease in fat, a 2-5% increase in protein and a 4-9% increase in moisture content between the 0% diet and the 20 and 30% inclusive diets. In the study by Hopkins *et al.* (1999) they did not investigate the effect of grazing saltbush on total fat and protein but found no significant difference in GR site fat depth between the treatments.

In other studies, Walker *et al.* (1971) demonstrated clear changes to the carcass composition of two year old merino wethers following the consumption of saline water containing 1.3% NaCl for 14 weeks (Table 2.1). The saline water regime resulted in a significant 8% and 11% increase in the proportion of protein and water respectively and a significant 31% decrease in the proportion of fat in the carcass compared to the tap water treatment.
Table 2.1 Carcass Composition of Sheep

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water</th>
<th>Protein</th>
<th>Fat</th>
<th>Ash</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Control</td>
<td>56.0</td>
<td>17.7</td>
<td>25.2</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>10% Salicornia by-product</td>
<td>56.4</td>
<td>17.4</td>
<td>25.2</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>20% Salicornia by-product</td>
<td>58.4</td>
<td>18.1</td>
<td>22.4</td>
<td>0.97</td>
<td>(Kraidees et al. 1998)</td>
</tr>
<tr>
<td>30% Salicornia by-product</td>
<td>60.9</td>
<td>18.5</td>
<td>19.6</td>
<td>0.95</td>
<td></td>
</tr>
</tbody>
</table>

Significance: P<0.05 P>0.05 P<0.01 P>0.05

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water</th>
<th>Protein</th>
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<tr>
<td>Tap water</td>
<td>52.8</td>
<td>16.8</td>
<td>24.0</td>
<td>5.6</td>
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<tr>
<td>Saltwater</td>
<td>59.0</td>
<td>18.3</td>
<td>16.4</td>
<td>5.9</td>
<td>(Walker et al. 1971)</td>
</tr>
</tbody>
</table>

Significance: P<0.01 P<0.05 P<0.05 n.s.

Based on the work by Kraidees et al. (1998) and Walker et al. (1971) it seems likely that a similar effect on carcass composition could be observed in sheep grazing saltbush. Saltbush is also a halophyte like Salicornia and contains similar levels of sodium. In addition, similar physiological effects of ingesting 1.3% sodium in the drinking water have been observed when sheep consume 10% sodium in the feed (Peirce 1957; Wilson 1966a). This is approximately the daily level of consumption by a sheep grazing saltbush as the sole feed source (Wilson 1966b).

2.3.2.1.1. Possible causes of changes in carcass composition with high salt intake

2.3.2.1.1.1. The energy to protein ratio

The carcass composition of a sheep is dependant on intrinsic factors such as the liveweight and genetic potential for lean tissue deposition but also the supply and ratio of dietary energy to
protein (Campbell 1988). Firstly, sufficient levels of both need to received to meet the basal requirements of the animal. Protein provides the building blocks for the growth of lean tissue whilst energy is required for conversion of this protein into lean tissue (Noblet et al. 1999). Beyond the minimum requirement the protein:energy ratio can be optimised so that animals deposit lean tissue as close to genetic potential as possible with a minimum simultaneous deposition of fat. Generally, by increasing the protein:energy ratio, growth is partitioned into an increase in lean tissue deposition and a reduction in fat deposition (Zimmerman et al. 1973).

There are two reasons why sheep grazing halophytes or a high salt diet in combination with a supplement may have a higher protein: energy ratio than sheep fed a low salt diet.

Quality and quantity of protein:

Saltbush contains high levels of crude protein (12.5-19%) and the high salt load may further increase the quantity as well as the quality of protein available for absorption in the small intestine. The high salt and resultant increase in water intake will increase the rate of passage of feed through the rumen, decreasing the rate of fermentation of dietary protein in the rumen. This increases the quantity and quality of bypass protein that is presented to the small intestine for absorption (Goodwin et al. 1986; Hemsley et al. 1975; Thomson et al. 1978).

Energy supply:

The increased rate of passage will also decrease the fermentation of organic matter in the rumen. In combination with the low metabolisable energy content of saltbush and lower feed intake, the
production and absorption of VFAs will be reduced. This is expected to result in a reduced energy supply to the sheep (Hemsley et al. 1975; Weston et al. 1970).

The energy supply to the animal is further reduced by an increased energy utilisation. Arieli et al. (1989) demonstrated that when sheep ingested saltbush or a high salt diet energy expenditure increased by 5kJ for every extra 1g intake of minerals. Digestive and renal processing of salt is an energy expensive process. The sodium ion uptake by the intestinal wall in the rumen is an active, energy requiring process (Jorgenson 1977). A large part of the sodium load is absorbed by the rumen hence increasing the sodium load to the gut will increase energy expenditure. The processing of the sodium by the kidney also requires energy. Up to 98% of the salt from the saltbush is excreted in the urine and most of the energy consumed by the kidneys is associated with the sodium pump (Summers et al. 1988). An additional energy expense is the production of proportionately more acetate than propionate in the rumen of sheep grazing saltbush or high salt diets (Arieli et al. 1989; Croom Jr et al. 1985; Hemsley 1975). Acetate directed fermentation is less efficient than propionate production (McRae et al. 1982). Acetate (and butyrate) production involves the formation of hydrogen which is used in conjunction with carbon dioxide to produce methane. Methane is a waste product of fermentation as it does not contribute to the animals’ energy requirements. Johnson et al. (1995) reported that methane loss as a proportion of gross energy intake reduced from 6-7% for a diet with an acetate directed fermentation to 2-3% for a diet with a propionate directed fermentation both offered at near ad libitum intake.

In conclusion, there is good evidence that the high salt in the saltbush will increase the quantity and quality of protein absorbed from the small intestine while reducing the energy available for production. Together these changes will result in a high protein: energy ratio and are consistent with increased protein: fat in the carcass.
2.3.2.1.1.2. **Hormonal effects**

Hormones facilitate changes in carcass composition as a result of changes in nutrient availability. The effect of ingesting a high salt or halophyte diet on the hormonal status of grazing sheep is unknown. Of the many hormones that affect lean tissue and fat deposition, this thesis will monitor the following as possible avenues for carcass compositional change:

- **Insulin** is the primary hormone involved in fat deposition. Increased availability of gluconeogenic precursors such as propionate or glucose will increase insulin secretion and stimulate lipogenesis (Abdul-Razzaq *et al.* 1988; Breier 1999; Chaturvedi *et al.* 1995; Chaturvedi *et al.* 2000; Gregory *et al.* 1980; Oddy *et al.* 1987).

- **Insulin like growth factor (IGF)** is also stimulated by an increased plane of nutrition and higher IGF values are associated with increased rate of growth resulting from an increase in muscle deposition and the reduction of muscle degradation (Cameron 1993).

- **Growth hormone (GH)** in contrast is stimulated by symptoms of malnutrition, namely hypoglycaemia and a low concentration of free fatty acids in the blood. Growth hormone has a specialised role for fat mobilisation primarily achieved by reducing the insulin response (Adams *et al.* 1996). It stimulates triglyceride breakdown and then enhances the conversion of fatty acids to acetyl CoA and subsequent utilisation of these for energy (Breier 1999). It can also increase the deposition of protein by increasing the transfer rate of amino acids into cells to be used for protein synthesis as well as decrease the catabolism of proteins (Lobley 1998; Oddy 1993).
Leptin is secreted by adipose tissue to regulate food intake. Decreased leptin concentrations due to lower levels of fat indicate that the body is not storing enough fat and this will stimulate the hypothalamus to increase feed intake (Blache et al. 2000; Prolo et al. 1998; Wilding 2001; Wilson et al. 1998; Woods et al. 1998).

Cortisol is stimulated by physical or neurogenic stress. It causes the mobilisation of fatty acids and protein for energy. Ingesting a high salt diet may increase the physical or neurogenic stress levels in a sheep (Fordham et al. 1989; Horton et al. 1996; James 1992; Pignatelli et al. 1998).

Triiodothyronine (T3) and thyroxin (T4) and are hormones that control the metabolic rate of the animal. They do not have a direct effect on fat and lean tissue deposition but they do increase the production of IGF and GH to increase the mobilisation of fatty acids for energy and promote protein deposition (Chaturvedi et al. 1995; Chaturvedi et al. 2000). The ingestion of a high salt load may increase the metabolic rate thus increase T3 and T4.

2.3.2.2. Other compounds in saltbush that may influence protein and fat deposition

Plants growing in inhospitable environments often contain secondary compounds that play a role in their survival (Masters et al. 2001). For salt tolerant plants these compounds may assist the plant to tolerate the soil, water and climatic stress (eg salt, betaine) or may act as deterrents to grazing herbivores (eg tannins, oxalates, coumarins, nitrates). There are other compounds and minerals that may also accumulate in saltbush such as triterpenoids, steroids, alkaloids and trace elements such as chromium (Gihad et al. 1994). Of these compounds, the literature suggests that chromium and betaine may have some influence on fat and protein deposition.
2.3.2.2.1. Chromium

The saltbush species *Atriplex halimus* has been traditionally used as an antidiabetic agent among the Arabs living near the Dead Sea in Israel. This plant has been reported to contain high levels of chromium (Degen 1993; Mirsky *et al.* 1999). Less is known of the concentration of chromium in other *Atriplex spp.* There is evidence that chromium picolinate supplementation can decrease subcutaneous fat thickness in pigs and increase loin eye area in pigs (Page *et al.* 1993). Kitchalong *et al.* (1995) and Gardner *et al.* (1998) also observed that supplementary chromium decreased subcutaneous fat depth in young sheep, suggesting a reduction in the rate of lipogenesis in this fat depot.

The role of chromium for carcass composition may be due to its potentiation and augmentation of insulin action (Andersen 1997; Kegley *et al.* 1999; Morris 1999). Increased insulin action should lead to an increased uptake and utilisation of glucose, leading to greater rates of lipogenesis (Prior *et al.* 1979). However lower fat levels are observed with chromium supplementation. The heightened insulin action may also increase protein synthesis and reduce protein degradation in preference to lipogenesis. Page *et al.* (1993) showed that chromium supplementation can increase nitrogen retention. The decrease in fat depth may be due to the diversion of energy yielding nutrients to support increased protein synthesis. The augmentation of insulin action might have also promoted a shift in the site of lipogenesis away from the subcutaneous fat depots to sites that are more insulin sensitive explaining the results of Kitchalong *et al.* (1995) and Gardner *et al.* (1998). Abdominal fat has been shown to be more insulin-sensitive than subcutaneous fat resulting in greater fat deposition in these areas (Leibel *et al.* 1989).
2.3.2.2.2.  Betaine

There is some evidence in the literature that betaine may affect fat deposition. Fernandez et al. (1998; 2000) demonstrated that feeding diets enriched with betaine could decrease GR fat thickness and lipid content of intramuscular fat in lambs. Betaine is a naturally occurring trimethyl nitrogen compound found in all plants and animals but in particularly high levels in salt tolerant plants (0.14-6.8%) (Randell et al. 1996; Storey et al. 1977). Betaine functions as a methyl group donor (Baker et al. 1985; Ekland et al. 2005; Lobley et al. 1996) assisting in the recycling of the essential amino acid methionine and in the production of choline. Methionine is an amino acid that is often the first limiting in ruminants and is important for lean tissue growth. Betaine contributes directly to methionine recycling, making it important in times of low methionine availability (Lobley et al. 1996). Cholines are the main components of cell membranes and are also an essential part of the lecithin molecule. Lecithin facilitates the transport of fat through the body and choline can be partially replaced in the diet by betaine or methionine (Baker et al. 1985; Saunderson et al. 1990). The reduction in fat deposition may be due to the improvement in the efficiency of methionine use for protein synthesis and the facilitation of choline activity (Ekland et al. 2005; Fernandez et al. 1998). There is also evidence in pigs that betaine has a more pronounced effect when dietary energy is limiting and so offers a means of improving meat quality through ensuring the provision of additional energy (Suster 2003). More research is needed to confirm this finding in sheep.

Betaine also has an osmoregulatory function in plants which specifically enables salt tolerant plants to tolerate high osmotic pressure while growing in highly saline soils (Randell et al. 1996; Storey et al. 1977). Betaine may also perform a similar osmotic function in animals and prevent
dehydration of cells under stressful conditions (Ekland et al. 2005). Specifically betaine may protect against the dehydration effects of preslaughter transport and lairage and prevent detrimental losses in carcass weights. Betaine may also have potential to increase the water holding capacity of the meat and improve tenderness and juiciness. These claims have encouraged the use of betaine in the livestock feed industry in recent years (Remus 1998) despite the lack of scientific evidence. Betaine is used in the pig and poultry industry with claims that it makes the meat less dry and also for the management of heat stress in the cattle industry. Clearly further work is needed to examine the role of natural sources of betaine such as saltbush on carcass composition and dehydration prevention.

2.3.3. Impact of a high salt load on the fatty acid composition of the meat

Customers from Australia, Europe and North America are increasingly selecting meat with lower levels of fat for health reasons. This needs to be balanced with a minimal level (around 3-4%) for optimal eating quality (Diaz et al. 2004; Lawrie 1998). Intramuscular fat is positively correlated with increased tenderness and juiciness and associated with flavour development (Hopkins et al. 1985; Melton 1990; Warriss 2000). The composition of the fatty tissue is also of importance with consumers demanding less saturated meat.

The effects of grazing saltbush on the fatty acid composition of sheepmeat have not been previously investigated. However, Walker et al. (1971) demonstrated changes in the fatty acid composition following the prolonged consumption of saline water compared to tap water (Table 2.2). There was a trend for the reduction of saturated fat in the omental and perirenal fat depots with the saline water treatment.
Table 2.2. Fatty acid composition of sheep offered differing salt concentrations in drinking water (Walker et al. 1971)

<table>
<thead>
<tr>
<th>Fat and treatment</th>
<th>Palmitic (16:0) %</th>
<th>Stearic (18:0) %</th>
<th>Oleic (18:1) %</th>
<th>Linoleic + Linolenic (18:2 +18:3) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omental Fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap water</td>
<td>23.03</td>
<td>29.58</td>
<td>27.3</td>
<td>5.55</td>
</tr>
<tr>
<td>Saltwater</td>
<td>22.25</td>
<td>24.05</td>
<td>26.95</td>
<td>7.78</td>
</tr>
<tr>
<td>Perirenal fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tap water</td>
<td>23.03</td>
<td>29.35</td>
<td>28.9</td>
<td>6.05</td>
</tr>
<tr>
<td>Saltwater</td>
<td>22.68</td>
<td>28.65</td>
<td>25.43</td>
<td>7.15</td>
</tr>
</tbody>
</table>

a Fatty acid nomenclature e.g. 16:0 = 16 carbon atoms and no unsaturated bonds, 18:2 = 18 carbon atoms and 2 unsaturated bonds

b Significant difference exists between in the concentration of stearic acid concentration in the omental fat between the two treatments.

The composition of fatty acids in the depot fat is dependant on the degree of hydrolysis and biohydrogenation in the rumen (Bas et al. 2000; Beam et al. 2000; Jenkins 1993). Unsaturated fatty acids consumed in the diet are extensively hydrolysed by rumen lipases to yield free fatty acids that in turn undergo biohydrogenation to yield saturated end products to the duodenum (Jenkins 1993). These products are absorbed and deposited in body tissues such as fat. Reducing the rate of hydrolysis and biohydrogenation in the rumen may result in a lower degree of saturated fat in the depot fat.
In Walker et al. (1971), the lower degree of saturation of the depot fat in sheep consuming the saline water regime may have been due to the high salt load and resultant high water intake (Hemsley et al. 1975) reducing hydrolysis and biohydrogenation in the rumen and final fat deposition. Similar digestive fermentation patterns are likely to exist in sheep grazing saltbush and therefore these animals may have a reduced saturation of depot fat.

There are significant commercial benefits of producing meat with a lower degree of depot fat saturation. The negative health implications of saturated fat have been widely researched and heavily publicised through the community (Hopkins et al. 1998; Jimenez-Colmenero et al. 2001; Pearson et al. 1983). There is an increasing demand for ‘healthier’ meat with higher levels of unsaturated fats. If saltbush meat does contain lower levels of unsaturated fat in the depot fat it may be branded as ‘healthy meat’ which will only add to its commercial acceptability.

2.3.4. Saltbush and meat colour

Meat colour is important because it is a major criterion the consumer can use to judge the acceptability of meat at purchase. Consumers prefer meat with a light pink to bright red colour and they strongly reject dark coloured meat believing it is from an old or sick animal or that it is bad or contaminated (Fabiansson et al. 1988). Furthermore, large losses in the meat industry due to meat colour deterioration have been reported in other countries (McDowell et al. 1996) but no data is available to confirm this perception in Australia. Value deterioration due to the conversion of meat from red to brown is due to product price markdown, product conversion and rework, product discard, lowered stock inventory and inefficient use of labour (McDowell et al. 1996).
The colour of meat is attributable due to both the oxidation state of the myoglobin present and the way the incident light is absorbed and scattered (Lawrie 1998). Metmyoglobin is the brown oxidised form of red myoglobin and its formation makes meat appear brown and undesirable (Boles 1995). Lipids (specifically polyunsaturated fatty acids of PUFA like linolenic acid) contained in the muscle provide the substrate for the oxidation of myoglobin to metmyoglobin (Greene, 1971).

The absorption and scattering of light is also influenced by the ultimate pH of the meat (Warriss 2000). Ultimate pH is determined by the level of glycogen in the muscle at the time of slaughter, a function of glycogen storage in the muscles prior to transport and lairage and the amount lost during transport and lairage. In the absence of oxygen post slaughter, glycogen is broken down to produce lactic acid, in a process called glycolysis (Lawrie 1998). The greater the store of glycogen in the muscles, the greater the build up of lactic acid and therefore lower ultimate pH. Increased lactic acid build up will ensure a faster pH drop and a lower final pH of the meat. The higher the pH of the meat the further apart the spacings between the muscle fibrils (Lawrie 1998). This allows for the incident light to penetrate the meat deeply without being scattered so it is absorbed by the myoglobin causing the meat to appear very red to purplish dark (Swan 1993). Light does not penetrate low pH meat so deeply before being scattered; thus the myoglobin cannot absorb much light and meat appears lighter in colour.

The effect of grazing saltbush on the oxidation state and ultimate pH of meat is unknown. If the ingestion of saltbush does result in a higher protein: energy ratio available for production as well as resultant changes in fatness and levels of metabolic hormones these outcomes may have important implications for glycogen storage and subsequent meat quality. Lower insulin levels and a reduced availability of glycogen substrates due to a lower energy intake can result in less
glycogen storage (Gardner et al. 2001; McVeigh et al. 1982; Pethick et al. 1996; Pethick et al. 1994). Lower levels of glycogen storage will result in elevated ultimate pH due to lower levels of lactic acid production during rigor mortis development (Lawrie 1998). A low ultimate pH (around 5.5) is commercially desirable, as it is associated with lighter meat colour and increased palatability. The significant commercial importance of meat colour warrants the investigation of the effect of grazing saltbush on ultimate pH and meat colour.

2.4. Saltbush and eating quality

Many consumers define meat eating quality in terms of tenderness and juiciness, with more tender and juicy meat having higher quality. However, in most instances, flavour of meat is also used to help determine acceptance. Sheepmeat flavour and odour is receiving increased interest from researchers probably because sheepmeat is gaining export importance (Rousset-Akrim et al. 1997). Sheepmeat characteristic odour and flavour is one reason why its consumption is very low in affluent markets such as North America and Japan (Prescott et al. 2001; Wong et al. 1975).

There are anecdotal claims that meat from sheep grazing saltbush is tastier and juicier. However there is a paucity of information on the role of saltbush on eating quality. If saltbush does change the eating quality attributes of sheepmeat it is important to make sure these changes are acceptable and favourable across a wide cross section of the community. This will allow for the advocation of saltbush as a beneficial feed source for meat quality thus ensuring consumer confidence in the final product. This section of the review will focus on how grazing saltbush based pasture systems may affect eating quality.
2.4.1. Sheepmeat flavour and aroma

2.4.1.1. Dietary effects

Diet can significantly affect sheepmeat flavour and aroma. Meat derived from animals raised on pasture has a characteristic flavour that sets it apart from meat from equivalent animals fed on concentrates (Batcher et al. 1969; Cramer et al. 1967; Mottram 1998; Park et al. 1972a; Park et al. 1972b; Park et al. 1972c; Shorland et al. 1970; Tudor et al. 1982; Wong et al. 1975; Young et al. 1996). For example, Park et al. (1972a) showed that a trained taste panel could significantly differentiate meat from lambs grazing legumes, lucerne (*Medicago sativa*) and or silverleaf (*Desmodium uncinatum*) even when fed for short periods of time from the meat of control lambs fed grass. The intensity of the characteristic lucerne taint described as a sharp or pungent odour and a dirty or sticky flavour was found to increase with increasing length of grazing lucerne.

Nawaz et al. (1992) examined the effect of a feeding regime consisting of 0, 24, 50, 75 and 100% *Atriplex amnicola* (river saltbush) and/or Sudex (sorgum x sudan grass hybrid) for 12 weeks on the organoleptic value of sheepmeat. No differences in flavour and aroma were observed between the treatment groups. Hopkins et al. (1999) carried out a limited sensory analysis of organoleptic characteristics and assessment of meat quality from animals fed either saltbush supplemented with oats, saltbush supplemented with lucerne hay or lucerne hay only. Tenderness, juiciness and the liking and strength of aroma and flavour (as well as tenderness and juiciness) were examined. The only significant finding of this study was increased aroma strength of meat from both groups given saltbush compared to the lucerne only group. No differences in liking of aroma were seen however. This observation may have been confounded by the use of lucerne as a control. Park et al. (1972a), Field and Kunsman (1973) and Nixon...
(1981) all demonstrated that grazing lucerne caused changes to the flavour and aroma of the meat compared to sheep grazing grass-like pastures. Had Hopkins et al. (1999) compared the saltbush treatments with sheep grazing a ‘control’ grass-like pasture system or hay/grain ration different eating quality results may have been observed. Therefore a comparison of meat from sheep fed saltbush needs to be made with sheep grazing a ‘control’ that is unlikely to independently influence flavour and aroma development.

2.4.1.2. Compounds implicated in sheepmeat flavour and aroma

A vast amount of work has been done to identify the chemicals that cause the distinctive sheepmeat flavour and aroma. Over 1000 volatile compounds responsible for meat flavour have been identified and some of them can be influenced by dietary constituents (Mottram 1998). Branched chain fatty acids and other odour volatiles such as sulphur-containing compounds, various pyrazines and pyridines and a range of phenolic compounds are the cause of characteristic sheepmeat odour and flavour (Cramer 1983; Ford et al. 1980; Mottram 1998). This thesis is not concerned with the identification of the compounds implicated in sheepmeat flavour and aroma. However it is important to note the possible influence on grazing saltbush on these compounds.

2.4.1.2.1. Branched chain fatty acids

The primary contributors to the undesirable characteristic sheepmeat flavour and aroma are branched chain fatty acids (BCFA) (Wong et al. 1975). The particular compounds are 4-methyl substituted C9 and C10 fatty acids called 4-methyloctanoic acid and 4-methylnonanoic acid. The dietary origins of BCFA are clear: they derive from ruminal propionate (Priolo et al. 2001). Ruminal propionate is the main substrate for liver gluconeogenesis. However when levels
exceed the capacity of the liver to metabolise it, the production of BCFA is stimulated. Simply, the accumulation of BCFA are associated with imbalanced high-energy, restricted roughage, high grain (typical feedlot) diets (Garton et al. 1972). The low energy high roughage content of the saltbush based diet may result in lower propionate formation and therefore a lower concentration of BCFA in the fat (Young et al. 1997). This review had already highlighted that high salt diets and saltbush reduce propionate production. Therefore sheep grazing saltbush may produce lower levels of these undesirable BCFA.

2.4.1.2.2.  Skatoles

The presence of BCFA is highly correlated with the presence of 3-methylindole or skatole, a faecal- smelling compound (Young et al. 1997). Skatole is a product of degradation of the amino acid tryptophan by rumen microbes. Why the level of skatole is higher in ruminants finished on pasture is not clear. Young et al. (1999) suggests that the higher ratio of protein/non fibrous carbohydrate, characteristic of grass diets, may enhance skatole production through a higher deamination of protein amino acids by rumen microbes. The diet may also have an important effect on microorganism species present in the ruminal liquor (Smith 1969) and that these different microorganisms may degrade tryptophan differently and therefore produce skatole. With regards to saltbush, it does have a high crude protein/non fibrous carbohydrate ratio (Correal et al. 1990; Davis 1981; Warren et al. 1990) and this may result in increased protein deamination and therefore skatole production. However, the high rate of digesta flow through the rumen due to the high fluid intake has been shown to reduce ruminal protein breakdown.
2.4.1.2.3. Oxidative products of linolenic acid

The oxidation of linolenic acid (18:3) and its derivatives have been associated with species flavours of meat as a result of the formulation of volatile compounds during cooking (Cramer 1983; Mottram 1998; Turner et al. 2002). Two derivatives of linolenic acid include eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6) (Wood et al. 1997). EPA and DPA are polyunsaturated fatty acids (PUFA) which are more prone to oxidation compared to oleic (18:1) or linoleic (18:2) acids (Elmore et al. 2000).

Sheep grazing pasture have a higher proportion of linolenic acid and its derivatives in their fat compared to those fed grain concentrated diets (Bas et al. 2000). This occurs because pasture diets are a rich source of linolenic acid and lower levels of hydrogenation occur in the rumen of grass fed ruminants (Larick et al. 1989).

The effect of grazing saltbush on the accumulation rates of linolenic acid and its derivatives is unknown. The high water intake of sheep grazing saltbush may affect the rates of hydrogenation in the rumen and increase the absorption of linolenic acid. If grazing saltbush does result in increased levels of linolenic acid in the fat, this has very important implications for the development of off flavours and aromas. There are however some benefits of increased levels of linolenic acid and its derivates in meat for human nutrition. Increased consumption of the derivatives of linolenic acid (EPA and DHA) has been recommended by health officials (Jimenez-Colmenero et al. 2001).
2.4.1.2.4. Sulphur compounds

The production of hydrogen sulfide during cooking of meat is associated with undesirable flavours and aromas (Nixon et al. 1979). Many sulphur compounds such as thiazoles, thiophens and sulfides contribute to the production of hydrogen sulfide. Cramer (1983) proposed that sheep have evolved a specific mechanism for the storage of sulphur unique to the species to maintain wool growth during low-sulphur stress periods. The rapid release of sulphur from sheep adipose tissue suggests this area may be a species specific store for sulphur compounds. This storage of sulphur could supply compounds or precursors of compounds that would make the cooking odour of lamb different to other species. Saltbush does contain high amounts of sulphur (Morecombe et al. 1991) and may increase the storage of sulphur compounds in the adipose tissue and lean meat.

2.4.1.3. The effect of grazing saltbush on flavour and aroma compounds

The potential effect of ingesting saltbush on the production of BCFA, skatole, linolenic acid or sulphur compounds and the resultant sheepmeat flavour and aroma has been discussed. Additionally, saltbush may also contain phenolic compounds capable of flavouring meat. A variety of secondary compounds with both antinutritional and osmoregulatory functions are found in saltbush (Masters et al. 2001). Assuming these compounds have the potential to be deposited in the fat or lean tissue of the sheep, upon cooking they may become volatile and contribute to sheepmeat flavour and aroma. The time the sheep need to be fed saltbush to allow for the accumulation of compounds that may affect flavour and aroma compounds in the meat is unknown. Previous studies suggest 8 weeks is the minimum time for adequate flavour development with feeding systems such as grain (Shorland et al. 1970). Meat fat is well documented to be the principal source of sheepmeat flavour and odour compounds (Cramer
Producing a leaner carcass by grazing saltbush may result in less flavoursome meat which may be undesirable in certain export markets. This is an important aspect to investigate.

2.4.2. Tenderness

Many consumers consider tenderness the most important palatability attribute because if meat is tough other sensory properties become less important. There are many factors that affect tenderness such as the length of the muscle fibres, the concentration of heat stable cross links (also referred to as collagen or connective tissue), the extent and rate of pH fall in the muscle post slaughter and the rate of proteolysis postslaughter (Lawrie 1998).

The rate and extent of pH fall of the meat will predominantly affect the tenderness (Dransfield 1981; Hopkins et al. 1998; Swan 1993). Optimal levels of tenderness will occur with an ultimate pH between 5.8 and 6.2. To reach this window, sufficient glycogen storage in the muscles prior to transport and lairage and minimal loss of glycogen during this time is essential (further discussion in section 2.3.4). The rate of pH fall, largely affected by post-slaughter abattoir practices such as electrical stimulation will also affect tenderness. If the decline is too slow this will result in a condition called ‘cold shortening’ where the muscles are stimulated to contract by the release of calcium ions from the sarcoplasmic reticulum of the muscles activating the actomyosin ATP-ase (Lawrie 1998). This muscle contraction will reduce the length of the muscle fibres (called sarcomeres) and decrease the tenderness of the meat. Too fast a decline will cause the denaturation of sarcoplasmic muscle proteins causing them to precipitate onto muscle fibres and become insoluble (Lawrie 1998). A fast pH fall will also decrease the potential for ageing or proteolysis post slaughter by denaturing the ageing proteins.
The higher the amounts of heat stable cross links (collagen or connective tissue) present, the tougher the meat. Principally the concentration of connective tissue increases with an increase in age but can also be modified by the nutritional regime of the growing animal. Animals on a high plane of nutrition will deposit greater proportions of fat and this fat is deposited in place of connective tissue. Fat first accumulates in the subcutaneous and intermuscular sites (Lawrie 1998). Following this, the fat then accumulates in the muscle in the perimysial connective tissue and is called intramuscular or marbling fat. The dilution of fibrous protein by soft fat decreases the resistance to shearing and chewing thus appears more tender. Also fat expansion in the perimysial connective tissue can open up muscle structure (Wood et al. 1990). If the sheep grazing saltbush receive an adequate protein and energy supply, then the intramuscular fat content will be increased and the intramuscular collagen decreased resulting in more tender meat. Alternatively, if the saltbush sheep are leaner then the consequences may be a greater concentration of connective tissue and therefore tougher meat. Kristensen et al. (2002) has shown that there is an increase in shear force and a consumer panel assessed perception of toughness in meat obtained from animals that have been selected to be lean or are leaner because of dietary manipulation.

The influence of grazing saltbush on the amount of connective tissue and the extent and rate of pH fall in the muscle of sheepmeat is unknown. Additionally important is the ability of sheep grazing saltbush to store sufficient levels of glycogen in the muscles to enable a pH of below 5.8 to be reached at a desirable rate. The commercial importance of tenderness to eating quality necessitates investigation into the effects of grazing saltbush on tenderness.
2.4.3. **Juiciness**

Juiciness is an important eating quality attribute as it increases the flavour intensity and helps soften meat making it easier to chew. Juiciness depends on the amount of water retained in the cooked meat product and the water holding capacity of the meat (Lawrie 1998; Warriss 2000). These aspects are influenced by the concentration of intramuscular lipids and extent and rate of pH fall.

A higher concentration of intramuscular fat will loosen up the muscle microstructure thus allowing more water to be retained in these areas (Lawrie 1998). Upon cooking meat with a higher intramuscular fat content contains more water and is juicier. Eating quality assessments by Mandell *et al.* (1997) and Owens and Gardner (1999) demonstrated that juiciness was positively related to fat concentration in the *longissimus* muscle. Steaks with 2-3% intramuscular lipid concentration were less juicy than steaks with 3.5-5% lipid content. If the saltbush meat is leaner, this may have adverse consequences on the juiciness.

The higher the ultimate pH of the meat, the greater the ability of the muscle proteins to bind water (Swan 1993). This increase in the water holding capacity of the meat will allow for less water to be liberated upon eating making the meat appear drier. However if the rate of pH decline is too great, protein denaturation will occur thereby reducing the water holding capacity of the meat so that more water will be lost as drip or during cooking making the meat drier. If grazing saltbush results in high stores of muscle glycogen this will result in a lower ultimate pH which will decrease the water holding capacity and make the meat juicier. Again, the rate of fall is largely dependent on the post slaughter practices of the abattoir but if the saltbush diet does encourage a fast rate of decline, cooking and drip loss may be increased making the meat drier.
2.5. Conclusions

This review has investigated the role of saltbush in producing a superior meat product. Both the benefits and detriments of such a grazing system have been discussed.

Grazing saltbush has potential to produce a carcass with a reduced level of fat and increased lean tissue content. Similar effects were observed in sheep consuming saline water. There are a variety of ways that grazing saltbush can affect carcass composition. Firstly, the high salt intake may affect the protein: energy ratio available for production. The high salt intake will increase the rate of passage of feed through the digestive tract thus increasing the amount of protein presented to the small intestine to be used for postprandial protein deposition. Grazing saltbush also increases the energy expenditure of the animal, a result of the necessary and energy expensive excretion of sodium and the acetate directed fermentation pattern. If energy intake requirements are not met, fatty tissue may be used to provide the energy substrates thus resulting in a decline in the fat stores of the animal. The current literature also suggests that saltbush and high salt diets favour the production of acetate which is correlated with lower rates of fat deposition. The high chromium and betaine content may also affect carcass composition. Furthermore, grazing saltbush may change the hormonal status of the animal. Such changes may facilitate alterations in fat and protein deposition.

The impact of grazing saltbush on the eating quality of sheepmeat is also unknown. There is some evidence that grazing saltbush on non-saline land will change the aroma of sheepmeat. There are also a variety of compounds such as branched chain fatty acids, skatoles, oxidative products of linolenic acid and sulphur compounds that may all have the potential to be deposited in the fat or lean tissue of the sheep, and upon cooking they may become volatile and contribute to sheepmeat flavour and aroma. It is essential to investigate the commercial acceptability of
any changes to flavour and aroma from grazing saltbush because of the commercial implications of changes in these variables for export markets. The production of leaner animals by grazing saltbush may be detrimental to tenderness and juiciness because a higher intramuscular fat content will improve these attributes.

Other benefits of grazing saltbush may include the reduction of saturated fat in the fat depot. Saturated fat is undesirable in the current health conscious market. The potential to advocate saltbush as a ‘healthy’ meat product will only add to its acceptability. In addition, grazing saltbush prior to slaughter may prevent dehydration and subsequent losses in carcass weight by promoting an increased water intake in lairage. The prevention of carcass weight loss will benefit both farmers and processors.

It was advocated throughout the review that for optimal meat production it is necessary for saltbush to be fed in conjunction with a high energy supplement such as barley. Grazing saltbush solely has been shown to reduce the performance of sheep and may be detrimental to meat quality. Optimal nutrition whilst on saltbush will ultimately achieve optimal liveweight gains to achieve high carcass weights. In addition, a high level of nutrition will enable sufficient glycogen stores in the muscles so that the ultimate pH can drop towards the optimum of 5.5. A lower ultimate pH of the meat will ensure improved tenderness, juiciness, meat colour and prevent the formation of off flavours and aromas.

In conclusion, there appears to be great potential for saltbush based pasture systems to produce a superior meat product. Therefore it is essential to investigate these how ingesting saltbush affects carcass and eating quality so that (i) cost-effective production systems to be implemented
for both farmer and meat processor and (ii) consumer confidence of the final product can be guaranteed.

2.6. General aims

The general aim of this thesis was to determine whether saltbush based saline pasture systems can be used to produce carcasses of high value and high eating quality.

2.7. General hypotheses

The general hypotheses examined in this thesis were:

1. Grazing saltbush based saline pasture systems will result in a high quality carcass
2. Grazing a saltbush based saline pasture systems can be used to produce meat with high eating quality
Chapter 3

General materials and methods

3.1. Introduction

The following sections outline the sampling, analytical and evaluation techniques common to two or more experiments. The techniques peculiar to a particular study are described in the relevant chapter.

3.2. Liveweight and liveweight gain

The body weight of each sheep in animal house and field experiments was recorded using a portable crate (Tru-test Australia) and electronic scales (SR 2000, Tru-test Australia) calibrated with a reference weight.

Liveweight gain was calculated as the slope of liveweight change over the period of the experiment using the regression function in Excel (Microsoft 2000).

3.3. Laboratory analysis

3.3.1. Sodium and potassium in urine, serum and plasma

The sodium and potassium concentration in the urine, plasma, serum and suint was analysed using the standard operating methods for the atomic absorption spectrophotometer (SpectrAA-30/40, Varian Techtron Pty Ltd).
3.3.2. **Creatinine, urea, albumin and lactate**

Creatinine, urea, albumin and lactate in the plasma, serum or urine were all analysed on a Cobas Mira (F. Hoffmann-La Roche, Switzerland) according to the standard operating procedures. All were analysed using Sigma Infinity Cobas Mira kits (Sigma-Aldrich).

3.3.3. **Urine Specific Gravity**

Urine was analysed for urine specific gravity with a Reichert Veterinary Refractometer model 10436 (Cambridge Instruments Inc., Buffaloe, NY, 14215, USA).

3.3.4. **Muscle Dry Matter**

Muscle dry matter percentage was calculated by weighing a muscle sample (approximately 2 g), drying it in a 70ºC oven for approximately 48 hours, or to constant weight. Muscle dry matter percentage was calculated as:

\[
\text{Muscle Dry Matter \%} = \frac{\text{Muscle Dry Weight}}{\text{Wet Weight}} \times 100
\]

3.3.5. **Fractional excretion rates**

Fractional excretion rate of sodium was determined by the following equation with all variables in the same units.

\[
\text{Fractional excretion rate of sodium (\%) = } \frac{\text{(Urinary sodium / serum sodium)}}{\text{(Urinary creatinine/ serum creatinine)}}
\]
3.4. **Sensory evaluation**

3.4.1. **Design**

The meat cut used for eating quality assessment in this thesis was the *m. longissimus thoracis et. lumborum* (LL) or loin as it is also called. The length of this piece of meat allowed for six 4 cm x 2.5 cm samples to be cut from the 12th rib (narrowest) end with muscle fibres running longitudinally. Each of the six 4 x 2.5 cm samples was considered on an individual basis.

Each panelist was given 1 sample of cooked meat and asked to complete an assessment sheet. This process was repeated 6 times at each evaluation session. Therefore each panelist received 6 pieces of meat. The allocation of each individual sample to a panelist and run was a modified latin square design. Additional allocation rules include:

- Treatments were represented equally i.e. there was an equal number of samples represented per treatment and an equal number of treatments in each run.
- A panelist sampled each treatment an equal number of times but not consecutively and samples were randomly allocated to panelist and run according to these constraints.

Powerplant (CSIRO Australia) was used to determine the number of samples needed in each chapter to detect a difference in eating quality with a power of 80% and above (Appendix 1). The numbers of samples needed was specific to an experiment and details of numbers used are in the experimental chapters. Before conducting a sensory evaluation of meat collected from the experiments, a test run was conducted. Appendix 1 details the results of this test run and confirms the validity of the technique developed.
3.4.2. Sample preparation and cooking

The samples were thawed for 24h at 4°C prior to cooking for presentation to panelists. All subcutaneous fat was removed. From each loin six (4 cm x 2.5 cm) samples were cut from the 12th rib (narrowest) end with muscle fibres running longitudinally. These six samples from each loin were numbered 1 to 6 with 1 being the sample cut closest to the narrow end working up to six. Samples were cooked on a well oiled (olive oil was used) silex grill for 2 minutes and ten seconds at 180°C according to methods devised by Williams et al. (2000). Samples were presented on plastic plates. Between each sample apple juice and brown bread was offered to cleanse the palate.

3.4.3. Sample assessment

Sensory evaluation of meat collected from the experiments described in chapter 4 and 6 was conducted using staff from the CSIRO. Panelists were asked to assess each sample for the following attributes (Table 3.1):
Table 3.1 Attributes and scale of assessment for eating quality traits

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strength of flavour</td>
<td>Very weak to very strong</td>
</tr>
<tr>
<td>Liking of flavour</td>
<td>Extremely dislike to extremely like</td>
</tr>
<tr>
<td>Strength of aroma</td>
<td>Very weak to very strong</td>
</tr>
<tr>
<td>Liking of aroma</td>
<td>Extremely dislike to extremely like</td>
</tr>
<tr>
<td>Tenderness</td>
<td>Very tough to very tender</td>
</tr>
<tr>
<td>Juiciness</td>
<td>Very dry to very juicy</td>
</tr>
<tr>
<td>Residual mouth feel</td>
<td>Very weak to very strong</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>Extremely dislike to extremely like</td>
</tr>
</tbody>
</table>

Panelists scored each attribute on a continuous 150 mm scale. The lines were measured and converted to a score out of 10. In addition, each panelist was invited to comment about each sample. Mean scores for attributes are presented.

Prior to start of the 6 runs a sample was offered to the panelists that was not experimental meat. This ‘dummy’ piece was used to assist the panellists to understand the assessment procedure. Panellists were not aware this was a test piece and they were asked to rate it in accordance with the other pieces. For the dummy run, beef rump was used. This dummy run was not included in any statistical analysis and removed from the data set.

3.4.4. Statistical analysis of sensory evaluation data

A General Linear Model (GLM) procedure using Systat (SPSS 2000) was used to analyse the sensory evaluation data. The GLM contained fixed effects for panelist, run, steak position (order
from the 12th rib), diet then tag and first order interactions of diet*steak number, diet*run and diet*panelist. Non-significant interactions (P>0.05) were sequentially removed from the model until the final model for each dependant was obtained. LSD values were used to compare treatment means.

3.5. **Pasture/feed analysis**

The drying procedure unless stated otherwise for feed and faeces was 65°C for 48 hours. The grinding procedure involved ground sub samples being passed through a 1 mm screen using a Tecator Cyclone© mill.

3.5.1. **Determination of dry matter of feed, pasture and faecal samples**

Previously ground feed and faecal samples (approximately 2 g) were weighed into preweighed and predried (overnight at 100°C) ceramic crucibles and dried to a constant weight for approximately 48 hours in a fan forced oven at 60°C. Samples were removed, cooled in a desiccator and then reweighed. Dry matter was calculated from:

\[ \text{Dry Matter} \% = \frac{\text{Dry Weight}}{\text{Wet Weight}} \times 100 \]

3.5.2. **Determination of organic matter and total ash**

Organic matter and total and soluble ash were determined on samples as described by AOAC (2005)
3.5.3. **Determination of nitrogen and mineral content of feed**

The mineral analysis was conducted by a commercial laboratory (Wesfarmers CSBP Ltd). Total N was determined by combustion using a Leco FP-428 N Analyser (Sweeney et al. 1987). Phosphorus (P), potassium (K), sulphur (S), Na, calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), boron (B) and molybdenum (Mb) were measured by Thermo Iris Intrepid Duo ICP-AES (McQuaker et al. 1979). Chloride (Cl) and nitrate were measured using an Lachat Flow Injection Analyser by the method of Zall et al. (1959). Feed was digested by the method of McQuaker et al. (1979) prior to the analysis.

3.5.4. **Determination of neutral detergent fibre and acid detergent fibre**

Neutral detergent fibre and acid detergent fibre were determined using methods detailed in AOAC (2005) Norman et al. (2004).

3.6. **Slaughter and dissection procedures**

Unless stated otherwise, the following procedures were undertaken for each slaughter.

Sheep were head stunned prior to exsanguination using a Thornton stunner handset model 1 (Cat no. T05202, Thornton Engineering group, Auckland New Zealand). Within 20 minutes of slaughter all sheep were electrically stimulated by a high voltage electrical stimulation tunnel for 60 seconds, peak voltage 1000V, 15Hz. Immediately following slaughter all carcasses were weighed to determine their hot carcass weight (HCW) and GR (total tissue depth over the 12th rib 110 mm from the midline). All carcasses were held in the same 5°C chiller for 24 hours. The decline in temperature and pH was determined in all carcasses every hour for 5 hours starting within an hour of slaughter. Temperature was determined using Cox recorders (Belmont, NC,
USA) and probes were inserted into the centre of the *m. longissimus thoracis et lumborum* (LL). pH measurements were taken using an Orion 250A pH meter (Cat. No. 0250A2, Orion Research Inc., Boston, Masset., USA) with a glass body, spear-tipped probe (Cat. No. 8163BN, Orion Research Inc., Boston, Masset., USA), coupled with a temperature probe. A fresh incision to remove subcutaneous fat was made over the muscle tissue, into which the pH probe was inserted. The pH meter was calibrated with standard solution and kept at room temperature (roughly 20°C).

Twenty-four hours after slaughter, ultimate pH, temperature and colour were determined. Colour was determined by first slicing across the fibres at the end of the LD adjacent to the first lumbar vertebrae. The cut area was left exposed for 30 minutes at room temperature. Meat colour was measured using a Minolta Model CR-200 chromameter set on the L*, a*, b* system (where L* measures relative lightness, a* relative redness and b* relative yellowness).

Dressing percentage calculated as:

Dressing percentage (%) = Hot carcass weight (kg) / Final liveweight prior to slaughter (kg) x 100

### 3.7. Estimating carcass composition

The evaluation of carcass composition is fundamental in the evaluation of protein and fat deposition for carcass grading in value based marketing. Chemical analysis is the more accurate technique for the measurement of body composition but is destructive, time consuming and expensive. Other indirect techniques that are quick and practical, such as single site backfat measurements, are fraught with inaccuracy when fat distribution is not constant between
animals Kelly et al. (1998). One reliable, convenient, accurate, cheaper and non-invasive method for determining both total and regional fat, lean tissue and bone mineral composition is dual energy x-ray absorptiometry (DXA), the principles of which have been discussed by Suster et al. (2003), Lukaski et al. (1999) and Kelly et al. (1998).

For this research project, carcass composition was measured using the DXA. DXA scans were performed using a Hologic QDR 4500 Fan Beam X-Ray Bone Densitometer (Hologic, Inc., Waltham, MA, USA). Calibration of the unit was performed with the Step Phantom (supplied by Hologic Inc.) once every week. The data from the step phantom calibration scan was automatically stored on the hard drive, and used during the whole body analysis, to ensure accurate fat/lean composition results. Spine calibration scans, using Spine Phantom (supplied by Hologic Inc.) were performed daily to minimise baseline drift and ensure accurate BMC measurement. Measurements made by DXA included total tissue mass (TTM), lean tissue mass (LTM), fat tissue mass (FTM) and bone mineral mass (BMM) in grams. The whole body scan mode was used for all animals and scan times were ~ 2 minutes depending on the length of the carcass. Regional analysis was not performed in the DXA software, but for whole carcass analysis, the entire DXA carcass image was placed in the left arm region of the regional grid.
Chapter 4

Effect of ingesting a saltbush and barley ration on the
carcass and eating quality of sheepmeat

4.1. Introduction

Forage halophytes such as saltbush are being widely used to revegetate saline land and can also provide a medium quality fodder source (Warren et al. 1990). There is widespread anecdotal evidence that sheep grazing on saltbush are leaner, tastier and juicer. Despite these claims and large areas of saltbush being planted on saline land there has been little research to assess the carcass and eating quality. Hopkins et al. (1999) investigated the effect of ingesting saltbush and oats, saltbush and lucerne hay or lucerne on carcass and eating quality of sheepmeat. The only significant finding was an increase in aroma strength from sheep grazed on the saltbush and lucerne treatment and no change to carcass quality. The study was conducted on saltbush grown on non-saline marginal land and its relevance to a saline land system is not known. Furthermore, a comparison with lucerne may be inconclusive as lucerne alone has been shown to affect the sensory properties of meat (Park et al. 1972a).

Previous studies indicate that there is potential to utilise saline land saltbush to produce leaner carcasses. Walker et al. (1971) and Kraidees et al. (1998) have both shown that the ingestion of a high salt load from the consumption of either halophytes or saline water reduces the fat
content of the carcass. Walker et al. (1971) also showed that the consumption of water high in salt resulted in less saturated fat in the fat depots and speculated that this was the result of less biohydrogenation of fat in the rumen due to the high water intake that occurs in sheep fed the high salt diets.

Chromium and betaine, compounds present in high quantities in saltbush may also affect carcass composition. Kitchalong et al. (1995) and Gardner et al. (1998) demonstrated that supplementary chromium decreased subcutaneous fat depth in young sheep. Chromium potentiates and augments insulin action to discourage fat deposition but also increases protein synthesis and reduces protein degradation. A reduction in fat thickness and lipid content of intramuscular fat has also been shown in lambs fed a diet enriched in betaine (Fernandez et al. 1998; Fernandez et al. 2000). The mechanisms of betaine action on fat deposition are believed to be mediated through an improvement in the efficiency of methionine use for protein synthesis and the facilitation of choline activity (Fernandez et al. 1998).

The use of saltbush with a suitable energy supply such as barley may be a strategy to produce acceptable carcass weights. Chriyaa et al. (1997) and Franklin-McEvoy (2002) both showed that a 60:40 saltbush and barley combination allowed over 60 g/d liveweight gain.

Ingesting a saltbush based ration prior to slaughter may also reduce carcass weight loss at slaughter. Failure to drink whilst in lairage has been suggested as the reason for loss in carcass weight and the consequent commercial costs (Jacob et al. 2005b). Sheep consuming a high salt diet prior to transport may maintain a high water requirement and drink more in lairage.
Finally, there is evidence that sheep ingesting high salt rations will have higher clean wool growth and fibre diameter (Hemsley 1975). The consumption of a high salt diet increases the rate of passage and therefore the amount of protein presented to the small intestine for absorption (Hemsley et al. 1975). Wool growth is responsive to an increase in protein absorption in the small intestine.

The aim of this chapter was to investigate the effects of ingesting saltbush from saline land on carcass and eating quality. Saltbush was hand collected and fed to sheep in an animal house. A control diet was used for comparison but also a control plus salt diet which contained similar levels and components of salt in the saltbush. The control plus salt diet was used to determine if it was the salts or the saltbush having an effect on carcass and eating quality.

The hypotheses for this chapter are:

1. That ingesting a saltbush and barley ration will result in a leaner carcass and change fatty acid composition compared with sheep fed a low salt control diet
2. That ingesting a saltbush and barley ration will improve sheep meat eating quality compared with sheep fed a low salt control diet
3. That ingesting a saltbush and barley ration will enable suitable liveweight gains to achieve acceptable carcass weights compared with sheep fed a low salt control diet
4. That ingesting a saltbush and barley ration will increase water consumption during simulated lairage and increase carcass weight as a proportion of final liveweight compared with sheep fed a low salt control diet
5. That ingesting a saltbush and barley ration will increase clean wool growth and fibre diameter compared with sheep fed a low salt control diet
4.2. Materials and Methods

4.2.1. Animal management

Thirty nine, 14-month old Merino hogget wethers (initial liveweight $38.8 \pm 0.28$ kg) were individually penned in an animal house at the CSIRO in Perth for 13 weeks. Each sheep was weighed weekly prior to feeding and liveweight gain calculated using methods detailed in chapter 3.

4.2.2. Experimental diets

For the first 19 days (days –19 to –1), all sheep were fed a standard ration (20% lupins, 77% oaten hay, 3% Siromin (refer to White et al. 1992 for comprehensive mineral breakdown)). For the following 10 weeks (days 0-69) the sheep were randomly allocated into 3 groups after stratification for equal liveweight and fed 1 of 3 diets:

1. Old man saltbush (*Atriplex nummularia*) (60%) plus barley (40%) (S+B)
2. Lupin (33%), barley (25%) and oaten hay (42%) (C)
3. Lupins (30%), barley (22%), oaten hay (38%), NaCl (5%), KCl (2%), MgCO$_3$ (1%) and CaSO$_4$ (2%) (C+S)

The saltbush was collected by hand stripping from a site near Tammin, 200 km east of Perth, Western Australia and dried immediately on return to Perth for 3 days at 45°C. The material collected was leaves and small stems (<2mm diameter) only. Six sampling periods were conducted between May and July 2001. The barley and lupin components were both in whole grain form. Barley was the chosen form of grain over other grains such as wheat as it significantly less likely to cause ruminal acidosis (McDonald et al. 1995).
The ingredients in each of the 3 diets were mixed together into a loose ration. Mixing was conducted using a commercial feed mixer (PressMatic Feed Mixer, Parkes Industries, USA). The hay was hammer milled through a 3.8 cm sieve and the saltbush was mulched using a commercial mulcher (Caravaggi BIO 200 chipper/shredder, Parklands Trading Australia) prior to feed mixing.

4.2.2.1. **Diet formulation and feeding allowances**

Using estimated and determined values for metabolisable energy and protein for the components of the S+B and C diet, the C diet was formulated to be of similar nutritional value to the S+B diet.

Prior to the start of the experiment, samples of each diet were analysed for digestibility and nitrogen content. The energy content was calculated from the digestibility of the ration (Standing Committee on Agriculture 1990).

The protein content was determined by first determining the nitrogen content using methods described in section 3.5.3. The crude protein (CP) content was described as CP=nitrogen x 6.25.

The S+B diet was also analysed for mineral content prior to the commencement of the experiment using methods detailed in chapter 3. The C+S diet was then formulated to replicate the mineral content of the S+B diet using NaCl, KCl, MgCO₃ and CaSO₄ (Redox Chemicals, Perth, Australia).
Using the values for metabolisable energy and protein of each diet, Grazfeed® was used to determine the level of feed intake required for each of the 3 diets to allow 60 g/d of liveweight gain. Average amounts of feed offered per group are shown in Table 4.1.

Table 4.1 Average weights of feed offered for days 0-70.

<table>
<thead>
<tr>
<th></th>
<th>S+B</th>
<th>C+S</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh matter offered (kg/d) (A)</td>
<td>1.25</td>
<td>1.19</td>
<td>1.03$^a$</td>
</tr>
<tr>
<td>Feed dry matter content (% fresh weight) (B)</td>
<td>93</td>
<td>94.1</td>
<td>93.2</td>
</tr>
<tr>
<td>Dry matter offered (kg/d) (A*B)</td>
<td>1.16</td>
<td>1.11</td>
<td>0.96</td>
</tr>
<tr>
<td>Feed organic matter content (% dry weight) (C)</td>
<td>86.8</td>
<td>91.2</td>
<td>94.7</td>
</tr>
<tr>
<td>Organic matter offered (kg/d) (C*(A*B))</td>
<td>1.00</td>
<td>1.01</td>
<td>0.90</td>
</tr>
</tbody>
</table>

$^a$The calculations for the fresh matter required by the control group were miscalculated and for the first 3 weeks the control group received 0.89 kg/head.d. This was changed to the correct amount at the end of week 3 to 1.1 kg.

4.2.2.2. Nutritive value and mineral content

The nutritive value and mineral composition of the S+B and C diets was analysed using methods detailed in chapter 3 (Table 4.2). Chromium analysis was done by a separate commercial laboratory (CSIRO Analytical Services). Samples were oven dried, digested, diluted and internal standard added for analysis according to standard operating protocols of ICP-MS (X series ICP-MS, Thermo Electron Cooperation, US).
Table 4.2 Nutritive value and mineral content of experimental diets (% dry matter)

<table>
<thead>
<tr>
<th></th>
<th>S+B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein (%)</td>
<td>11.7</td>
<td>12.1</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>86.8</td>
<td>94.7</td>
</tr>
<tr>
<td>ADF (%)</td>
<td>23.3</td>
<td>27.9</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>37.4</td>
<td>44.3</td>
</tr>
<tr>
<td>IVD (%)</td>
<td>73.7</td>
<td>72.9</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>3.01</td>
<td>0.67</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>1.8</td>
<td>0.83</td>
</tr>
<tr>
<td>Sulfur (%)</td>
<td>0.30</td>
<td>0.16</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.50</td>
<td>0.19</td>
</tr>
<tr>
<td>Copper (mg/kg DM)</td>
<td>7.1</td>
<td>6.4</td>
</tr>
<tr>
<td>Zinc (mg/kg DM)</td>
<td>27.7</td>
<td>18.0</td>
</tr>
<tr>
<td>Iron (mg/kg DM)</td>
<td>176</td>
<td>117</td>
</tr>
<tr>
<td>Chromium (mg/kg DM)</td>
<td>4.08</td>
<td>2.03</td>
</tr>
</tbody>
</table>

4.2.2.3. **Feeding and feed intake**

Feeding was once daily in the morning at approximately 9.00am. Feed intake was measured on individual sheep for days 0 to 69. Feed residues were weighed daily and bulked for that week. A subsample of fresh feed was collected daily and bulked for that week. Both residues and weekly bulked feed samples were dried at 75°C for 3 days to allow calculation of dry matter, ash and organic matter intake for each sheep. All residues were bulked weekly. The sheep were allowed free access to fresh tap water from nipple drinkers.
4.2.3. Sampling procedures

4.2.3.1. Blood and muscle sampling

A 40 mL of blood was collected from each sheep by jugular venipuncture on days 15, 38 and 59 into (1) heparinised and centrifuged tubes (32 mL), (2) EDTA and (3) centrifuged (5 mL) and heparinised and not centrifuged (3 mL) tubes. Samples for urea, creatinine, lactate, albumin, potassium, leptin, thyroxin and thriiodothyronine were collected in heparinised tubes whereas samples for insulin like growth factor (IGF) analysis were collected in EDTA tubes. All tubes were placed on ice immediately. The heparinised and EDTA tubes were centrifuged at 3000 rpm within 30 minutes of collection, plasma was decanted and stored at –20°C until analysis. Glucose was measured immediately upon collection of blood using a Mediscence Glucose Meter®. Packed cell analyses were performed on the heparinised and unspun blood within 2 hours of collection.

Blood samples (10 mL) were also collected on day 59 through a jugular catheter into heparinised tubes. Samples were taken every 20 mins for 6 hours and 20 mins. All tubes were placed on ice and centrifuged at 3000 rpm within 30 mins of collection, plasma was decanted and stored at –20°C until analysis. Samples taken every 20 minutes were assayed for growth hormone (GH). Samples taken at hourly intervals for the first 6 hours were assayed for insulin and cortisol.

Muscle biopsies using the method of Gardner et al. (1998) were collected on days 10, 30 and 56 for glycogen analysis.
4.2.3.2. Wool quality assessment using wool patches

Wool patches were used to measure the wool growth of the sheep. A wool patch of 10x10cm (approximately 100 cm²) was collected from the midside of the animal (Langlands et al. 1968). Wool was clipped using small animal clippers (Oster—Model A500, Milwaukee, USA) with a fine cutting blade (Oster A5, size 40). A clear plastic bag was placed over patch and an outline of the area shaved was drawn using a permanent marker. The area of the patch was later determined to assist in the correction for differences in patch area. Wool samples were taken from all experimental sheep on days -13, 10 and 65.

4.2.4. Analyses

4.2.4.1. Growth hormone

Analysis for growth hormone was by the method described by Adams et al. (1996). All samples were measured in a single assay. The intra-assay coefficient of variation (CV) for the 4 pools averaging 3.3, 5.2, 9.3 and 15.6 ng/mL were 0.4, 0.32, 0.5 and 0.9 respectively. The specific binding (Bo) was 34%, the non-specific binding (NSB) was 6.5%, the minimal detectable concentration was 0.7 ng/mL and the 50% maximal displacement on the standard curve (ED50) was 5.3 ng/mL.

4.2.4.2. Insulin

Insulin concentrations in the plasma were analysed using the methods described by Adams et al. (1996). All samples were measured in a single assay. The intra-assay CV for the 3 pools averaging 0.1, 0.5, 1.4 and 3.5 ng/mL were 0.03, 0.04, 0.07 and 0.16 respectively. The Bo was
29%, the NSB was 1.9%, the minimal detectable concentration was 0.09 ng/mL and the 50% maximal displacement on the standard curve (ED50) was 0.83 ng/mL.

4.2.4.3. Insulin-like growth factor (IGF)

IGF was analysed using the methods described by Breier et al. (1991) and Adams et al. (1996). All samples were measured in a single assay. The intra-assay CV for the 3 pools averaging 0.63, 0.96 and 3.8 ng/mL were 0.14, 0.01 and 0.42 respectively. The Bo was 47%, the NSB was 3.6%, the minimal detectable concentration was 0.08 ng/mL and the 50% maximal displacement on the standard curve (ED50) was 3.84 ng/mL.

4.2.4.4. Cortisol

Plasma cortisol was analysed using the method described by Atkinson and Adams (1988). All samples were measured in a single assay. The intra-assay CV for the three pools averaging 1.65, 2.7 and 4.0 ng/mL were 0.34, 0.52 and 0.8 respectively. The Bo was 33%, the NSB was 1.3%, the minimal detectable concentration was 0.08 ng/mL and the 50% maximal displacement on the standard curve (ED50) was 0.5 ng/mL.

4.2.4.5. Leptin

Plasma leptin concentrations were analysed using the method described by Blache et al. (2000).

4.2.4.6. Thyroxin (T4) and Triiodothyronine (T3)

Plasma thyroxin and thriiodothyronine was analysed using the methods of Zhang et al. (2005).
4.2.4.7. **Urea, creatinine, lactate, sodium and potassium**

Plasma urea, creatinine, lactate, sodium and potassium were analysed using methods detailed in chapter 3 (section 3.3.1 and 3.3.2).

4.2.4.8. **Packed cell volume**

Packed cell volume of whole blood was determined using the micro-hematocrit centrifuge method of Anon (2000).

4.2.4.9. **Fatty acid composition of subcutaneous and intramuscular fat**

The fatty acid composition of the subcutaneous and intramuscular fat was prepared and analysed using the methods of Folch *et al.* (1957). The results are expressed as the % in the total. There were some fatty acids that could not be identified.

4.2.4.10. **Muscle glycogen**

Muscle glycogen concentration was analysed using the method of Chan and Exton (1976) modified by removing the filter paper step (Gardner *et al.* 1998).

4.2.4.11. **Wool analysis**

4.2.4.11.1. **Yield**

Sub samples (between 1.5g and 2.0g) of each wool patch were weighed and conditioned as described by Masters *et al.* (1998). The wool samples were weighed again and then placed individually in small nylon bags and washed in a Bendix (Model No. W1062B) washing
machine modified to automatically deliver the following sequence: 1) 5 minutes washing with agitation in approximately 20 L of detergent (14 mL of wool scouring compound [Dolmar Chemicals, Canning Vale, Western Australia] plus 20L of water) at 60°C; 2) 2 warm water (50°C) rinsing cycles of 2 minutes each; 3) a 3 minute spin cycle (Masters et al. 1998). The samples were weighed after drying at 105°C. Yield was calculated as:

\[
\text{Yield (\%)} = \left( \frac{\text{Weight of wool clean}}{\text{Weight of wool greasy}} \right) \times 100
\]

4.2.4.11.2. **Clean wool growth**

The sample area was calculated using a leaf area index meter and the total weight of each greasy wool patch sample was then weighed. The clean yield from each sample (see above) was then used to calculate the clean wool growth (CWG) in grams of clean wool per 100 cm² per day. CWG was calculated using the wool grown between patches taken at day 10 and 65. The wool was removed for the sheep for the first 10 d of the experiment because during this week the sheep were not on 100% of their allocated diets and this adjustment period may influence the wool growth. The wool collected from the first wool patch at day -13 was used as a covariate for the third patch at day 65.

4.2.4.11.3. **Wax and suint extraction**

Sub samples of approximately 1.5 g of the wool samples taken at 65 d were conditioned and weighed out for each patch sample as described previously. Wax and suint were extracted using the methods of Hemsley and Marshall (1984).
4.2.4.11.4. Suint analysis

The sodium and potassium concentration of the suint samples from the wool samples taken at 65 days was determined using the standard operating methods for the atomic absorption spectrophotometer (SpectrAA-30/40, Varian Techtron Pty Ltd). The concentrations were converted to a sample percentage. This was used to calculate the sodium and potassium concentration in g/100 g per clean fibre.

4.2.4.11.5. Diameter testing

The wool fibre diameters were measured using the OFDA 2000 (OFDA, (Baxter 2001)). All samples from all collection days were firstly conditioned and washed as described above. Samples were then prepared by cutting fibres into 2 mm lengths and spreading them onto a 70 mm glass plate. Counts of over 2000 were considered acceptable. The grease correction factor, GCF, was set to zero. Fibre diameter was determined from wool from the patch taken on day – 13 and 65. The wool collected from the first wool patch at day -13 was used as a covariate for the third patch at day 65.

4.2.4.11.6. Calculation of clean wool growth per animal per day

Clean wool growth per animal per day (CWGA) was calculated using the following steps:

4. Clean wool growth was converted from g/100 cm².d into g/m².d

5. The surface area of wool producing skin was calculated using equation by Lines et al. (1931) using a average body weight of animal between days 16-55.

   \[ \text{Surface area (m}^2\text{)} = 0.0909 \times \text{Body weight (kg}^{2/3}\text{)} \]
7. The surface area of the animal was multiplied by clean wool growth in g/m².d to give clean wool growth per animal (g/head.d) (CWGA)

4.2.4.11.7. Calculation of clean wool growth per animal per liveweight gain and organic matter intake

To calculate CWGA per unit of liveweight gain and per unit of organic matter intake the following equations were used:

- CWGA per liveweight gain = CWGA / average liveweight gain
- CWGA per organic matter intake = CWGA / average organic matter intake

The average liveweight gain and organic matter intake used were from days 16-55.

4.2.5. Total body water determination

The determination of body water content was conducted on day 59. Blood (10 mL) was collected from each sheep prior to an injection into muscle of backleg with approximately 5 g of deuterium oxide (²H₂O, 99.9% atom %. Sigma-Aldrich, USA) at 8.00 am. All syringes were weighed before and after injection to determine the exact amount injected. The sheep were bled a second time after equilibration of the deuterium oxide with the body water pool (approximately 6 hours). Sample preparation for GCMS analysis followed the method described by Liu (2002). The analyses were carried out on a HP 6890/5973 GCMS system. Samples were analysed at 70 eV under chemical ionization with methane as the reagent gas. The selected ions monitored were m/z 61 and 63. The calibration curve was obtained by a linear regression analysis of theoretical ²H-enrichment in standards (ranged from 50 to 250 ppm) against measured m/z 63 : 61 ratios. Samples were injected in duplicates. The deuterium oxide enrichment was measured in 10 of the pre-injection samples (randomly selected) to provide a
mean background and all of the post injection blood samples to determine deuterated water space. Deuterated water space was converted to total body water using the equations from Searle (1970).

While the methods for determination of body water were similar to those previously published, the preparation of the sheep was modified. Total body water is usually determined as a first step in the prediction of body composition of the sheep (lean, fat and ash). In this experiment, the aim was to determine the additional amount of water retained by sheep fed high salt diets. For this reason, fasting the sheep overnight would have given an inaccurate indication of the proportion of liveweight attributable to water retention in grazing sheep. The sheep were allowed access to feed and water up to the time of deuterium oxide injection but not during the period of equilibration. The sheep were fed following the second bleed.

4.2.6. Digestibility

From days 21 to 27, 7 randomly chosen sheep per treatment group were placed into individual metabolism crates and fitted with faecal collection harnesses. Faeces was collected from days 23-27. Faeces were collected daily during this period, weighed and approximately 10% subsampled and dried in an oven at 65°C overnight. The dried faeces were bulked over the 4 day period for each sheep. Any feed residues were weighed and bulked over the 4 days individually for each sheep. A subsample of each of the diets was also collected daily and bulked. Water intake was measured for individual sheep from day 24-27. Bulked subsamples of feed, feed residues and faeces (approximately 1 kg of each) were subsequently ground and dry matter, organic matter and sodium content determined using the methods detailed in chapter 3 (section 3.5). Dry matter digestibility (DMD), organic matter digestibility (OMD), digestible organic
matter intake (DOMI), digestible organic matter in the dry matter (DOMD) and metabolisable energy (ME) were calculated by standard methodology (Standing Committee on Agriculture 1990). Calculations used were:

\[
\text{DMD} \, (\%) = \frac{(\text{DMI} - \text{faeces DM})}{\text{DMI}} \times 100
\]

\[
\text{OMD} \, (\%) = \frac{(\text{OMI} - \text{faeces OM})}{\text{OMI}} \times 100
\]

\[
\text{DOMI} \, (\text{kg/d}) = \text{OMD} \times \text{OMI}
\]

\[
\text{DOMD} \, (\%) = \frac{(\text{OMI} - \text{faeces OM})}{\text{feed DM}} \times 100
\]

\[
\text{Metabolisable energy} \, (\text{MJ/kg DM}) = (0.18 \times \text{DOMD}\%) - 1.8
\]

4.2.7. Lairage simulation study

This study was conducted on days 45-46. The animals were not fed on these days but allowed *ad libitum* access to water in buckets in each pen. Water intake was measured for all sheep over the 48 hour period. Following the 48 hour period, the sheep were fed and allowed access to water from the nipple drinkers located in the individual pens.

4.2.8. Slaughter and dissection procedures

At 11 am on day 69, all sheep were weighed and then loaded onto a truck and transported to Hillside Abattoir in Narrogin 200 km from Perth. Here they were held in the lairage yards for 24 hours before being slaughtered at 11 am on day 71. Whilst in lairage all sheep had access to water in a trough but no access to feed.

The slaughter and dissection procedures are detailed in chapter 3 (section 3.6). Hot carcass weight, GR depth, decline in temperature and pH, ultimate pH and colour were measured. Within an hour of slaughter samples of both *m. semimembranosis* (SM) and *m. semitendinosis*
(ST) muscles were collected for glycogen analysis. These samples were snap frozen in liquid nitrogen, after having all fat tissue removed, and stored at -40°C. A 100 g sample of the subcutaneous fat from over the top of the SM and ST muscles was taken and also frozen in liquid nitrogen. The samples were analysed for fatty acids. The *m. longissimus thoracis et lumborum* (LL) muscle was removed 24 hours after slaughter and immediately vacuum packed. This sample was then kept in a 2°C chiller for 5 days before being frozen at -20°C prior to sensory analysis. A 250 g sample of the LL was also removed for fatty acid analysis and immediately frozen at -20°C. A 2 g sample of the LL was also taken at slaughter for dry matter determination using methods detailed in chapter 3 (section 3.6).

### 4.2.9. Carcass composition

Half of each carcass was cut into thirds and scanned with a DXA for estimation of the relative fat, lean, and bone content of the carcass as described in chapter 3. (section 3.7)

### 4.2.10. Sensory analysis

The sensory analysis in this experiment was assessed using consumer taste panels and the *m. longissimus thoracis et. lumborum* (LL). Consumer taste panel protocols were developed for this project and the methodology and the results from a test run of the technique are detailed in chapter 3 and appendix 1 (section 3.4).

### 4.2.11. Statistical analyses

All statistical analysis in this chapter was done using Systat (SPSS Version 9.01). The statistical analysis of the data from the sensory evaluation is detailed in chapter 3 (section 3.4).
Analysis of variance (ANOVA) was used to examine the effect of treatment on pre-slaughter liveweight, liveweight gain, hot carcass weight, GR depth, ultimate pH, colour, fat, lean and bone content, fatty acids and muscle dry matter content, the effect of treatment on clean wool growth, fibre diameter, wax and suint content, clean wool growth per liveweight gain and clean wool growth per organic matter intake and the effect of treatment on organic matter and dry matter digestibility, digestible organic matter intake, digestible organic matter in the dry matter, daily metabolisable energy intake and sodium and water intake.

The results from the lairage study was analysed using both a repeated measures analysis as well as a comparison of individual time points.

For analysis of the insulin and growth hormone results repeated measures over the sampling period was conducted as well as comparison of individual sampling times using ANOVA. For IGF a repeated measures analysis over the 3 sampling times was conducted as well as a comparison of individual time points.
4.3. Results

Results are only reported here for the S+B and C groups. There are 2 reasons for this:

1. As this was a loose mix diet, satisfactory mixing of the high levels of salt was difficult. During mixing much of the salt settled to the bottom on the mixture and the diet was not homogeneous.

2. The loose mix created further difficulties during the experiment with sheep showing an ability to selectively leave salt in the feed bin.

4.3.1. Liveweight and liveweight gain

There was no significant difference in liveweight between the two treatment groups at the start of the experiment (Figure 4.1). All groups increased in liveweight over the experimental period and no difference in liveweight gain (both 62 ± 0.6 g/d) existed between the treatments (P>0.05). There was a significant difference in liveweight on day 69.

Figure 4.1 Liveweight changes of sheep fed S+B or C treatments (Mean ± standard error)
4.3.2. Digestibility

Digestibility and intake data are shown in Table 4.3. The sheep fed the C diet had a higher digestible organic matter intake than sheep fed the S+B diet. The metabolisable energy intake per day was significantly lower in the S+B fed sheep compared to the C diets. The daily sodium and water intake and sodium excretion was significantly different between treatment groups (Table 4.3).

Table 4.3 Digestibility of feed sources calculated during 4 day digestibility study, feed intakes over 10 week period, daily metabolisable energy content of S+B or C diets and the sodium and water intake (Mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>S+B</th>
<th>C</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter intake (DMI) (kg/d)</td>
<td>1.11 ± 0.01</td>
<td>0.94 ± 0.02</td>
<td>0.002</td>
</tr>
<tr>
<td>Organic matter intake (OMI) (kg/d)</td>
<td>0.955 ± 0.006</td>
<td>0.896 ± 0.006</td>
<td>0.001</td>
</tr>
<tr>
<td>Dry matter digestibility (DMD) (%) A</td>
<td>69.5 ± 0.94</td>
<td>76.0 ± 1.17</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Organic matter digestibility (OMD) (%) A</td>
<td>67.1 ± 1.86</td>
<td>77.8 ± 2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Digestible organic matter intake (DOMI) (kg/d) A</td>
<td>0.64 ± 0.02</td>
<td>0.69 ± 0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Digestible organic matter in the DM (DOMD) (%) A</td>
<td>59.9 ± 2.4</td>
<td>66.2 ± 2.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ME content of feed (MJ/kg DM) A</td>
<td>8.9 ± 0.13</td>
<td>10.1 ± 0.15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ME per day (MJ/d) B</td>
<td>9.97 ± 0.2</td>
<td>10.7 ± 0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sodium intake (g/d) C</td>
<td>33.4 ± 1.6</td>
<td>6.3 ± 0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Water intake (L/d)</td>
<td>6.36 ± 0.8</td>
<td>2.66 ± 0.5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

A Calculated using equation in section 4.2.6.
4.3.3. Carcass quality

4.3.3.1. Carcass characteristics

There was no significant difference between the treatments for HCW, GR depth or dressing percentage (P>0.05) (Table 4.4).

Table 4.4 Carcass characteristics of sheep fed S+B or C diet prior to slaughter (Mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>S+B</th>
<th>C</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot carcass weight (kg)</td>
<td>17.2 ± 0.3</td>
<td>17.9 ± 0.3</td>
<td>0.07</td>
</tr>
<tr>
<td>GR fat depth (mm)</td>
<td>3.92 ± 0.6</td>
<td>4.9 ± 0.46</td>
<td>0.01</td>
</tr>
<tr>
<td>Dressing percentage (%)</td>
<td>41.1 ± 0.44</td>
<td>41.5 ± 0.39</td>
<td>0.76</td>
</tr>
</tbody>
</table>

4.3.3.2. Carcass composition

Sheep fed the S+B diet had a significantly lower fat content than the C fed sheep (P=0.055), significantly higher bone content and no difference in lean content (Table 4.5). There was a trend for the S+B group to have higher total body water content compared to the sheep fed the C diet.
Table 4.5 Fat, lean and bone content of carcasses fed S+B or C diets (Mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>S+B</th>
<th>C</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat content (% in carcass)</td>
<td>15.4 ± 0.005</td>
<td>16.6 ± 0.004</td>
<td>0.055</td>
</tr>
<tr>
<td>Lean content (% in carcass)</td>
<td>81.3 ± 0.4</td>
<td>80.7 ± 0.4</td>
<td>0.28</td>
</tr>
<tr>
<td>Bone content (% in carcass)</td>
<td>3.1 ± 0.001</td>
<td>2.8 ± 0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>Total body water content (kg)</td>
<td>31.9 ± 0.9</td>
<td>29.3 ± 0.9</td>
<td>0.06</td>
</tr>
</tbody>
</table>

4.3.3.3. Meat Biochemistry

There was no significant effect of diet on colour, glycogen content at slaughter and ultimate pH (Table 4.6) (P>0.05).

Table 4.6 Colour and ultimate pH 24 hours post slaughter and glycogen content at slaughter for sheep fed S+B or C diet prior to slaughter (Mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>S+B</th>
<th>C</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour (L* value)</td>
<td>37.8 ± 0.48</td>
<td>37.0 ± 0.65</td>
<td>0.62</td>
</tr>
<tr>
<td>Colour (a* value)</td>
<td>21.5 ± 0.6</td>
<td>21.9 ± 0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Colour (b* Value)</td>
<td>9.5 ± 0.42</td>
<td>10.0 ± 0.42</td>
<td>0.16</td>
</tr>
<tr>
<td>Glycogen content SM (g/100g)</td>
<td>1.32 ± 0.09</td>
<td>1.43 ± 0.08</td>
<td>0.5</td>
</tr>
<tr>
<td>Glycogen content ST(g/100g)</td>
<td>0.98 ± 0.06</td>
<td>0.95 ± 0.05</td>
<td>0.35</td>
</tr>
<tr>
<td>Ultimate pH SM</td>
<td>5.61 ± 0.03</td>
<td>5.55 ± 0.03</td>
<td>0.42</td>
</tr>
<tr>
<td>Ultimate pH ST</td>
<td>5.74 ± 0.06</td>
<td>5.70 ± 0.06</td>
<td>0.21</td>
</tr>
<tr>
<td>Ultimate pH LD</td>
<td>5.62 ± 0.06</td>
<td>5.60 ± 0.03</td>
<td>0.83</td>
</tr>
<tr>
<td>Dry matter LD (%)</td>
<td>26.8 ± 1.3</td>
<td>28.4 ± 1.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>
4.3.4. Sensory evaluation

Treatment had no significant effect on any of the eating quality attributes (Table 4.7).

Table 4.7 Sensory evaluation of meat from sheep fed a S+B or C diet prior to slaughter. (Mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>S+B</th>
<th>C</th>
<th>P value</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour strength</td>
<td>5.12 ± 0.25</td>
<td>4.63 ± 0.26</td>
<td>&gt;0.1</td>
<td>0=weak, 10=strong</td>
</tr>
<tr>
<td>Liking of odour</td>
<td>6.02 ± 0.19</td>
<td>5.98 ± 0.20</td>
<td>&gt;0.1</td>
<td>0=dislike, 10=like</td>
</tr>
<tr>
<td>Tenderness</td>
<td>6.44 ± 0.25</td>
<td>6.37 ± 0.27</td>
<td>&gt;0.1</td>
<td>0=tough, 10=tender</td>
</tr>
<tr>
<td>Juiciness</td>
<td>5.76 ± 0.25</td>
<td>5.67 ± 0.23</td>
<td>&gt;0.1</td>
<td>0=dry, 10=juicy</td>
</tr>
<tr>
<td>Flavour strength</td>
<td>5.35 ± 0.23</td>
<td>5.24 ± 0.22</td>
<td>&gt;0.1</td>
<td>0=weak, 10=strong</td>
</tr>
<tr>
<td>Liking of flavour</td>
<td>6.35 ± 0.23</td>
<td>6.28 ± 0.23</td>
<td>&gt;0.1</td>
<td>0=dislike, 10=like</td>
</tr>
<tr>
<td>Residual fatty mouth feed</td>
<td>4.29 ± 0.25</td>
<td>4.62 ± 0.24</td>
<td>&gt;0.1</td>
<td>0=weak, 10=strong</td>
</tr>
<tr>
<td>Overall acceptance</td>
<td>6.43 ± 0.23</td>
<td>6.2 ± 0.26</td>
<td>&gt;0.1</td>
<td>0=dislike, 10=like</td>
</tr>
</tbody>
</table>

4.3.5. Lairage study

There was an effect of treatment on the consumption of water over a 48 hour period of feed deprivation (P<0.05) (Figure 4.2). The total water consumption over the 48 hour period was 7.00 ± 0.31 L/d for the S+B treatment and 5.61 ± 0.3 L/d for the C treatment. There was an effect of time on water intake (P<0.05) with both treatments decreasing with an increase in period of feed withdrawal. There were significant differences between the treatments up till hour 4 but by hour 24 no differences in the water consumption rates existed.
4.3.6. Fatty acids

The individual fatty acid composition of the intramuscular and subcutaneous fat deposits are described in table 4.8. Differences existed between the treatments for individual fatty acids. There was a significant difference in the sum of unsaturated fatty acids ($P<0.004$) between the treatments in both fat depots. The S+B sheep had a significantly lower overall unsaturated fatty acid content compared to the C treatment. No differences in the saturated fat content were observed between treatments. The percentage of unidentified fatty acids was significantly higher in the S+B fat compared to the C treatments ($P<0.001$) in both fat depots.
Table 4.8 Individual fatty acid composition of the intramuscular and subcutaneous fat deposits in sheep fed S+B or C diets (Mean ± standard error)

<table>
<thead>
<tr>
<th>Fatty acid (%)</th>
<th>Intramuscular fat</th>
<th>Subcutaneous fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S+B</td>
<td>C</td>
</tr>
<tr>
<td>C 6:0</td>
<td>3.21 ± 0.33</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>C 10:0</td>
<td>0.3 ± 0.03</td>
<td>0.28 ± 0.02</td>
</tr>
<tr>
<td>C 12:0</td>
<td>0.3 ± 0.03</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>C 13:0</td>
<td>0.31 ± 0.04</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>C 14:0</td>
<td>1.9 ± 0.08</td>
<td>1.86 ± 0.07</td>
</tr>
<tr>
<td>C 15:0</td>
<td>0.52 ± 0.02</td>
<td>0.43 ± 0.02</td>
</tr>
<tr>
<td>C 16:0</td>
<td>19.3 ± 0.48</td>
<td>20.0 ± 0.46</td>
</tr>
<tr>
<td>C 17:0</td>
<td>1.33 ± 0.07</td>
<td>1.2 ± 0.02</td>
</tr>
<tr>
<td>C 18:0</td>
<td>17.5 ± 0.44</td>
<td>17.9 ± 0.43</td>
</tr>
<tr>
<td>C 20:0</td>
<td>0.87 ± 0.06</td>
<td>0.73 ± 0.06</td>
</tr>
<tr>
<td>C 24:0</td>
<td>0.40 ± 0.01</td>
<td>0.30 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Value 1 ± Error</td>
<td>Value 2 ± Error</td>
</tr>
<tr>
<td>--------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>C 14:1</td>
<td>0.33 ± 0.03</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.94 ± 0.05</td>
<td>0.98 ± 0.04</td>
</tr>
<tr>
<td>C 18:1 trans-9</td>
<td>0.46 ± 0.02</td>
<td>0.44 ± 0.01</td>
</tr>
<tr>
<td>C 18:1, cis-7</td>
<td>1.72 ± 0.09</td>
<td>1.4 ± 0.09</td>
</tr>
<tr>
<td>C 18:1, cis-9</td>
<td>28.2 ± 0.71</td>
<td>31.8 ± 0.71</td>
</tr>
<tr>
<td>C 18:1 total</td>
<td>30.4 ± 0.93</td>
<td>33.7 ± 0.64</td>
</tr>
<tr>
<td>C18:2</td>
<td>4.45 ± 0.21</td>
<td>3.85 ± 0.21</td>
</tr>
<tr>
<td>C18:3</td>
<td>1.00 ± 0.06</td>
<td>0.78 ± 0.06</td>
</tr>
<tr>
<td>C 20:1</td>
<td>0.34 ± 0.08</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>C 20:2</td>
<td>0.56 ± 0.11</td>
<td>0.42 ± 0.04</td>
</tr>
<tr>
<td>C 20:5</td>
<td>0.54 ± 0.11</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>C 24:1</td>
<td>0.63 ± 0.19</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>C 22:5</td>
<td>0.67 ± 0.09</td>
<td>0.63 ± 0.06</td>
</tr>
<tr>
<td>C 22:6</td>
<td>0.54 ± 0.14</td>
<td>0.45 ± 0.06</td>
</tr>
<tr>
<td>TOTAL (%)</td>
<td>75.8 ± 0.12</td>
<td>79.3 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Unidentified (%)</td>
<td>10.5 ± 0.7</td>
<td>8.5 ± 0.6</td>
</tr>
<tr>
<td>Unsaturates (%)</td>
<td>41.6 ± 0.52</td>
<td>43.5 ± 0.49</td>
</tr>
<tr>
<td>Saturates (%)</td>
<td>45.5 ± 0.6</td>
<td>45.8 ± 0.6</td>
</tr>
</tbody>
</table>
4.3.7. Hormones and Metabolites

4.3.7.1. Insulin

Using repeated measures analysis there was a significant effect of diet on plasma insulin concentration (P<0.05) (Figure 4.3). The sheep fed the S+B diet had significantly lower plasma insulin concentration than sheep fed the C diet (P<0.02). The average insulin concentration for the sheep fed the S+B diet was $23.2 \pm 1.4$ and $37.1 \pm 2.0$ ng/mL for the C group. Time of sampling had no effect on insulin concentration (P>0.5). At the individual time points, the S+B sheep had significantly lower insulin in the plasma than the C treatment at 0 (P<0.05), 180 (P<0.02), 240 (P<0.002) and 300 (P<0.03) minutes after the start of sampling.

Figure 4.3 The effect of diet on plasma insulin concentration (Mean ± standard error)
4.3.7.2. Growth hormone

There was a significant difference in plasma growth hormone concentration between treatments using a repeated measures analysis (P<0.001) (Figure 4.4). There was also an effect of time (P<0.05). The average concentration for sheep fed S+B was 4.82 ± 0.18 and 3.71 ± 0.3 ng/mL for the C treatment. There were significant differences between the S+B treatment and the C at 60, 80, 120, 180, 220 and 320 minutes after the start of sampling.

Figure 4.4 The effect of diet on plasma growth hormone concentration (Mean ± standard error)
4.3.7.3. **Insulin-like Growth Factor**

There was a significant effect of diet on IGF concentration (P=0.04) using repeated measures analysis (Figure 4.5). The S+B sheep had lower overall concentration of IGF compared to the C treatment at day 15 and 38. There was no effect of sampling time on IGF secretion (P>0.05).

Figure 4.5 The effect of diet on plasma IGF concentration (Mean ± standard error)
4.3.7.4. Plasma urea and creatinine

Treatment had a significant effect on plasma urea concentrations (P<0.001) (Figure 4.6). The sheep fed the C treatment had significantly higher plasma urea concentrations than the S+B treatment at each time point. There was a significant effect of time on plasma urea (P<0.001).

Treatment had no overall significant effect on plasma creatinine concentration (P=0.6) (Figure 4.6). There was a significant effect of time (P<0.05) and a significant interaction between time and diet (P<0.05).

Figure 4.6 The effect of diet on plasma urea and creatinine concentration (Mean ± standard error)
4.3.7.5. Muscle glycogen content

Treatment had an effect on the glycogen content of the SM muscle (P<0.05) (Figure 4.7). There was an effect of time on glycogen concentration (P=0.04) with C treatment increasing till day 38 then decreasing thereon. There was a significant difference between treatments on day 15 and 38.

Figure 4.7 The effect of diet on SM glycogen concentration (Mean ± standard error)

Treatment had no effect on the glycogen content of the ST muscle (P>0.86) (Figure 4.8). There was an effect of time on glycogen concentration (P<0.001) with both treatments increasing with duration of experiment. There was no significant difference between treatments at any of the individual time points.
4.3.7.6. **Other hormones and metabolites**

There was no significant difference between treatments for cortisol, leptin, T3, T4 or sodium, potassium and lactate concentration in plasma (P>0.01).

4.3.8. **Wool growth and fibre diameter**

There was a treatment effect on clean wool growth with the sheep fed the S+B diet having the highest growth (Table 4.9). There was a significant effect of diet on fibre diameter and yield with the sheep fed the C ration having a significantly lower fibre diameter and yield (P<0.02). The suint content of the wool from the S+B fed sheep was significantly lower than the C treatments (P<0.01). The efficiency of wool growth expressed as CWGA per liveweight gain was significantly higher in the sheep fed S+B compared to the C diet (P<0.003).
<table>
<thead>
<tr>
<th></th>
<th>S+B</th>
<th>C</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean wool growth (g/100 cm²/d)</td>
<td>0.109 ± 0.006</td>
<td>0.092 ± 0.005</td>
<td>0.04</td>
</tr>
<tr>
<td>Fibre diameter (µm)</td>
<td>19.7 ± 0.25</td>
<td>18.4 ± 0.25</td>
<td>0.02</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>68.3 ± 0.64</td>
<td>65.2 ± 0.64</td>
<td>0.04</td>
</tr>
<tr>
<td>Wax (g/100g clean fibre)</td>
<td>18.5 ± 0.56</td>
<td>18.9 ± 0.56</td>
<td>0.56</td>
</tr>
<tr>
<td>Suint (g/100g clean fibre)</td>
<td>9.9 ± 0.93</td>
<td>14.6 ± 0.93</td>
<td>0.03</td>
</tr>
<tr>
<td>CWGA per live weight gain (g/g)</td>
<td>0.301 ± 0.06</td>
<td>0.159 ± 0.02</td>
<td>0.003</td>
</tr>
<tr>
<td>CWGA per organic matter intake (g/head.kg OMI)</td>
<td>11.2 ± 0.64</td>
<td>10.4 ± 0.59</td>
<td>0.18</td>
</tr>
</tbody>
</table>
4.4. Discussion

4.4.1. Carcass quality

4.4.1.1. Fatness

The hypothesis that ingesting a saltbush and barley diet would result in beneficial changes to carcass composition was supported. The S+B fed sheep had a significantly lower proportion of fat and lower GR depth and a trend for a higher proportion of lean compared to the sheep fed the C diet. This is a commercially desirable result for processors because the costs of fat denudation are high. Farmers also increase their farm productivity from stock depositing less fat for every kilogram gain in liveweight.

The lower proportion of fat on the carcasses of the S+B fed sheep is reflective of a higher protein to energy ratio available for production for the sheep in this group. It has been shown that higher protein: energy ratios will result in less deposition of fat (Andrews et al. 1970; Campbell 1988; Campbell et al. 1984; Searle et al. 1982). The saltbush fed sheep probably have a higher protein:energy ratio due firstly to an increase in the availability and possibly the quality of protein absorbed in the small intestine compared to the control fed sheep. This has been shown to be a consequence of the increased consumption of water associated with a high salt intake increasing the rate of passage of feed through the digestive tract (Hemsley et al. 1975). The conclusion of a higher rate of protein reaching the small intestine in the S+B fed sheep is supported by a lower plasma urea concentration in the S+B group. An increase in rumen undegradable protein will result in a decrease in the concentration of urea in the plasma (Bohnert et al. 2002; McDonald et al. 1995). Secondly the sheep fed the S+B diet may also have a decreased energy availability due to the reduced fermentation of organic matter in the rumen.
and lower absorption of VFAs. This may also be associated with a higher water consumption but Weston et al. (1970) also suggested that saltbush intake was associated with decreased VFA absorption. The S+B group also had a significantly lower ME intake compared to the C group. In addition, the higher energy requirements to process the salt (Arieli et al. 1989) would decrease the availability of energy away from lipogenesis. The increase in protein absorption and decreased VFA absorption and energy intake of the saltbush fed sheep offers an explanation for the increased ratio of protein: energy and lower deposition of fat in this group.

These observations in fatness are supported by the changes in metabolic hormones. The S+B group had consistently lower levels of insulin and IGF but higher GH during the experiment. These results are reflective of the higher plane of nutrition of the C group as indicated by the significantly higher DOMI and ME intake of this group. Insulin is the primary hormone involved in the regulation of fat storage with higher levels resulting in greater fat deposition (Breier 1999; Guyton et al. 2000). Insulin is indirectly stimulated by increased production of propionate, the gluconeogenic precursor of glucose. Sheep consuming higher levels of ME will have an increased production of propionate (Abdul-Razzaq et al. 1988; Bailey 1989) therefore the lower circulating levels of insulin in the S+B group are consistent with lower propionate absorption. In contrast growth hormone stimulates triglyceride breakdown and then enhances the conversion of fatty acids to acetyl CoA and subsequent utilisation of these substrates for energy (Breier 1999). Growth hormone also increases the deposition of protein by increasing the transfer rate of amino acids into cells to be used for protein synthesis as well as decreasing the catabolism of proteins (Lobleay 1998; Oddy 1993).

The high levels of chromium in the S+B diet compared to the C diet may also contribute to a lower level of fat on the carcasses of the S+B group. Chromium supplementation decreased GR
fat depth in pigs (Page et al. 1993) and in mature wethers (Gardner et al. 1998) fed chromium 0.1-0.2 and 1 mg/kg.head dry matter respectively. The primary function of chromium is that it increases insulin response and glucose utilisation in sheep (Andersen 1997; Kegley et al. 1999; Morris 1999). However, the chromium-initiated insulin response does not result in increased fat deposition as would be expected rather it acts to increase protein synthesis and therefore protein accretion by diverting energy yielding substrates for this purpose (Gardner et al. 1998; Kegley et al. 1999; Morris 1999; Page et al. 1993; Sano et al. 1997). In a study that may also be associated with the chromium content of saltbush, (Mertz et al. 1973) showed that the ash extracted from saltbush (Atriplex halimus) could increase insulin response of adipose tissue in vitro. The only related in vivo work involved feeding leaves from Atriplex halimus to the sand rat (Psammomys obesus) residing in the Arabic region. When fed a standard ration the rats become insulin resistant but this was corrected by feeding the saltbush leaves (Mertz et al. 1973).

The high betaine content of the saltbush may also be associated with decreased fat deposition (Fernandez et al. 1998; Fernandez et al. 2000). Fernandez et al. (1998; 2000) demonstrated that feeding diets enriched with 2 g/kg dry matter of betaine could decrease GR fat thickness and lipid content of intramuscular fat in lambs. Although the betaine content was not measured in this experiment, previous researchers have demonstrated that saltbush contains high quantities of betaine (1.4-68 g/kg dry matter) (Randell et al. 1996; Storey et al. 1977). Betaine influences lean deposition by improving the efficiency of methionine use, an essential amino acid for lean tissue growth and improving choline activity to reduce fat deposition. The lower energy intake of the S+B group may amplify betaine action. There is evidence in pigs that betaine has a more pronounced effect when dietary energy is limiting and so offers a means of improving meat quality through ensuring the provision of additional energy (Suster 2003). Further work in this
area with sheep is needed confirm the link between low energy intake and improved betaine action.

The lower proportion of fat on the carcass corresponds with a higher proportion of total body water in the carcass. Panaretto (1963) and Searle (1970) observed that sheep displaying lower rates of fat synthesis had higher amounts of body water compared to those depositing greater levels of fat at the same liveweight. In this experiment, the S+B fed sheep had a higher amount of total body water than the C fed sheep.

In concluding this section, the combination of higher protein:energy ratio, chromium and betaine content of the S+B diet in combination with decreased insulin and IGF and increased growth hormone all explain the lower rates of fat deposition of the sheep fed the S+B diet compared to the C diet.

4.4.1.2. Fatty acid composition

The hypothesis that the increased consumption of water by sheep on the S+B diet would result in less biohydrogenation of fat in the rumen and therefore less saturated fat in the fat deposits was not supported. Ingesting S+B resulted in a significantly lower percentage of unsaturated fat and equal levels of saturated fat with the C treatment. Eichhorn et al. (1985) and De Smet et al. (2000) found that sheep fed a high energy diet and with a higher body fat had an increased percentage of unsaturated fat in the fat deposits. They also found that lower energy intakes are associated with an increased saturated fat content. The consumption of a high energy diet and a higher level of fatness is also associated with higher levels of 18:1 and a reduction in 18:2, 20:4
and 18:0 (De Smet et al. 2000; Eichhorn et al. 1985). Similarly, the S+B fed sheep showed a reduction in 18:1 and an increase in 18:2; 20:4 and no change to 18:0.

The significantly higher levels of linolenic acid (C 18:3) in the intramuscular fat deposits of the sheep fed the S+B diets may have commercial significance. The products of oxidation of linolenic acid and its derivatives have been associated with species specific flavours of meat as a result of the formulation of volatile compounds during cooking (Mottram 1998). However there was no significant difference between treatments for the products of the oxidation of linolenic acid (docosahexaenoic acid (C 22:6) and eicosapentaenoic acid (C 20:5)) suggesting that these fatty are not being oxidised.

4.4.1.3. **Meat biochemistry**

The differences in ME intake and resultant changes in fatness and levels of metabolic hormones may also have important implications for glycogen storage and subsequent meat quality. Lower insulin levels and a reduced availability of glycogen substrates due to a lower energy intake in the S+B fed sheep could result in less glycogen storage (Gardner et al. 2001; McVeigh et al. 1982; Pethick et al. 1996; Pethick et al. 1994). Lower levels of glycogen storage will result in elevated ultimate pH due to lower levels of lactic acid production during rigor mortis (Lawrie 1998). A low ultimate pH (around 5.5) is commercially desirable, as it is associated with lighter meat colour and increased palatability.

In this experiment there were no difference between treatments for muscle glycogen concentrations during the experiment or at slaughter and no differences in ultimate pH. Hopkins et al. (1999) also observed no differences in glycogen concentrations or ultimate pH of sheep
ingesting saltbush and oats, saltbush and lucerne hay or lucerne. The ultimate pH of both treatments was below 5.8, ensuring optimal meat quality. There was also no effect of treatment on meat colour. The L* values indicate the darkness of the meat and values below 34 are more commercially desirable. Interestingly both the a* and b* colour values were higher in this experiment than in the study by Hopkins et al. (1999). Higher a* values indicate that the meat is redder, a positive finding as consumers prefer redder meat. Whereas higher b* values indicate an increase in meat yellowness, a trait which is not commercially desirable by consumers. It is possible that the saltbush used in this experiment contained compounds that may influence meat redness and yellowness that were not contained in the saltbush on non-saline land used in Hopkins et al. (1999). Overall, no negative effects on glycogen content, colour or ultimate pH were observed despite the differences in ME intake, fatness and metabolic hormones.

4.4.1.4. **Growth performance and hot carcass weight**

The hypothesis that ingesting saltbush and barley would result in improved hot carcass weights was not supported. There was no difference in hot carcass weight or dressing percentage (carcass weight as a proportion of final liveweight) between the treatments. Processors are demanding higher carcass weights than those achieved in this experiment. There was no difference between treatments for dressing percentage despite the S+B sheep having a lower final liveweight than the C fed sheep. This finding may have occurred because the sheep fed the S+B diet retained fluid in the tissues which buffered against losses in liveweight between leaving animal house and slaughter thus reducing the loss in carcass weight of this treatment. The fluid retention may be the result of the high salt load consumed prior to slaughter resulting in fluid retention in the tissues (Michell 1985) or alternatively the S+B group drank more water in lairage and maintained fluid stores above those of the C group. Over the 48 hour lairage
stimulation period the S+B sheep were shown to consume more water compared to the C group in the absence of feed, a finding likely to occur in lairage.

An interesting observation from the lairage simulation experiment was that the greatest proportion of this fluid was drunk in the first 0-8 hours and was not sustained over the 48 hour period. After this point there was no difference in water intake. The high water intake in the first 0-8 hours is stimulated by the intake of high levels of salt prior to the removal of feed. It is likely that all the excess salt is all excreted after 24 hours thus removing the stimulus to drink. Whether or not this higher initial water intake will benefit carcass quality is unknown. It can be speculated that this outcome is best utilised if sheep are slaughtered within 24 hours of water and food deprivation.

The 62 g/d liveweight gain observed in the S+B fed group is a positive result suggesting that the combination of barley and saltbush can be used to elicit liveweight gains. However opportunities to increase liveweight gains whilst on saltbush need to be investigated if saltbush to be considered a viable option to finish sheep. The age classification of sheep represented in this study could achieve higher liveweight gains if fed greater energy and protein levels. The equal liveweight gain of both groups occurred despite a significantly lower ME and DOMI intake and resultant lower circulating IGF concentrations by the S+B group. Further field experiments may clarify the growth performance of ingesting saltbush.

4.4.2. Eating quality

The hypothesis that ingesting a S+B ration prior to slaughter would result in improved eating quality was not supported. There was no treatment effect on any of the eating quality attributes
assessed. Although there was no improvement in the liking of flavour and aroma, tenderness, juiciness and overall acceptance there were also no negative effects on these attributes. Therefore the results indicate that saltbush can be used for meat production but the anecdotal claims that the meat is tastier and juicer are not supported.

The lower level of fat on the carcasses of sheep fed the S+B diet may have confounded any changes to eating quality. Intramuscular fat is positively correlated with increased tenderness and juiciness and associated with flavour development (Hopkins et al. 1985; Melton 1990; Rousset-Akrim et al. 1997; Warriss 2000). In the case of tenderness and juiciness, a decrease in fat can increase the proportions of intramuscular collagen- conducive to greater toughness and decrease the amount of water entrained in the muscles making the meat drier (Lawrie 1998; Lushbough et al. 1963). Meat fat is also the principal source of sheepmeat flavour and odour compounds but the lower levels of fat observed in the carcasses in this experiment did not cause less flavour in the meat. Had the saltbush fed sheep had a higher carcass fat content, it is possible that the eating quality attributes may have been changed. An important finding is that the low carcass fat content was not detrimental to eating quality.

The high water intake and therefore the resultant increased rate of passage of feed could result in compounds for flavour and odour development being flushed out or not adequately digested to allow for accumulation in the fat. While there is no published evidence to support this theory, it is highly likely a change in rate of passage will influence digestion of a range of compounds.

The length of time the animals were fed the diets may also have had an effect on eating quality (Crouse et al. 1981; Crouse et al. 1983; Hocquette et al. 1999). The South Australian ‘Saltbush Dija’ product is based on minimum 300 days grazing and the New South Wales product for a
minimum 12 weeks. The sheep in this experiment were fed for only 10 weeks and this may not have been a long enough time period for sufficient deposition of the flavour and aroma influencing compounds into the fat and lean tissue. Rousset-Akrim et al. (1997) observed that 90 days of pastoral feeding versus grain feeding only slightly increased sheepmeat flavour and aroma as assessed by a consumer sensory panel. After 215 days the flavour and aroma was significantly exacerbated. Pethick et al. (2005) also observed no difference in overall liking, liking or strength of flavour of meat as assessed by a consumer taste panel of sheep fed an irrigated ryegrass-clover-kikuyu based pasture compared to sheep fed on moderate to high energy pelleted concentrate rations for 77 days. Therefore, future field experiments will involve longer grazing periods to increase the potential for deposition of flavour and aroma compounds. Longer grazing periods may also counteract the effect of a reduced rate of fat deposition in the S+B on flavour and aroma deposition.

The possible effect of drying the saltbush on flavour and aroma compounds available for deposition in the meat and fat also requires further investigation. Drying was necessary in the current experiment to allow for mixing and storage of the diets. It was not logistically possible to provide fresh, undried feed so the saltbush had to be harvested and brought from its country location prior to the start of the experiment and dried to prevent spoilage. The saltbush was dried at a low temperature to reduce potential volatilisation of any compounds. It is therefore essential that this experiment be replicated using fresh saltbush in situ to confidently conclude that the lack of effect on eating quality was not simply due to the drying of the saltbush.
4.4.3. **Wool quality**

This experiment has supported the hypothesis that feeding a saltbush and barley ration can increase clean wool growth and fibre diameter. This finding is consistent with those of Hemsley *et al.* (1975) and Masters *et al.* (2005) who found that wool growth is highly responsive to an increase in bypass protein due to the consumption of a high salt diet. This current experiment indicated higher wool growth efficiency expressed as CWGA per liveweight gain. Higher values indicate that sheep are partitioning a higher proportion of protein towards fibre production than liveweight gain. The sheep fed the S+B ration had a significantly higher CWGA per liveweight gain compared to the C diet.

An additional theory why the sheep fed the high salt diets had a higher CWG may be due to the higher sulphur content of the diets. Saltbush contains twice as much sulphur as green pastures and four times as much as dried annual pastures (Morecombe *et al.* 1991). This would aid the synthesis of sulphur rich proteins required for wool production.

The sheep fed the S+B cut wool with a significantly higher clean wool yield than the control treatment. This may be due to the lower suint production in these animals. Suint is the sweat present in the wool fleece. The lower suint production by the S+B fed sheep suggests that they are sweating less or have a different sweat conformation. This may be related to the high water intake of this group. When an animal consumes water it needs to heat this water up to body temperature which is energy consuming. This process is an advantage for thermoregulation in hot environments and may possibly reduce sweating (Lundin *et al.* 1925). Lower suint production is related to less fleece yellowing and less fleece rot (Aitken *et al.* 1994).
4.5. Conclusions

The following conclusions were made from this chapter:

- Sheep fed the S+B diet had lower levels of fat on the carcass compared to the C diet. This may be due to the higher protein:energy ratio and higher chromium and betaine content of the diets as well as a decreased insulin and IGF and increased growth hormone levels.

- The lower ME intake and level of fatness observed by sheep fed S+B were associated with reduced concentrations of unsaturated fatty acids in the fat deposits compared to the C fed sheep.

- The consumption of the S+B diet did increase water intake during a 48 hour period of water deprivation but mostly in the first 8 hours. This outcome is best utilised if sheep are slaughtered within 24 hours of water and food deprivation.

- Feeding the S+B ration enabled 60 g/d of liveweight gain however the final liveweight of the S+B fed sheep was slightly lower than the sheep fed the C diet.

- The higher body water content of the S+B group buffered against dehydration and prevented carcass weight loss to result in similar hot carcass weights and dressing percentages despite the S+B group having a lower final liveweight.

- There was no effect of ingesting S+B on any eating quality attributes possibly due to the lower level of carcass fat and drying of the saltbush. This work needs to be repeated in the field.

- The consumption of S+B increased clean wool growth and fibre diameter through a higher wool growth efficiency in this group. The S+B fed sheep also had a higher wool yield which appears to be related to the lower suint production. The lower suint production indicates that the S+B fed sheep were sweating less possibly due to the high water intake improving thermoregulation.
Chapter 5

A sodium to creatinine ratio in a spot sample of urine for the prediction of daily sodium intake

5.1. Introduction

The conclusions from the previous chapter demonstrate that grazing experiments need to be performed to further quantify the carcass and eating quality of animals grazing saltbush based saline pasture systems. One limitation to conducting grazing trials to assess carcass and eating quality is in quantifying the level of intake of the saltbush and understorey in the paddock.

There are techniques available that quantitatively estimate herbage intake of a grazing animal and also identify the botanical composition of their intake. The most commonly used procedure for estimating herbage intake and botanical composition of the consumed herbage is the alkane method (Dove et al. 2000; Dove et al. 1996; Mayes et al. 1986). Alkanes are plant wax components and every plant species has a unique alkane profile or ‘fingerprint’. These alkanes are recovered in the faeces and intake and diet composition determined from the alkane profile. The alkane analysis is expensive and time consuming and is believed not well suited to pasture systems with more than four different pasture species present (Dove et al. 1996). Given that saltbush pasture systems may contain over 20 plant species (Masters et al. 2001) this technique is not appropriate.
Another alternative is $^{22}$Na, a radioactive isotope of sodium. This has been used very effectively to estimate food intake (Anon. 2000; Green et al. 1989). A decline in the specific activity of the sodium in the blood, following an injection with the isotope allows a measure of the sodium influx per unit time. If the sodium content of the animals’ diet is known-then the food intake can be estimated. There are problems with the use of this method in the field. It is expensive and treated animals are not permitted to enter the human or animal food chain. It is therefore not appropriate for meat quality experiments.

A third alternative arises from work by Wilson et al. (1969) and is based on the use of water intake to estimate saltbush intake. Their hypothesis was that because the water intake is closely related to the sodium intake an increase in water intake could be used to calculate the amount of saltbush eaten. This technique is open to some errors. There are seasonal differences in the water intake of a ruminant and some times of the year all water is obtained from pasture and not tanks or troughs. For this reason this method is only reliable in the summer months when pastures have a low dry matter content.

The limitations of these three options justify the exploration of other techniques to estimate feed intake. As the focus of this thesis is to understand the effects of saltbush on the carcass and eating quality of sheepmeat any method developed only needs to indicate the intake of saltbush and not other low salt plants in the paddock.

Old Man Saltbush contains high levels of sodium within the range of 4-8% (% dry weight) (Arieli et al. 1989; Morcombe et al. 1996; Pol 1980; Wilson 1966b). Sheep excrete roughly 98% of the sodium they ingest, of which 5-10% is excreted in the faeces and 90-95% in the urine (Hemsley et al. 1975; Tomas et al. 1972; Wilson 1966b) hence the sodium intake is almost the
same as the urinary output. Therefore urinary sodium output per day can be used to predict the sodium intake of the animal with a high level of confidence. Furthermore by determining the average sodium content of the saltbushes in the paddock the approximate quantity of saltbush being consumed per day can be estimated. This calculation assumes negligible amounts of salt are consumed from other sources.

To determine the daily output of sodium it would be necessary to collect urine over a 24 hour period. In the field it is not practical to make full 24 hour collections. An alternative is to estimate the daily excretion of sodium from the concentration of sodium in a spot sample of urine and use this with a compound or marker that is excreted at a constant rate. Gonda and Lindberg (1994), Chen et al. (1995) and Faichney et al. (1995) have all used urinary creatinine as an internal marker for urinary output. The use of creatinine in a ratio with sodium could allow for the sample to be standardised for differences in urine concentration.

Using creatinine in a ratio to predict the daily level of excretion of compounds of interest is not new. Allantoin, albumin and urea are examples of compounds that have been successfully measured. For example, Chen et al. (1995) used the allantoin to creatinine ratio in a spot sample of urine to estimate daily microbial protein supply to the ruminant animal with an $r^2$ of 0.84. Gonda et al (1994) found that the urea:creatinine ratio could be successfully used to determine the dietary nitrogen utilisation in ruminants.

Based on the assumption that the renal clearance of sodium approaches that of creatinine, the molar ratio of sodium to creatinine (Na:creat) in spot urine samples could be used to assess daily sodium excretion. The validity of this ratio depends on it having; (1) a small diurnal variation and (2) a high correlation with the daily measurement of sodium intake. With spot urine
samples, it is also essential to adopt a measure that is independent of the unknown daily urine volume (Chen et al. 1995). These aspects will be investigated.

The hypotheses for this chapter are:

1. That the Na:Creat ratio in urine can be used to predict daily sodium excretion
2. That the sodium excretion predicted from a spot sample of urine can be used to predict sodium intake

The experimental component of this chapter will be divided in two parts. Part 1 details the validation and development of this technique and aims to validate the use of the Na:Creat ratio to estimate daily sodium excretion and then evaluate the validity of using daily predicted sodium excretion to estimate actual sodium excretion. Part 2 is the validation of this method by testing samples collected from a number of other unrelated animal house experiments.

5.2. Development and verification of the Na:Creat ratio

5.2.1. Materials and methods

5.2.1.1. Animals and Diets

Thirty six Merino wethers (approximately 6 months of age; average liveweight $34.9 \pm 0.25$ kg at the start of the experiment) were housed in individual pens in the animal house facilities at the CSIRO Floreat Park. The sheep were allocated to one of 4 diets. The diets had similar constituents but differed in their level of sodium addition (0, 0.87, 2.18, 3.48 moles/kg DM, equivalent to 0, 20, 50 and 75 g/kg DM). Compositions of the diets are shown in table 5.1. The diets were pelletised to reduce selection and the sheep were allowed free access to tap water from nipple drinkers. The sheep were fed the diet for 5 weeks prior to sampling. Feeding was
once daily in the morning (9am). Feed residues were weighed each day and both residues and feed were dried at 75°C for 3 days to allow calculation of dry matter intake for each sheep.

Table 5.1 Composition of experimental diets

<table>
<thead>
<tr>
<th>Constituent (in g/kg DM)</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hammer milled oaten hay</td>
<td>534</td>
<td>506.8</td>
<td>466.4</td>
<td>425.8</td>
</tr>
<tr>
<td>Lupinseed</td>
<td>50</td>
<td>47.5</td>
<td>43.6</td>
<td>39.8</td>
</tr>
<tr>
<td>Oat grain</td>
<td>400</td>
<td>379.7</td>
<td>349.1</td>
<td>318.6</td>
</tr>
<tr>
<td>Ca(OH)₂</td>
<td>6</td>
<td>5.7</td>
<td>5.2</td>
<td>4.8</td>
</tr>
<tr>
<td>NaCl</td>
<td>0</td>
<td>51</td>
<td>127</td>
<td>203</td>
</tr>
<tr>
<td>Na mol/kg DM</td>
<td>0.07</td>
<td>0.99</td>
<td>2.00</td>
<td>3.27</td>
</tr>
</tbody>
</table>

A Determined by chemical analysis of the diet.

5.2.1.2. Sample collection

A purpose built harness was used to collect urine. The harnesses consisted of a moulded latex cup held under the pizzle, which drained into a 5 litre plastic bottle using silicone tubing (see Appendix 2, plate 1). The collection bottles were monitored and the contents transferred to storage bottles as necessary.

Urine was collected for 4 days. For the first 24 hours a sample was collected every two hours from all 36 sheep. At this time amount of urine present was measured and the volume recorded. A 4 mL sample was taken and added to a 10 mL tube containing 6 mL of 2% H₂SO₄. The acid was to stop any microbial growth. The samples were immediately frozen at −20°C prior to analysis. The remaining urine was discarded.
Over the next 3 days, spot samples were taken from all 36 sheep. Spot samples of urine (over 10mL) were collected from all 36 sheep at 7.00 am on day 2, 10.00 am on day 2, 12.00 pm on day 3 and 4.00 pm on day 3. Every 30 mins prior to the designated spot sampling time, all urine was removed from the bottle and placed in a separate collection bottle collecting the daily urine voidance. From each spot sample a 4 mL sample was taken and added to a 10 mL tube containing 6mL of 2% H₂SO₄. The samples were immediately frozen prior to analysis.

Complete 24 hour urine collections were also performed over 3 consecutive days. A 10% (by volume) subsample of the daily urine collection was acidified with 10 mL of 2% H₂SO₄ and stored at -20°C until analysis.

Feed intake data was available and from this and the sodium analysis of the diet, total sodium intake was calculated.

The daily output of faeces was also collected for three days. The sheep were fitted with a faecal collection harness during this time. Each day, the faeces were weighed and a 10% subsample dried at 75°C for 72 hours. The dried faeces were weighed again to allow calculation of faecal dry matter.

5.2.1.3. Analysis

Samples of each diet were collected daily and bulked. Mineral and nutritional analysis were as previously described in chapter 3 (section 3.5).
All urine samples was analysed for sodium and creatinine as described in section 3.3. The faeces samples were also analysed for sodium. A subsample was passed through a 1 mm screen using a Tecator Cyclone© mill then sodium (Na), were assayed in a commercial laboratory (CSBP Soil and Plant Laboratory, Bibra Lake, WA) using the standard operating methods for the Thermo Iris Intrepid Duo ICP following digestion by the method of McQuaker et al. (1979) prior to analysis.

5.2.1.4. Interpretation and analysis of data

For the urine collected over the first 24 hours the sodium and creatinine concentration (in g/L) were corrected for dilution and the original volume of urine collected was then used to calculate total sodium and creatinine for each urination. The sum of all urinations from the 24 hour collection were added together for each animal to give daily sodium and creatinine excretion in g/d. The total sodium and creatinine excretion on days 2-4 was estimated from sodium and creatinine concentrations and total urine excretion each day. These summations are the daily observed values for sodium and creatinine excretion.

The sodium to creatinine ratio for each spot sample was determined by dividing the concentration of sodium by the content of creatinine (Na:Creat) (in g/L). The Na:Creat ratios from the spot samples over the three days was then used to predict daily sodium excretion via the formula:

Daily predicted sodium excretion (g/d) = Na:Creat*PCreat
Where: Na:Creat = sodium:creatinine ratio of the spot sample and PCreat = predicted creatinine excretion (g/d). The predicted daily excretion of creatinine (PCreat) was determined using an equation derived by Brody (1964): 

\[ PCreat (\text{g/d}) = 0.0527g \times (\text{Weight of the animal}^{0.75}) \]

According to Brody (1964), creatinine is excreted in proportion to lean body mass within a wide range of body weights and is independent of the diet. Therefore, the consistency of excretion and production means the total daily excretion per kg of bodyweight can be estimated from a urine spot sample.

The values for the daily predicted sodium excretion were then compared against the daily observed sodium excretion and daily observed sodium intake from day 2-4 only using a non-linear and linear regression to obtain the best \( r^2 \) value. The following best fit equation was formulated and had an \( r^2 \) value calculated:

\[
\text{Daily observed sodium intake} = a \times (\text{daily predicted sodium excretion}) + b
\]

OR:

\[
\text{Daily observed sodium intake} = a \times (\text{Na:Creat} \times PCreat) + b
\]

Paired t-tests were used to compare actual sodium intake and predicted sodium intake within diet and at each time of spot sampling. A paired t test was also used to compare actual sodium intake with average predicted sodium intake for each diet.

Analysis of variance was conducted to determine if the Na:Creat ratio differed between times within a diet and also if daily predicted creatinine excretion differed from daily observed creatinine excretion.
5.2.2. Results

5.2.2.1. Intake

Average sodium intakes over the 4 day collection period were significantly different between diets (table 5.2) (P value= 0.01).

Table 5.2 Daily observed sodium and dry matter intake

<table>
<thead>
<tr>
<th>DIET</th>
<th>Average dry matter intake (g/d)</th>
<th>Intake of sodium (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>677</td>
<td>1.42</td>
</tr>
<tr>
<td>2</td>
<td>710</td>
<td>14.6</td>
</tr>
<tr>
<td>3</td>
<td>772</td>
<td>35.9</td>
</tr>
<tr>
<td>4</td>
<td>762</td>
<td>56.9</td>
</tr>
</tbody>
</table>

5.2.2.2. The relationship between actual sodium intake and actual urinary sodium excretion

There was a significant relationship between sodium intake and sodium excretion ($r^2=0.81$, P<0.05) (Figure 5.1). The equation derived from this relationship was:

\[ \text{Sodium excretion} = 0.927 \times \text{sodium intake} + 1.55 \]
5.2.2.3. The relationship between predicted sodium excretion and actual sodium excretion

There was a significant relationship between actual sodium excretion and daily predicted sodium excretion using the Na:Creat ratio and predicted daily creatinine excretion calculated using the equation by Brody (1964) \( r^2=0.92, P<0.05 \) (Figure 5.2).

The equation generated from this relationship was:

\[
\text{Actual daily sodium excretion} = 1.803^* (\text{Predicted daily sodium excretion})^{0.8853}
\]

OR:

\[
\text{Actual daily sodium excretion} = 1.803^* (\text{Na:creat*PCreat})^{0.8853}
\]
5.2.2.4. **The relationship between predicted sodium excretion and actual sodium intake**

There was a significant relationship between predicted sodium excretion and actual sodium intake ($r^2=0.82$, $P<0.05$) (Figure 5.3).

The equation generated from this relationship was:

\[
\text{Actual daily sodium intake} = (0.935 \times \text{Predicted daily sodium excretion}) + 1.3
\]

Or:

\[
\text{Actual daily sodium intake} = (0.935 \times \text{Na:creat} \times \text{Pcreat}) + 1.3
\]
5.2.2.5. The use of a spot sample of urine to predict daily sodium intake

The values for predicted sodium intake determined from the spot sample at 10 am were not significantly different to the actual daily sodium intake for any diet ($P>0.05$) (Table 5.3). The predicted values at the other timeslots and the average predicted intake (across sampling times) were all significantly different to actual intake for at least one of the diets. There were significant differences between the Na:Creat ratios across times within treatments ($P<0.05$).
Table 5.3 Prediction of sodium intake from daily predicted sodium excretion in spot samples of urine (Mean ± standard error)

<table>
<thead>
<tr>
<th>Na:Creat in spot samples of urine</th>
<th>Predicted sodium intake from spot samples$^A$ (g/d)</th>
<th>Average predicted sodium intake$^B$G</th>
<th>Actual sodium intake (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10am$^E$</td>
<td>4pm$^F$</td>
<td>7am$^F$</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10am</td>
<td>2.14$^{ac}$ ±</td>
<td>4.60$^b$ ±</td>
<td>5.89$^b$ ±</td>
</tr>
<tr>
<td>4pm</td>
<td>0.74 ±</td>
<td>0.93 ±</td>
<td>1.67 ±</td>
</tr>
<tr>
<td>7am</td>
<td>13.6$^a$ ±</td>
<td>26.6$^b$ ±</td>
<td>18.4$^{ab}$ ±</td>
</tr>
<tr>
<td>12pm</td>
<td>1.70 ±</td>
<td>3.49 ±</td>
<td>5.93 ±</td>
</tr>
</tbody>
</table>

$^A$ Determined from $= (0.935*\text{Na:Creat}*\text{Pcreat})+1.3$, $^B$ Average across four collection times, $^C$ For Na:Creat in spot samples of urine numbers across the row that are not followed by the same superscript are significantly different (P<0.05), $^D$ Compared with actual sodium intake (paired comparison) significantly different values have different superscripts (P<0.05), $^E$ No significant difference exists between the predicted sodium intake in this timeslot overall and actual sodium intake (P<0.05), $^F$ Significant difference exists between the predicted sodium intake in this timeslot overall and actual sodium intake, $^G$ Average predicted intake across the timeslots within diets was not significantly different to the actual sodium intake.
5.2.2.6. Excretion pattern of the sodium:creatinine ratio, urinary sodium and creatinine and urine volume

Statistical differences existed between all four diets in the Na:Creat ratio with higher ratios from higher sodium diets (Figure 5.4, $P = 0.001$). The repeated measures analysis indicated that diurnal variation did exist within the diets ($P = 0.001$). There was also a diet by time interaction with an increasing trend for diets 2 and 3 but not for diet 1 or 4.

Figure 5.4 Diurnal variation of the Na:Creat ratio (Mean ± standard error)

There were significant differences in urinary sodium excretion ($P = 0.001$) but not for creatinine excretion ($P = 0.386$) (Figures 5.5 and 5.6). There was a significant effect of times within diets for both sodium and creatinine excretion ($P = 0.006$ and 0.01 respectively). All diets follow a similar trend; steady until 4pm then increasing after this till a steady decline from 3am.
The output of creatinine and sodium in the urine followed urine volume (Figure 5.7). Over the 24 hour collection period the urine volume increased from 2pm onwards which is consistent with the increases in sodium and creatinine excretion. Significant differences in urine volume existed between diet 1 and diets 2, 3 and 4 and between diet 2 with diets 3 and 4 (P<0.05).
Figure 5.7 Diurnal variation in urine volume (Mean ± standard error)
5.2.2.7. The relationship between faecal sodium excretion and observed sodium intake

There was no relationship between actual faecal sodium excretion and actual sodium intake ($r^2=0.025$, $P<0.05$) (Figure 5.8).

Figure 5.8 The relationship between sodium intake and faecal sodium excretion

5.2.2.8. The relationship between predicted creatinine excretion and observed creatinine excretion

There was a significant relationship between predicted and observed creatinine excretion ($r^2=0.79$, $P<0.05$) (Figure 5.9). The equation derived from this relationship was:

$$\text{Observed creatinine excretion} = 0.89 \times \text{predicted creatinine excretion} + 0.138$$
Figure 5.9 Relationship between actual daily creatinine excretion and predicted daily creatinine excretion over three-day estimated from three day urine collection
5.3. Validation of the Na:Creat ratio

5.3.1. Materials and methods

Samples were taken from other experiments to validate the use of the Na:creat ratio. These experiments were being conducted by others so the aims will not be discussed only the diets and animals. Again, the urine harnesses were fitted to the animals at a specified time to obtain a spot sample for sodium and creatinine analysis.

5.3.1.1. Validation 1

This experiment was designed to study the effect of high salt intake on appetite control and was part of an Honours project by Micaela Grandison (Grandsion, 2003). Twelve wether sheep were fed one of two diets ad libitum for two weeks (table 5.5), either with or without sodium. Urine samples were collected from the sheep 7 days after they started on the experimental diets at 11am and analysed for sodium and creatinine. Storage and analysis of urine and feed was described previously.
Table 5.4 Diet composition of validation experiment 1

<table>
<thead>
<tr>
<th>Constituent (in g/kg DM)</th>
<th>Control diet (g/kg DM)</th>
<th>High salt diet (g/kg DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hammer milled oaten hay</td>
<td>534</td>
<td>425.8</td>
</tr>
<tr>
<td>Lupinseed</td>
<td>50</td>
<td>39.8</td>
</tr>
<tr>
<td>Oat grain</td>
<td>400</td>
<td>318.6</td>
</tr>
<tr>
<td>Ca(OH)$_2$</td>
<td>6</td>
<td>4.8</td>
</tr>
<tr>
<td>NaCl</td>
<td>0</td>
<td>203</td>
</tr>
<tr>
<td>Na$^A$</td>
<td>1.7</td>
<td>75.3</td>
</tr>
</tbody>
</table>

$^A$ Determined by chemical analysis of the diet.

5.3.1.2. Validation 2

This experiment was designed to determine if feeding straw or saltbush would alter ruminal microbial activity and was part of an Honours thesis by Diane Mayberry (Mayberry, 2003). Four mature wether sheep were fed either a 100% saltbush based diet or a 100% cereal straw diet for 3 weeks. The saltbush diet contained 7.06% sodium and the straw diet contained 0.6% sodium. Urine samples were collected from the sheep 7 days after they started on the experimental diets at 2pm and analysed for sodium and creatinine. Storage and analysis of urine and feed were described previously.

5.3.1.3. Interpretation and analysis of the data

For both validation experiments feed intake data was available, from this and the sodium analysis of the diet, total sodium intake was calculated.

The sodium and creatinine content of the spot samples of urine were analysed as previously described and the Na:Creat ratio of the spot sample determined. The total daily excretion of
creatinine was determined using an equation derived by Brody (1964). Using the equation developed in section 5.1.1.4, the Na:Creat ratio and the daily calculated value for creatinine excretion were then used to predict sodium intake.

5.3.2. Results

Table 5.6 summarises the average Na:Creat ratios found in the spot samples for both validation experiments. The equation developed in section 5.3.1.4 under predicted daily sodium intake by sheep on the salt treatments 18 and 10% for validation 1 and 2 respectively.
Table 5.5 Prediction of sodium intake from spot samples of urine from validation experiments (Mean ± SEM)

<table>
<thead>
<tr>
<th>No of Sheep</th>
<th>Na:Creat Ratio</th>
<th>Predicted creatinine excretion&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Predicted sodium excretion&lt;sup&gt;B&lt;/sup&gt; (g/d)</th>
<th>Actual sodium intake (g/d)</th>
<th>Predicted sodium intake&lt;sup&gt;C&lt;/sup&gt; (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 6</td>
<td>6.28 ± 1.2</td>
<td>0.87 ± 0.014</td>
<td>5.52 ± 1.09</td>
<td>6.24 ± 0.52</td>
<td>6.46 ± 1.72</td>
</tr>
<tr>
<td>Salt 6</td>
<td>69.72 ± 7.7</td>
<td>0.82 ± 0.014</td>
<td>58.07 ± 7.2</td>
<td>68.37 ± 10.50</td>
<td>55.59 ± 8.17</td>
</tr>
<tr>
<td>Control 2</td>
<td>4.66 ± 0.001</td>
<td>1.13 ± 1.08</td>
<td>5.25 ± 1.16</td>
<td>4.18 ± 0.47</td>
<td>6.20 ± 1.82</td>
</tr>
<tr>
<td>Salt 2</td>
<td>48.7 ± 0.001</td>
<td>1.18 ± 1.53</td>
<td>57.43 ± 1.63</td>
<td>61.43 ± 5.3</td>
<td>54.99 ± 2.42</td>
</tr>
</tbody>
</table>

<sup>A</sup> Predicted creatinine excretion in g/d using equation by Brody (1964) = 0.5268* (liveweight<sup>0.75</sup>)

<sup>B</sup> Predicted sodium excretion using the equation = Na (g/d) = Na:Creat x PCreat (g/d)

<sup>C</sup> Predicted using the equation: Daily sodium intake = (0.935 *predicted daily sodium excretion) + 1.3
5.4. Discussion

The first hypothesis that the Na:Creat ratio in urine can be used to estimate daily sodium excretion was supported. There was a strong relationship between the predicted sodium excretion calculated using the Na:Creat ratio and actual sodium excretion with an $r^2$ of 0.92 (Section 5.2.1.3). The second hypothesis that the predicted sodium excretion could accurately predict daily sodium intake was also supported with an $r^2$ of 0.82 (Section 5.2.1.4) from the equation

$$\text{actual daily sodium intake} = (0.935 \times \text{Na:creat*Creat}) + 1.3).$$

However, significant variation in predicted sodium intake within sheep indicates that this technique is not suitable for predicting daily sodium intake of individual sheep. Under the conditions of this experiment, the technique still provided a good predictor of group sodium intakes particularly when differences in sodium intake were large. In the field where sheep graze on saltbush dominated pasture systems compared with dry pasture there are significant differences in the potential sodium intake. The sodium intake of sheep grazing saltbush is likely to be an order of magnitude higher than the sodium intake of sheep grazing pasture or stubble. Therefore under field conditions the technique will be useful to differentiate between treatment groups and provide a quantitative estimate of the mean sodium intakes of groups of sheep.

Significant variation was observed in sodium intake predictions depending on the time of collection of urine for the spot test. It was shown that the best time to take a sample most reflective of daily observed sodium intake was at 10 am. Predicted values at 10 am for all diets were not significantly different to actual values whereas sampling at the other timeslots resulted in predicted values significantly different to actual values for at least one of the diets.

The variation between sampling times for the spot test is attributable to diurnal variation in the Na:Creat ratio. The ratio was not constant throughout the day due to proportionately greater
increases in sodium excretion compared to creatinine excretion from 4pm onwards. The differences in excretion rates indicate differing rates of renal clearances of sodium and creatinine. The differences in renal clearances of sodium and creatinine may have been caused by the feeding patterns in the experiment. The sheep were fed once a day and this may have resulted in a variable amount of sodium being presented to the kidneys for excretion. This variation could increase the renal clearance of sodium compared to creatinine at certain times of the day and therefore result in an elevated Na:Creat ratio for much of the 24 hour period. If the sheep receives a more constant flow of feed either as a result of a higher frequency of feeding in the animal house or a more regular grazing pattern in the field, the Na:Creat ratio may have been less variable throughout the day. It seems likely that feeding once a day has provided an example of the worst performance of the test, conclusions therefore on the usefulness of the technique are conservative. For the purpose of the field experiments, this discrepancy between the predicted and observed values may be reduced if further work to determine the optimal spot sampling time is conducted. These samples were taken at random times during the day but by pinpointing a more appropriate time the predictions may be more reliable.

The use of the sodium content from faeces was also evaluated an alternative method to estimate daily sodium intake. However, the comparison between faecal sodium content and sodium intake showed a poor relationship between these variables (Figure 5.8). The poor relationship between faecal sodium excretion and sodium intake is due to the preferential partitioning of sodium into the urine rather than the faeces (Meintjes et al. 1993; Michell 1985).

An additional aspect to be considered is the possibility of dietary effects on creatinine excretion. Faichney et al. (1995) and Van Niekerk et al. (1963b) found that large increases in protein intake and starvation decreased creatinine excretion and attributed these results to changes in body composition. In this experiment, no differences in daily creatinine excretion were observed. This may be due to each treatment group having an equal protein intake and no depression in
liveweight possibly due to an above maintenance energy intake. Grazing saltbush however may directly affect creatinine excretion as it has a high protein content, low metabolisable energy and has been shown to change body composition (Van Niekerk et al. 1963a).

The success of the Na:Creat technique to estimate the levels of excretion has been replicated by other researchers. The use of this technique to estimate allantoin, albumin and urea excretion has been successfully used by Chen et al. (1995), Faichney et al. (1995), Gonda et al. (1994), Han et al. (1992). The use of the excretion data to estimate intake is a novel approach and will only work if the intake closely reflects that of output as did in this experiment.

In conclusion, ratio of Na:Creat in spot sample of urine of sheep fed diets with different sodium contents was subject to diurnal variation but was highly correlated to the daily output of sodium in the urine. Predicted sodium excretion also showed a moderate degree of accuracy to predict daily sodium intake. Therefore the ratio of spot urine samples could be used under field conditions as a practical indicator of daily sodium excretion and intake.
Chapter 6

Carcass and eating quality of sheep grazing saltbush based saline pasture systems

6.1. Introduction

Chapter 4 demonstrated that ingesting a saltbush and barley ration containing dried saltbush did not affect eating quality when compared with a ‘control’ barley grain and hay based diet. It is possible that the drying of the saltbush reduced or changed the compounds in the saltbush that may be responsible for changes in flavour and aroma or that the length of the experiment may have not been sufficient for the flavour and aroma compounds to infiltrate the fat and lean. Despite the lack of eating quality effects, benefits to carcass quality were observed. The sheep fed the saltbush and barley ration grew at 60 g/d and had significantly less fat on the carcass compared to the control ration. This reduction in fat content has benefits for the processor, farmer and consumer. There was also no change in meat biochemical attributes. All these findings need to be confirmed in the field. Therefore the ultimate aim of this chapter is to investigate if grazing saltbush-based pasture systems would influence meat eating quality and carcass characteristics.

This experiment will also examine further the effects of ingesting saltbush on hydration status. Chapter 4 demonstrated that despite the saltbush plus barley group having a lower final liveweight than the control sheep, there was no difference in the dressing percentage (carcass weight as a proportion of final liveweight) or hot carcass weight. This result suggests that the higher total body water content may be buffering against losses in liveweight between leaving
animal house and slaughter thus reducing the loss in carcass weight. This experiment will investigate if a similar result occurs under field conditions.

This chapter will also investigate the vitamin E status of sheep grazing saltbush. Saltbush is green throughout the year and vitamin E content is higher in green rather than dry plants (Puls 1994; Tramontano et al. 1993). The consumption of saltbush high in vitamin E may be beneficial during the summer and autumn period in Western Australia when livestock are dependent on dry, senesced pastures, cereal grain and stubbles that are low in vitamin E. Vitamin E (or the tissue active form of this vitamin; α-tocopherol) deficiency can cause nutritional myopathy in weaner sheep and can also reduce the shelf life of sheepmeat (Guidera et al. 1997; McDowell et al. 1996; Mitsumoto et al. 1998). Both nutritional myopathy and a reduction in the shelf life of sheepmeat are the result of lipid peroxidation of muscle membranes (Hidiroglou et al. 1991; McDowell et al. 1996; Rice et al. 1988). In 2003–04, over 1 million sheep in Western Australia alone were supplemented with vitamin E (G. Smith, pers. comm.). These synthetic supplements are costly and the administration is labour intensive. Natural grazing strategies that improve the vitamin E status of sheep during summer and autumn would be preferable.

The hypotheses for this chapter are:

1. That grazing saltbush with a barley supplement will decrease fat in the carcass compared to sheep grazing pasture
2. That grazing saltbush with a barley supplement will increase hot carcass weight and dressing percentage compared to sheep grazing pasture
3. That grazing saltbush with a barley supplement will improve sheep meat eating quality compared to sheep grazing pasture
4. That grazing saltbush with a barley supplement will increase the vitamin E status of sheep compared to sheep grazing pasture
6.2. Year one

6.2.1. Materials and Methods

6.2.1.1. Experimental design

Sheep were either grazed on a saltbush based pasture system or a ‘control’ plot which consisted of barley stubble and senesced pasture. Both treatment groups were supplemented with barley grain with the aim of causing equal liveweight gain. Sheep were stratified based on liveweight prior to the start of the experiment.

6.2.1.2. Experimental site

This study was conducted 20 km from Goomalling, Western Australia (mean annual rainfall is 365 mm, Latitude 31°30'S and Longitude 116°82’E) on the property of Geoff and Debbie White. Fifty (2x25) 6 month old merino hogget wethers (average starting liveweight 38 kg) were grazed on either a saltbush or control plot for roughly 14 weeks. All sheep were given free access to scheme water. Analysis of the water using a conductivity-salinity-temperature meter (TPS Model WP-84, Brisbane, Australia), indicated a conductivity of 80 mS/m which is within the recommended range for livestock. This experiment took place during the summer-autumn period from 5 January to 3 May, 2003 (number of days=110). The experiment will be referred to as Goomalling 2003. See Appendix 2, plate 3 and 4 for photos of grazing site. The sheep were shorn during the experimental period on day 91.

Two saltbush species were present in the saltbush plot; old man saltbush (*Atriplex nummularia*) and wavy leaf saltbush (*Atriplex undulata*). The species between the rows of saltbush was predominantly (estimated at 80%) barley grass (*Hordeum leporinum*) with the remainder being a combination of annual ryegrass (*Lolium multiforum*), rumex (*Rumex acetosella*) and silvergrass (*Vulpia bromoides*). The control plot consisted predominantly of (estimated at 90%) barley
stubble (*Hordeum vulgare*) with the remaining pasture being annual ryegrass, barley grass and capeweed (*Arctotheca calendula*).  

**6.2.1.3. Supplementation protocol**

Sheep were supplemented with barley grain with the aim of allowing equal liveweight gain between the two treatments. The choice of barley grain enables the field experiments to be consistent with the animal house experiments. The amount of barley fed to each plot was adjusted weekly according to the weight gains of the animals (Table 6.1). Sheep were fed the grain in troughs every 2-4 days.
Table 6.1 Barley supplement offered (kg/head.day)

<table>
<thead>
<tr>
<th>Day</th>
<th>Saltbush</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>0.075</td>
<td>0.07</td>
</tr>
<tr>
<td>7</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>9</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>12-15</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>18</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>21</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>24–36</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>40</td>
<td>0.36</td>
<td>0.3</td>
</tr>
<tr>
<td>44-48</td>
<td>0.3</td>
<td>0.24</td>
</tr>
<tr>
<td>52</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>56</td>
<td>0.24</td>
<td>0.2</td>
</tr>
<tr>
<td>60-64</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>68-76</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>80-110</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

6.2.1.4. Sampling

6.2.1.4.1. Liveweight and liveweight gain

Liveweight was recorded on day 0, 6, 17, 27, 42, 58, 71, 91, and 100. Liveweight gain was calculated as described in chapter 3.
6.2.1.4.2. Estimation of pasture and saltbush on offer

Saltbush on offer was determined using the ‘Adelaide Technique’ described by Andrew et al. (Andrew et al. 1979) on days 0, 27, 58 and 100. Briefly, a ‘unit’ was taken from a random plant, representing 15-20% of the average shrub size. Approximately 30 shrubs per plot were scored for the number of equivalent ‘units’ that each bush contained, with 2 people simultaneously stating their estimate for each bush, then re-estimating if necessary to reach a consensus. Following estimation of units per plant, the leaves of the ‘unit’ were stripped, along with stems finer than 3mm in diameter. This edible component was dried at 80°C for 48 hours and weighed to determine the dry matter (DM) of the ‘unit’. The number of saltbushes per plot was counted and multiplied by the average units per plant, to calculate saltbush on offer. Separate saltbush on offer values were estimated for the old man and river species and then added together. Pasture of offer in the interrow of the saltbush paddock and throughout the control paddock was determined on days 0, 27, 58 and 100 of the grazing period. Twelve 0.3 m² random quadrant cuts were taken from each plot. The pasture samples were oven dried at 80°C for 48 hours and weighed to determine dry matter per 0.3 m² and subsequently converted to DM per hectare.

6.2.1.4.3. Sodium and saltbush intake

Saltbush intake was estimated using the sodium:creatinine ratio (Na: Creat) in a spot sample of urine (Chapter 5) on day 27 and 58. A spot sample of urine was taken from 10 sheep per treatment group. Urine was collected using a rubber pizzle harness with a 50 mL collection bottle (see Appendix 2, plate 4 for picture). A 10 mL subsample was collected and stored at –20°C prior to analysis. The sodium and creatinine concentration of the urine sample was analysed using methods detailed in chapter 3. Both values were converted to g/L and sodium divided by creatinine to give the sodium to creatinine ratio.
An estimate of the daily sodium intake was made using the formula:

\[ \text{Daily sodium intake} = 0.935^a \times (\text{Na:Creat}^b \times (\text{PCreat}^c)) + 1.3^a \]

Where:

^a This equation was derived in chapter 5

^b Na:Creat is the ratio of sodium and creatinine in the urine sample

^c PCreat is the daily creatinine excretion of an animal predicted as: \(0.0527 \times \text{Lwt}^{0.75}\) (Brody 1964)

Lwt is the liveweight of the animal at the time of sampling.

The intake of saltbush was then estimated:

\[ \text{Daily saltbush intake (kg DM/day)} = \frac{\text{Daily sodium intake}^d \,(g/d)}{\text{sodium content of the saltbush}^e \,(g/kg)} \]

Where:

^d Daily sodium intake derived from previous equation

^e Average sodium content of the old man and wavy leaf saltbush in the plot (Table 6.2)

### 6.2.1.4.4. Nutritive value and mineral content of pasture and saltbush on offer

Pasture samples from both the control and saltbush plots were collected, using random quadrants as outlined in section 6.2.1.4.2, on the same days as pasture and saltbush on offer were assessed. For each site and category and sample collection time the nutritive value and mineral composition was analysed using methods detailed in chapter 3. Samples were dried at 65°C for 48 hours.
6.2.1.5. Analyses

6.2.1.6. Slaughter and dissection procedures

On day 118, all 50 lambs were slaughtered at a commercial abattoir after undergoing a 12 hour period in farm curfew (held in sheep yards) followed 3 hours of transport to the abattoir and 12 hours in lairage.

All sheep had access to water in a trough in the corner of each pen but no access to feed whilst in lairage. The slaughter and dissection procedures are detailed in chapter 3. Hot carcass weight, GR depth, decline in temperature and pH were recorded within an hour of slaughter. Ultimate pH and colour were measured 24 h post slaughter. Also within an hour of slaughter a 2 g samples of the m. semimembranosus (SM) and the m. semitendinosus (ST) were taken and used to calculate muscle dry matter percentage (DM%) from each sheep. The bladders of each sheep were also collected after slaughter and the urine extracted, weighed and a sample taken to determine urine specific gravity (USG). Methods of DM and USG analysis are detailed in chapter 3. The m. longissimus thoracis et lumborum (LL) was removed 24 hours after slaughter from all sheep and immediately vacuum packed. This sample was then kept in a 2°C chiller for 5 days before being frozen in a −20°C to await sensory analysis.

6.2.1.7. Carcass composition

Half of each carcass was scanned using a DXA for yield estimates of the relative fat, lean and bone content of the animal according to methods in chapter 3.

6.2.1.8. Eating quality

A sensory analysis on the LL muscle was conducted using methods detailed in chapter 3. The meat remained frozen until sensory analysis. 20 loins were randomly chosen from the 25 animals
that were slaughtered from each treatment groups. Therefore the numbers of samples were: 40 * 
6 = 240. There were 40 panelists at each sitting and each panellist participated in 6 runs.

6.2.1.9. Statistical analysis

All statistical analysis in this chapter was carried out using Systat (SPSS Version 9.01). The 
statistical analytical procedures of the data from the sensory evaluation are detailed in chapter 3 
(section 3.4.4).

Analysis of variance was used to examine the effect of treatment on pre-slaughter liveweight, 
liveweight gain, hot carcass weight, GR depth, ultimate pH, colour at 24 hours, relative fat, lean 
and bone content urine specific gravity and dry matter content.
6.2.2. Results

6.2.2.1. Nutritive value and mineral content

Table 6.2 Nutritional and mineral content of saltbush and pasture on offer in control and saltbush plots (Average and range) (per unit of dry matter).
<table>
<thead>
<tr>
<th></th>
<th>Old man saltbush</th>
<th>Wavy saltbush</th>
<th>Saltbush pasture</th>
<th>Control pasture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ave</td>
<td>Range</td>
<td>Ave</td>
<td>Range</td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td>35.2</td>
<td>30.9-41.3</td>
<td>86.9</td>
<td>77.9-91.3</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>75.9</td>
<td>71.2-84.6</td>
<td>78.1</td>
<td>67-9-87.3</td>
</tr>
<tr>
<td>In vitro digestibility (%)</td>
<td>71.6</td>
<td>69.8-72.3</td>
<td>69.0</td>
<td>55.4-72.2</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>14.4</td>
<td>10.9-17.8</td>
<td>16.2</td>
<td>9.0-23.6</td>
</tr>
<tr>
<td>Neutral detergent fibre (%)</td>
<td>34.4</td>
<td>28.9-36.9</td>
<td>35.5</td>
<td>33.5-37.8</td>
</tr>
<tr>
<td>Acid detergent fibre (%)</td>
<td>19.1</td>
<td>16.1-22.3</td>
<td>21.1</td>
<td>18.3-29.3</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>4.22</td>
<td>3.9-4.4</td>
<td>3.99</td>
<td>2.5-4.5</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>3.54</td>
<td>3.53-3.59</td>
<td>2.95</td>
<td>1.2-5.6</td>
</tr>
<tr>
<td>Sulphur (%)</td>
<td>0.46</td>
<td>0.455-0.457</td>
<td>0.38</td>
<td>0.07-1.4</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.15</td>
<td>0.08-0.2</td>
<td>0.19</td>
<td>0.09-2.9</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.71</td>
<td>0.68-0.71</td>
<td>0.83</td>
<td>0.76-0.98</td>
</tr>
<tr>
<td>Chloride (%)</td>
<td>7.37</td>
<td>6.9-7.7</td>
<td>6.89</td>
<td>5.5-7.9</td>
</tr>
</tbody>
</table>
6.2.2.2. Liveweight and liveweight gain

There was no significant difference in liveweight between the groups at the start of the experiment (Figure 6.1). Both groups increased in liveweight from the start of experimental period until day 71 when shearing occurred. At the end of the experiment there was a significant difference in liveweight between the treatment groups with sheep fed the control treatment being heavier.

Figure 6.1 Liveweight of sheep in the control or saltbush paddock (Mean ± standard error)
6.2.2.3. Sodium and saltbush intake

The estimated saltbush intake of sheep was 0.89 kg DM at week 4 and 0.71 kg DM at week 8 (Table 6.3).

Table 6.3 Sodium and saltbush intake (Mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>Saltbush</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Intake (g/head.d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>35.6 ± 1.6</td>
<td>5.23 ± 0.09</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Week 8</td>
<td>28.4 ± 2.3</td>
<td>4.18 ± 0.04</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Saltbush Intake (kg/head.d DM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>0.89 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 8</td>
<td>0.71 ± 0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6.2.2.4. Saltbush and pasture on offer

There was a steady decline in availability of saltbush, saltbush understory and pasture on offer in the paddocks over the period of the experiment (Figure 6.2).
6.2.2.5. Carcass Quality

There was no difference between treatments for hot carcass weight (Table 6.4). The saltbush fed sheep had a lower GR depth compared to the control sheep. The saltbush fed sheep had a significantly lower fat and bone content and significantly higher lean content than the control sheep. There were no differences in meat biochemical attributes between treatments.
Table 6.4 Carcass characteristics of sheep grazing saltbush or control plots (Mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>Saltbush</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final liveweight (kg)</td>
<td>35.4 ± 0.7</td>
<td>38.4 ± 0.7</td>
<td>0.04</td>
</tr>
<tr>
<td>Hot carcass weight (kg)</td>
<td>14.5 ± 0.3</td>
<td>14.8 ± 0.4</td>
<td>0.9</td>
</tr>
<tr>
<td>GR fat depth (mm)</td>
<td>1.4 ± 0.2</td>
<td>2.1 ± 0.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Dressing percentage (%)</td>
<td>40.9 ± 0.7</td>
<td>38.5 ± 0.5</td>
<td>0.15</td>
</tr>
<tr>
<td>Fat content (% on carcass)</td>
<td>12.4 ± 0.4</td>
<td>14.3 ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Lean content (% on carcass)</td>
<td>84.4 ± 0.4</td>
<td>82.3 ± 0.3</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td>Bone content (% on carcass)</td>
<td>3.23 ± 0.08</td>
<td>3.47 ± 0.07</td>
<td>0.04</td>
</tr>
<tr>
<td>Colour L*</td>
<td>34.3 ± 0.4</td>
<td>34.9 ± 0.4</td>
<td>0.31</td>
</tr>
<tr>
<td>Colour a*</td>
<td>19.9 ± 0.3</td>
<td>19.9 ± 0.3</td>
<td>0.99</td>
</tr>
<tr>
<td>Colour b*</td>
<td>8.3 ± 0.2</td>
<td>8.5 ± 0.2</td>
<td>0.51</td>
</tr>
<tr>
<td>Ultimate pH SM</td>
<td>5.6 ± 0.02</td>
<td>5.6 ± 0.04</td>
<td>0.47</td>
</tr>
<tr>
<td>Ultimate pH ST</td>
<td>5.8 ± 0.05</td>
<td>5.8 ± 0.05</td>
<td>0.36</td>
</tr>
<tr>
<td>Ultimate pH LD</td>
<td>5.6 ± 0.04</td>
<td>5.7 ± 0.04</td>
<td>0.18</td>
</tr>
</tbody>
</table>

6.2.2.5.1. **Hydration status**

The saltbush fed sheep had a significantly lower USG compared to the control sheep (Table 6.5). The dry matter content of the SM, ST and LL muscles was significantly lower in the saltbush fed group. The saltbush fed sheep had a significantly higher urine weight.
Table 6.5 USG, muscle dry matter % and urine weights for lambs grazing saltbush or control plots (Mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>Saltbush</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>USG</td>
<td>1.016 ± 0.001</td>
<td>1.03 ± 0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>SM Muscle DM%</td>
<td>23.5 ± 0.28</td>
<td>24.6 ± 0.24</td>
<td>0.001</td>
</tr>
<tr>
<td>ST Muscle DM%</td>
<td>22.8 ± 0.26</td>
<td>23.6 ± 0.28</td>
<td>0.002</td>
</tr>
<tr>
<td>Urine weight (g)</td>
<td>73.1 ± 14.6</td>
<td>30.1 ± 6</td>
<td>0.001</td>
</tr>
</tbody>
</table>

6.2.2.6. Eating Quality

Grazing saltbush had no significant effect on eating quality (Table 6.6).

Table 6.6 Eating quality on a score out of ten (Mean ± standard error)

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Saltbush</th>
<th>Control</th>
<th>P value</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour strength</td>
<td>5.0 ± 0.9</td>
<td>5.0 ± 0.9</td>
<td>&gt;0.1</td>
<td>0=weak, 10=strong</td>
</tr>
<tr>
<td>Liking of odour</td>
<td>5.4 ± 0.6</td>
<td>6.1 ± 0.6</td>
<td>&gt;0.1</td>
<td>0=dislike, 10=like</td>
</tr>
<tr>
<td>Flavour strength</td>
<td>4.2 ± 0.7</td>
<td>5.6 ± 0.7</td>
<td>&gt;0.1</td>
<td>0=weak, 10=strong</td>
</tr>
<tr>
<td>Liking of flavour</td>
<td>5.9 ± 0.6</td>
<td>5.7 ± 0.6</td>
<td>&gt;0.1</td>
<td>0=dislike, 10=like</td>
</tr>
<tr>
<td>Tenderness</td>
<td>5.4 ± 0.8</td>
<td>5.4 ± 0.8</td>
<td>&gt;0.1</td>
<td>0=tough, 10=tender</td>
</tr>
<tr>
<td>Juiciness</td>
<td>5.8 ± 0.8</td>
<td>5.4 ± 0.8</td>
<td>&gt;0.1</td>
<td>0=dry, 10=juicy</td>
</tr>
<tr>
<td>Residual mouth feel</td>
<td>4.0 ± 0.7</td>
<td>3.9 ± 0.7</td>
<td>&gt;0.1</td>
<td>0=weak, 10=strong</td>
</tr>
<tr>
<td>Overall acceptance</td>
<td>5.5 ± 0.7</td>
<td>5.9 ± 0.7</td>
<td>&gt;0.1</td>
<td>0=dislike, 10=like</td>
</tr>
</tbody>
</table>
6.3. Year two

6.3.1. Materials and methods

6.3.1.1. Experimental design, site and animals

In year 2, sheep were again grazed on a saltbush based pasture system or a ‘control’ plot which consisted of barley stubble and senesced pasture on the property of Ashley Lewis in Wickepin (to be referred to as Wickepin 2004). Eighty (2x40) 18 month old merino hogget wethers (average liveweight 48 kg) were used. Grazing period was 14 weeks, from 5 January to 28 April 2004 (number of days=114). All treatment groups were supplemented with barley grain with the aim of achieving equal liveweight gain across the 2 treatments. For the 2 treatments, sheep were stratified based on liveweight prior to the start of the experiment. All sheep were given free access to scheme water with a conductivity of 76 mS/m. See appendix 2, plates 5, 6 and 7 for pictures. The sheep were shorn during the experimental period on day 93.

Immediately prior to the start of the experiment all sheep were given:

- 5 mL oral anthelmintic drench (Scanda, Coopers Animal Health, Schering-Plough, Baulkham Hills NSW)
- 1 mL of Glanvac 5 in 1 vaccine with added selenium (CSL Ltd, Australia).

Two saltbush species were present in the saltbush plot; old man saltbush and river saltbush. The species between the rows of saltbush were 80% barley grass with the remainder being a combination of puccinellia (*Puccinellia ciliata*), mintweed (*Salvia reflexa*), annual ryegrass (*Lolium multiforum*), rumex (*Rumex acetosella*) and silvergrass (*Vulpia bromoides*). The control plot consisted of 50% capeweed (*Arctotheca calendula*), 10% barley stubble, 10% woolly clover (*Trifolium tomentosum*) with the remaining pasture being annual ryegrass and barley grass (*Hordeum leporinum*).
At the start of the experiment, all animals were also orally supplemented with 2500 IU of dl-\(\alpha\)-tocopherol acetate in 6.25 mL of solution (equivalent to 1520 mg of \(\alpha\)-tocopherol) (Advanced Feeds, WA). Judson et al. (1991) gave a similar dose and observed a peak in plasma concentrations within 4 days after administration and a return to untreated levels 7–21 days later. The liver concentrations peaked 7 days after administration and were back to levels similar to those at the start of the experiment after 64 days. These results suggested that in the absence of dietary vitamin E the tissue levels would have returned to levels at or below the levels at the start of the experiment. Therefore, we believed that this dose was sufficient to prevent clinical vitamin E deficiency, but still low enough to allow detection of any response to additional vitamin E.

6.3.1.2. Supplementation

Sheep were supplemented with barley grain to allow for equal liveweight gain between the two treatments. The amount of barley fed to each plot was adjusted weekly according to the weight gains of the animals (Table 6.7). Sheep were fed the grain in troughs every 4 days.
Table 6.7 Barley supplement offered to each treatment group (kg/head.day) for Wickepin 2004.

<table>
<thead>
<tr>
<th>Day</th>
<th>Saltbush</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 4</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>7</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>9</td>
<td>0.09</td>
<td>0.09</td>
</tr>
<tr>
<td>12- 15</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>18</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>21</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>24</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>28</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>32-60</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>64-92</td>
<td>0.3</td>
<td>0.25</td>
</tr>
<tr>
<td>94-103</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

6.3.1.3. **Sampling**

6.3.1.3.1. **Liveweight and liveweight gain**

Liveweight was recorded on day 0, 15, 28, 42, 53, 69, 89, 103 and 108. Liveweight gain was calculated as described in chapter 3.

6.3.1.3.2. **Estimation of pasture and saltbush on offer**

Estimation of pasture and saltbush on offer was measured on days 0, 28, 53 and 103 using methods detailed in section 6.2.1.4.2.
6.3.1.3.3.  Sodium and saltbush intake

Estimation of sodium and saltbush intake was measured on days 0, 28, 53 and 103 using methods detailed in section 6.2.1.4.3.

6.3.1.3.4.  Nutritional value and mineral and vitamin content of pasture and saltbush on offer

Pasture samples and samples of the old man and river saltbush were collected on days 0, 28, 53 and 103. For each plot and category the samples were bulked and the nutritional and mineral composition was analysed using methods detailed in chapter 3. Samples were dried at 65°C for 48 hours prior to analysis.

A combined sample of old man and river saltbush and a barley grain sample were collected at the end of the experiment and chilled at 4°C prior until analysis for α-tocopherol and polyunsaturated fatty acids (PUFA). Four week old green subterranean clover (*Trifolium subterraneum*) and annual ryegrass samples were collected in July 2004 and bulked for polyunsaturated fatty acid (PUFA) analysis.

6.3.1.3.5.  Blood sampling

Blood was collected by jugular venipuncture in heparinised tubes from the same 10 sheep in each of the 2 treatment groups on days 0, 28, 53 and 103. All tubes were placed on ice and centrifuged at 3000 rpm within 30 minutes of collection, plasma decanted and stored at –20°C prior to plasma α–tocopherol analysis.
6.3.1.4. Analysis

6.3.1.4.1. Determination of α-tocopherol concentration in muscle, plasma, liver and saltbush samples

The α-tocopherol content of the saltbush, barley, muscle, liver and plasma were measured using the HPLC method of McMurray et al. (1979). Feed and tissues were saponified by the method of Bieri et al. (1961) prior to the analysis.

6.3.1.4.2. Determination of polyunsaturated fatty acid content in feed

The extracted fatty acids were methylated using boron trifluoride and methanolic sodium hydroxide and then quantified by capillary gas chromatography using the method of Blight and Dyer (1959). The percentages of oleic (18:1), linoleic (C18:2, n-6) and linolenic (C18:3, n-3) acids in the plant were determined.

6.3.1.5. Slaughter and dissection procedures

On day 114, 25 sheep per treatment (n=50) were slaughtered at a commercial abattoir after undergoing a 24 hour period in farm curfew followed a 4 h transport period to the abattoir and 24 hours in lairage.

All sheep had access to water in a trough in the corner of each pen but no access to feed whilst in lairage. The slaughter and dissection procedures are detailed in chapter 3. Hot carcass weight, GR depth, decline in temperature and pH, ultimate pH and colour were obtained. After slaughter 2 g samples of the m. semimembranosus (SM), m. semitendinosus (ST) and m. longissimus thoracis et lumborum (LL) were taken and used to calculate muscle dry matter percentage (DM%) from each sheep. The bladders of each sheep were also collected after slaughter and the urine extracted, weighed and a sample taken to determine urine specific gravity (USG). Methods
of DM and USG analysis are detailed in chapter 3. The LL was removed 24 hours after slaughter from all sheep and immediately vacuum packed. This sample was then kept in a 2°C chiller for 5 days before being frozen in a –20°C to await sensory analysis.

A 200 g sample of the liver from all 50 animals was obtained at slaughter, kept chilled at 4°C and analysed for α-tocopherol within 5 days of slaughter. Twenty four hours after slaughter 100 g samples of the LL were collected for colour stability assessment from 15 sheep per treatment. Additionally 50 g samples were taken from the LL for α–tocopherol analysis and a 200 g sample for drip loss analysis. Drip loss and colour stability assessment were performed immediately as described below. Samples of the LL for α-tocopherol analysis were vacuum-sealed 3 hours after collection and kept chilled until analysis 7 days later.

6.3.1.6. Meat colour

Samples of the LL were placed on black foam trays, wrapped with polyvinyl (Vinylwrap Resinite, AEP Industries Australia) and heat sealed. The samples were stored at 4°C for 7 days under flourescent lights (100-1500 Lux).

Colour and reflectance readings were recorded each day over the 5 day period from each muscle sample using a Hunter Lab Mini Scan™ XE Plus (Cat. No. 6352, model No. 45/0-L, Hunter Associates Laboratory Inc., Reston, VA., USA), using C set as the light source with a aperture set to 10. The instrument was calibrated on a white tile and black glass as specified by the manufacturer. The "L a b" scores were read using the colour program.

The reflectance at wavelengths of 580 nm and 630 nm were read using the reflectance program. The daily ratio of reflectance values at 630 nm to 580 nm allows for an indirect estimate of surface metmyoglobin levels (Stewart et al. 1965; Strange et al. 1974).
6.3.1.7. **Drip and cooking loss**

The sample of LL was firstly weighed (A) then a 0.5 cm$^2$ commercial net material was tied around the sample and suspended in a plastic bag for 4 days at 4°C. The sample was weighed again after 4 days (B), placed in a plastic bag and tied so no fluid could escape and immersed in an 80°C water bath for 1 hour. Samples were then reweighed (C). Drip loss percentage was calculated as $[(A-B)/A]*100$. Cooking loss percentage was calculated as $[(B-C)/B]*100$.

6.3.1.8. **Carcass composition**

Half of each carcass was scanned by a DXA to yield estimates of the relative fat, lean and bone content of the animal according to methods in chapter 3.

6.3.1.9. **Eating quality**

Sensory analyses on the LL muscle from the Wickepin 2004 experiment were conducted using methods detailed in chapter 3 (section 3.4). The meat remained frozen until sensory analysis. Twenty loins were randomly chosen from the 25 animals slaughtered in each treatment group. Therefore the numbers of samples were: 40 * 6 = 240 samples. There were 40 panelists each sitting and each panellist participated in 6 runs.

6.3.1.10. **Statistical analysis**

All statistical analysis in this chapter was carried out using Systat (SPSS Version 9.01). The statistical analytical procedures of the data from the sensory evaluation are detailed in chapter 3 (section 3.4).

Analysis of variance was used to examine the effect of treatment on:
• Pre-slaughter liveweight, liveweight gain, hot carcass weight, GR depth, ultimate pH, colour at 24 hours, relative fat, lean and bone content urine specific gravity and dry matter content.

• Muscle and liver \( \alpha \)-tocopherol concentrations and drip and cooking loss

• Plasma \( \alpha \)-tocopherol concentrations at each time point

• \( L, a, b \) and reflectance ratio results at individual times and as a repeated measures analysis.
### 6.3.2. Results

#### 6.3.2.1. Nutritive value and mineral content

Table 6.8 Nutritional and mineral content of old man and river saltbush and pasture on offer in control and saltbush plots (Average and range) (per unit dry matter).

<table>
<thead>
<tr>
<th></th>
<th>Old man saltbush</th>
<th>River saltbush</th>
<th>Saltbush pasture</th>
<th>Control pasture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (%)</td>
<td>30.3</td>
<td>52.3</td>
<td>65.0</td>
<td>65.7</td>
</tr>
<tr>
<td></td>
<td>23.7-43.6</td>
<td>43.9-62.7</td>
<td>56.8-76.7</td>
<td>63.9-69.4</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>77.8</td>
<td>81.3</td>
<td>67.1</td>
<td>89.1</td>
</tr>
<tr>
<td></td>
<td>74.3-82.3</td>
<td>78.4-83.6</td>
<td>54.4-78.3</td>
<td>87.3-90.6</td>
</tr>
<tr>
<td>In vitro digestibility (%)</td>
<td>69.5</td>
<td>60.1</td>
<td>51.6</td>
<td>61.3</td>
</tr>
<tr>
<td></td>
<td>63.7-74.5</td>
<td>52.7-62.5</td>
<td>49.2-53.7</td>
<td>60.7-61.7</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>11.0</td>
<td>8.4</td>
<td>5.9</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>8.1-13.1</td>
<td>6.75-9.3</td>
<td>5.25-6.5</td>
<td>1.58-1.72</td>
</tr>
<tr>
<td>Neutral detergent fibre (%)</td>
<td>37.2</td>
<td>46.9</td>
<td>60.5</td>
<td>53.9</td>
</tr>
<tr>
<td></td>
<td>29.9-44.7</td>
<td>44.1-48.1</td>
<td>56.8-63.8</td>
<td>51.4-55.8</td>
</tr>
<tr>
<td>Acid detergent fibre (%)</td>
<td>20.5</td>
<td>27.0</td>
<td>43.9</td>
<td>36.1</td>
</tr>
<tr>
<td></td>
<td>17.5-24.8</td>
<td>25.8-27.7</td>
<td>36.1-49.8</td>
<td>33.8-37.4</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>4.33</td>
<td>3.83</td>
<td>0.68</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>1.3-5</td>
<td>3.3-4.5</td>
<td>0.18-1.08</td>
<td>0.1-0.23</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>2.52</td>
<td>1.87</td>
<td>0.46</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>1.4-2.8</td>
<td>1.5-2.1</td>
<td>0.1-0.7</td>
<td>0.32-0.7</td>
</tr>
<tr>
<td>Sulphur (%)</td>
<td>0.41</td>
<td>0.29</td>
<td>0.17</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>0.36-0.45</td>
<td>0.28-0.29</td>
<td>0.15-0.21</td>
<td>0.11-0.13</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.11</td>
<td>0.11</td>
<td>0.12</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>0.09-0.12</td>
<td>0.09-0.12</td>
<td>0.89-0.16</td>
<td>0.12-0.13</td>
</tr>
<tr>
<td></td>
<td>0.54</td>
<td>0.4-0.63</td>
<td>0.56</td>
<td>0.54-0.57</td>
</tr>
<tr>
<td>----------</td>
<td>-------</td>
<td>----------</td>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium (%)</td>
<td>0.55</td>
<td>0.4-0.62</td>
<td>0.46</td>
<td>0.39-0.51</td>
</tr>
<tr>
<td>Chloride (%)</td>
<td>7.97</td>
<td>6.6-8.4</td>
<td>6.26</td>
<td>5.2-7</td>
</tr>
</tbody>
</table>
6.3.2.2. Liveweight and liveweight gain

There were no significant differences in liveweight between the groups at the start of the experiment (Figure 6.3). At the end of the experiment there were no significant differences in liveweight between the treatment groups. There was a significant difference at day 20 and 40 with the saltbush sheep having a higher liveweight.

Figure 6.3 Liveweight of sheep grazing saltbush or control diet (Mean ± standard error)

6.3.2.3. Sodium and saltbush intake

The estimated saltbush intake of sheep increased from an initial 0.85 kg dry matter per day at week 2 to a maximal intake of 1.2 kg/day at week 8 and then declined after this time to 0.7 kg at week 12 (Table 6.9).
### Table 6.9 Estimated sodium and saltbush intake (Mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>Saltbush</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sodium intake (g/head.d)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>34 ± 1.6</td>
<td>4.8 ± 1.0</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Week 4</td>
<td>44 ± 4.4</td>
<td>3.9 ± 0.6</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Week 8</td>
<td>48 ± 4.0</td>
<td>3.2 ± 0.5</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Week 12</td>
<td>28 ± 2.8</td>
<td>2.9 ± 0.3</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Week 12</td>
<td>0.85 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Saltbush Intake (kg DM/head.d)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 4</td>
<td>1.1 ± 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 8</td>
<td>1.2 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 12</td>
<td>0.75 ± 0.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 6.3.2.4. Saltbush and pasture on offer

There was a steady decline in availability of saltbush, saltbush understory and pasture on offer in the paddocks over the period of the experiment (Figure 6.4).
6.3.2.5. Carcass Quality

The saltbush fed sheep had a significantly lower dressing percentage and trend for a lower hot carcass weight compared to the control sheep (Table 6.10). The saltbush fed sheep had a lower GR depth compared to the saltbush fed sheep. The saltbush fed sheep had a significantly lower fat content and significantly higher lean content than the control sheep. The ultimate pH of the SM muscle for the saltbush fed sheep was significantly higher than the control fed sheep.
Table 6.10 Carcass characteristics of sheep fed saltbush on control diet for the Wickepin 2004 experiment (mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>Saltbush</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final liveweight (kg)</td>
<td>47.1 ± 1.1</td>
<td>47.7 ± 0.9</td>
<td>0.89</td>
</tr>
<tr>
<td>Hot carcass weight (kg)</td>
<td>18.8 ± 0.3</td>
<td>19.4 ± 0.3</td>
<td>0.18</td>
</tr>
<tr>
<td>GR fat depth (mm)</td>
<td>1.9 ± 0.3</td>
<td>4.1 ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Dressing percentage (%)</td>
<td>36.5 ± 0.4</td>
<td>38.0 ± 0.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Fat content (% on carcass)</td>
<td>15.6 ± 0.7</td>
<td>18.0 ± 0.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Lean content (% on carcass)</td>
<td>80.2 ± 0.7</td>
<td>77.6 ± 0.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Bone content (% on carcass)</td>
<td>4.2 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>0.52</td>
</tr>
<tr>
<td>Ultimate pH SM</td>
<td>5.77 ± 0.03</td>
<td>5.68 ± 0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Ultimate pH ST</td>
<td>6.17 ± 0.06</td>
<td>6.04 ± 0.06</td>
<td>0.15</td>
</tr>
<tr>
<td>Ultimate pH LD</td>
<td>5.78 ± 0.03</td>
<td>5.68 ± 0.03</td>
<td>0.055</td>
</tr>
</tbody>
</table>

6.3.2.5.1. **Hydration status**

The saltbush fed sheep had a significantly lower USG compared to the pasture fed sheep (Table 6.11). The dry matter content of the SM, ST and LL muscles was significantly lower in the saltbush fed group. The saltbush fed sheep had a significantly higher urine weight.
Table 6.11 USG, muscle dry matter % and urine weights for sheep fed saltbush or control diet (Mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>Saltbush</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>USG</td>
<td>1.019 ± 0.001</td>
<td>1.029 ± 0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>SM Muscle DM%</td>
<td>24.1 ± 0.3</td>
<td>25.8 ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td>ST Muscle DM%</td>
<td>23.5 ± 0.29</td>
<td>24.1 ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td>LL muscle DM%</td>
<td>26.8 ± 0.59</td>
<td>28.5 ± 1.72</td>
<td>0.04</td>
</tr>
<tr>
<td>Urine weight (g)</td>
<td>61.6 ± 8.1</td>
<td>34.7 ± 7.2</td>
<td>0.02</td>
</tr>
</tbody>
</table>

6.3.2.5.2. Drip and cooking loss

There were no significant differences in drip and cooking loss between the treatments (P>0.05) (Table 6.12).

Table 6.12 Drip and cooking loss of LL (%) (Mean±standard error)

<table>
<thead>
<tr>
<th></th>
<th>Saltbush</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drip loss</td>
<td>1.38 ± 0.26</td>
<td>1.21 ± 0.24</td>
<td>0.32</td>
</tr>
<tr>
<td>Cooking Loss</td>
<td>30.53 ± 1.44</td>
<td>30.8 ± 1.28</td>
<td>0.54</td>
</tr>
</tbody>
</table>

6.3.2.5.3. Meat colour stability

There was a significant effect of treatment and time on lightness (L*), redness (a*) and yellowness (b*) (P<0.05) (Figure 6.5). For both treatments the L* value increased with time but was significantly higher for the lambs fed saltbush than lambs fed pasture (P < 0.04) over the entire 5 day assessment period. The a* values were significantly higher for the sheep fed saltbush on days 4 and 5 compared to sheep fed pasture (P < 0.001). The a* values increased up to day 2 then declined to levels similar to those at the start for both groups. The yellowness (b*)
of the fat increased with time for both groups but was significantly higher at day 4 for the saltbush fed sheep compared to the pasture fed sheep. There was a significant effect of treatment, time and interaction between time and treatment for the reflectance ratio ($P < 0.05$). The reflectance ratio of the pasture fed sheep was significantly ($P < 0.01$) lower than for saltbush fed lambs at days 4 and 5. The ratio decreased over time for both groups however crossed over from day 1 to 2.
Figure 6.5 L*, a* and b* values and reflectance ratios of sheep fed saltbush and control diet in Wickepin 2004 experiment post slaughter (Mean±standard error).
6.3.2.6. Eating Quality

Grazing saltbush had no significant effect on any of the eating quality characteristics (Table 6.13).

Table 6.13 Eating quality on a score out of ten (mean ± standard error)

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Saltbush</th>
<th>Control</th>
<th>P value</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour strength</td>
<td>4.8 ± 1.2</td>
<td>4.7 ± 1.2</td>
<td>&gt;0.1</td>
<td>0=weak, 10=strong</td>
</tr>
<tr>
<td>Liking of odour</td>
<td>6.0 ± 0.9</td>
<td>6.3 ± 0.9</td>
<td>&gt;0.1</td>
<td>0=dislike, 10=like</td>
</tr>
<tr>
<td>Flavour strength</td>
<td>5.6 ± 0.9</td>
<td>5.7 ± 0.9</td>
<td>&gt;0.1</td>
<td>0=weak, 10=strong</td>
</tr>
<tr>
<td>Liking of flavour</td>
<td>6.7 ± 0.9</td>
<td>6.6 ± 0.9</td>
<td>&gt;0.1</td>
<td>0=dislike, 10=like</td>
</tr>
<tr>
<td>Tenderness</td>
<td>6.7 ± 0.9</td>
<td>6.7 ± 0.9</td>
<td>&gt;0.1</td>
<td>0=tough, 10=tender</td>
</tr>
<tr>
<td>Juiciness</td>
<td>6.5 ± 0.9</td>
<td>7.1 ± 0.9</td>
<td>&gt;0.1</td>
<td>0=dry, 10=juicy</td>
</tr>
<tr>
<td>Residual mouth feel</td>
<td>2.8 ± 0.9</td>
<td>3.3 ± 0.9</td>
<td>&gt;0.1</td>
<td>0=weak, 10=strong</td>
</tr>
<tr>
<td>Overall acceptance</td>
<td>6.5 ± 0.8</td>
<td>7.2 ± 0.8</td>
<td>&gt;0.1</td>
<td>0=dislike, 10=like</td>
</tr>
</tbody>
</table>

6.3.2.7. Vitamin E status

6.3.2.7.1. Vitamin E and PUFA content of feed

The vitamin E content of river saltbush, old man saltbush and barley was 116 mg/kg DM, 139 mg/kg DM and 6 mg/kg DM respectively. The percentage (expressed as a percentage of total fatty acids) of oleic (18:1), linoleic (C18:2, n-6) and linolenic (C18:3, n-3) acids in the old man saltbush was 8.24, 23.98 and 34.9 %, in the river saltbush was 3.98, 24.26 and 46.3 % and in the green clover, ryegrass mix was 2.6, 11.8 and 67.8%. The sum of PUFA was 58.5 %, 70.5 % and 81.7 % in the old man saltbush, river saltbush and clover ryegrass mix respectively.
6.3.2.7.2. Muscle and liver $\alpha$-tocopherol levels

The saltbush grazed sheep had significantly higher LL muscle and liver $\alpha$-tocopherol concentrations compared to the control group at the end of the experiment ($P < 0.001$) (Table 6.14).

Table 6.14 LL muscle and liver $\alpha$-tocopherol concentrations (mg/kg fresh weight) (Mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>Saltbush</th>
<th>Control</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL Muscle $\alpha$-tocopherol</td>
<td>6.3 ± 0.18</td>
<td>2.4 ± 0.29</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Liver $\alpha$-tocopherol</td>
<td>11.3 ± 0.79</td>
<td>1.8 ± 0.17</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

6.3.2.7.3. Plasma $\alpha$-tocopherol levels

The plasma concentrations of $\alpha$-tocopherol were not significantly different between treatments at the start of the experiment or at week 4. The concentrations increased for both groups until week 4 for the control group and week 8 for the saltbush group. There were significant differences between treatments at week 8 ($P < 0.001$) and week 12 ($P < 0.05$) (Figure 6.6).

Figure 6.6 Plasma $\alpha$-tocopherol concentration (Mean ± standard error)
6.4. Discussion

6.4.1. Carcass quality

6.4.1.1. Fatness

For both the Goomalling 2003 and Wickepin 2004 experiments the saltbush fed sheep had significantly higher proportion of lean and significantly less fat than sheep grazing the control pasture plot. These results in the field support the findings in the animal house experiment in chapter 4. The reasons for this result are likely to be different between the two field experiments.

For the Wickepin 2004 experiment, the lower deposition of fat at the same carcass weight may be due to a higher protein:energy ratio of the sheep grazing saltbush compared to those on the control plot. A higher protein:energy ratio has been shown to decrease the rate of fat deposition and increase the rate of protein deposition, providing adequate energy intake to facilitate the conversion of protein into lean tissue (Campbell 1988). The ratio of protein:energy would have been higher firstly because saltbush generally contains high quantities of protein and the increased rate of passage would have increased the quality and quantity of protein delivered to the small intestine for absorption. Secondly, the combination of reduced VFA production and absorption in the rumen and an increased energy output due to the processing of the high salt load would have also contributed to a reduced energy supply to the animal. No hormonal studies were conducted as part of this experiment but it can be assumed that similar results to those in chapter 4 may have been observed. The hormonal results in chapter 4 were significantly lower circulating insulin and IGF and higher growth hormone concentration. The lower insulin levels would result in less fat deposition and the higher growth hormone levels would favour increased lean tissue deposition. However the low IGF levels would result in lower overall levels of growth of lean tissue and liveweight. Therefore the overall increase in the protein:energy ratio and hormonal patterns preventing fat deposition would have resulted in leaner carcasses.
For the Goomalling 2003 experiment, the weight loss over the last few weeks confounded the carcass composition of the sheep. Although there was a difference in fat content between groups at slaughter, the saltbush sheep did have significantly greater rate of liveweight loss that may have been mostly fat loss during this period. Based on these results it is not possible to conclusively say that grazing saltbush has resulted in a decreased rate of fat deposition and increased rate of protein deposition although the results are consistent with carcass results in other experiments.

### 6.4.1.2. Hot carcass weight and growth performance

The hypothesis that grazing a saltbush based saline pasture system would improve hot carcass weight was not supported. For the Wickepin 2004 experiment, grazing saltbush resulted in similar hot carcass weights compared to sheep grazing the pasture/stubble control plot. Despite this finding the liveweight gains in the saltbush group were not impressive. An overall liveweight gain of 20 g/head.day for the saltbush sheep and 31 g/head.day for the control plot was observed.

In agreement, the hot carcass weights of the sheep grazing saltbush in the Goomalling 2003 experiment were similar to those on the control plot. Both groups lost weight in the final weeks of the experiment but the loss was more pronounced in the saltbush fed sheep. A logical explanation for these results is that the prediction of feed on offer and therefore the feasible length of the grazing period was inaccurate. This was an unfortunate finding because up until this point the sheep were steadily gaining weight well on the saltbush. An additional problem may have been that the sheep were not shorn until day 91. A frequent practice used by farmers when finishing animals is to shear the sheep at least 6 weeks before slaughter. Its believed that
shearing will enable the sheep will direct nutrients towards liveweight gain rather than wool growth. The increases in liveweight following shearing are an example of this.

The growth results in this study are consistent with those in the study by Hopkins et al. (1999) who also observed low growth rates of approximately 50 g/head.day (D.L. Hopkins, Pers. Comm as results not published) despite supplementation with hay or grain. The low growth rates on saltbush are also supported in the studies by Chriyaa et al. (1997) and Franklin-McEvoy (2002). The likely reasons for the low growth rates from saltbush are the low feed intake due to the high salt content of the saltbush, low edible biomass available and poor energy utilisation due to the processing of the high salt load. Reflective of the low growth rates is the pattern of liveweight change over the experiment with the sheep only gaining weight over the first 6 days in experiment 1 and 30 d for experiment 2 and were just at or below maintenance for the reminder of the experiments. Although speculative, it is possible that the liveweight gain for the first 6/30 days is attributable to the retention of fluid following the consumption of a high salt diet. For the remainder of the grazing period, the maximum fluid retention has occurred and no more liveweight is gained. This finding further indicates that the ration fed was low in digestible nutrients and energy utilisation was poor.

A potentially valuable finding from this chapter is that the use of older animals, as in the hoggets in Wickepin 2004, in which higher liveweight gains were achieved with thos group of animals, may be a more successful strategy than using lambs. The combination of processing excess salt and growing appears to be counter-productive in utilising saltbush to finish lambs. Further research to understand the potential of finishing mutton or hogget aged animals on saline land saltbush may be more commercially valuable, providing there are niche markets available for this product.
6.4.2. Hydration status

The hypothesis that the consumption of saltbush prior to slaughter would improve hydration status at slaughter and result in improved hot carcass weights and dressing percentages compared to sheep grazing the control plot was not supported in either experiment. However, the consumption of saltbush did result in increased fluid content in the muscles of the saltbush fed sheep in both experiments as indicated by a significantly lower muscle dry matter content. There are two reasons why the saltbush fed sheep had a higher amount of retained fluid compared to the control fed sheep. Firstly and most likely, the increased fluid retention is due to the higher lean content of the carcass. Higher levels of lean on an animal are associated with increased body water content (Michell 1985; Searle 1970) (and Section 4.3.3.2) which may be reflected as a higher fluid content in the muscles at slaughter. Secondly, the high salt and betaine intake from consuming saltbush may also result in increased fluid retention in the muscles. Salt has been shown to increase fluid retention (Searle 1970), a homeostatic process to maintain blood tonicity. Betaine is speculated also to assist in the retention of fluid in the cell to due to its role in maintaining osmotic pressure under conditions of dehydration (Ekland et al. 2005). Despite the significantly higher muscle dry matter in the saltbush grazed sheep this did not reflect into higher carcass or muscle weights probably because the fluid difference (calculated to approximately 300g) was not great enough between the treatments (i.e. average muscle dry matter content was 24.1% for the saltbush and 25.8% for the control which represents a 75.9 and 74.2% fluid content, multiply this value by the liveweight of the animal= 35.7 kg and 35.4 kg of water in the carcasses of the saltbush and control treatments respectively).

The consumption of saltbush also resulted in a significantly lower urine specific gravity and significantly higher urine weight at slaughter compared to the control fed sheep. The decreased specific gravity and increased urine weight could either be due to fluid consumption in lairage stimulated by the consumption of a high salt load on farm. Alternatively, the excretion of fluid originating from the consumption and subsequent retention of high amounts of water on farm
may also reduce the urine concentration and increase the amount of urine excreted. In the absence of measurements of water intake in lairage it is difficult to confirm the cause of the lower urine concentration and higher urine weight of the saltbush fed sheep.

This chapter has not indicated any benefits for improved carcass quality via changes to the hydration status from consuming saltbush over a long term period. However under conditions where the body composition of the animals remains the same, short term strategic feeding of saltbush or salt and betaine may reduce carcass weight loss and increased dressing percentages. The consumption of the high salt and betaine load in the saltbush whilst on farm could result in increased water consumption in lairage but also fluid retention that could essentially buffer the animals against dehydration. Further work is needed to elucidate of the potential benefits of short term consumption of salt and or betaine prior to slaughter.

6.4.3. **Vitamin E status**

This study has demonstrated that saltbush is a potential source of vitamin E for grazing sheep, with levels of \(\alpha\)-tocopherol of 139 mg/kg dry matter and 116 mg/kg in old man and river respectively. Saltbush provided equal if not higher levels of vitamin E to those found in high quality green pasture. Gabbedy *et al.* (1977) and Tramontano *et al.* (1993) reported the vitamin E content of green pasture ranged from 50-200 mg/kg DM. In contrast, the levels in dry senesced pasture available during the summer and autumn period in areas with a Mediterranean climate are between 2-20 mg/kg DM (Hardy *et al.* 1983; Tramontano *et al.* 1993). The barley grain was also very low in vitamin E, containing 6 mg/kg of \(\alpha\)-tocopherol. Clearly, saltbush has the potential to provide vitamin E during periods where dry senesced pasture, grains and stubbles are the dominant feed source.
The daily vitamin E intake of the saltbush grazed sheep was estimated to be 106 mg/d from the saltbush at week 4 (average daily vitamin E intake was determined by multiplying daily intake of saltbush in kg/d by average \( \alpha \)-tocopherol content of the saltbush in mg/kg), 150 mg/d at week 8 and 93 mg/d at week 12. The pasture grazed sheep received approximately 2-20 mg/d of vitamin E (assuming 1 kg of pasture intake per day). The National Research Council (1985) recommends a daily intake of 15 mg/kg DM.

The high vitamin E intake of the sheep grazing saltbush elevated tissue concentrations of vitamin E. \( \alpha \)-Tocopherol concentrations in the plasma over the period of the experiment and liver and muscle samples at slaughter in the saltbush grazed sheep were significantly higher than in the control group. The observed levels in the saltbush group were above the threshold concentration required to prevent nutritional myopathy. Adequate levels are 1.2 mg/kg, 1.8 mg/kg and 1 mg/L in liver, muscle and plasma respectively (Hidiroglou 1987; Judson et al. 1991). The control group had levels close to the threshold for all tissues studied although no sheep displayed any obvious signs of subclinical or clinical myopathy. This may have been due to the vitamin E treatment prior to the start of the experiment.

The liver is a short-term store of \( \alpha \)-tocopherol. \( \alpha \)-Tocopherol is bound to a carrier protein in the liver and this complex is then released into circulation (Machlin 1980). The liver is often used as a measure of vitamin E status (Puls 1994). Significantly higher liver concentrations (11.8 mg/kg wet weight) were observed in the saltbush grazed sheep compared to the livers from sheep fed dry pasture and stubble (1.8 mg/kg wet weight). In comparison, Hidiroglou et al. (1994) demonstrated that adding dl-\( \alpha \)-tocopherol acetate to the diet of lambs at a rate of 300 mg/head.day for 60 days resulted in liver concentrations of 16 mg/kg wet weight of \( \alpha \)-tocopherol compared to 3 mg/kg in lambs given a diet supplying approximately 17 mg vitamin E/head.day.
The muscle concentration of α-tocopherol at slaughter was also significantly higher in the saltbush grazed animals. Ochoa et al. (1992) reported concentrations of 15 mg/kg of α-tocopherol in the LL muscle of sheep fed 1000 mg/head.day of dl-α-tocopherol acetate for 56 days.

Plasma α-tocopherol concentrations in both treatment groups at the start of the experiment averaged 0.8 mg/L. Grazing saltbush resulted in significantly higher plasma concentrations from week 4 onwards and maintained plasma concentrations above deficiency levels throughout the experiment. The use of an oral drench at the start of the experiment probably prevented this difference becoming more pronounced earlier on. The pattern of plasma α-tocopherol concentration followed the pattern of saltbush intake during the experiment. The decline in availability of saltbush resulted in a decrease in the potential vitamin E intake. Fry et al. (1993) fed lambs 100 mg/head.day of α-tocopherol acetate and observed plasma concentrations of 2.5 mg/L. These concentrations were higher than recorded in the current experiment but still indicate that saltbush is a significant source of vitamin E.

Overall, the combined results of the plasma, liver and muscle samples indicates that grazing saltbush significantly elevated vitamin E status over time and compared to grazing dry senesced pasture and stubble. In contrast, the sheep grazing pasture, stubble and grain only, had concentrations of vitamin E in plasma, liver and muscle that were borderline adequate for the prevention of nutritional myopathy. Importantly this was observed even though all sheep were given a vitamin E treatment prior to the experiment. There is potential for the high vitamin E content in saltbush to be used for the prevention of vitamin E deficiencies reducing the need for expensive and labour intensive synthetic supplements. Further work is needed to determine the minimal grazing times needed to observe these beneficial changes to vitamin E status.
It is pertinent to note that this study dealt with the vitamin E status of 18-month-old sheep. There has been very little research with sheep of this age because the problem of vitamin E deficiency is usually associated with weaner sheep (Machlin 1980). The effects of nutritional myopathy are not fully described in older animals but it is reasonable to predict that the vitamin E requirements, tissue capacity, and physiological pathways may be different in animals whose growth and development are near completion (Hidiroglou et al. 1991). Younger animals are believed to have higher vitamin E requirements due to the high growth rates (Hidiroglou et al. 1970; McDowell et al. 1996; Rice et al. 1988). Clearly, further work is required to investigate the potential of saltbush to improve the vitamin E status of weaner sheep.

Saltbush may also be a superior natural source of vitamin E compared to green pasture. The benefit of saltbush may be due to its lower relative content of PUFA and therefore lower vitamin E to PUFA ratio compared to the green pasture (based on predicted values of vitamin E content in green pasture from Gabbedy et al. (1977) and Tramontano et al. (1993). The vitamin E to PUFA ratio was 1.98 for the river saltbush, 1.96 for old man saltbush and 2.44 for green pasture. The ratio of vitamin E to PUFA in the plant will determine the degree of oxidative challenge for the animal. The higher the PUFA content, the higher the vitamin E requirement to protect these lipids from peroxidation (Rice et al. 1988). Upon consumption by the sheep, most but not all PUFA is hydrogenated by the rumen (Priolo et al. 2001). However any that escapes rumen hydrogenation will be absorbed and deposited by the animal presenting an oxidative challenge to the animal (Rice et al. 1988). The lower oxidative challenge from saltbush may reduce the rate of depletion of the tissue α-tocopherol, slowing the onset of nutritional myopathy as well as playing a role in the promotion of meat colour stability.
6.4.4. **Meat colour stability and drip loss**

The results from the Wickepin 2004 experiment indicate that grazing saltbush had a positive effect on the colour of lamb meat. In this study the meat from lambs fed saltbush was lighter than the meat from lambs fed pasture over the 5 day assessment period. Consumers generally prefer meat to be lighter in colour, although this can depend on the experience of the consumer. High ultimate pH is usually associated with darker colour (Dransfield 1981) but the saltbush meat had a higher ultimate pH and was lighter in colour. This lighter result may have been due to differences in hydration status of the lambs as the LL from lambs fed saltbush had a lower dry matter than the LL from lambs fed pasture. Jacob *et al.* (2005a) found that lamb meat was darker when lambs were dehydrated prior to slaughter.

Consumers also prefer meat to be redder in colour. The meat from the saltbush fed sheep was redder on day 4 and 5 compared to the pasture fed sheep as indicated by the higher $a^*$ value. A slower decline in the $a^*$ value over time by sheep elevated vitamin E status is consistent with findings by Wulf *et al.* (1995), Guidera *et al.* (1997) and Turner *et al.* (2002). The $a^*$ value did elevate over the first 3 days, a finding also observed by Macit *et al.* (2003).

An increase in yellowness as indicated by the $b^*$ value will reduce consumer acceptability. The yellowness was also significantly higher for the saltbush fed sheep at day 4. Grasses or forages rich in carotenoids increases internal fat yellowness (Muramoto *et al.* 2003; Yang *et al.* 2002). There is some speculation that saltbush is high in vitamin A and possibly vitamin A precursors such as carotenoids. An increased carotenoid content of the saltbush would contribute to the increased yellowness of the fat.

The ratio of the reflectance of light at the wavelength of 630 nm compared to 580 nm is used as an indirect estimate of surface metmyoglobin levels (Greene 1971; Strange *et al.* 1974). A low ratio close to 1 indicates the conversion of myoglobin to metmyoglobin which is the cause of
brown colour in meat (Faustman et al. 1989a; Greene 1971; Strange et al. 1974). The interaction between time and treatment in Wickepin 2004 indicates that the rate of metmyoglobin formation was lower in the saltbush fed group than the pasture fed group. This reduced rate of accumulation of metmyoglobin was confirmed by a* values being lower at days 4 and 5. This improvement in colour stability can be explained by the differences between treatments for vitamin E concentration in the LL. Vitamin E is a powerful antioxidant that is thought to prevent oxidation of myoglobin to metmyoglobin both directly and indirectly by preventing the oxidation of lipids in cell membranes.

There may also have been other antioxidants in the saltbush such as carotenoids, phytic acid, and flavonoids that could also contribute to the protection against lipid oxidation in saltbush-grazed animals (Mercier et al. 2004). In addition, the lower polyunsaturated fatty acid content of the saltbush may reduce oxidative stress from the saltbush (as described in section 6.4.2) and promote meat colour stability (Turner et al. 2002; Wulf et al. 1995; Yang et al. 2002).

Reports in the literature on the beneficial effects of vitamin E supplementation for extending the shelf-life of meat are mostly related to beef although there have been some studies done with sheepmeat. For beef, the concentration of vitamin E in muscle must reach a threshold level before an economically beneficial effect is observed. Liu et al. (1996) proposed a threshold level of 3.5 mg/kg wet weight for beef and Faustman et al. (1989b) cite a level of 3-3.3 mg/kg. Interestingly in this study the concentration of vitamin E in the LL was above this threshold for lambs fed saltbush and below this threshold for the lambs fed dry pasture. Grazing saltbush could therefore be used by farmers as a strategy to improve the shelf life of lamb meat in the summer autumn period, when the only alternative feed source is dry annual pasture and grain.

Drip loss was also examined as part of this study. The meat from the saltbush grazed sheep had a lower muscle dry matter content compared to the pasture fed sheep but there was no significant
effect of grazing treatment on drip or cooking loss. High muscle vitamin E concentrations have been shown to reduce drip loss by stabilising cell integrity and enhancing the ability of muscle to retain sarcoplasmic components and therefore hold on to the fluid in the muscles tightly (Mitsumoto et al. 1998). In this case, the high vitamin E concentrations in the muscle of the saltbush grazed sheep may be promoting the retention of water without allowing for it to be lost as drip or when cooked.

6.4.5. Eating quality

The hypothesis that grazing saltbush with a barley supplement prior to slaughter would result in improved eating quality has not been supported. There was no treatment effect on any of the eating quality attributes assessed. Despite there being no improvement in terms of the liking of flavour and aroma, tenderness, juiciness and overall acceptance there was also no detrimental effects on these attributes. These findings are consistent with those in chapter 4 and by Hopkins et al. (1999), where no differences in eating quality were observed in sheep grazing saltbush on non-saline land. Interestingly, there was a 33% difference between flavour strength between the two treatments but this was not significant. Had the SEM been lower and in the range of what was observed in chapter 4 (figure 4.7), there may have been a significant difference between treatments for flavour strength. The higher SEM for the field experiments compared to the animal house experiment may be due to the production of a greater range in meat quality under such grazing conditions.

In agreement with chapter 4, the lack of effect on eating quality could be due to the high water and salt intake affecting the metabolism and subsequent deposition of compounds responsible for the changes in flavour and aroma. Also the low carcass fat content may also have masked the any changes to eating quality (Hopkins et al. 1985). A minimal level of carcass fat is required to ensure tenderness and juiciness.
The expected decrease in tenderness and juiciness commonly associated with low levels of carcass fat did not occur in this experiment. The high vitamin E levels may have counteracted the detrimental effect of low carcass fat. Geay et al. (2001) showed that vitamin E supplementation reduced the toughness of muscles by decreasing the formation of hydroxyllysyl pyridinolin, a protein associated with the collagenic toughness of meat. The high vitamin E levels may have also influenced juiciness. Juiciness is dependant on the amount of retained water in the cooked meat product and is negatively correlated to drip loss. This chapter demonstrated that the saltbush fed sheep, possibly due to the high vitamin E content, had a reduced potential for drip and cooking loss despite a higher muscle fluid content, a finding also confirmed by others who have fed supranutritional levels of vitamin E (Faustman et al. 1989a; Mitumoto et al. 1998; Yang et al. 2002). Further work is needed to understand the benefits of vitamin E in improving tenderness and juiciness of meat from lean sheep. With current industry support driving the move towards leaner carcasses it is essential to take necessary precautions to prevent any detriment to eating quality. Feeding diets high in supplemental vitamin E may be an avenue to ensure tenderness and juiciness.

The high muscle vitamin E levels will also prevent the development of rancid flavours and odours specifically attributed to the oxidative products of polyunsaturated fatty acids (PUFA) (docosahexaenoic acid (C 22:6) and eicosapentaenoic acid (C 20:5) (Guidera et al. 1997; Wulf et al. 1995). Deposition of these oxidative products in the fat is associated with species specific flavours of meat as a result of the formulation of volatile compounds during cooking (Cramer 1983; Mottram 1998; Turner et al. 2002). Lower concentrations of oxidative products of linolenic acid in the fat depots of the S+B fed sheep were observed in chapter 4 possibly due to the protection of linolenic acid from oxidation from the high vitamin E intake in this experiment. Saltbush also contains lower amounts of PUFA. A lower ratio of PUFA:vitamin E will further prevent undesirable lipid oxidation. So instead of the saltbush meat containing compounds which impart beneficial flavour and aroma volatiles as previously claimed by the suppliers and
marketers of the saltbush meat products in NSW and SA, the improved eating quality may simply be due to the inhibition of off flavour and aroma development compared to meat from sheep grazed on dry pasture. Had the meat assessed in this chapter been aged for longer, as is the case with the SA and NSW product (both minimum 1 week aging) then differences in flavour and aroma intensity and liking may have been more pronounced.

6.5. Conclusions

The following conclusions can be made from this chapter:

- Sheep that had grazed saltbush had significantly less fat on the carcass. Providing adequate levels of protein and energy were met, this result was attributed to a higher dietary protein: energy ratio of the saltbush fed sheep resulting in less fat and more protein deposition.
- Both field experiments demonstrated sub-optimal liveweight gains therefore establishing that grazing saltbush to finish sheep is not appropriate for the achievement of commercially desirable carcass weights. Frequent and careful monitoring of animal production and feed intake enabled equal liveweight gains and hot carcass weights of sheep grazing saltbush compared to sheep grazing pasture.
- The long term consumption of saltbush and barley prior to slaughter did not result in decreases in carcass weight loss and improvement in dressing percentages due to an improved hydration status at slaughter. Body composition appears to have confounded the effects of saltbush on carcass fluid content. However, these experiments did show that grazing saltbush resulted in increased urine weight and decreased urine concentration. This was attributed to either an increased consumption of water in lairage or to the excretion of fluid originally drunk and retained in the body compartments whilst on farm and consuming high levels of sodium.
The high level of vitamin E in the saltbush elevated plasma, liver and muscle concentrations in the sheep grazing saltbush compared to those grazing dry stubble and pasture. The high muscle concentrations of vitamin E in the saltbush grazed sheep improved colour stability but did not influence the drip and cooking loss of the meat despite a decrease in the muscle dry matter of the meat.

There was no detectable improvement or decline in eating quality resulting from grazing saltbush. The high meat vitamin E content may have counteracted the negative effects of low carcass fat content on tenderness and juiciness.

It is hypothesised that the saltbush does not impart beneficial flavour and aroma volatiles as previously thought instead the high vitamin E levels inhibit off flavour and aroma development compared to meat from sheep grazed on dry pasture. This effect may be more pronounced in aged meat product. The low levels of polyunsaturated fatty acids in the saltbush may also potentially reduce the amount of tainting oxidative products being deposited in the fat.
Chapter 7

The use of sodium chloride and betaine to improve the hydration status of lambs at slaughter

7.1. Introduction

Under commercial conditions lambs are routinely denied access to water immediately prior to and during transport from farms to the abattoir. Water is then made available in the lairage yards at the abattoir. Jacob et al. (2006b) monitored commercial consignments to abattoirs and found that lambs are often dehydrated at the time of slaughter. Dehydration at the time of slaughter corresponds to reductions in the fluid content of meat causing liveweight loss (Cole 1995) and reduced muscle weight, dressing percentage and hot carcass weight (Jacob et al. 2006a). Jacob et al. (2006b) showed that sheep that do not drink in lairage can lose up to 2kg in carcass weight. Importantly, Jacob et al. (2006b) showed that dehydration can be averted if the sheep drink in lairage.

The sequence of physiological responses to water deprivation firstly involves the reduction in the urine volume to conserve fluid to maintain blood volume but also the loss of electrolytes to protect against increased tonicity of the body fluids. The retention of plasma volume is essential to maintain blood pressure and is the overriding physiological process during a period of water deprivation. The body defends the plasma volume first by drawing fluid from the interstitial fluid and then at the expense of intracellular fluid as dehydration progresses (Cole 1995; Fitzsimons 1979; Guyton et al. 2000). The changes in osmotic pressure between the fluid compartments drives the movement of water. Based on these responses, commonly commercially-used indices of dehydration or hydration status can include lowered urine volume, increased urine osmolarity...
and/or urine specific gravity (USG), decreased muscle fluid content and/or decreased plasma volume indicated by an increase in serum osmolarity (Jacob et al. 2005b).

Results reported in chapter 6 indicated that saltbush grazed sheep produced a higher volume of urine with a low USG and osmolarity as well as having a higher muscle fluid content in comparison to sheep grazing pasture/stubble. Based on these observations it appears that the saltbush fed sheep were significantly less dehydrated than the stubble fed sheep. Although the long term feeding of saltbush did not improve dressing percentage or increase hot carcass weight compared to sheep fed the control diet possibly due to confounding changes in body composition, different results may be observed if saltbush or a combination of salt and betaine is fed on a short term strategic basis.

These observations of a change in hydration status of the saltbush fed sheep could be the result of the high salt and/or high betaine content of the saltbush. Saltbush contains 15-30% salt which will result an increased plasma osmolarity due to the absorption of sodium across the gut wall into the plasma. The increase in plasma osmolarity will both stimulate the movement of fluid from the interstitial fluid into the plasma thus increasing the plasma volume and blood pressure and also stimulate the hypothalamic neurons to increase water intake (Cheek 1961; Fitzsimons 1979; Guyton et al. 2000; Hansard et al. 1953; Harper et al. 1997b; Kirkendall et al. 1976; Whittow 1968). Increased blood pressure will trigger the baroreceptors in the hypothalamus and heart to lower anti-diuretic hormone (ADH) and the renin-angiotensin-aldosterone system (RAA) to reduce plasma volume (Guyton et al. 2000; Guyton et al. 1982; McKnight et al. 1994; Vander et al. 1970). Lower ADH will reduce water reabsorption by the kidney (Robertson 1992; Whittow 1968; Young et al. 1976). Lower RAA will reduce sodium reabsorption by the kidney (Guyton et al. 2000; Wessen 1961). Sodium excretion is coupled with the simultaneous excretion of water. In addition the secretion of atrial natriuretic peptides (ANP) is increased which act to reduce circulating angiotensin and aldosterone and increase the glomerular filtration rate.
The physiological result of these concurrent processes is the excretion of high volumes of urine with a high sodium content but low osmolarity and thus USG.

The higher plasma volume due to the consumption of the high salt diet may be advantageous when an animal is exposed to a period of water deprivation. The need to withdraw fluid from the stores in the muscle interstitial and intracellular spaces to maintain the blood volume would be reduced. Hence if the depletion of the muscle fluid stores is reduced, higher levels of fluid would be present at slaughter resulting in less carcass and liveweight loss. In addition, there are high volumes of water in the gastrointestinal tract of sheep fed high salt diets and this may also act as a water reserve during times of water deprivation. Hix et al. (1953) showed that when sheep are fed a high salt diet prior to a period of water deprivation, they have a higher loss of extracellular fluid (incorporates interstitial, gut and plasma fluid) than intracellular during this time. The loss of extracellular fluid was more easily corrected and weight recuperation was faster in the high salt fed sheep.

Betaine is a naturally occurring compound found in high quantities in halophytes (up to 6.8%) (Randell et al. 1996; Storey et al. 1977). In plants, betaine has an osmoregulatory role in the control of the movement of sodium and potassium across cell membranes assisting in the maintenance of osmotic balance. In animals, betaine may also have osmoregulatory properties and assist in the retention of water in the muscles. There are also suggestions that it can reduce water requirements when given as a supplement during heat stress (Masters et al. 2001). Betaine is regularly used in commercial feed for the poultry and swine industries. Betaine has also been shown to have lipotropic properties that can reduce fat deposition (Fernandez et al. 1998; Kettunen et al. 2001; Saunderson et al. 1990; Silander-Rasi et al. 2003). There is very little in the literature about the benefits of betaine for ruminants particularly under conditions of dehydration.
The role of strategic feeding of sodium chloride and/or betaine in a finishing system to prevent dehydration could be potentially beneficial in two different commercial scenarios. Firstly, it is common for some sheep not to drink any water between leaving the farm and slaughter due to the high stress environment and high pen densities. In these instances where water intake is low or minimal, feeding salt and/or betaine on farm may maintain the hydration status by providing a buffering effect against dehydration. The use of salt would increase the animals’ water intake prior to leaving the farm essentially ‘loading’ plasma and gastrointestinal fluid with water. Additionally betaine may also help prevent the loss of water from the muscle as a result of its osmoregulatory function. In a second scenario where sheep do have access to water in lairage, the high salt diet may stimulate water intake above normal levels whilst in lairage. An increase in water intake in combination with the use of the betaine to retain the water in the muscles could prevent dehydration. The additional retention of fluid in the plasma and gastrointestinal tract prior to transport and lairage may also be beneficial in this situation.

The study reported here evaluates the addition of sodium chloride at the rate of 5% and/or betaine at the rate of 0.6% to a diet fed prior to consignment. The first experiment investigated the potential benefits of feeding these diets prior to a period of water deprivation before slaughter. The second experiment examined the benefits when these diets were fed prior to commercial slaughter where water is made available in lairage.

The hypotheses for this chapter are:

1. That the addition of sodium chloride and/or betaine to the diet will improve the liveweight, hot carcass weight, dressing percentage, muscle dry matter and muscle weight if fed prior to a period of water deprivation before slaughter
2. That the addition of sodium chloride and/or betaine to the diet will improve the liveweight, hot carcass weight, dressing percentage, muscle dry matter and muscle weight if fed prior to commercial slaughter where water is made available in lairage
7.2. Feeding sodium chloride and betaine prior to a period of water deprivation to prevent dehydration in lambs at slaughter: Experiment 7.1

7.2.1. Materials and Methods

7.2.1.1. Experimental design

The experiment was a 2 x 2 factorial design with two levels of sodium chloride (referred to as salt in this chapter) added (0 and 5%) and two levels of betaine (0 and 0.6%) (Table 7.1). Due to housing limitations the sheep were split into four groups of 24. There were 6 sheep for each treatment group in each time replicate. The four groups were then sequentially taken through the experimental treatments. The experiment was therefore structured to have four time replicates (n=4 x 24= 96).

Table 7.1 Description of treatments

<table>
<thead>
<tr>
<th>Sodium Chloride (salt)</th>
<th>Betaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>- (0%)</td>
<td>- (0%)</td>
</tr>
<tr>
<td>+ (5%)</td>
<td>+ (0.6%)</td>
</tr>
<tr>
<td>-salt and -betaine</td>
<td>-salt and -betaine</td>
</tr>
<tr>
<td>+salt and -betaine</td>
<td>-salt and +betaine</td>
</tr>
<tr>
<td>+salt and +betaine</td>
<td>+salt and +betaine</td>
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7.2.1.2. Animals and Diets

Ninety-six poll dorset-merino cross 8-month old wether lambs with a starting mean liveweight of 45.9 ± 0.17 kg were used in this experiment.

Upon arrival at the Murdoch University farm, all lambs were held in a pasture paddock. The allocation to each time replicate was made using liveweight stratification. This ensured group weights were equal for each replicate. At the start of each of the four time periods, 24 lambs in
the group were placed in a pen for acclimatisation to the pellets. The pellets were fed in conjunction with barley and oaten hay. The animals were allocated 1.5 kg/head.d of ration fed in a group trough (Table 7.2).

Table 7.2 Pellet acclimatisation

<table>
<thead>
<tr>
<th>DAY</th>
<th>-salt-betaine pellets (%)</th>
<th>Barley (%)</th>
<th>Hay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Following the three day pellet acclimatisation, the lambs were moved into individual pens for 14 days. At this point, each animal was allocated to a treatment using liveweight stratification to ensure that average liveweight was equal in each treatment group. The next 7 days were an introduction period with each lamb being gradually introduced to the treatment diet as shown in Table 7.3.

Table 7.3 Treatment introduction to salt diets

<table>
<thead>
<tr>
<th>DAY</th>
<th>Treatment pellet (%)</th>
<th>-salt-betaine pellet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>50</td>
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<tr>
<td>4</td>
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<td>40</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>
After the 7-day introductory period, the sheep were fed the treatment diets for an additional 7 days. During both the acclimatisation and treatment period each animal received 1.5kg/head.d of feed. All lambs were allowed free access to tap water from nipple drinkers.

Table 7.4 and 7.5 detail the ingredient and nutritional composition of each of the four diets.

Table 7.4 Diet composition

<table>
<thead>
<tr>
<th>Constituent</th>
<th>-salt-betaine</th>
<th>+salt-betaine</th>
<th>-salt+betaine</th>
<th>+salt+betaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>(in g/kg DM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steam cut oaten chaff</td>
<td>399.4</td>
<td>378.9</td>
<td>398.7</td>
<td>375.7</td>
</tr>
<tr>
<td>Barley</td>
<td>395</td>
<td>375</td>
<td>392</td>
<td>373</td>
</tr>
<tr>
<td>Lupins</td>
<td>150</td>
<td>143</td>
<td>149</td>
<td>141</td>
</tr>
<tr>
<td>Extruded canola meal</td>
<td>40.0</td>
<td>38.0</td>
<td>38.0</td>
<td>38.0</td>
</tr>
<tr>
<td>Limestone</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Calcium hydroxide</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Sulphur flakes</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral premix\textsuperscript{a}</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Betaine\textsuperscript{b}</td>
<td>0</td>
<td>0</td>
<td>6.7</td>
<td>6.7</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>50</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Siromin\texttrademark

\textsuperscript{b}The betaine was donated by Feedworks (Melbourne, Australia) a commercial supplier of betaine and they have recommended this level of betaine suitable for use with lambs.
Table 7.5 Chemical analysis (%) of the diets used in this experimenta

<table>
<thead>
<tr>
<th>Constituent (%)</th>
<th>-Salt-Betaine</th>
<th>+Salt-Betaine</th>
<th>-Salt+Betaine</th>
<th>+Salt+Betaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>2.4</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.22</td>
<td>1.9</td>
<td>0.2</td>
<td>1.6</td>
</tr>
<tr>
<td>In vitro digestibility</td>
<td>74.5</td>
<td>74.9</td>
<td>72.4</td>
<td>73.1</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>95.4</td>
<td>90.0</td>
<td>95.2</td>
<td>90.9</td>
</tr>
</tbody>
</table>

a Analysis as detailed in chapter 3.

7.2.1.3. Water deprivation period

Following the 7 day treatment period the 24 sheep were randomly allocated to group pens in one of 2 temperature controlled rooms (i.e. 12 sheep per room/3 per treatment group). All sheep remained in these rooms for 48 hours without food or water, after which they were commercially slaughtered.

The temperature in the temperature controlled rooms was modified throughout the day to replicate conditions experienced during commercial transport and lairage conditions in the summer period. At 9am the temperature was increased in the rooms to 35°C and turned down at 3pm to 28°C.
7.2.1.4. Sampling and measurements

Water intake was measured over the last 3 days of the 7 day treatment period.

The lambs were all weighed upon arrival at Murdoch University, immediately before starting the 7 day adjustment in the animal house; after finishing the 7 day treatment period prior to entering the temperature controlled rooms and; after 48 hours in the temperature controlled rooms just before slaughter.

Urine was collected using purpose built pizzle harnesses to hold a rubber collector over the pizzle. Five mL samples of urine were taken from all sheep after finishing the 7 day treatment period prior to entering the temperature controlled rooms (0 hours after removal of feed and water) and 24 hours after entering the temperature control rooms (24 hours). The bladders were collected at slaughter and a urine sample taken (48 hours). The urine samples were immediately frozen at –20°C prior to analysis. Urine samples were analysed for urine specific gravity creatinine and sodium concentration as described in chapter 3 (section 3.3) and glucose as described in chapter 4 (section 4.2.3.1).

Blood was collected by jugular venipuncture into a non-heparinised container. Samples were taken after finishing the 7 day treatment period prior to entering the temperature controlled rooms (0 hours after removal of feed and water); 24 hours after entering the temperature control rooms (24 hours) and; an hour prior of slaughter (48 hours). The samples were spun at 3000 rpm for 15 mins and serum collected and stored at –20°C prior to analysis. Serum samples were analysed for sodium, potassium, creatinine and urea concentrations as described in chapter 3.
7.2.1.5. **Calculations**

The urinary and serum concentrations of sodium and creatinine were used to calculate fractional excretion rate of sodium as described in chapter 3.

An estimate of serum osmolarity was calculated using the formula (Stockman *et al.* 2002) (All units are mmol/L):

\[
\text{Serum osmolarity} = 1.86\times ([\text{Sodium}] + [\text{Potassium}] + [\text{Glucose}] + [\text{urea}])
\]

An estimate of urine osmolarity was made using the following equation (Watson 1998):

\[
\text{Urine osmolarity} = (\text{USG}-1)\times36000
\]

7.2.1.6. **Slaughter and dissection procedures**

All 96 sheep were slaughtered in the Murdoch University abattoir. Lambs were head stunned prior to exsanguination using a Thornton stunner handset model 1 (Cat no. T05202, Thornton Engineering group, Auckland, New Zealand).

Immediately following slaughter all carcasses were weighed to determine their hot carcass weight (HCW) and GR (total tissue depth over the 12\textsuperscript{th} rib, 110mm from the midline).

All carcasses were held in the same 5°C chiller for 24 hours after slaughter. Twenty-four hours later, ultimate pH was determined as previously described in chapter 3.
From each animal, the whole *m. semimembranosis* (SM) and *m. semitendinosis* (ST) muscles on one leg were dissected out, weighed and a 2 g sample taken. Muscle dry matter was determined on these samples using methods described in chapter 3.

7.2.1.7. **Data analysis**

Analysis of variance was used to examine the main effects of salt and betaine on measured traits. To take account of variation due to replication over time, the ANOVA included a replicate as a block. The effect of treatment at each time within replicate for urine specific gravity, fraction excretion rate of sodium, urinary and serum sodium concentration was determined using ANOVA. Repeated measures was used to identify the effects of time separately from treatments. The software package Systat (SPSS Version 9.01) was used to perform the analysis.
7.2.2.   Results

There was a significant effect of sodium chloride (P<0.01) but not of betaine (P>0.05) on water intake (Table 7.6) with higher levels of sodium chloride increasing water intake. There was no primary effect of either sodium chloride or betaine on liveweight, liveweight loss after 48 hours of water deprivation, hot carcass weight (HCW), SM weight, ST weight, SM and ST dry matter content or dressing percentage (Table 7.6 and 7.7). There was a significant interaction between the effects of salt and betaine for GR depth (P<0.02).
Table 7.6 The effect of sodium chloride and betaine on liveweight loss and carcass characteristics (means ± standard error)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-salt</td>
<td>+salt</td>
</tr>
<tr>
<td>Water intake (Litres/lamb.day)</td>
<td>5.4±0.33</td>
<td>8.3±0.69</td>
</tr>
<tr>
<td>Liveweight prior to start of 7 day feeding period (kg)</td>
<td>45.3±0.42</td>
<td>44.9±0.39</td>
</tr>
<tr>
<td>Liveweight prior to the water deprivation period (kg)</td>
<td>46.1±0.27</td>
<td>45.5±0.38</td>
</tr>
<tr>
<td>Liveweight loss after 48 h water deprivation period (kg)</td>
<td>4.6±0.42</td>
<td>4.9±0.18</td>
</tr>
<tr>
<td>Hot carcass weight (kg)</td>
<td>20.9±0.18</td>
<td>20.5±0.20</td>
</tr>
<tr>
<td>GR fat depth (mm)</td>
<td>12.1±0.41</td>
<td>12.2±0.60</td>
</tr>
<tr>
<td>Dressing percentage (%)</td>
<td>50.2±0.5</td>
<td>51.1±0.5</td>
</tr>
</tbody>
</table>
Table 7.7  The effect of sodium chloride and betaine on muscle dry matter, muscle weight and ultimate pH (means ± standard error)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-salt -betaine</td>
<td>+salt +betaine</td>
</tr>
<tr>
<td>Fresh muscle weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>301±4</td>
<td>305±4</td>
</tr>
<tr>
<td>ST</td>
<td>105±2</td>
<td>105±2</td>
</tr>
<tr>
<td>Muscle dry matter (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SM</td>
<td>27.8±0.2</td>
<td>27.9±0.2</td>
</tr>
<tr>
<td>ST</td>
<td>27.6±0.31</td>
<td>28.3±0.31</td>
</tr>
<tr>
<td>Ultimate pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>5.64±0.02</td>
<td>5.64±0.02</td>
</tr>
<tr>
<td>SM</td>
<td>5.56±0.01</td>
<td>5.56±0.01</td>
</tr>
<tr>
<td>ST</td>
<td>5.64±0.01</td>
<td>5.66±0.01</td>
</tr>
</tbody>
</table>
There was a significant effect of sodium chloride (P<0.001) but no effect of betaine (P>0.5) on USG (Figure 7.1). A difference existed between the high sodium chloride and low sodium chloride treatments at 0 and 24 h of water deprivation with the high sodium chloride group having a lower USG. Time off water had a significant (P<0.001) effect for all treatments on USG. USG increased as the length of water deprivation increased.

There was a significant effect of sodium chloride (P<0.05) but no effect of betaine (P>0.8) on the urinary sodium concentration (Figure 7.1). This significant difference between the high sodium chloride and low sodium chloride treatments existed at 0 h with the sheep fed the high sodium chloride diets having a high urinary concentration of sodium. At 24 and 48 h the effect was reversed and the sheep fed the low sodium chloride diet had a higher urinary sodium concentration. There was a significant time x treatment interaction (P<0.001).

There was a significant effect of sodium chloride (P<0.05) but no effect of betaine (P>0.8) on the urinary creatinine concentration (Figure 7.1). This significant difference between the high sodium chloride and low sodium chloride treatments existed at 0 h with the sheep fed the high sodium chloride diets having a lower urinary concentration of creatinine.

There was a significant effect of sodium chloride (P<0.05) but no effect of betaine (P>0.8) on the urinary urea concentration (Figure 7.1). This significant difference between the high sodium chloride and low sodium chloride treatments existed at 0 h
with the sheep fed the high sodium chloride diets having a lower urinary concentration of urea.
Figure 7.1 The effect of sodium chloride and/or betaine on urine specific gravity (USG), urinary sodium, urea and creatinine concentration after 0, 24 and 48 h of water deprivation (Mean ± standard error)
There was a significant effect of sodium chloride (P<0.05) but no effect of betaine (P>0.8) on the urinary sodium:creatinine ratio (Figure 7.2). This significant difference between the high sodium chloride and low sodium chloride treatments existed at 0 h with the sheep fed the high sodium chloride diets having a high urinary sodium:creatinine ratio.

There was a significant effect of sodium chloride (P<0.05) but no effect of betaine (P>0.9) on fractional excretion rate of sodium (Figure 7.2). This significant difference between the high sodium chloride and low sodium chloride treatments existed at 0 h only with no difference detected between any of the treatments after 24 or 48 h of water deprivation. There was a significant time x treatment interaction (P<0.001). The fractional excretion rate of sodium decreased as the length of water deprivation increased for all treatments but to a greater degree in the high sodium chloride groups.

There was no significant effect of sodium chloride or betaine (P=0.065) (P>0.1) on urine osmolarity (Figure 7.2). However, there were differences between the high and low sodium treatments at 0 and 24 h (P<0.05). The sheep fed the high sodium diets had a lower osmolarity at these times. Time off water had a significant (P<0.001) effect for all treatments on urinary osmolality. The osmolality increased as the length of water deprivation increased.
Figure 7.2 The effect of sodium chloride and/or betaine on the fractional excretion rate of sodium and serum sodium concentration after 0, 24 and 48 h of water deprivation (Mean ± standard error)
There was a significant effect of sodium chloride (P<0.05) but no effect of betaine (P>0.3) on serum sodium concentration (Figure 7.3). This significant difference between the high sodium chloride and low sodium chloride treatments existed at 24 and 48 h after the onset of water deprivation. The sheep fed the high sodium diets had a lower concentration of sodium in the serum at these times. Time off water had a significant (P<0.001) effect for all treatments on serum sodium concentration. The concentration of sodium in the serum increased as the length of water deprivation increased.

There was no effect of sodium chloride or betaine on serum osmolarity (P>0.1) (Figure 7.3). Time off water had a significant (P<0.001) effect for all treatments on urinary osmolarity. The osmolarity increased as the length of water deprivation increased.
Figure 7.3 The effect of sodium chloride and/or betaine on serum and urinary osmolarity concentration after 0, 24 and 48 h of water deprivation (Mean ± standard error)
7.2.3. **Discussion**

The addition of sodium chloride and/or betaine to the diet fed prior to a period of water deprivation did not result in a reduction in liveweight or prevent carcass loss at slaughter. No differences between the treatments for preslaughter liveweight, liveweight loss, hot carcass weight or muscle weight were observed. Importantly no differences in muscle dry matter were observed between treatments, thus expectedly there was no difference in carcass weights.

The ingestion of a high sodium chloride and betaine diet was expected to preserve the water in the interstitial and intracellular spaces at the expense of maintaining blood volume and blood pressure during a period of water deprivation by either increasing fluid in the plasma and gut prior to water deprivation or by maintaining fluid in the muscles. At 0 h indirect blood measures indicated that the high salt group did have a higher plasma and gut fluid volume than the low salt group. However by 48 h the high salt group had excreted all the excess fluid and both groups had conserved similar levels of fluid. The similar plasma volume and equal fluid content in the muscles between the treatments indicates that both groups had a similar hydration and physiological status at 48 h. A similar physiological and hydration status between treatments occurred because the high salt group: (1) were driven to actively excrete the excess fluid and (2) delayed the initiation to conserve fluid, compared to the low salt group. The following explanation outlines how and why these processes occurred.

The drive to excrete the excess fluid occurred because the high salt groups had an elevated plasma volume content at 0 h. The higher plasma volume was due to the increase in salt intake increasing plasma sodium content due to the absorption of
sodium across the gut wall. This would have caused the movement of fluid from the interstitial fluid and gut into the plasma to maintain the osmolarity pressure gradient between compartments (Harper et al. 1997a; Heer et al. 2000; Kirkendall et al. 1976; Whittow 1968) thus increasing blood volume. The serum sodium concentration was significantly lower in the high salt group at 0 h despite a higher sodium content of the plasma reflecting an increased plasma volume to achieve equilibrium. Similar findings in sheep fed high sodium chloride diets were observed by Goodwin and Williams (1986) and Meintjes and Engelbrecht (1993). Gut volume is increased due to increased fluid intake stimulated by an increase in plasma osmolarity following the consumption of high levels of sodium chloride and may have also helped to maintain blood volume. Volume was not measured in this experiment but observed by other authors who have fed high salt diets (Goodwin et al. 1986).

The consequence of an elevated blood volume is an increase in blood pressure above the homeostatic levels. To maintain a normal homeostatic blood pressure the baroreceptors in the hypothalamus and heart lower anti-diuretic hormone (ADH) and renin-angiotensin-aldosterone (RAA) secretion to reduce plasma volume (Michell 1985; Mills 1970). Lower ADH reduces water reabsorption by the kidney (Robertson 1992) and lower RAA will reduce sodium reabsorption by the kidney (Graudal et al. 1998; Michell 1985; Mills 1970) which is coupled with the simultaneous excretion of water (Guyton et al. 2000). The high urine output to reduce plasma volume was indicated in our study by a significantly lower urine specific gravity and urine osmolarity despite a higher urinary concentration and content of sodium. The high excretion rate of sodium occurs because the increased salt intake raises plasma osmolarity. Plasma osmolarity is also homeostatically controlled and any increase will
stimulate the secretion of atrial natriuretic factor (ANP) (McKnight et al. 1994; Rosenzweig et al. 1991) to increase sodium excretion by the kidney through a reduction in tubular reabsorption (Kirkendall et al. 1976; Mills 1970). The significantly higher fractional excretion rate of sodium (FER) for the high salt group at 0 h compared to the low salt group indicates that less sodium was conserved by the kidney compared to the low sodium chloride fed sheep. Similar serum and urinary measures and fractional excretion rates have been observed in sheep fed high sodium chloride containing diets by Goodwin and Williams (1986) and Meintjes and Engelbrecht (1993). In contrast, the low salt group at hour 0 h had a normal plasma volume and excretion of fluid and sodium. This group were not consuming high levels of salt so no fluctuations in blood volume were expected. Serum and urinary indices were all within the normal range for sheep in normal physiological state as defined previously (Michell (1985); Brueue and West (1990); Puls (1994) and Suttle and Jones (2000)).

The high salt fed sheep did not start conserving fluid until later than the low salt fed sheep due to the elevated blood volume at 0 h. A decrease in blood volume is an essential trigger to stimulate the secretion of hormones critical for the conservation of fluid by the kidney (Guyton et al. 2000; Whittow 1968). By 24 h the blood volume of the high salt fed sheep had dropped below homeostatic levels due to the excretion of all the excess fluid in the plasma and gut and the absence of any exogenous water intake (Hix et al. 1953; Meyer et al. 1955). Evidence of the drop in blood volume was shown by the decrease in urine volume, indicative of fluid conservation. Indicators of a reduced urine volume are an increase in USG, urinary osmolarity and urinary sodium concentration. Similar changes in urinary indices during water deprivation
were observed by Jacob et al. (2006a). A decrease in urine volume occurs because the baroreceptors detect the decrease in blood pressure and stimulate ADH secretion to increase water reabsorption by the kidney (Guyton et al. 2000; Young et al. 1976) lowering urine output. In contrast, the low sodium chloride fed sheep were actively conserving fluid from 0 h as shown by similar changes in urinary indices from 0 h onwards. This group also exhibited naturesis of sodium between 0-24 h primarily to reduce the osmolarity of the blood (Michell et al. 1995). The naturesis is evident by the significant increase in urinary sodium concentration between 0-24 h.

By 48 h there was no difference in the muscle dry matter content between the treatments indicating that there is no difference in the fluid content of interstitial and intracellular fluid spaces. This result indicates that both groups lost and conserved similar levels of fluid in these compartments. Alternatively, a similar fluid content may have occurred because the high salt group started with a lower fluid level in these compartments at 0 h and had no fluid loss over the 48 h period compared to the low salt group. Movement of fluid from the interstitial and intracellular fluid into the plasma to reduce plasma osmolarity (raised from the high salt intake) at 0 h may have occurred without any change in total body water content as demonstrated by Heer et al. (2000). Therefore, the consequence of this process was a fluid deficit in the interstitial and intracellular fluid of the high sodium chloride group compared to the low sodium chloride group at 0 h. However as compartment fluid volumes were not measured this hypothesis cannot be confirmed.

At slaughter the blood volume also appeared speculatively be equal between groups. This assumption has been made because: (1) plasma volume has been shown not to
change over a 3 day water deprivation period in sheep fed low sodium levels typical to that used in this experiment (Cole 1995; Macfarlane et al. 1961) and (2) the high excretion rate of fluid over the 0-48 h period indicates that the high salt group lost all the excess blood fluid and may have returned to a normal homeostatic level similar to that of the low salt group. In the absence of known compartment fluid volumes this finding is difficult to confirm as serum sodium concentration and serum osmolarity, previously used to define blood volume, are incoherent. The serum sodium concentration at 48 h was lower for the high salt fed sheep despite similar serum osmolarity between the groups. This finding indicates either or both (1) an equal blood volume and lower sodium content of the serum of the high salt group compared to the low salt group due to the over-excretion of sodium or (2) a higher blood volume of the high salt group. McKinley et al. (1983) demonstrated that dehydration induced a considerable increase in sodium excretion to place the animal in a sodium deficit. In combination with the additional loss of sodium to facilitate the loss of excess fluid between 0-48 h this may be why the high salt group had a greater sodium deficit than the low salt group.

In summary, the combination of metabolising a high salt load whilst lacking fluid intake will firstly result in the excretion of excess fluid in the plasma to resume normal blood volume and only after this point will the body then begin to conserve fluid. Had the stimulus to conserve fluid been triggered earlier then it is possible that the muscles would have had a higher fluid content. In addition, the use of these diets may be more beneficial when used in combination with water intake in lairage. Meyer et al. (1955) fed +/- sodium chloride diets at similar levels to those used in this experiment in combination with a 36 h water deprivation period and observed that
following the resumption of water intake after 36 h, quicker body weight gain occurred in the high sodium chloride group compared to the low sodium chloride group. This finding was attributed to a greater loss of extracellular fluid specifically from the interstitial fluid in the high salt group compared to the higher loss of intracellular fluid from the low salt fed sheep during the water deprivation period. The extracellular fluid loss was believed to be more easily corrected whereas intracellular loss in the low sodium chloride group was not. In this current experiment, a greater loss of fluid from the extracellular fluid has also occurred from the high salt group. The availability of water after 24 h may increase the effectiveness of these diets at reducing liveweight loss. However the benefits of any liveweight recuperation to carcass weight were not studied by Meyer et al. (1955). Thus further work is needed to determine if a similar outcome would occur with the diets used in this current study.

The only effect of betaine was an increased tissue depth (GR depth). The reasons for this finding are unclear as there were no associated changes in urine, blood or carcass parameters. If betaine were to change cell volume then presumably it would lead to a change in muscle and carcass weight unless tissue density was changed as well.
7.3. Feeding sodium chloride and betaine to lambs prior to commercial slaughter when water is available in lairage: Experiment 7.2

7.3.1. Materials and methods

7.3.1.1. Experimental design

The experiment was a 2 x 2 factorial design to test the effects of sodium chloride and betaine addition to the diet (Table 7.1).

7.3.1.2. Animals and diets

The diets used in this experiment were of the same chemical and nutritional composition as experiment 7.1 (table 7.4 and 7.5). Two hundred and four merino wether lambs with a starting mean liveweight of $43.1 \pm 0.35$ kg were used. The lambs were allocated to 1 of 12 group pens located at the CSIRO Yalanbee Research Station near Northam, Western Australia in groups of 17. The sheep were stratified for liveweight so that each pen had equal average liveweights. For the first three days the lambs were acclimatised to pellets by feeding in conjunction with barley and oaten hay as described in table 7.2.

After this period each pen of lambs was randomly allocated to 1 of the 4 experimental diets with three pens (replicates) each receiving the same treatment (i.e. $3 \times 17 \times 4 = 204$ animals). The feed allocated was 1.5 kg/head.d. The sheep were allowed *ad lib* access to clean, fresh scheme water in a self filling concrete trough.
Following 3 days of acclimatisation to pellets, lambs allocated to a high sodium chloride diet were introduced to their treatment pellet over a three day period according to the regime described in table 7.8.

Table 7.8 Treatment introduction

<table>
<thead>
<tr>
<th>DAY</th>
<th>Treatment pellets (%)</th>
<th>-salt - betaine pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

The sheep were then fed their treatment diet for 7 days. At 10am on the final day (day 15), the lambs were mustered into 1 large group and placed in a holding pen with no feed or water. At 5pm the sheep were loaded onto a truck and transported in the morning to a commercial abattoir. The truck arrived at 9am on day 16 and the sheep were unloaded immediately into covered lairage yards where they had ad lib access to water available in 1 trough per pen. The lambs were then slaughtered at 10am on day 17, hence a total period of 48 h off feed and with a 24 h period immediately prior to slaughter with free access to water.

7.3.1.3. Sampling and measurements

The lambs were all weighed prior to entering the feedlot pens prior to the start of the 7 day acclimatisation period, after finishing the 7 day acclimatisation period prior to start of 7 day feeding period and immediately prior to transportation to the abattoir.
Urine was collected from live lambs using a purpose built pizzle harness. Five mL samples of urine were taken at end of treatment feeding period (0 h after removal of feed and water) from 15 sheep per treatment, upon arrival at the abattoir 24 h (24 h) from 10 of the 15 sheep sampled at 0 h. The bladders were also collected at slaughter and a urine sample taken from all sheep (48 h). The urine samples were immediately frozen at –20°C prior to analysis.

Blood was collected by jugular venipuncture into a non-heparinised container. Samples were taken at the end of treatment feeding period (0 h after removal of feed and +/- water) from 15 sheep per treatment and immediately after slaughter from all sheep (48 h). The samples were spun at 3000rpm for 15 mins and serum collected and stored at –20°C prior to analysis.

7.3.1.4. Slaughter and dissection procedures

As described in experiment 7.1.

7.3.1.5. Sample analysis

As described in experiment 7.1.

7.3.1.6. Data analysis

Analysis of variance was used to determine the main effects of salt and betaine. The effect of treatment at each time for urine specific gravity, fraction excretion rate of sodium, urinary and serum sodium concentration was determined using ANOVA at each time. Repeated measures was used to identify the effects of time separately from
treatments. The software package Systat (SPSS Version 9.01) was used to perform the analysis.
7.3.2. Results

There was no effect (P>0.05) of treatment on liveweight, hot carcass weight or dressing percentage. Betaine was associated with a significant reduction in GR depth (P= 0.05) but there was no effect of sodium chloride (P>0.05). There was no effect of treatment (P>0.05) on proportion of dry matter of the SM or ST (Table 7.9 and 7.10).
Table 7.9 The effect of treatment on liveweight, hot carcass weight, dressing percentage and GR depth (Mean ± standard error)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-salt</td>
<td>+salt</td>
</tr>
<tr>
<td></td>
<td>-betaine</td>
<td>+betaine</td>
</tr>
<tr>
<td>Liveweight prior to 7 day feeding period (kg)</td>
<td>42.7±0.67</td>
<td>43.0±0.67</td>
</tr>
<tr>
<td>Liveweight prior to leaving farm (kg)</td>
<td>43.5±0.77</td>
<td>43.9±0.77</td>
</tr>
<tr>
<td>HCW (kg)</td>
<td>16.7±0.33</td>
<td>16.7±0.33</td>
</tr>
<tr>
<td>Dressing percentage (%)</td>
<td>38.4±0.65</td>
<td>38±0.65</td>
</tr>
<tr>
<td>GR Depth (mm)</td>
<td>5.9±0.38</td>
<td>7.2±0.38</td>
</tr>
</tbody>
</table>
Table 7.10 The effect of treatment on muscle dry matter and ultimate pH (Mean ± standard error)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-salt</td>
<td>+salt</td>
</tr>
<tr>
<td>Muscle dry matter (%)</td>
<td>-betaine</td>
<td>-betaine</td>
</tr>
<tr>
<td>SM</td>
<td>25.0±0.3</td>
<td>24.7±0.3</td>
</tr>
<tr>
<td>ST</td>
<td>24.3±0.3</td>
<td>24.2±0.3</td>
</tr>
<tr>
<td>Ultimate pH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL</td>
<td>6.03±0.05</td>
<td>6.07±0.05</td>
</tr>
<tr>
<td>SM</td>
<td>5.92±0.03</td>
<td>5.95±0.03</td>
</tr>
<tr>
<td>ST</td>
<td>5.93±0.03</td>
<td>5.96±0.03</td>
</tr>
</tbody>
</table>
There was a significant effect of sodium chloride (P<0.001) but no effect of betaine (P>0.5) on urine specific gravity (Figure 7.4). A difference existed between the high sodium chloride and low sodium chloride treatments on farm, in lairage and at slaughter. Time off water had a significant (P<0.001) effect for all treatments on USG. The USG increased as the length of time from leaving the farm increased.

There was a significant effect of sodium chloride (P<0.05) but no effect of betaine (P>0.1) on the urinary sodium concentration (Figure 7.4). This significant difference between the high sodium chloride and low sodium chloride treatments existed on farm with the sheep fed the high sodium chloride diets having a high urinary concentration of sodium. Upon reaching lairage the urinary sodium in the high sodium chloride group had dropped and the low sodium group had elevated. No significant difference existed between the groups in lairage or at slaughter. There was a significant time x treatment interaction (P<0.001).

There was a significant effect of sodium chloride (P<0.05) but no effect of betaine (P>0.1) on the urinary creatinine concentration (Figure 7.4). This significant difference between the high sodium chloride and low sodium chloride treatments existed on farm with the sheep fed the high sodium chloride diets having a lower urinary concentration of creatinine. No significant difference existed between the groups in lairage or at slaughter. There was a significant time x treatment interaction (P<0.001).
Figure 7.4 The urine specific gravity and concentration of sodium and creatinine in the urine on farm, during lairage and at slaughter (Mean ± standard error)
The fractional excretion rate of sodium (Figure 7.5) was higher in sheep fed a high sodium chloride diet on farm compared to sheep fed a low sodium chloride diet on farm (P<0.01). However there was no effect of sodium chloride intake on fractional excretion rate of sodium at the time of slaughter (P<0.05). There was no effect of betaine on fractional excretion rate of sodium at either time. There was a significant time x treatment interaction (P<0.001). The fractional excretion rate of sodium decreased as the length of time from leaving the farm increased but to a greater degree in the high sodium chloride group.

There was no significant effect of time, sodium chloride (P>0.1) or betaine (P>0.6) on serum sodium concentration (Figure 7.5).
Figure 7.5 Fractional excretion rate and concentration of sodium in the serum on farm and at slaughter (Mean ± standard error)
7.3.3. Discussion

Feeding sodium chloride and/or betaine prior to commercial slaughter with access to water in lairage did not result in less liveweight and carcass loss at slaughter. Despite this finding, the results indicate that the high salt group drunk proportionately more fluid than the low salt group from hour 24-48 h whilst in lairage.

There was a drop in urinary sodium levels between lairage and slaughter providing evidence that both groups were drinking in lairage. In the absence of fluid intake, urinary sodium typically increases (Jacob et al. 2006a). A higher water intake in lairage by the high salt group is indicated by the significantly lower USG and lower urine osmolarity, at slaughter compared to the low salt group. A decrease in both indices has been shown to correspond with an increased urine volume and dilution of solutes in the urine (Jacob et al. 2006a). A high water intake of the high salt group may have been due to the physiological conditioning of the sheep in this group to frequently drink with this practice continuing in lairage. An unexpected conclusion however is that the low salt group also consumed water, although not as much as the high salt group. It is possible that the low salt groups observed the frequent drinking pattern of the high salt group and were encouraged to drink. Interestingly, both the high and low sodium chloride groups had USG levels below 1.045, with levels above 1.045 indicative of severe dehydration. Levels were significantly lower than those in experiment 7.1 indicating that water consumption in lairage can avert this degree of dehydration.
It appears that when access to water was restored at 24 h both groups were at a similar hydration status and had conserved similar levels of fluid. As a result water intake from 24 h had a similar outcome in both treatment groups and was likely to have maintained blood volume. It may be assumed at this point that similar fluid levels were present in the interstitial and intracellular spaces between both groups. Therefore no difference in fluid content of the carcass between treatments was likely at slaughter and no treatment differences in carcass weight and dressing percentage would be expected. Any extra water intake as in the case of the high salt group is simply excreted. This finding rejects the hypothesis developed in experiment 7.1 that preventing loss of fluid from the intracellular spaces during the water deprivation period would allow for greater weight recovery upon resumption of water intake in lairage and increased carcass weight. Both groups are simply replenishing stores at equal rates.

Similarly to sheep in experiment 7.1, between 0-24 h the high salt groups excreted all the excess fluid and salt and conserved a similar level of fluid to the low salt group. This was evident by the change from a low USG, high fractional excretion rate and high urinary sodium concentration at 0 h to a significantly higher USG, a urinary sodium concentration similar to that of the low salt group at 0 h (normal) and no difference in the fractional excretion rate indicating a similar rate of sodium conservation at this point.

Future experiments may also investigate whether slaughtering sheep fed a high salt diet earlier than 48 h after removal from feed and water would result in increased carcass weights. This experiment has shown that at 24 h, the high salt group had a higher body
fluid content compared to the low salt group due to the loss of excess fluid only. It is postulated that this may have resulted in increased fluid content of the muscles and therefore heavier hot carcass weights.

Feeding a high sodium chloride diet prior to curfew was also counterproductive in relation to keeping sheep clean and dry during transport. The truck driver made the comment that this consignment of lambs was a “very wet” load. Although no measurements were taken of the volume of urine produced during transport, this observation would be consistent with the lambs on the high sodium chloride diet producing a high volume of dilute urine for 24 h after the commencement of fasting.

In other observations in this experiment, betaine was associated with a lower GR tissue depth for the +salt+betaine. Reasons for this finding are unclear especially considering GR depth was higher for sheep on the betaine diets in experiment 1. Betaine has been shown to decrease carcass fatness when fed for extended periods of time (Silander-Rasi et al. 2003) however these diets were only fed for 7 days and this time period was unlikely to produce similar results. An alternative theory is that the tissue density may have been changed by the addition of betaine to the diet. There was a trend (P=0.13) for higher muscle dry matter of the muscle in sheep receiving the betaine. The lower fluid content of the muscle is essentially ‘shrinking’ the tissue depth thus reducing the density and final tissue depth. This theory conflicts with the initial proposal that betaine would retain the water in the muscles thus preventing dehydration.
7.4. Conclusions

Under two different commercial scenarios the benefits of feeding sodium chloride and or betaine for 7 days prior to farm curfew, lairage and slaughter was investigated.

Feeding these diets prior to a 48 hour water deprivation period prior to slaughter did not provide a buffering effect against a period of water deprivation. No differences in liveweight loss, carcass weight, muscle dry matter or muscle weights were observed between the treatments. The consumption of the high salt diet did result in higher fluid content in plasma and gut at the start of the 48 h water deprivation period but by slaughter this excess fluid was lost plus a similar amount to that lost by the low salt group. It may be assumed at this point that similar fluid levels were present in the interstitial and intracellular spaces between both groups. Therefore both groups were at a similar physiological and hydration status by 48 h resulting in no difference in carcass fluid content between treatments.

If water is provided in lairage, these diets also offered no benefit for liveweight and carcass weight or muscle weights. By 24 h and reaching lairage both groups were at a similar hydration status and had conserved similar levels of fluid. As a result any water intake from 24 h had a similar outcome in both treatment groups and is likely to have been used to maintain blood volume. Therefore no difference in fluid content of the carcasses between treatments was likely at slaughter and no treatment differences in carcass weight could be expected.
In experiment 2, the high salt group appeared to drink more water in lairage than the low salt group. The low salt group also consumed water in lairage, possibly imitating the high salt group. In studies by Jacob et al. (2006b; 2005b) it has been reported sheep do not drink in lairage. If this had been the case the correct comparisons should have been between the high salt fed sheep in experiment 2 with the low salt sheep in experiment 1. Using such a comparison it is clear from USG and muscle dry matter content that the sheep fed the high salt diets were more hydrated. The high salt fed sheep had significantly lower USG (1.032 vs 1.05 respectively) and muscle dry matter content (24.9 vs 28.0 % for the SM muscle and 24.5 and 27.9% for the ST muscle) than the low salt at slaughter. Further research is needed to determine if the low salt sheep drank in lairage as a learned response from the high salt group or if both groups drank because of other reasons such as placement and density of water outlets.

In both experiments, betaine did not offer any clear benefit to hot carcass weight or muscle dry matter. This may be because levels in the diets were not high enough or because the osmotic role of betaine is specifically for non-ruminants.
Chapter 8

General Discussion and Conclusions

The experiments reported in this thesis have provided a unique insight into the carcass and eating quality of sheep grazed on saltbush based saline pasture systems. Some fundamental biochemical and physiological questions on saltbush ingestion and a high salt load were also answered. However the application of many of the findings in this thesis still requires future research to understand how they may be of benefit.

The central hypotheses were:

1. Grazing saltbush based saline pasture systems will result in a high quality carcass
2. Grazing a saltbush based saline pasture systems can be used to produce meat with high eating quality

The central hypotheses are discussed in this chapter.
8.1. Discussion of central hypotheses:

8.1.1. Hypothesis 1: Grazing saltbush based saline pasture systems will result in a high quality carcass

The hypothesis that grazing saltbush would result in a carcass of high quality was partially supported.

8.1.1.1. Fatness and fatty acid composition

Ingesting saltbush resulted in carcasses with lower proportion of fat and higher proportion of lean compared to sheep grazing either a grain-hay based diet or a pasture-stubble paddock in both the animal house and field experiments respectively. Importantly this finding occurred without any difference in hot carcass weight between the saltbush and pasture-stubble treatments. This is a commercially desirable result for processors, farmers and the consumer. There is a commercial focus by processors to produce higher yielding, leaner carcasses with premiums being paid for products meeting these specifications. Greater profits can be made by processors due to the reduced costs of fat denudation and an increase the product available for sale. Farmers can also increase their farm productivity from depositing less fat and more lean for every kilogram gain in liveweight. Furthermore, consumers are also demanding less fatty meat.

The reasons for this reduction in fat deposition and increase in lean content on the carcasses in the saltbush fed sheep in both the animal house and field experiments was attributed primarily to the high protein:energy ratio available for production compared with
traditional grain and forage diets, but may also be associated with secondary compounds in saltbush such as chromium and betaine.

The changes in body composition were consistent with the hormonal changes measured in sheep fed saltbush in the animal house. The sheep had lower levels of insulin and higher GH levels. Lower insulin levels result in greater fat deposition (Breier 1999; Guyton et al. 2000). Higher GH stimulates triglyceride breakdown (Breier 1999) and increases the deposition of protein by increasing the transfer rate of amino acids into cells to be used for protein synthesis as well as decreasing the catabolism of proteins (Lobley 1998; Oddy 1993).

Ingesting the saltbush and barley diet in the animal house resulted in a significantly lower percentage of unsaturated fat content in the fat depots and unchanged levels of saturated fat compared to the sheep fed the control diet. This may have commercial implications as consumers are demanding meat with higher levels of ‘healthy’ unsaturated fat. This finding may be associated with the higher energy content of the control diet and the higher proportion of fat on the carcass of the control fed sheep (Eichhorn et al. 1985). The combined consumption of saltbush and a high level of supplementation with a high energy source in the field may prevent the deposition of high levels of saturated fat.
8.1.1.2. **Hot carcass weight**

This research has demonstrated that the use of saltbush to finish sheep for slaughter is not appropriate to attain a commercially desirable carcass weights unless accompanied by supplementation with a high energy source. In the animal house experiment (chapter 4) a 60:40 saltbush and barley diet (equivalent to 480 g/head.d barley) enabled 60 g/d liveweight gain. Whereas in the Wickepin 2004 field experiment where sheep were fed low levels of barley (below 250 g/head.d on average), liveweight gains of only 20 g/head.day for the saltbush fed sheep and 31 g/head.day for the pasture grazed sheep were observed. These growth rates resulted in hot carcass weights of only 18 kg. The class of sheep used in this experiment were hoggets and an 18 kg hogget carcass is not commercially acceptable. Likewise in the Goomalling 2003 field experiment where sheep were also fed a low level of grain supplementation (below 200 g/d.head on average), sheep in both treatments lost weight. Low and undesirable carcass weights of 14 kg were achieved. Therefore, the use of saltbush in the field to produce commercially desirable carcass weights of above 18 kg may only be successful if high levels of grain supplementations are used in combination.

The growth results in this study are consistent with those in the study by Hopkins *et al.* (1999), Chriyaa *et al.* (1997) and Franklin-McEvoy (2002). The low growth rates can be attributed to a decreased availability of energy substrates, low feed intake and increased energy output of sheep on high salt diets.

This research has also demonstrated that sheep grazing saltbush were less dehydrated than sheep fed wheat stubble residues at slaughter. The saltbush fed sheep produced a higher
volume of urine with a low USG and osmolarity as well as having a higher muscle fluid content in comparison to sheep grazing pasture/stubble. Although saltbush did not improve dressing percentage or hot carcass weight these results suggested that saltbush ingestion may delay the onset of dehydration prior to slaughter.

Feeding components of saltbush on a short term basis to improve hydration status was also investigated. Mimicking saltbush, sodium chloride and betaine inclusive diets were fed for a week prior to slaughter under two different commercial scenarios (Chapter 7). In the first experiment the sheep were fed these diets prior to a 48 h period of water deprivation before slaughter whereas in the second experiment these diets were fed prior to a 48 transport and lairage period but access to water was available from 24-48 h. The use of salt was expected to increase the animals’ water intake prior to leaving the farm ‘loading’ the plasma and gastrointestinal fluid with water. Additionally betaine was expected to help prevent the loss of water from the muscle as a result of its osmoregulatory function. The consumption of salt increased fluid retention in the extracellular spaces for both experiments. However after a 48 hour transport and lairage period, with and without access to water in lairage, the fluid content in the muscles was no different to sheep on a low salt diet. Therefore no differences in hot carcass or muscle weights could be expected. Importantly the high salt group did drink in lairage during the second experiment and were hydrated at slaughter. Unexpectedly in this experiment the low salt group also appear to have drunk in lairage. Therefore further investigation of salt to manage dehydration is warranted.
In conclusion, the use of saltbush alone to finish sheep will not provide commercially acceptable carcass weights. A combination of saltbush and concentrates however can be used to achieve some liveweight gains.

8.1.1.3. Meat appearance and biochemistry

Meat from saltbush grazed sheep was (1) redder on day 4 and 5 as indicated by the higher a* value, (2) lighter than the meat from the pasture fed lambs over the assessment period and (3) yellowier on day 4 compared to meat from the pasture fed sheep.

The higher degree of redness of the saltbush fed meat on day 4 and 5 is most likely due to the higher vitamin E content of the loin (LL). Vitamin E is a powerful antioxidant that is thought to reduce the rate of meat browning by reducing the oxidation of myoglobin to metmyoglobin.

There are important commercial implications of reducing the rate of meat browning. Value deterioration due to the conversion of meat from red to brown results in product price markdown, product conversion and rework, product discard, lowered stock inventory, and inefficient use of labour (McDowell et al. 1996). Supermarkets are observing a reduction in the shelf life of meat over the summer and autumn period and this may be due to the low vitamin E intake during this period. This research has demonstrated that grazing saltbush could be used by farmers as a strategy to improve the shelf life of lamb meat in the summer autumn period or to avoid the use of expensive commercial vitamin E products.
In both the animal house and field experiments the ultimate pH was below 5.8, essential to ensure optimal meat quality. This indicates that saltbush and/or salt did not influence glycogen storage.

8.1.2. **Hypothesis 2: Grazing a saltbush based saline pasture systems can be used to produce meat with high eating quality**

The hypothesis that ingesting a saltbush prior to slaughter would result in improved eating quality has not been supported. There was no treatment effect on any of the eating quality attributes assessed in either the animal house or field experiments. Despite there being no improvement in terms of the liking of flavour and aroma, tenderness, juiciness and overall acceptance there was also no negative effects on these attributes. This result indicates that farmers can finish sheep on saltbush and not cause any taint in the meat.

Although this research did not support the anecdotal claims that saltbush reared meat is tastier and juicer, there some indications that meat eating quality may be changed in saltbush fed sheep under some circumstances. Results in this thesis may substantiate the claims made by the suppliers and marketers of these products that the product is of superior eating quality. The high muscle vitamin E levels due to the consumption of saltbush may prevent the development of rancid flavours and odours specifically the oxidative products of polyunsaturated fatty acids such as linolenic acid (C 18:3) (Guidera *et al.* 1997; Wulf *et al.* 1995). So instead of the saltbush meat containing compounds which impart beneficial flavour and aroma volatiles as previously claimed, the improved eating quality may simply be due to the inhibition of off flavour and aroma development compared to meat from
sheep grazed on dry pasture. Importantly, saltbush also has a lower overall polyunsaturated fatty acid content which would reduce the potential oxidising compounds available for deposition. This change in eating quality may be more pronounced in meat that has been aged for longer than tested in this research allowing more time for oxidation.

It is essential to note that there are differences between the saltbush meat niche products from South Australia (SA) and New South Wales (NSW) and the meat generated in this chapter, so that these results are not entirely comparable. The SA and NSW products utilised saltbush grown on pastoral and non-saline land respectively. The potentially higher salt content of saltbush used in the experiments in this thesis may have affected the deposition of flavour and aroma compounds in the meat and fat. Both commercial products also used older animals (mutton) whereas this research used only lambs and hoggets. An older animal does have a more intense flavour and aroma profile (Cramer 1983; Ford et al. 1980) and these basal levels may have contributed to the intense game-like and lasting flavour and not the ingestion of saltbush. The grazing periods used in this research were also shorter than those used for the SA and NSW products. The SA product involves grazing for the entire lifetime of the animal and the NSW product for a minimum of 14 weeks. Had the experiments been run for longer then it is possible that the eating quality would have been different.

8.1.3. Conclusion

The potential for saltbush to result in improvements to carcass composition, meat colour stability with no detriment to eating quality may encourage farmers to consider previously unproductive land planted to saltbush to be a useful enterprise. A potentially useful way to
incorporate these results into an Australian farming system is to use saltbush on a short term basis, not for the length of period grazed in this thesis. The definition of short term will need to be determined by further research but it is essential that minimal liveweight be lost during the grazing period. Hypothetically this time period could range from 2 to 6 weeks. The short term use of saltbush should provide sufficient grazing time for an elevation of vitamin E levels in the muscle to improve meat colour stability, increase the amount of lean and decrease fat levels of a carcass all without changing eating quality. For example; sheep may be grazed on saltbush for a short period prior to entering a feedlot or before slaughter to change carcass composition and boost muscle vitamin E levels and improve meat quality instead of using expensive vitamin E supplements. In addition saltbush could be grazed over short term period to boost vitamin E status in grazing sheep. Particular questions that need to be therefore addressed include how long sheep need to be grazed on saltbush to boost vitamin E levels and change carcass composition. Further work is needed to ensure that these benefits can be achieved without compromising animal production and eating quality.
8.1.4. **Future research**

A number of key areas have been identified in this thesis which warrant further investigation. Future areas of research could assess:

- The minimum grazing period required to achieve the beneficial changes in carcass and fat composition and whether or not these results can be achieved without any detriment to liveweight gain and eating quality.
- The minimal grazing times needed to observe benefits to vitamin E status and meat colour stability from grazing vitamin E rich saltbush.
- The potential presence of other antioxidants in saltbush and the role that may play on improving the health and meat colour stability.
- The benefits of Vitamin E in improving tenderness and juiciness of meat from lean sheep.
- The potential for finishing mutton-aged animals on saltbush on saline land.
- Did the low salt sheep drunk in lairage as a learned response from the high salt group or if both groups drank because of other reasons such as placement and density of water outlets.
- The validity of the sodium:creatinine ratio for estimating daily sodium intake in the field.
- The link between low energy intake and improved betaine action.
References


Anon (2004) 'Renal Clearances: Laboratory Practical Book for Veterinary Physiology.'(Murdoch University)


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Fitzsimons JT (1979) 'The physiology of thirst and sodium appetite.'(Cambridge University Press: Cambridge)


Hardy B, Frape DL (1983) 'Micronutrients and Reproduction, No 1895.'(Hoffman-La Roche: Basel)


Hopkins AF, Congram ID, Shorthose WR (1985) 'Australian consumer requirements for beef and lamb: consumer preferences for three common cuts from lamb carcasses of varying weights and fatness and subject to different rates of chilling.' Part 2 (Meat and Livestock Australia: Australia)


Puls R (1994) 'Vitamin levels in animal health.' (Sherpa International: Canada)


Remus J (1998) 'Betafin- background and practical uses.' (Finnfeeds Ltd: UK)


Warren BE, Casson T (1996) Saltbushes for forage - where have we been and where are we going. *Australian Journal of Soil and Water Conservation* **9**, 41-44.


Warriss PD (2000) 'Meat Science: An Introductory Text.'(CABI Publishing)


Wood JD, Fisher AV (Eds) (1990) 'Reducing fat in meat animals.' (Elsevier Applied Science)


Young OA, Braggins TJ, West J, Lane GA (Eds) (1999) 'Animal production origins of some meat colour and flavour attributes.' Quality attributes of muscle foods (Kluver Academic/Plenum Publishers: New York)


APPENDIX 1

Development of eating quality protocols and test run taste panel results

Methods of assessment

The use of sensory measurements to define eating quality is highly important in new product development, quality assurance, product improvement and determining consumer acceptability. Measurement of palatability can be performed by either trained or consumer (i.e. untrained) taste panels. Trained taste panels determine where an experimental product differs from a standard in respect to a given character (Roessler et al. 1956). Trained taste panel scores will have a smaller variance, but can often be biased by the training procedure. On the other hand a consumer panel is unbiased by definition, but with a larger variance. Having large numbers of panellists and averaging the data can reduce this variance. Establishing a trained sensory panel is expensive and involves rigorous testing of candidates to ensure they have the ability to detect all the basic tastes, a variety of flavours, odours and textures.

The use of consumer sensory evaluation panels does not yield as much information as trained taste panel but are reliable for determining if a change in ingredients, processing, packaging or storage affected the sensory properties of the meat (Bett 1993). Consumers
use a broad spectrum of sensory input including appearance, aroma, flavour and texture to make purchasing and eating decisions related to meat. It makes sense to use consumer perception over a diversity of members of the public as a means of establishing the most important quality factors describing the product (Chambers et al. 1993). Consumer sensory panels were used in this research project.

A variety of testing procedures including qualitative and quantitative consumer measures can be used to generate different information. Qualitative systems include preference or liking tests to identify the acceptability of the product. These tests usually use a scaling system where the panelists assign an intensity rating to each sample across a number of attributes. Quantitative tests can also use a scaling system and there will allow for specific sensory attributes of products to be defined and differentiated. Both of these systems are used in consumer taste panels only commonly for market research for new product development, and direction for improvement.

An important aspect of conducting consumer taste panels is the tasting design. Taste panels on meat products differ from most other manufactured food products, in that the researchers are trying to ascertain both between treatment and between animal variations in the product. If all panelists cannot taste samples from all animals then an incomplete randomised block design is generally used. Because each panelist can only taste between five to 10 samples before fatigue sets in, the randomisation of samples across sessions and presentation order is critical. As scores are given relative to other product being tested, it is important that the full range of treatments be offered at each tasting session.
There most common techniques used with consumer panels are the difference tests; triangle tests and paired comparisons. Triangle tests are used to determine if there is a difference between 3 samples, 2 samples are the same and the third is different. The panellists identify the sample that is different. These tests give no information on liking or preference or indicate how big the difference is. Paired comparisons are used to determine the direction of a difference that exists between two samples with respect to a specific attribute eg; which is saltier. These systems are good to use when a difference is known but neither is appropriate in the assessment of meat products. There are many factors that affect meat quality and as a result, no meat sample will be the same or have one specific attribute that is different.

Meat and Livestock Australia (MLA) have developed a successful commercial consumer tasting protocol (Thompson et al. 2005). The protocols in used are based on those used by Australian sensory groups and the American Meat Science Association. Simply, the MLA system recruits consumer panellists from a broad range of socio-economic backgrounds to participate in consumer taste panels. The protocols for preparation of grilling samples have been described by Thompson et al. (2005). 25mm steaks were cut and grilled to a standard degree of doneness. At each tasting session, a consumer was presented with 7 grilled half steaks, over a 35 minute interval. Consumers were asked to score each sample for tenderness, juiciness, flavour and overall liking. The scoring sheet comprised four 100 mm lines, anchored with the words very tough/very tender for tenderness, very dry/very juicy for juiciness and extremely dislike/extremely like for both, flavour and overall acceptability. Each sensory score was the mean of 10 individual consumer scores.
The use of the MLA commercial consumer taste panels for the assessment of meat is highly expensive and will not be used in this research project. Therefore, the establishment of a successful taste panel protocol must be established to meet the aims of this PhD.

**Material and Methods**

The design, sample preparation, assessment and statistical analysis are detailed in chapter 3 (section 3.4).

Prior to conducting sensory evaluation on experimental meat a test run was conducted on supermarket bought meat. This was done to determine the logistics of the operation and possibly identify problem areas prior to the real runs. The three treatments used in the test run were lamb legs, mutton backstraps and beef rump.

These treatments were chosen to represent differences in flavour, aroma, tenderness, juiciness and overall quality between different types and cuts on meat. The lamb legs were expected to be low in flavour and overall poor quality (hence the assumption was made that they would not be very juicy or tender). The mutton backstraps were anticipated to high quality and be high in flavour and aroma due to the age of the animal. The beef rump was average quality and low in flavour.

40 panelists took part in this test run. There were 6 runs. Therefore the number of samples was 40 * 6 = 240 samples with 80 samples per treatment.
Results

For odour and flavour strength there was a significant difference between the sheep meats and the beef with the sheep meats having a higher level of flavour and aroma. There were also differences between the degree of liking of these qualities (table 9.1)

For tenderness and juiciness- which are considered the key indicators of quality- there were also significant differences between the three treatments. Tenderness was correlated to quality, the higher the quality the higher the score. The mutton product was rated considerably higher than the other two products. For juiciness there was a very large significant difference between the sheep meats compared to the beef.

Panelists also rated the sheep meats, even the poor quality lamb, higher than the beef.
Table 9.1. Sensory evaluation scores (Mean ± standard error)

<table>
<thead>
<tr>
<th></th>
<th>Lamb</th>
<th>Mutton</th>
<th>Beef</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1 (odour strength)</td>
<td>4.94a* ± 0.26</td>
<td>5.21a ± 0.26</td>
<td>3.97b ± 0.26</td>
<td>0.003</td>
</tr>
<tr>
<td>Q2 (like odour)</td>
<td>6.55a ± 0.21</td>
<td>5.98a ± 0.21</td>
<td>6a ± 0.21</td>
<td>0.117</td>
</tr>
<tr>
<td>Q3 (tenderness)</td>
<td>4.72a ± 0.29</td>
<td>6.74b ± 0.3</td>
<td>5.45a ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Q4 (juiciness)</td>
<td>6.73a ± 0.24</td>
<td>6.87a ± 0.24</td>
<td>4.03b ± 0.24</td>
<td>0.001</td>
</tr>
<tr>
<td>Q5 (flavour strength)</td>
<td>5.66a ± 0.23</td>
<td>5.42a ± 0.23</td>
<td>4.14b ± 0.23</td>
<td>0.001</td>
</tr>
<tr>
<td>Q6 (like flavour)</td>
<td>6.33a ± 0.24</td>
<td>6.35a ± 0.24</td>
<td>5.4b ± 0.24</td>
<td>0.011</td>
</tr>
<tr>
<td>Q7 (residual)</td>
<td>4.3a ± 0.25</td>
<td>3.86a ± 0.25</td>
<td>3.8a ± 0.25</td>
<td>0.373</td>
</tr>
<tr>
<td>Q8 (overall acceptance)</td>
<td>5.95a ± 0.28</td>
<td>6.48a ± 0.28</td>
<td>4.9b ± 0.28</td>
<td>0.001</td>
</tr>
<tr>
<td>Q9 (rating)</td>
<td>2.3ab ± 0.1</td>
<td>2.6b ± 0.1</td>
<td>1.9a ± 0.1</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Numbers across the row that aren’t followed by same superscript are significantly different (P<0.05)

Discussion

The test run was a successful endeavour and the technique developed is believed appropriate to use for the remaining eating quality work in this thesis.

The results show that the average consumer is reasonably apt in distinguishing between meat types. Not only have the consumers picked the difference between the sheep and beef meats but also between different degrees of quality.
APPENDIX 2: Pictures

Figure 1. Collection of urine using a rubber pizzle harness and 5 L collection bottle
Figure 2 and 3. Saltbush grazing sites for Wickepin 2004.
Figure 3. Collection of spot sample of urine in the field using rubber harnesses
Figure 5 and 6: Saltbush grazing sites for Goomalling 2003
Figure 7: Typical grazing outcome for saltbush. Wickepin 2004