Faculty of Science and Engineering
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The Effect of Supplementation of Mediterranean Browse Species on Nutritive Properties of Sheep Diets and Predicting these Properties from Faecal Analyses

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This thesis is presented for the Degree of
Doctor of Philosophy
of
Curtin University

March 2023

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council Australian code for the care and use of animals for scientific purposes 8th edition (2013). The proposed research study received animal ethics approval from the Curtin University Animal Ethics Committee.

Date: 14/03/2023

Dedication

To loving sheep (Manju, Yoga, Gamini and the crew) who supported this research.

Abstract

Leguminous *Acacia saligna* (saligna) and *Chamaecytisus palmensis* (tagasaste) and halophyte *Atriplex amnicola* (river saltbush) and *Atriplex nummularia* (oldman saltbush) are major cultivated browse forage species used for ruminant feeding systems in Western Australia (WA). *Rhagodia eremaea* (tall saltbush) is also a halophyte browse species, indigenous to the southern rangelands of WA, and continues to be investigated as a potential forage species for cultivation. Investigations were undertaken on the effects of supplementation and limitations of these browse species (grown in the Mediterranean environment of WA) when incorporated into sheep diets containing oaten (*Avena sativa*) chaff. The potential of faecal attributes (composition) to predict nutritional characteristics (composition, digestibility, metabolisable energy) of sheep diets that included these browses was also investigated.

The chemical composition, biological effect of tannin and *in vitro* nutritive value of these selected browse species (and oaten chaff) were determined prior to the commencement of feeding trials. A total of five feeding trials were undertaken and each involved feeding the experimental diets to individually penned, mature Merino wethers for 7 d adaptation followed by 5 d total collection of feed refusals and faeces for determination of apparent digestibility of the diets. Freeze-dried forage/diet samples and oven-dried faecal samples associated with each feeding trial were ground and subsequently analysed for proximate composition. Forage samples were also analysed for total phenolics (TP), total tannins (TT) and mineral contents and 24 h *in vitro* gas production (GP), with and without the addition of polyethylene glycol (PEG), using the rumen buffer gas fermentation technique. Nutrient digestibility, metabolisable energy (ME) content and short chain fatty acid (SCFA) production of diets were also determined using 24 h net GP.

The reference data and faecal near infrared spectrum pairs (n=240) originating from all of the feeding trials (total of 40 experimental diets) were pooled to derive faecal near infrared reflectance spectroscopy (*f*NIRS) calibrations to predict nutritional (chemical and functional) attributes of browse-containing sheep diets.

The effect of treatments (forage species, PEG, level of supplementation) was tested by analysis of variance (ANOVA) procedure. Appropriate mean comparison procedures (Duncan's new multiple range test, least significant difference test or t test) were employed to compare means of the treatments. Limitations to inclusion of the browses in the diet were assessed with the help of Pearson pair wise correlation coefficient (r). Stepwise regression procedure was used to develop multiple regression models to predict the nutritional characteristics of the diets from faecal chemical composition. The predictive model with the highest coefficient of determination (R^2) and lowest residual standard deviation (RSD) was selected as the best-fit predictive model. The nutritional characteristics of the diets used in the validation experiment were predicted from respective faecal indices by best-fit predictive regression model. The estimates (slope, intercept, R^2) of the regression between measured and predicted nutritional characteristics of the validation experiment were used to test the validity of the best-fit predictive model. Modified partial least squares procedure was used to develop fNIRS calibrations for chemical and functional attributes of sheep diets. The precision of calibrations was evaluated by the R^2 of the calibrations (R^2_c>0.80) and standard error of calibration (SEC). The predictive ability of calibrations was evaluated by standard error of cross-validation (SECV), standard error of prediction (SEP), slope of the validation regression and the ratio of the standard deviation of the reference data to the SECV (RPD>3).

The crude protein (CP) content of all the browse species was higher than that of oaten chaff. The CP content of oaten chaff, *C. palmensis*, *A, saligna*, *A. amnicola*, *A. nummularia* and *R. eremaea* were 52.5, 121.3, 134.5, 96.7, 133.8 and 216.7 g/kg DM, respectively. *Acacia saligna* contained the highest concentrations of acid detergent lignin (ADL) and TT (123.2 and 28.9 g/kg DM, respectively). The TT content was moderate in *C. palmensis* (8.9 g/kg DM) and very low in the selected halophyte species (0.9 - 2.0 mg/kg DM) as well as in oaten chaff (1.3 g/kg DM). While the halophytes were extremely high in ash (156.9 to 178.8 g/kg DM), all browse species were rich in most minerals including calcium (Ca), magnesium (Mg) and zinc (Zn), which were in much lower concentrations in oaten chaff (basal diet). *In vitro* organic matter digestibility (OMD) was 582.2, 667.5, 452.5, 494.9, 526.6 and 492.9 g/kg DM and *in vitro* metabolizable energy (ME) was 8.7, 10.0, 6.6, 7.2, 7.6 and 7.0 MJ/kg DM for oaten chaff, *C. palmensis*, *A. saligna*, *A. amnicola*, *A. nummularia* and *R. eremaea*, respectively. Only *A. saligna* showed significant improvements in *in vitro* GP, OMD and ME due to the addition of PEG. The *in vivo* (apparent) DM digestibility (DMD), OMD and CP digestibility (CPD) of *C. palmensis* were 567, 563 and 699 g/kg DM while those of *A. saligna* were 446, 449 and 290 g/kg DM, respectively. The *in vivo* digestible OM (DOM) and digestible CP (DCP) contents of *C. palmensis* were 508 and 91 g/kg DM while those of *A. saligna* were 392 and 33, respectively. The *in vivo* DOM and DCP contents of oaten chaff varied between 477 and 501, and 13 and 19 g/kg DM, respectively.

The inclusion level of browse had significant effects on the digestible nutrient contents and ME of the total diet, with digestible DM (DDM) content of the diets decreasing with increasing substitution of fresh browse for oaten chaff in the diet. More importantly, the level of DCP steadily increased with increasing browse supplementation. Increasing the

inclusion of *A. saligna* and *A. amnicola* in the diet resulted in decreases in both DOM content and ME. However, these dietary parameters initially increased and then decreased with increasing inclusion of *C. palmensis* in the diet.

Results of this study showed the potential of browse species to improve the nutritive value of roughage-based (in this case oaten chaff) sheep diets. However, the level of inclusion of the browse species was limited due to their low ME. The low ME of *C. palmensis* and *A. saligna* was due to poor digestibility likely associated with their high ADL and tannin contents. The low ME of *A. amnicola* was associated with its low nitrogen (N) to sulphur (S) ratio (N:S) and high levels of ash. Based on the results of this study, the recommended supplementation levels of leaves and edible twigs of *A. saligna, C. palmensis* and *A. amnicola* for low quality roughage diets (oaten chaff) were 479, 831 and 496 g/kg DM, respectively.

The best-fit predictive regression model for dietary CP (g/kg DM) from faecal composition had a very low R^2 (0.21). The best-fit regression models predicting dietary TP (g/kg DM), TT (g/kg DM), OMD (g/kg DM), SCFA (mmol/40 mL) and ME (MJ/kg DM) from faecal N (g/kg DM), ash (g/kg DM) and ADL to neutral detergent fibre (NDF) ratio had R^2 greater than 0.78 and low RSD. In the validation experiment, regressions of predicted TP and TT against measured TP and TT had positive intercepts and low R^2. The slopes of these regressions were much lower than 1.0. Intercepts of the regressions of predicted OMD, SCFA and ME against their measured values did not differ from zero. The R^2 of these regressions were very high and slopes were close to 1.0. The CP content of browse-containing diets could not be predicted from faecal fibre, ADL and ash contents. However, these faecal indices could collectively predict the OMD, SCFA production and ME of browse-containing sheep diets. These predictive models could

effectively be used to formulate supplementary feeding programmes for sheep reared in WA rangelands where the nutritive value of forage browse is not directly measurable and thus have broad application.

The R^2_c was greater than 0.80 for all *f*NIRS calibrations (CP, TP, TT, protein precipitating capacity (PPC), phosphorus (P), *in vivo* DMD, *in vivo* OMD, *in vivo* CPD, *in vitro* OMD, *in vitro* ME and *in vitro* SCFA). The SEC was close to the respective SECV. Slope of the validation regressions did not deviate from 1.0 for chemical attributes but deviated from 1.0 for functional attributes (except for SCFA). The RPD ratio of *in vivo* DMD and *in vivo* OMD was less than 3.0 while the ratio was greater than 3.0 for other (CP, TP, TT, PPC, P, *in vivo* CPD, *in vitro* OMD, *in vitro* ME, *in vitro* SCFA) calibrations. Data derived from the feeding trials could be used to derive robust *f*NIRS calibrations to predict important chemical attributes (CP, TP, TT, PPC of tannin, P) of browse-containing sheep diets. Although, *f*NIRS calibrations predicting dietary *in vitro* functional properties (digestibility and ME) were superior to those predicting *in vivo* functional properties, both were not so robust. Statistics of *f*NIRS calibrations derived using reference data originating from *in vitro* methods needs to be carefully interpreted.

Faecal chemical composition and NIRS-based calibrations are recommended to predict functional and chemical attributes of browse-containing sheep diets, respectively.

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Acknowledgements

Firstly, I extend my immense gratitude to my principal supervisor, Assoc Prof. Gaye Krebs, for her friendly supervision, guidance, patience and support throughout this doctoral program. I especially appreciate the fact that you trusted and motivated me so much during this period, without which completion of this task would be so difficult.

I would also like to thank my Associate Supervisor, Mr. Peter McCafferty and co-researchers of the research group, Mr. Ken Dods, Mr. Tom Naumovski, Dr. Kevin Ho and Mr. Richard Stephens of ChemCentre (Western Australia) for their support and active involvement in the *f*NIRS component of the research project.

Appreciation is also rendered to Curtin University of Technology for providing the Endeavour International Postgraduate Research Scholarship (EIPRS) to undertake a PhD at the university and the Rural Industries Research and Development Corporation (now AgriFutures) for funding the research program.

The support of Prof. Graeme Robertson (formerly of Curtin University), Assoc. Prof. Peter Batt (formerly of Curtin University), Assoc. Prof. Sarita Bennet, Dr. Franca Jones and Prof. Damien Arrigan is greatly appreciated, especially during the difficult times associated with completing this thesis.

I would also like to recognise Prof. L.H.P. Gunaratne of the University of Peradeniya, Sri Lanka and my friends Suriyagoda and Chalinda for their generous help in the research program.

Special thanks are given to Bryden Krebs and Mrs. Beverley Pratt for helping me to keep the experimental sheep healthy. Many thanks must be given to staff of the former School

of Agriculture and Environment (at Muresk), including Karen Shackleton, Terrie Sheridan and Zen Birkholze for their kind assistance.

Lionel Martin, Angelo, Daya aiya and Ramani akka are always thanked for making our life comfortable in Northam.

I must thank all the sheep who helped in the trials; without you friends the trials would not have happened. Please forgive me for the inconvenience caused by experimenting with you. How can I ever pay off your scarifies?

I am thankful to my loving parents for growing me up and teaching me to face many challenges in life. Last but by no means least, from the bottom of my heart I thank my wife Harshi for your time with my experiments and patience during my studies.

If I have forgotten anyone else, my sincere apologies.

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Glossary of Abbreviations

ADF	Acid detergent fibre
ADFD	Acid detergent fibre digestibility
ADL	Acid detergent lignin
ANOVA	Analysis of variance
ARC	Agricultural Research Council
BSA	Bovine serum albumin
BW	Body weigh(s)
CEL	Cellulose
CELD	Cellulose digestibility
CF	Crude fibre
CP	Crude protein
CPD	Crude protein digestibility
CT	Condensed tannins (also known as proanthocyanidins)
CV	Coefficient of variation
CWC	Cell wall content
DADF	Digestible acid detergent fibre
DCEL	Digestible cellulose
DCP	Digestible crude protein
DDM	Digestible dry matter
DDMI	Digestible dry matter intake
DE	Digestible energy
DHCEL	Digestible hemicellulose
DIG	Digestibility
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
DNDF	Digestible neutral detergent fibre
DNMRT	Duncan’s new multiple range test
DOM	Digestible organic matter
ED	Effective degradability
EE	Ether extract

f	Faecal
FAO	Food and Agriculture Organisation
FM	Fresh matter
*f*N	Faecal N
*f*NIRS	Faecal near infrared reflectance spectroscopy
GE	Gross energy
GP	Net gas production (from *in vitro* fermentation)
HCEL	Hemicellulose
HCELD	Hemicellulose digestibility
HT	Hydrolysable tannins
IAEA	International Atomic Energy Agency
ICP-AES	Inductively coupled plasma atomic emission spectrophotometer
ILRI	International Livestock Research Institute
ISP	Insoluble proanthocyanidin
LSD	Least significant difference
Max	Maximum
ME	Metabolisable energy
Min	Minimum
MPLS	Modified partial least squares
MPT	Multipurpose tree
NDF	Neutral detergent fibre
NDFD	Neutral detergent fibre digestibility
Ndg	Nitrogen degradability
NE	Net energy
NEL	Net energy for lactation
NRC	National Research Council
OM	Organic matter
OMD	Organic matter digestibility
OMdg	Organic matter degradability
PD	Potential degradability
PEG	Polyethylene glycol
PPC	Protein precipitation capacity
PVPP	Polyvinyl polypyrrolidone

R^2_c	Coefficient of determination of calibration
R^2_{cv}	Coefficient of determination of cross validation
RPD	Ratio of the standard deviation of the original data to standard error of cross-validation
RSD	Residual standard deviation
SA	Saponin
SCFA	Short chain fatty acid(s)
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SEC	Standard error of calibration
SECV	Standard error of cross-validation
SEP	Standard error of prediction
SIP	Simple phenolics
SP	Soluble phenolics
$S_{x,y}$	Residual error
T	Tannin
TEA	Triethanolamine
TO	Total oxalate
TP	Total phenolics
TSP	Total soluble proanthocyanidins
TT	Total tannins
WA	Western Australia

Chapter 1: Introduction

Trees and shrub foliage, often called browse, are important source of nutrients in ruminant production systems in many tropical, subtropical and Mediterranean environments. Browse has long been considered important for the nutrition of grazing ruminants in Australia, particularly in areas prone to extended periods of below average rainfall or drought. In these areas native and introduced browses provide additional green feed when grasses and other herbaceous species are dry and of low nutritional quality. During severe drought when all other forage is absent, browse becomes the only source of protein and energy for grazing animals (Lefroy et al., 1992; Gutteridge and Shelton, 1994). In Australia, there are approximately 200,000 ha of cultivated browse species (Lefroy, 2002) and native species of browse, such as *Acacia aneura* F. Muell. Ex Benth. (mulga) in Queensland and northern New South Wales.

1.1 Browse species

There are a large number of browse species, both in Australia and internationally, that have been utilised as feeds for ruminant animals. With specific reference to Australia, considerable research has been undertaken on the use of a number of trees and shrubs as sources of forage (Norton, 1994a; 1994b; Monjardino et al., 2010; Vandermeulen et al., 2018), particularly *A. aneura* (McMeniman et al., 1974; Burrows et al., 1990; Goodchild, 1990; Pritchard et al., 1992; Lowry et al., 1996; Dynes et al., 2002; Robins and Brooker, 2005) and *Leucaena leucocephala* (Jones et al., 1984; Quirk et al., 1988; Goodchild, 1990; Shelton & Dazell, 2007; Dalzell et al., 2012; Taylor et al., 2016; Wildin, 2019). However, the focus of the research presented in this thesis were five other browse species of known or potential feeding value for small ruminants in Australia, viz. *Chamaecytisus*

palmensis, Acacia saligna, Atriplex amnicola, Atriplex nummularia and *Rhagodia eremaea.*

Chamaecytisus palmensis (H. Christ) F.A. Bisby and K.W. Nicholls, is a tree legume belonging to the family Fabaceae and genus *Chamaecytisus* (tagasaste, Figure 1.1). The species is native to islands of La Palma in the Canary Islands, was first introduced in 1879 and has since become naturalised in southern Australia. In Australia, the species is also known as tree lucerne. It is a shrub or small tree growing to about 5-7 m in height with a crown diameter of about 5 m. There are erect and prostrate types. The branches are long, drooping, and leafy. The leaves are trifoliate and grey-green colour, with 70 mm long leaflets (Newcomb, 1999; George, 2003; Cook et al., 2005). Being a deep-rooted (10 m) perennial species, which absorb moisture from subsoil, *C. palmensis* is an evergreen plant. It is recommended for sandy, hilly, gravelly soils in drought-prone areas where it can provide good quality forage all year round. However, because of the relatively slow growth rate and recovery after harvesting during winter, the species best provides high quality fodder during summer and early autumn (George, 2003). Owing to its high forage value (Assefa et al., 2015; Sulas et al., 2016; Melesse et al., 2019), it is one of the two cultivated browse species that has gained commercial acceptance as a sole source of forage during seasons of feed shortages (Lefroy et al., 1992). Where it has established in areas with deep sandy soils, it is a profitable and sustainable addition to annual pasture systems (Lefroy et al., 1997).

Figure 1.1: *Chamaecytisus palmensis* (tagasaste, tree lucerne)

Acacia saligna (Labill.) H.L. Wendl is a broad-leaved, perennial, woody, tree legume shrub (Figure 1.2) that belongs to the family fabaceae and genus *Acacia*, and is indigenous to Western Australia (WA). The species is commonly known as saligna, golden wreath wattle, orange wattle and Western Australian golden wattle. The plants are single or multi-stemmed and thornless. These small trees are generally 2-6 m tall, but may grow up to 9 m. Where it has become naturalised, it is commonly a dense bush which may be wider than it is high. The dark green to blue-green, 80-250 mm long, narrow, phyllodes leaves are either straight or sickle in shape (Vercoe and McDonald, 1987; Whibley and Symon, 1992). It grows on a range of soil types (deep sand to mildly saline and waterlogged sand clay) across southern Australia. The leaves are palatable to livestock when fresh (Sallam et al., 2017) or dried (Zegeye et al., 2016; Yirdaw et al., 2017; Golender et al., 2021). The plant regrows well after grazing/harvesting, although, the nutritive value of the regrowth will vary depending on the season of grazing/harvesting and height of cutting. Krebs et al. (2003) found that regrowth from spring-cut plants generally had higher crude protein (CP) content and organic matter digestibility (OMD)

than regrowth from autumn- or winter-cut plants. Kandle and Sharief (2017) found cutting established *A. saligna* plants at 30 cm height increased the foliage yield and its quality. Although it is grazed by sheep, its incorporation into ruminant feeding systems in Australia is much less than that of *C. palmensis* (Lefroy et al., 1992), presumably due to its relatively lower feeding value as well as its potential to become invasive without appropriate management (Nsikani et al., 2017; Cohen et al., 2018). *Acacia saligna* has only moderate CP and fibre contents (George et al., 2007). Previous research has shown that the concentrations of condensed tannins (CT) and phenolics (Krebs et al., 2007) and the protein precipitation capacity (PPC) of these tannins (George et al., 2007) are high in *A. saligna* and this also limits its feeding value for ruminants (Degen et al., 1995; Krebs et al., 2007).

Figure 1.2: *Acacia saligna* (saligna, golden wreath wattle)

Atriplex amnicola Paul G. Wilson belongs to the family Amaranthaceae and genus Atriplex and is commonly known as river saltbush or swamp saltbush (Figure 1.3). The species is a multi-branched shrub that grows as either an erect or prostrate shrub while

the branches spread along the ground. The shrub may grow up to 2.5 m high and 4 m wide under ideal conditions. The bluish-green colour leaves are covered with fine silvery hairs. The leaves vary in size and shape but are often 10-20 mm long and spear-shaped (Mitchell and Wilcox, 1994). *Atriplex amnicola* is endemic to WA and is considered to be among the most productive browse species in the Australian rangelands (Masters et al., 2010). Since the species is highly salt tolerant it is used to rehabilitate saline and other degraded areas in Australia (Commander et al., 2013). When grown on saline soil, it has the best long-term survival and growth compared to other saltbush species. Further, well mature shrubs of the species are relatively tolerant to drought and waterlogging conditions. It has a relatively high CP content (Ben Salem et al., 2002a; 2002b) and is low in tannins (Ahmed et al., 2015), although, its nutritive value and palatability could be influenced by the salinity of the soil and the surrounding water (Masters et al., 2010). The species is highly favoured by sheep and recovers well from grazing (Mitchell and Wilcox, 1994; Barrett-Lennard and Malcolm, 1995).

Figure 1.3: *Atriplex amnicola* (river saltbush)

Atriplex nummularia Lindl is also a saltbush species belongs to Amaranthaceae family and *Atriplex* genus (Duilio Iamonico, 2012). The large woody shrub is commonly known as oldman saltbush (Figure 1.4). It is one of the most important perennial halophyte forage shrub species suitable to alkaline and saline lowlands (Emms, 2008). The species is extremely hardy and able to thrive under harsh conditions. *Atriplex nummularia* is native to Australia and occurs in every State of the country. It is the largest species of Australian saltbush, typically growing 2–4 m wide and up to 3 m tall in either a sprawling or erect arrangement (Bauder et al., 2008). The bush develops woody stems which branches from close to the ground and also has a moderate to deep taproot system (Duilio Iamonico, 2012). The irregular, simple alternate, 10-50 mm long, oval to round leaves have silvery grey coating on both sides with a scaly texture (Emms, 2008). *Atriplex nummularia* is much valued for its ability to provide year-round, good quality (Norman et al., 2008) forage for grazing by extending feed availability into dry periods (Li et al., 2018).

Figure 1.4: *Atriplex nummularia* (oldman saltbush)

Rhagodia eremaea Paul G. Wilson is another halophyte browse species indigenous to WA (Mitchell and Wilcox, 1994). The species belongs to Chenopodiaceae family and genus *Rhagodia.* It grows well on sand, clayey or sandy loam, and often stony soils. The rounded, straggly shrub is 0.6-2 m high and commonly known as tall saltbush (Figure 1.5). On average, the leaves are about 20 mm long and 10 mm wide. They are spear-head shaped and sometimes lobed at the base. Very short waxy hairs on the leaves give tall saltbush its silver grey-green appearance. During the drought the shrub sheds some leaves (Mitchell and Wilcox, 1994). *Rhagodia eremaea* is moderately palatable to livestock based on pastoralists' observations of sheep grazing and the evidence of utilisation observed in the field (Mitchell and Wilcox, 1994; Russell and Fletcher, 2003). It is resistant to heavy grazing and able to persist when more palatable species are eliminated (GBIF Secretariat, 2021).

Figure 1.5: *Rhagodia eremaea* (tall saltbush)

Establishment of halophytic plant species such as Atriplex remains one of the few feasible opportunities to revegetate salinity-affected grazing landscapes in Australia (Masters et

al., 2001). The forage of these species is high in CP and a rich source of minerals (Islam and Adams, 2000; Norman et al., 2004b). However, Atriplex species are typically low in metabolisable energy (ME) as evidenced by the improvements in total digestibility (Ben Salem et al., 2004; Ben Salem et al., 2005a) and microbial protein synthesis (Ben Salem et al., 2002a; 2002b) when livestock fed these browses are supplemented with cereal grain or other energy sources (Norman et al., 2008; Shawket et al., 2010; Khattab and Anele, 2021) Both *A. nummularia* and *A. amnicola* are widely used in commercial grazing systems on saline land (Norman et al., 2004a). Currently, *R. eremaea* is not commercially cultivated and its chemical composition and functional properties have not been previously reported.

The potential of these browses as supplementary feeds for sheep has not been adequately investigated, particularly with regards to the impact of level of inclusion on the nutritional characteristics of the total diet. Investigation of the effect of level of supplementation would give valuable information about any limitations of inclusion of these browses in diets for sheep. With knowledge of the optimal level of inclusion in the diet, grazing systems with appropriate stocking rates could then be designed to encourage sheep to select these browses at the appropriate level.

1.2 Potential of faecal attributes to predict dietary attributes

Being able to predict the nutritive value of the diet of grazing animals in the field would greatly enhance the ability to decide suitable supplementary feeding regimens to improve animal production. Varying degrees of associations between faecal chemical composition and attributes of ruminant diets have been reported in the past. The nutritive value of browse-containing sheep diets is affected by the PPC of tannins that are found

in many browse species. The concentration of tannins varies widely and is largely unpredictable (Makkar, 2003a). Condensed tannins bind to fibre and protein in the ruminant digestive tract (Degan et al., 1995; Makkar et al., 1995), increasing faecal excretion of N and fibre (Kaitho et al., 1998c; Ben Salem et al., 2005c; Krebs et al., 2007). Those browse species high in tannins also tend to be high in lignin (Gasmi-Boubakker et al., 2005). Faecal fibre, lignin and N contents could thus potentially indicate the level of phenolics, tannin and PPC of tannin present in the diet. Previous research suggests that faecal fibre fractions have the potential to predict digestibility and energy content of the diet (Vera, 1973; Hodgman et al., 1996). Faecal N has been shown to be closely associated with dietary N (Holecheck et al., 1982a; Mubanga et al., 1985), OMD (Boval et al., 2003) and ME (Kamler and Homolka, 2005) of typical grass/legume diets. However, faecal N is less useful for predicting the digestibility (Holecheck et al., 1982a; 1982b) and N content (Nastis and Malecheck, 1981; Mould and Robbins, 1981) of diets high in soluble phenolics or tannins. Therefore, including multiple faecal components such as faecal fibre, lignin and N may improve predictability of the nutritive and phenolics-related attributes of browse-containing diets for sheep.

Faecal near infrared reflectance spectroscopy (*f*NIRS) is a non-invasive technique that has been used to predict nutritive attributes of cattle (Boval et al., 2004; Dixon and Coates, 2005; Coates and Dixon 2007; 2008; 2011; Dixon and Coates, 2015), deer (Kamler et al., 2004; Showers et al., 2006; Tellado et al., 2015) and small ruminant diets. Up until recently, *f*NIRS calibration equations had to be developed for each diet and animal species under consideration. Variable success has been achieved with regard to developing either generic or multispecies *f*NIRS calibrations for assessing dietary attributes (Jancewicz et al., 2016; Villamuelas et al., 2017; Tolleson et al., 2021). With specific references to small

ruminants (as opposed to cattle and deer), as they were the focus of the research reported in this thesis. Landau et al. (2004) used *f*NIRS equations to predict the CP content, *in vitro* dry matter digestibility (DMD) and polyethylene glycol (PEG) binding tannin of diets containing lucerne hay, concentrate and browse species fed to Mediterranean goats whilst Li et al. (2007) derived *f*NIRS equations to predict CP and *in vivo* digestible organic matter (DOM) contents of diets containing grass hay, forbs and browse species fed to sheep in North America. More recently, Núñez-Sánchez et al. (2016) found *f*NIRS could be used to accurately predict the botanical composition, proportions of forage ingredients and chemical composition of diets consumed by ewes having free access to a variety of feed ingredients. *f*NIRS predictions would be more widely useful if more 'generic' type predictive equations could be developed. By merging databases of feeding experiments conducted in Belgium and France, a *f*NIRS equation was derived to predict *in vivo* OMD of a wide range of temperate forages for sheep (Decruyenaere et al., 2009). Data mining of past digestibility trials conducted in France, Italy and Israel has resulted in the development of *f*NIRS equations to predict an array of nutritive attributes including CP, OMD and CP digestibility (CPD) of sheep diets (Landau et al., 2008). Kneebone and Dryden (2015) found *f*NIRS equations derived from a dataset that included representatives of 25 diets, successfully predicted the characteristics of diets that included forages fed alone and with the type of supplements used in tropical Australia. *f*NIRS calibrations predicting diet quality have not been derived for sheep in Western Australia (WA). The most useful *f*NIRS equations would be those that predicted the nutritive attributes of a range of diets fed to sheep.

1.3 Hypotheses and objectives

Acacia saligna, C. palmensis, A. amnicola, A. nummularia, R. eremaea are Mediterranean browse species known to be high in CP thus are invaluable source of N supplements for sheep. However, the nutritive value of sheep diets which included the browse species may be influenced due to the presence of phenolics in particular the tannins in the species. Therefore, investigating the biological effects of supplementation of these Mediterranean browse species on the nutritive attributes of sheep diets and the potential of predicting these attributes from faecal analysis were the focus of the research presented in this thesis. The most biologically important nutritive attributes of forage are OMD, ME and ruminal microbial protein synthesis. The over-arching null-hypotheses (h_0) of the experiments conducted were as follows:

- The nutritive value of the browse species (*A. saligna, C. palmensis, A. amnicola, A. nummularia, R. eremaea*) and oaten chaff used for the experiments are not significantly different.
- There is no effect of supplementation with browse species (*A. saligna, C. palmensis* or *A. amnicola*) on the nutritive value of oaten chaff-based diets fed to sheep.
- Nutritive attributes of browse-supplemented diets fed to sheep can not be predicted either from faecal chemical composition or from *f*NIRS analysis.

The specific objectives of the studies presented in the thesis were to:

- Investigate and compare the chemical composition, *in vitro* nutritive value and biological effect of tannin of *A. saligna, C. palmensis, A. amnicola, A. nummularia* and *R. eremaea* grown in the Mediterranean environment of WA.

- Investigate the effect of level of supplementation with either *A. saligna*, *C. palmensis* or *A. amnicola* on the total nutritive value of oaten chaff-based diets fed to sheep.
- Derive multiple regression models to predict the nutritive and phenolics related attributes of browse-containing sheep diets from fibre, lignin and N contents of faeces.
- Investigate the usefulness of data and faecal samples generated from the experiments reported in this thesis to derive *f*NIRS calibrations to predict the chemical and nutritive attributes of browse-containing sheep diets.

Chapter 2: Literature Review

2.1 Scope of the review

Arid and semi-arid zones cover more than 70% of the land area of Australia. Indigenous trees and shrub species are the most important forages in the nutrition of grazing and browsing ruminants in these zones, particularly where the quantity and quality of pasture declines during seasonal dry periods. Acacia shrublands cover about 26% of the Australian land mass and it is one of the three main vegetation types that occur in arid and semi-arid zones. Out of the total area of 20,000 – 30,000 ha of shrub fodder species being cultivated in the country, 5,000 ha is estimated to be planted with the indigenous legume, *A. saligna* (formerly known as *Acacia cyanophylla* Lindl). The tree legume, *C. palmensis* was introduced to Australia in 1879 and is now naturalised in southern Australia on freely drained soils in areas receiving more than 500 mm annual rainfall. More than 10,000 ha in WA has been planted with this species, predominantly in deep sandy soils. *Chamaecytisus palmensis* is one of the two cultivated species that has gained commercial acceptance as a sole source of forage (Lefroy et al., 1992). Establishment of halophyte species such as Atriplex is one of the few options to revegetate over one million hectares of salt affected land in southern Australia (Masters et al., 2001). *Atriplex amnicola* and *A. nummularia* are indigenous to the salt lakes and drainage lines of dry temperate and semi-arid Australia (Lefroy et al., 1992; Mitchell and Wilcox, 1994).

The literature review consists of two main areas associated with browse feeding and nutrition for ruminants, and in particular sheep. Literature on the chemical composition, anti-nutritive factors and nutritive value of *A. saligna, C. palmensis, A. amnicola* and *A. nummularia* which were the browse species focus of the research is reviewed. Then the published information on predicting the nutritive value of the diets of grazing ruminants

using chemical composition and *f*NIRS technology and the challenges of such determinations particularly when the diet contains browse species are reviewed.

2.2 Assessing chemical composition of browse diets

The nutritional or feeding value of a feed can be considered in relation to a number of factors. The concentrations of nutrients in the feed are usually based on chemical analysis. The voluntary intake of feed, also referred to as palatability, is influenced by the chemical composition of the feed. The digestibility describes the availability of those nutrients to the animal. Additionally, the efficiency which the absorbed nutrients are utilised by the animal is considered in assessing the nutritional value of animal feed (Coleman and Henry, 2002).

2.2.1 Proximate composition

Proximate analysis and detergent-based systems are the most widely used systems to assess nutritional characteristics of feeds. The nutritional characteristics investigated in the research presented in this thesis included dry matter (DM), ash, CP, neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). Ash is defined as the residue remained after a sample is ignited at 550ºC until all carbon (C) has been removed. The residue represents the inorganic constituents of the forage. However, the residue may also contain material of organic origin such as sulphur (S) and phosphorus (P) from proteins. Some loss of volatile material in the form of sodium (Na), chloride (Cl), potassium (K), P and S takes place during ignition. Therefore, the residue ash is not truly representative of the inorganic material of the forage (McDonald et al., 2002). The CP is an estimate of the total amount of protein present as calculated from the total N present. The most widely used Kjeldahl method determines the N contained in protein as

well as non-protein such as free amino acids, nitrites, nitrates and certain cyclic N compounds. In the determination of CP content using the Kjeldahl method, it is assumed that all the N of the feed is present as protein and all feed protein contains 160 g/kg of N. Thus, the CP content is different from that of the true protein content (McDonald et al., 2002). Van Soest (1967) defined the NDF and ADF fractions of the fibre component in forages. Neutral detergent fibre is a measure of plant cell wall material and consists mainly of lignin, cellulose (CEL) and hemicellulose (HCEL), while ADF represents the crude lignin and CEL fractions of plant but also includes silicon (Si).

2.2.2 Anti-nutritional compounds

Anti-nutritional compounds (plant secondary metabolites, phytochemicals) are a diverse group of molecules that are involved in the adaptation of plants to their environment but are not part of the primary biochemical pathways of cell growth and reproduction. Common anti-nutritional factors found in browse include polyphenolic compounds, non-protein amino acids and glycosides (Teferedegne, 2000).

Tannins are polyphenolic substances that are broadly categorised into two major groups: hydrolysable tannins (HT) and CT, also known as proanthocyanidins. Hydrolysable tannins consist of a central core of carbohydrate to which phenolics carboxylic acids are bound by ester linkage. Condensed tannins consist of oligomers of two or more flavan-3-ols such as catechin, epicatechin or the corresponding gallocatechin (Makkar et al., 2007). Both CT and HT are present in tree leaves, but some tree leaves may contain predominantly CT whereas others contain predominantly HT (Kumar and Vaithiyanathan, 1990). The concentrations of tannins present in trees and shrubs varies widely (Ogunbosoye et al., 2015) and is largely unpredictable. Multiple phenolic hydroxyl groups of tannins lead to the formation of complexes, primarily with proteins

and to a lesser extent with metal ions, amino acids and polysaccharides (Makkar, 2003a). Hydrolysable tannins are potentially toxic to animals (Makkar et al., 2007). The effects of CT on ruminants are variable. The ingestion of plants containing high concentrations of CT may decrease nutrient utilisation particularly that of protein (Waghorn, 2008), ultimately resulting in a decrease in feed intake (Kozloski et al., 2012). On the other hand, low concentrations of CT have been associated with improved protein digestion and metabolism (Patra and Saxena 2011; Tedeschi et al., 2014) in ruminants, through the creation of rumen undegradable or bypass protein (Muir, 2011), and in protecting them against pasture bloat (McMahon et al., 2000; Min et al., 2006). Recently, Méndez-Ortiz et al. (2018) found that although CT intake did not impact on DM intake (DMI) or live weight change of growing sheep, when dietary CP was low, the presence of CT in the diet increased digestible CP (DCP) requirement, indicating a metabolic cost associated with the consumption of CT.

Alkaloids, terpenoids, oxalate and indospecine may also be found in the foliage of shrubs and trees (Aganga and Tshwenyane, 2003). Alkaloids are a diverse group of organic bases containing secondary, tertiary or cyclic amines. There are about 5,500 known alkaloids, comprising the largest single class of plant secondary metabolites. Chemically, they are a very heterogenous group ranging from simple compounds like coniine to the pentacyclic structure of strychnine. Many alkaloids are terpenoids in nature and some are steroidal; others are mainly aromatic compounds. The bitter taste of alkaloids in fresh leaf or fruits often impacts on their voluntary intake by ruminants (Makkar et al., 2007).

Oxalate is a common constituent in many plant species. Depending on the species, it may accumulate primarily as soluble oxalate, insoluble calcium (Ca) oxalate or a combination

of these two forms. Oxalates possess negative charges and thereby have high affinity for minerals such as Ca, magnesium (Mg) and zinc (Zn) (Makkar et al., 2007).

Tannins, terpenes and saponins form the three major classes of secondary compounds in Mediterranean shrubs (Rogosic et al., 2008). Saponins are glycosidic compounds composed of an aglycone and a sugar moiety varying in the number and type of monosaccharides (Majak, 2001). They are naturally occurring chemical compounds found in a wide variety of forage plants (Milgate and Roberts, 1995). Three major classes of saponins exist in the plants, viz. triterpene glycosides, steroids and steroidal alkaloids; triterpene glycosides are the most common in plants (Jenner et al, 2005). Interactions with cellular and membrane compounds are the primary biological effect of saponin (Makkar et al., 2007). Saponins have been reported in desert plants species (*Yucca schidigera*; Wanga et al. (1998)) and Atriplex species (El-Hyatemy et al., 1993).

2.3 Nutritive value of browse species

The nutritive value of browse varies widely between and within species. The CP content is usually high (12-30%; Norton, 1994) and can be influenced by several factors including genotype (subspecies variability), plant maturity and season of growth. In general, a greater proportion (often up to twice as much) of protein is found in the leaves of browse compared to the twigs and stems (Dicko, 1992). The digestibility of the CP contained in browse is; however, affected by the tannin content (Shayo and Uden, 1999). Browse plants, at any given stage of growth, contain higher levels of cell contents, lignin, secondary compounds and N compared to grasses, which contain higher levels of cell wall constituents such as cellulose and hemicellulose. Although the cell wall constitutes a smaller component of the browse plant overall, it contains a very high level of

completely indigestible lignin. As a result, the digestibility of browse can often be lower than that of grasses, which have a less lignified and thus more easily digestible cell walls (Gordon and Illius, 1994).

The chemical composition and feeding value of leguminous (*A. saligna, C. palmensis*) and halophyte (*A. amnicola, A. nummularia*) browse species for ruminants have been investigated in a number of studies. The published data presented in Table 2.1, Table 2.4 and Table 2.7 provides evidence that the concentrations of phenolics and tannin are greater in *A. saligna*, moderate in *C. palmensis* and low in both Atriplex species. However, due to the apparent inconsistency in the method of sample preparation and assays used in different experiments, comparison of contents of phenolics reported in the literature would not be perfect. Further, there are many factors affecting the composition and nutritive value of these species. Thus, it is essential to investigate the nutritive value of browse species grown under WA conditions in order to formulate appropriate feeding/grazing programmes to optimise the utilisation of the browse species in diets of sheep in this State.

The chemical, gravimetric, protein precipitation and biological methodologies for the quantification of phenolics and tannins in trees and shrubs foliage have been recommended by FAO/IAEA Co-ordinated Research Project (Makkar, 2003b).

2.3.1 Acacia saligna

2.3.1.1 Proximate composition

The proximate composition of *A. saligna* forage reported in some of the past research is summarised in Table 2.1. In addition, Getachew et al. (2002) reported 134 g CP/kg DM in the leaves of forage harvested in Zawai, Ethiopia. There is a marked variation in the

composition of the forage reported in these studies, although variations in the ADL (129 - 155 g/kg DM) and CP (88 - 149 g/kg DM) contents are relatively less. On the other hand, variations in the NDF (368 - 657 g/kg DM) and ADF (291 - 394 g/kg DM) contents are large and greatly attributed to the variation in HCEL and CEL contents of the forage. Both the CP and fibre (NDF, ADF) levels of the species have been reported as being moderate when grown in WA (George et al., 2007).

Stage of maturity and season (of harvest) also impact on the nutritive value of *A. saligna*. Degen et al. (1997) studied the difference in the chemical composition of *A. saligna* phyllodes harvested from mature and immature trees. The average CP content in mature trees was 111 g/kg DM, whereas, it ranged between 121 and 132 g/kg DM in mature trees. In contrast, the NDF (490 vs. 457 g/kg DM), ADF (266 vs. 221 g/kg DM) and ADL (144 vs. 107 g/kg DM) contents were higher in mature trees than young trees. The ash content of phyllodes from young trees was approximately twice as high as those from older trees. Salem (2005) studied the impact of season of harvesting on the chemical composition of *A. saligna* leaves. The CP content was lower in summer (143 g/kg DM) compared to that of autumn (171 g/kg), winter (177 g/kg DM) and spring (182 g/kg DM). The NDF, ADF, ADL and CEL contents were lower in winter (332, 258 and 133 g/kg DM respectively) than in spring (374, 298 and 154 g/kg DM, respectively) and intermediate values were observed in autumn (354, 274 and 143 g/kg DM, respectively) and summer (356, 276 and 142 g/kg DM, respectively). There were no differences between growth season in terms of ash (74 - 85 g/kg DM) and HCEL (74 - 79) contents of leaves.

Table 2.1: Chemical composition (g/kg DM) of *Acacia saligna* reported in past research.

Nature and origin of forage samples analysed	DM[a]	Ash	NDF	ADF	ADL	HCEL	CEL	CP	TP[b]	TT[c]	T[d]	CT[e]	PPT	Reference
Phyllodes from Negev desert, Israel		72	446	297	147	149	150	125	121			83		Degen et al. (1995)
Leaves from Negev desert, Israel			446	298	155									Makkar et al. (1995)
Fresh leaves and lush stems from Southern Sinai, Egypt	435	139	657	394	143	263	251	105						Abou El Nasr et al. (1996)
Leaves from ILRI Zawai site, Rift valley, Ethiopia		117	447	297	146			149	52			45		Kaitho et al. (1998d)
Leaves from ILRI Zawai site, Rift valley, Ethiopia		117	447	297	146			150						Kaitho et al. (1998e)
Foliage branches (<10 mm) from Negev desert, Israel		176.5	463.2	311.8	128.5			88.4	106.7	93.9		148.8		Degen et al. (2000)
Leaves from ILRI, Forage multiplication Centre, Zawai, Rift valley, Ethiopia								134.4	108.8		87.7	132.2		Getachew et al. (2002)
Leaves from Northern highlands, Ethiopia	340	118	482	314	147			126						Asefa and Tamir (2006)
Phyllode (<4 mm) from natural populations in Western Australia			367.8	290.7				134.4					88.2	George et al. (2007)
Foliage (<12 m old) from Western Australia	350	73						114	94.5			26.9		Krebs et al. (2007)
Leaves from University of Alexandria farm, Egypt		104	649	606					42.1	27.7		72.0		Sallam et al. (2017)

DM[a], Dry matter (g/kg fresh foliage); NDF, Neutral detergent fibre; ADF, Acid detergent fibre; ADL, Acid detergent lignin; HCEL, Hemicellulose; CEL, Cellulose; CP, Crude protein; TP[b], Total phenolics (in tannic acid equivalents); TT[c], Total tannins (in tannic acid equivalents); T[d], Tannins (in tannic acid equivalents); CT[e], Condensed tannins (in leucocyanidin equivalents); PPT, Protein precipitable tannins.

George et al. (2007) reported significant differences in the chemical composition among naturally occurring genetic groups of *A. saligna* in WA. These genetic groups included the typical variant, which is widespread and occurs predominantly in the wheatbelt region; the cyanophylla variant which is found along the Swan coastal plain near Perth; the forest variant which occurs in the southern forest region; and the Tweed River variant that is geographically restricted and largely occurs within the range of the Forest variant in WA. The cyanophylla/ Tweed River variants had higher CP content (150 g/kg DM) while the CP contents of the other variants were similar (125 g/kg DM). The typical variant had higher NDF (377 vs. 354 and 358 g/kg DM) while the cyanophylla group had lower HCEL (70 vs. 79 and 78 g/kg DM) content. The ADF content (288, 274 and 299 g/kg DM) did not vary significantly among the populations investigated. According to Rubanza et al. (2005), variations in the chemical composition among foliage of Acacia species may be partly due to genotypic factors that control accumulation of nutrients in forages.

2.3.1.2 Anti-nutritive compounds

For most of the experiments reported in Table 2.1 (except George et al., 2007 and Krebs et al., 2007), the *A. saligna* forage samples were taken from trees grown in arid or semi-arid African regions. Makkar and Becker (1998) found that the browse and shrubs in these regions contained more total phenolics (TP, 15.7 vs 6.0%), and had greater PPC (327.2 vs 56 mg of bovine serum albumin (BSA) precipitated per gram of sample) and operational activity of tannin (1.97 vs 0.66 mg BSA precipitated per gram of phenolics) compared to those from the subtropical region of the foothills of the north Himalayan range. Salem (2005) reported lower CT content in the *A. saligna* leaves collected in Egypt during winter (62.6 g/kg DM) compared to those collected in autumn, spring and summer

(77.1, 79.5 and 113 g/kg DM, respectively). Therefore, harsh environmental conditions appear to increase the concentrations of phenolics and tannins in *A. saligna* forage. Within the *A. saligna* populations in WA, a large range in the tannin content has been reported but this variation was not associated with climate (rainfall, temperature) or soil type (George et al., 2007). The age of trees from which foliage is harvested contributes to differences in the concentration of phenolics in the forage but often the age of the trees is not specified in studies. Degen et al. (1997) found that the TP and TT contents of the phyllodes of mature trees were greater than those of three years younger trees (150 vs. 103 and 125 vs. 95 g/kg DM, respectively in tannic acid equivalents). However, the relationship was inverse for CT content with maturity (88 vs. 156 g/kg DM, in leucocyanidin equivalents for mature and immature trees, respectively). George et al. (2007) reported that the protein precipitable tannin (PPT) content of *A. saligna* grown in WA did not differ between genetic groups but differed between the morphological variants. The populations of the 'Tweed River' morphological variant had significantly higher tannin content than the other variants (typical variant, cyanophylla variant, forest variant), which did not differ.

2.3.1.3 Palatability and intake

An overview of selected studies where the nutritive value of *A. saligna* forage was studied is presented in Table 2.2. Low intake of *A. saligna* is a consistent finding in a number of studies. Kaitho et al. (1997) reported a decreasing trend in the palatability of *A. saligna* over a 12-day feeding period. Secondary metabolites in browse species may have a negative effect on palatability (Claussen et al., 1990). Sheep and goats exhibit different preferences, particularly for the foliage (Kaitho et al., 1997). Degen et al. (2000) found that in comparison to sheep, goats had a higher palatability index (2.00 vs. 0.10) for the

forage indicating their greater preference for *A. saligna*. In addition, goats consumed more than sheep. In a feeding trial with *A. brevispica* and *Sesbania sesban*, Woodward and Reed (1995) found that the CT content of browse influenced palatability in sheep but not in goats. The level of tannins, particularly CT, is high in *A. saligna* (Table 2.1). Degen et al. (1997) found that the intake of forage from young trees which contained greater CT concentrations was lower than that of mature trees which had low CT concentrations. Therefore, as Assefa and Tamir (2006) suggested, astringency of CT of *A. saligna* could be a cause for low palatability and intake of the saligna forage, particularly in sheep.

2.3.1.4 Digestibility

Except for the succulent parts of *A. saligna*, the DM and OM digestibilities (312 - 408 and 304 - 438 g/kg DM, respectively) of the forage have been found to be low in both sheep and goats (Table 2.3). Supplementing maize stover with *A. saligna* has been shown to result in low NDF digestibility and low ruminal protozoa populations in sheep compared to those fed maize stover supplemented with other tree species (Odenyo et al., 1997). Krebs et al. (2007) found that protozoa were eliminated from ruminal fluid when sheep were fed a pure *A. saligna* diet. Ruminal protozoa play a significant role in fibre digestion, with digestibility reduced considerably in defaunated animals (Ushida and Jouany, 1990; Chaudhary et al., 1995). Defaunation results in low ruminal pH, ammonia-N and fibre (NDF, ADF, CEL) digestibilities (Santra et al., 2007). Krebs et al. (2007) found that neither ruminal pH nor ammonia concentrations were optimum for microbial fermentation when animals were fed *A. saligna*, with average ruminal ammonia concentrations much lower than the minimum threshold concentration (50 mg/L) for optimal fermentation activity of ruminal microbes (Satter and Slyter, 1974).

Table 2.2: An overview of selected studies where the nutritive value of *Acacia saligna* forage materials were studied.

Experimental forage species/ diets	**Animal species**	**Important findings related to biological effects of *Acacia saligna***	**Reference**
Phyllodes of A. saligna.	Female sheep and goats	The ME and DM intakes as well as DMD and OMD were low for both animal species and both were in negative N balance, with sheep losing more weight than goats.	Degen et al. (1995)
Fresh, air-dried or ensiled succulent parts of *A. saligna* or *A. nummularia*.	Adult male sheep	Intake of *A. saligna* was inferior to *A. nummularia*. Ensiled forage resulted in the highest DMI. Dry matter, CP and NDF were efficiently digested and utilised in the ensiled form compared to the other forms. Only ensiled forages provided maintenance requirements of DCP and total digestible nutrients.	Abou El Nasr et al. (1996)
Eragrostis tef straw 0.5 kg with 0.4 kg of wilted or 0.2 kg of dried 18 MPT forage species including *A. saligna*.	Wethers and bucks	There was a decreasing trend in daily DMI of *A. saligna* during the 12-day feeding period. *Acacia saligna* in the dried form was preferred over the wilted form by both species. The palatability index of *A. saligna* was greater for goats compared to sheep.	Kaitho et al. (1997)
Air-dried phyllodes of *A. saligna* or *A. salicina* from young or mature trees.	Sheep and goats	The DMI, especially of young trees, and digestibility were low for both Acacia species. The ME intakes were higher for phyllodes from mature trees compared to those from young trees. For all diets, DMD and OMD were low but generally higher for goats than for sheep. Both animal species showed negative N balance mainly due to high urinary-N losses. The diets did not meet the maintenance energy requirements and the animals lost weight.	Degen et al. (1997)

Table 2.2: *Continued*

Experimental forage species/ diets	Animal species	Important findings related to biological effects of *Acacia saligna*	Reference
Maize stover with 300 g/total DMI of MPT species including *A. saligna*.	Ruminal cannulated sheep	*Acacia saligna* supplementation reduced protozoa numbers but did not completely eliminate them in ruminal fluid. *Acacia saligna* supplementation caused a greater reduction in NDF digestibility compared to other MPT species and also resulted in a greater negative N balance in the animals.	Odenyo et al. (1997)
Powdered concentrate alone, powdered concentrate mixed with finely ground *A. saligna* phyllodes (400 g/kg), powdered quebracho (100 g/kg) or tannic acid (150 g/kg), without (periods 1 and 3) or with (periods 2 and 4) PEG. Each period was 10 d.	Ewe lambs	When fed *A. saligna*, the addition of PEG had inconsistent effects on both DMI and body weight.	Degen et al. (1998)
Edible portions (leaves, petioles, twigs) of 40 browse species including A. saligna.	Cannulated sheep and steers	*Acacia saligna* had high rumen bypass N and low N degradability compared to browse species containing low levels of tannin.	Kaitho et al. (1998e)
Natural herbage alone, natural herbage with fresh or wilted *A. saligna* forage (phyllodes, small stems); Natural herbage alone, natural herbage with A. saligna and water without or with PEG.	Sheep and goats	Wilting *A. saligna* did not increase DMI. The DMI was higher in goats than sheep. Administration of PEG increased *A. saligna* intake, and digestibility and intake remained high after PEG was subsequently withdrawn. Although, *A. saligna* intake was about 10% of maintenance requirement in both species, supplementation had a positive effect on body weight change and the effect appeared greater in sheep than goats.	Degen et al. (2000)

Table 2.2: *Continued*

Experimental forage species/ diets	Animal species	Important findings related to biological effects of *Acacia saligna*	Reference
Leaf samples of 37 tropical browse species including *A. saligna*.	*In vitro*	Addition of PEG to *A. saligna* increased *in vitro* GP. Positive correlations were evident between TP or TT contents and PPC or percentage increase in *in vitro* GP on addition of PEG. There was a strong positive relationship between *in vitro* GP and short chain fatty acid production.	Getachew et al. (2002)
Dried *A. saligna* leaves from all seasons.	Incubated in buffered rumen fluid from sheep, cattle and buffalo	*In vitro* GP from *A. saligna* was higher with buffalo ruminal fluid compared to sheep and cattle in all seasons except winter. Gas production from buffalo ruminal fluid was not affected by harvesting season but GP was higher for cattle and sheep ruminal fluid in winter. *In vitro* DMD was higher in winter and spring and lower in summer and autumn for all species but greater for buffalos.	Salem (2005)
Grass hay alone, grass hay (free choice) with fresh, wilted or sun-dried *A. saligna* leaves.	Lambs	Supplementation of *A. saligna* (fresh, wilted, dried) improved total DM (per metabolic body weight), OM and CP intakes. While lambs on hay alone diet lost body weight while lambs on supplemented diets gained weight. The best feed conversion efficiency was with the dried leaves supplemented diet.	Asefa and Tamir (2006)
Forage of *A. saligna* alone, *A. saligna* with PEG 4,000 or 6,000.	Sheep	Supplementation of PEG to *A. saligna* improved DM and N intakes. In all diets, mean ruminal ammonia concentration was well below the threshold level for maximal microbial growth. Negative N balance and weight loss were also evident for all Acacia diets. Feeding a pure *A. saligna* diet resulted in defaunation of the rumen.	Krebs et al. (2007)

Table 2.2: *Continued*

Experimental forage species/ diets	**Animal species**	**Important findings related to biological effects of *Acacia saligna***	**Reference**
Grass hay 700 g alone, grass hay 700 g with free choice of fresh, wilted or dried *A. saligna* leaves.	Male lambs	Inclusion of *A. saligna* improved total DM, OM and CP intakes but reduced NDF intake. *Acacia saligna* increased NDF digestibility but decreased DM, OM and CP digestibilities. Dried leaves increased the digestibility of DM, OM and CP more than the fresh leaves. While lambs fed with hay alone lost weight those fed with *A. saligna* gained weight.	Tamir and Asefa (2009)
Partial (50%) or total (100%) substitution of clover (*Trifolium alexandrina*) hay by fresh *A. saligna* leaves	Sheep	Linear decrease in DMI with increasing inclusion of *A. saligna* was evident. The digestion coefficients of DM, OM, CP, NDF and ADF also decreased. Nitrogen intake and urinary N were reduced, while faecal N was increased by *A. saligna* inclusion in the diet.	Sallam et al. (2017)

ADF, Acid detergent fibre; ADL, Acid detergent lignin; CP, Crude protein; DM, DCP, Digestible crude protein; Dry matter; DMD, Dry matter digestibility; DMI, Dry matter intake; GP, Gas production; ME, Metabolisable energy; MPT, Multipurpose tree(s); NDF, Neutral detergent fibre; OM, Organic matter; OMD, Organic matter digestibility; PEG, Polyethylene glycol; PPC, Protein precipitating capacity; TP, Total phenolics; TT, Total tannins.

Based on *in vivo* studies, there is a wide variation in DM and OM digestibilities (312 - 542 and 304 - 438 g/kg DM, respectively) for *A. saligna* (Table 2.3). These variations are marked between animal species. Neither sheep nor goats produce proline rich salivary proteins to defend against tannins (Austin et al., 1989; Distel and Provenza, 1991). However, goats digest *A. saligna* to a higher degree than sheep (Degen et al. 1995; 1997). They appear to detoxify tannins and degrade products of tannins better than sheep (Distel and Provenza, 1991). Thus, the potential of salivary proteins other than proline rich proteins having a high affinity for tannins cannot be neglected (Austin et al., 1989; Makkar and Becker, 1998). In addition, the higher total number of ciliate protozoa as well as holotrichs and spirotrichs present in the ruminal fluid of goats compared to sheep (Santra et al., 1998), might contribute to the better digestion of *A. saligna* by goats. Therefore, the defaunation by *A. saligna* tannins might be one of the reasons for the low digestibility of the forage in sheep. Monforte-Briceño et al. (2005) found *in vitro* GP and protozoa numbers were negatively correlated with CT content of several tropical fodder trees when incubated in ruminal fluid collected from cattle. However, the effect on rumen protozoa numbers is not consistent across all tropical, tannin-containing trees (Bhatta et al., 2012; 2015; Malik et al., 2017; Piñeiro-Vázquez et al., 2018; Khejornsart et al., 2021).

Evaluation of *A. saligna* from *in vitro* studies typically report either ME or GP estimates. Based on incubation in ruminal fluid collected from cattle, Degen et al. (1995) reported a ME of 4.37 MJ/kg DM for phyllodes. In later studies involving foliage branches (< 10 mm), Degen et al. (2000) reported ME of 5.23 MJ/kg DM, increasing to 6.75 MJ/kg DM with the addition of PEG. Getachew et al. (2002) also reported significant responses to PEG, with GP at 24 h increasing from 42.4 mL to 80.3 mL with the addition of PEG to leaf samples incubated in ruminal fluid from cattle.

Table 2.3: Summary of the *in vivo* digestibility, metabolisable energy and N balance of ruminants fed *Acacia saligna* forage.

Forage	Animal species	DMD	OMD	CPD	DE	ME	ME intake	N balance	Reference
Phyllodes	Sheep	319	342			4.38	78.6	-2.95	Degen et al. (1995)
Phyllodes	Goats	408	429			5.99	121.9	-2.09	Degen et al. (1995)
Fresh succulent parts	Sheep	542		444					Abou El Nasr et al. (1996)
Air-dried phyllodes of young trees	Goats	402	438		382	5.0	69	-4.89	Degen et al. (1997)
Air-dried phyllodes of mature trees	Goats	312	325		288	4.6	122	-2.98	Degen et al. (1997)
Air-dried phyllodes of young trees	Sheep	383	398		346	4.6	30.1	-2.53	Degen et al. (1997)
Air-dried phyllodes of mature trees	Sheep	323	338		307	5.1	98.9	-1.93	Degen et al. (1997)
Forage, PEG −	Sheep	313	304					-4.5	Krebs et al. (2007)
Forage, PEG + 4,000	Sheep	368	321					-0.5	Krebs et al. (2007)
Forage, PEG + 6,000	Sheep	378	330					-0.3	Krebs et al. (2007)

DMD, Dry matter digestibility (g/kg DM); OMD, Organic matter digestibility (g/kg DM); CPD, Crude protein digestibility; DE, Digestible energy; ME, Metabolisable energy (MJ/kg DM); ME intake, Metabolisable energy intake (kJ/kg$^{0.75}$/d); N balance, Nitrogen balance (g/d); PEG −, Without polyethylene glycol; PEG +, With polyethylene glycol.

2.3.1.5 Nutrient availability and supplementary feeding

The CP content of *A. saligna* reported in the literature is in the range of 88.4 - 150 g/kg DM (Table 2.1). This level of CP is above the threshold CP requirement (69 g/kg DM) for adequate rumen microbial activity (ARC, 1980). However, negative N balance has been consistently reported for animals fed fresh and air-dried *A. saligna* forage (Table 2.3), with the exception of Mousa (2011), who reported positive N balance in sheep fed up to 40% dried leaves. It was suggested the absence of negative effects of tannin may have been due to the relatively low quantity of tannin ingested and/or to the effect of drying the acacia before feeding.

The differences in N balance between the forage from young and mature (low and high in CT, respectively; Degen et al., 1997) plant material, and between non-treated and PEG-treated forage (Krebs et al., 2007) confirms the negative effect of CT of *A. saligna* on N utilisation. Further, the improvement of *in vitro* GP (Degen et al., 2000) and ME (Gatachew et al., 2002) of the forage brought by PEG supplementation confirms the negative effect of tannins on the energy value of the forage. The ME requirement for maintenance of 40 kg yearling range ewes is 6.36 MJ/d (NRC, 2007), which is equivalent to 400 kJ/kg$^{0.75}$/d. As highlighted in Table 2.3, the ME intake of sheep fed sole *A. saligna* diets are well below the maintenance energy requirement of sheep. As presented in Table 2.2, less gain or loss of body weight of animals fed *A. saligna* diets were the consequences of high tannins leading to lower intake and digestibility of protein and energy. However, the effects vary between animal species; sheep are affected more than goats and buffaloes are the least affected (Salem, 2005). Ensiling (Abou El Nasr et al., 1996) and PEG supplementation (Degen et al., 1998; 2000) significantly improve the quality of *A. saligna* forage. Interestingly, improvements brought to the forage qualities remain even after

PEG supplementation has been withdrawn, indicating the potential adaptability of ruminal microbes to defend the anti-nutritive properties of *A. saligna*. However, responses to supplementation of PEG vary; Krebs et al. (2007) found that even with PEG supplementation *A. saligna* was inadequate as a sole source of nutrients for sheep. The anti-nutritive effects of CT are the major factors attributed to the reduced nutritive value of the forage. However, despite the limitations *A. saligna* forage is still suitable as a supplement for ruminants (Degen et al., 2000; Asefa and Tamir, 2006). Inclusion of dried *A. saligna* at the rate of 265 g/kg DM in grass hay basal diets was recommended to improve nutrient utilisation of yearling lambs (Tamir and Asefa, 2009). Shawket et al. (2010) reported lambs could gain satisfy growth when fed *A. saligna* supplemented with barley or date seed at a ratio 1:1 of ME requirements.

2.3.2 Chamaecytisus palmensis

2.3.2.1 Proximate composition

Researchers at the International Livestock Research Institute (ILRI) in Ethiopia have conducted a number of studies on the forage value of *C. palmensis*. A summary of the chemical composition of forage of the species reported in past publications is presented in Table 2.4. The ash, NDF, ADF, ADL and CP contents range between 50 and 114, 330 and 549, 217 and 361, 63.2 and 98 and 144 and 239.5 g/kg DM, respectively. Umnna et al. (1995), Kaitho et al. (1997, 1998a, 1998b, 1998c, 1998d), El hassan et al. (2000) and Mengesha et al. (2017) assessed leaves whereas Ventura et al. (2002), Assefa et al. (2008b) and Kitaw et al. (2012) assessed leaves and edible branches of *C. palmensis*. Thus, variation in the composition appears greatly attributed to the variation in the forage material assessed. The forage of *C. palmensis* was found to have lower CP (144 vs. 168 g/kg DM) and greater NDF (549 vs 339 g/kg DM) contents compared to those of *Sesbania*

sesban (Varvikko and Khalili, 1993). The forage (175 g/kg DM) also contained more CP than *Calluna vulgaris* (98 g/kg DM) and *Ulex europaeus* (168 g/kg DM) but less than that of *Sarothamnus scoparius* (189 g/kg DM). Compared to *Calluna vulgaris*, *Ulex europaeus* and *Sarothamnus scoparius* forages (464, 557 and 482 g/kg DM, respectively), *C. palmensis* had the lowest (389 g/kg DM) NDF content (Tolera et al., 1997). However, El hassan et al. (2000) reported greater NDF and ADF contents for *C. palmensis* forage (388 and 217 g/kg DM, respectively) compared to those of *Acacia angustissima* (364 and 179, respectively), *Leucaena leucocephala* (346 and 192 g/kg DM, respectively) and *Sesbania sesban* (170 and 117 g/kg DM, respectively). Similar findings were reported by Longland et al. (1995), who found that the level of non-starch polysaccharides such as arabinose, glucose and galactose of *C. palmensis* (26.4, 99.9 and 8.0 mg/g DM, respectively) forage was greater than that of many tropical forage legumes species including *Acacia cyanophylla* (10.2, 74.5 and 4.3 mg/g DM, respectively), *Leucaena leucocephala* (12.9, 53.8 and 6.2 mg/g DM, respectively), *Sesbania goetzei* (6.2, 90.1 and 5.5 mg/g DM, respectively) and *Sesbania sesban* (10.9, 48.2 and 4.4 mg/g DM, respectively).

4Table 2.4: Chemical composition (g/kg DM) of *Chamaecytisus palmensis* reported in past research.

DM[a]	Ash	NDF	ADF	ADL	CP	TP	SP[b]	TSP	ISP[c]	Nature and origin of forage samples analysed	Reference
	50	549	361	98	144		6.6		18.4	Forage from Ethiopia	Varvikko and Khalili (1993)
	77	365			207					Leaves from Ethiopia	Umunna et al. (1995)
	114	389			175					Browse from International Livestock Centre for Africa (ILCA), Ethiopia	Tolera et al. (1997)
	53	449	273		213					Leaves from ILRI, Seed multiplication site, Zwai, Rift valley, Ethiopia	Kaitho et al. (1997)
	60	449	255	81	209			0.8		Leaves from ILRI, Research farm, Debre Zeit, Ethiopia	Kaitho et al. (1998a)
	53	449	255	81	209	34		1		Leaves from ILRI, Seed multiplication Centre, Zwai, Rift valley, Ethiopia	Kaitho et al. (1998b)
	112	343	231	67	202	22		1.2		Leaves from ILRI, Research farm, Debre Zeit, Ethiopia	Kaitho et al. (1998d)
	53	449	255	81	213	34		1		Leaves from ILRI, Seed multiplication Centre, Zwai, Rift valley, Ethiopia	Kaitho et al. (1998e)
	59	388	217	76	208					Leaves from Ethiopia	El hassan et al. (2000)
331	81	438	302	77	174					Leaves and stems (< 3 mm) from Las Palmas de Gran Canaria, Spain	Ventura et al. (2002)
	62	330			198					Foliage from ILRI, Debre Zeit, Ethiopia	Hindrichsen et al. (2004)
269	61.1	398.1	218.6	63.2	239.5					Leaves and edible parts of forage from North Shewa Zone, Ethiopia	Becholie et al. (2005)
	60	400	220		240					Debre-Zeit Agricultural Research Station, Ethiopia	Bochelie and Tamir (2006)

Table 2.4: *Continued*

DM[a]	Ash	NDF	ADF	ADL	CP	TP	SP[b]	TSP	ISP[c]	Nature and origin of forage samples analysed	Reference
	51.2	350.6	220.9	68.6	215.3					Leaves and edible branches (<3 mm) from Holetta research Centre, Ethiopia	Assefa et al. (2008b)
	49.1	596.5	378.8		225.9					Leaf and edible branches, from Holetta research Centre, Ethiopia	Kitaw et al. (2012)
	60	360	240		167					Leaf, from local watershed villages in Debre Birhan, Ethiopia	Mengesha et al. (2017)

DM[†], Dry matter (g/kg fresh foliage); NDF, Neutral detergent fibre; ADF, Acid detergent fibre; ADL, Acid detergent lignin; CP, Crude protein; TP, Total phenolics, SP[b], Soluble phenolics (g/kg OM); TSP, Total soluble proanthocyanidins; ISP[c], Insoluble proanthocyanidins (g/g NDF).

Table 2.5: An overview of selected studies where the nutritive value of *Chamaecytisus palmensis* forage materials was studied.

Experimental forage species/ diets	Animal species	Important findings related to biological effects of *Chamaecytisus palmensis*	Reference
Native hay *ad libitum* and 4.5 kg/d concentrate supplement replaced at 0, 33, 66 and 100% by wilted *C. palmensis*.	Rumen cannulated cows	Cows refused to eat all *C. palmensis* at higher replacement levels (66, 100%), resulting in lower DM and CP intakes, milk yield and milk protein content. Replacing *C. palmensis* did not affect DMD and OMD but decreased CPD. Increased inclusion increased ruminal pH and acetic acid concentration and decreased ruminal propionic acid, butyric acid and ammonia concentrations. With increased supplementation, milk fat was not affected but yield and protein declined.	Varvikko and Khalili (1993)
Oats hay alone, hay and 250 g of *C. palmensis*, *Lablab purpureus*, wheat middlings or *Sesbania sesban*, oats straw with *L. purpureus*.	Sheep	Oats hay intake decreased markedly (substitution effect) by supplementation with *C. palmensis* (and *Lablab purpureus*). Supplementation increased total DM and OM intakes, microbial N supply, N retention and growth rate but DMD and OMD were not affected. *Chamaecytisus palmensis* supplementation did not affect N supply efficiency; however, the greater digestible OM intake was attributed to increased microbial N supply.	Umunna et al. (1995)
Predominantly *C. palmensis.*	Steers	Steers fed *C. palmensis* had low liveweight gain when phenolic levels were high in the dry season and had high weight gain when phenolic levels were low in the wet season. Liveweight gain patterns of all the groups of steers were similar regardless of their status of *Streptococcus caprinus* (tannin-resistant bacterium) in ruminal fluid.	McNeill et al. (1996)
Oven-dried (fan-assisted) branches of *C. proliferus* subspecies and varieties.	Chemical analysis	Although the total alkaloid content did not vary among the subspecies of *C. proliferus*, the most toxic alkaloid, sparteine (the major alkaloid in the species) was least in *C. palmensis*.	Muzquiz et al. (1996)

Table 2.5: *Continued*

Experimental forage species/ diets	Animal species	Important findings related to biological effects of *Chamaecytisus palmensis*	Reference
Chamaecytisus palmensis forage.	Cattle	*In sacco* digestibility was seasonally variable but remain relatively high throughout the year and should not be limiting animal liveweight gain. However, there was an inverse relationship between the level of TP and liveweight gain of cattle grazed on the forage.	Edwards et al. (1997a; 1997b)
Eragrostis tef straw 0.5 kg with 0.4 kg of wilted or 0.2 kg of dried 18 MPT forage species including *C. palmensis.*	Wethers and bucks	There was an increasing trend in daily intake of *C. palmensis* during the 12-day feeding period. The palatability index of *C. palmensis* was greater for goats compared to sheep (1.88 vs. 0.97).	Kaitho et al. (1997)
Eragrostis tef straw *ad libitum*, straw *ad libitum* supplemented with 190 g of dried leaves of six *Sesbania sesban* accessions, *Dolichos lablab*, *C. palmensis*, *Leucaena leucocephala* or *Sesbania goetze*.	Male sheep	Supplementation of browse increased total DM, OM and CP intakes. There was no substitution effect of *C. palmensis* on straw intake. Browse supplementation did not affect digestibilities (DM, OM. Conversely, NDF digestibility of the *C. palmensis* supplemented diet was less than that of the straw diet (514 vs. 573 g/kg DM, $P>0.05$). Sheep fed *C. palmensis* increased liveweight while those on *E. tef* diet lost weight (36.4 g/d vs. -17.5).	Kaitho et al. (1998d)

Table 2.5: *Continued*

Experimental forage species/ diets	Animal species	Important findings related to biological effects of *Chamaecytisus palmensis*	Reference
Eragrostis tef straw *ad libitum*, straw *ad libitum* supplemented with *L. leucocephala*, *Leucaena pallida*, *C. palmensis* or *S. sesban* at 15, 30, 45 and 60% of ration DM intake.	Fistulated and non-fistulated sheep	As inclusion increased the rate of substitution of straw also increased (10-134 g/kg supplement). DM and digestible OM intake increased with increasing level of browse supplementation. Sheep fed *E. tef* lost weight (-24 g/d) while those fed *C. palmensis* increased weight and the effect increased with increasing supplementation (6.5-35.1 g/d). Liveweight gain and level of supplementation were closely related. The optimum level of *C. palmensis* in terms of liveweight was 30% of total ration DM. The digestibilities of DM, OM and N increased with increasing level of supplementation. The NDF digestibility was not affected by browse supplementation; however, faecal and urinary N output and N retention increased as the level of supplementation increased.	Kaitho et al. (1998a)
Eragrostis tef straw *ad libitum*, straw *ad libitum* supplemented with 190 g of dried leaves of six *S. sesban* accessions, *D. lablab*, *C. palmensis*, *L. leucocephala* or *S. goetze*.	Male sheep	The supplemented sheep had significantly higher total DM and N intakes. The digestibility of N was also greater for supplemented diets. Supplementation increased both faecal N output and N retention. Although, N retention of *C. palmensis* was not different to that of some browse species (*D. lablab, L. leucocephala, S. goetze*) it was less than that of many *S. sesban* accessions. The estimated water-soluble fraction, truly undegradable protein, rumen escape CP, rumen degradable protein and intestinally digestible protein of *C. palmensis* forage were 214, 62, 397, 626 and 239 g/kg CP, respectively.	Kaitho et al. (1998c)

Table 2.5: *Continued*

Experimental forage species/ diets	Animal species	Important findings related to biological effects of *Chamaecytisus palmensis*	Reference
Leaves and small twigs of *Acacia angustissima, L. leucocephala* or *S. sesban*, leaves of *C. palmensis* or *Vernonia amygdalina* or whole plant of *Medicago sativa* hay included at 0, 15, 30 or 60% level in *Eragrostis abyssinica* straw.	Cannulated bulls, ruminal fluid from the same bulls	Substantial quantities of N were associated with NDF fraction in *C. palmensis* (also in *L. leucocephala, V. amygdalina* and *A. angustissima*) decreasing available N significantly. However, *C. palmensis* had high available N content (22.3 g/kg DM). Fibre (NDF, ADF) of *C. palmensis* (and other MPT spp.) showed greater *in vitro* degradability. *C. palmensis* was one of the two species reported with the most extensive *in situ* degradation. The rate of *in vitro* GP increased progressively with inclusion of *C. palmensis* (also other MPT spp. and *M. sativa*); however, the potential GP decreased with inclusion of *C. palmensis* (and other MPT spp.). *In vitro* total GP increased by *C. palmensis* at the 300 and 600 g/kg DM inclusion levels.	El hassan et al. (2000)
Stems (<3mm) with leaves of the *C. proliferus* subspecies in spring and autumn.	Cannulated female goats	Intake did not correlate with OM, CP or NDF content and ruminal effective degradability of OM or N. Alkaloid content correlated with intake but did not correlate with ruminal effective OM or N degradability.	Ventura et al. (2000)
Hand-plucked cuts of *C. proliferus* subspecies in four seasons.	Cannulated male goats, Tilley and Terry method	OM degradability, OM digestibility and net energy for lactation (NEL) were not different among subspecies or *Medicago arborea*. NEL was highest in summer and lowest in winter. Mean OM and CP degradability, OMD and NEL were 479-539, 604-684, 632-650 g/kg DM and 5.4 MJ/kg DM, respectively.	Ventura et al. (2002)

Table 2.5: *Continued*

Experimental forage species/ diets	Animal species	Important findings related to biological effects of *Chamaecytisus palmensis*	Reference
Maize stover *ad libitum* and 200 g of sun-dried foliage of *C. palmensis*, *Leucaena diversifolia*, or *L. purpureus*.	Male sheep	*Ad libitum* intake of maize stover when supplemented with *C. palmensis* did not differ from that *L. purpureus* and *L. diversifolia* supplementation. Total NDF-N intake of *C. palmensis* diet was not different to *L. diversifolia* diet but lower to that of *L. purpureus* diet. Total N and NDF-N digestibility of *C. palmensis* diet was greater than that of *L. diversifolia* diet. Ruminal protozoa and bacteria numbers did not differ among *C. palmensis*, *L. diversifolia* or *L. purpureus* supplemented diets. At a similar N intake, *C. palmensis* (and *L. purpureus*) resulted in higher urinary N losses but lower faecal NDF-N losses compared to *L. diversifolia*. However, total faecal N output tended to be higher in *L. diversifolia* resulting in lower N retention compared to *C. palmensis* (and *L. purpureus*). Sheep offered *C. palmensis* had higher particulate outflow rate than sheep offered *L. purpureus* or *L. diversifolia.*	Hindrichsen et al. (2004)
Fresh, slightly wilted, highly wilted, sun-dried *C. palmensis*; Grass hay supplemented with *C. palmensis* at 0, 3.8, 7.6, 11.4 or 15.2 g DM/kg body weight; *C. palmensis* supplemented at 0, 68, 128, 188 or 238 g DM/d.	Lambs	Animals refused to eat fresh *C. palmensis* and the DMI was greater for highly wilted and sun-dried treatments. Lambs lost weight irrespective of the wilting/ drying stage but the loss progressively declined from fresh to highly wilted state of fodder. Supplementation did not have a substitution effect on hay intake. The best weight and feed conversion efficiency were achieved at 178 g/d consumption level. The highest DM, OM and CP intakes was at 238.3 g/d consumption level (280 g/kg DM). The best digestibility of DM, OM, NDF and ADL was at 188 g/d consumption level.	Becholie et al. (2005)

Table 2.5: *Continued*

Experimental forage species/ diets	Animal species	Important findings related to biological effects of *Chamaecytisus palmensis*	Reference
Native grass hay and hay supplemented with 10, 20, 30 or 40% sun-dried *C. palmensis.*	Male lambs	Supplementation improved OM and CP intakes with the highest CP intake at 40% inclusion level. The DM, OM, NDF and ADF digestibilities of the total diet were also improved with supplementation and the highest was at 30% supplementation level.	Bochelie and Tamir (2006)
Natural pasture hay *ad libitum* and concentrate supplement of 200 g/d replaced at 0, 33, 67 or 100% level by *C. palmensis*.	Sheep, Steers	The potential and effective degradability of *C. palmensis* was greater than that of concentrate and natural hay. *Chamaecytisus palmensis* supplementation increased total DM, OM and ADF intakes per metabolic body weight. With increasing *C. palmensis* inclusion, CP intake per metabolic body weight increased but weight gain decreased. Digestibility of DM, OM, NDF, ADF, and CF decreased with increasing level of inclusion. The ME intake was less at 67 and 100% inclusion levels compared to lower levels.	Assefa et al. (2008a)

DM, Dry matter; OM, Organic matter; CP, Crude protein; TP, Total phenolics; NDF, neutral detergent fibre; ADF, Acid detergent fibre; ADL, Acid detergent lignin; CF, Crude fibre; EE, Ether extract; MPT, Multipurpose tree species; NE, Net energy.

Table 2.6: Summary of the digestibility, degradability and energy of *Chamaecytisus palmensis* forage reported in past research.

Forage	Animal species	*In vitro* DMD	*In vitro* OMD	*In sacco* PD	*In sacco* ED	*In sacco* OMD	*In sacco* ND	*In sacco* CPD	NEL	Reference
Air-dried leaves	Non-fistulated and rumen fistulated sheep			851	635					Kaitho et al. (1998a)
Shade-dried leaves	Rumen fistulated male sheep			874	686					Kaitho et al. (1998b)
Air-dried leaves	Cannulated bulls, ruminal fluid from the same bulls	678					228			El hassan et al. (2000)
Stems (<3 mm) with leaves, in spring	Female goats					524	652			Ventura et al. (2000)
Stems (<3 mm) with leaves, in autumn	Female goats					537	519			Ventura et al. (2000)
Hand-plucked forage, mean of all seasons	Male goats		638			539		604	5.3	Ventura et al. (2002)

DMD, Dry matter digestibility; OMD, Organic matter digestibility; CPD, Crude protein digestibility PD, Potential degradability; ED, Effective degradability; ND, Nitrogen degradability; CPD, Crude protein degradability; NEL, Net energy for lactation.

The CP content of *C. proliferus* subspecies (*C. palmensis, C.* meridionalis**,** *C. canariae*) averaged 165 g/ kg DM, being the highest in *C. palmensis* subspecies in spring and autumn (Ventura et al., 2002). Borens and Poppi (1990) studied the variation of chemical composition of *C. palmensis* leaves during maturity period of six months and found that the N content fell progressively after the first month (42.3 g/kg DM) but was still high at the sixth month (26.3 g/kg DM). The NDF content decreased over the first month (416 g/kg DM) and thereafter remained relatively constant (290 - 340 g/kg DM) up until the sixth month. The ash content was low throughout the entire period (39.0 - 54.3 g/kg DM).

Although, *C. palmensis* forage has a greater N concentration, a substantial quantity (11 g/kg DM) of that is associated with the NDF fraction, which significantly decreases the available N for ruminal fermentation (El hassan et al., 2000). Similar to other browse species investigated (*Leucaena leucocephala, Leucaena pallida, Sesbania sesban*) investigated, *C. palmensis* forage also had considerable quantities of NDF- (37 g/kg DM) and ADF- (12 g/kg DM) bound N (Kaitho et al., 1998b). However, *C. palmensis* recorded the lowest level of NDF-bound N (1.2 g/kg DM) among the species studied (*C. palmensis, Leucaena purpureus, Calliandra calothyrsus, Leucaena diversifolia, Vernonia amygdalina*) at ILRI (Hindrichsen et al., 2004). The interesting feature in the variation in the composition of *C. palmensis* forage is that the composition (CP, fibre) is affected only during early growth stages and thereafter remains relatively stable. This phenomenon is in contrast with spring/summer growth of grass/legume pastures which declines rapidly in CP content over 1-2 months (Holmes, 1980)

2.3.2.2 Anti-nutritive compounds

Chamaecytisus palmensis contains moderate concentrations of TP in leaves, as evidenced by the samples analysed at the ILRI (Table 2.4). Furthermore, it was one of the MPT species found to contain low concentrations of CT/proanthocyanidins (Kaitho et al., 1997). As reviewed by Edwards (2000), the TP concentration in edible leaf and stem material routinely varies from 5 - 50 g/kg DM (in tannin acid equivalents) in the cool, wet winter-spring growth period up to 100 - 120 g/kg DM in the hot, dry late summer-autumn period. Further, in response to a locust attack, the 25% higher TP concentration was associated with rejection of the forage by grazing sheep. Ventura et al. (2000) reported a variation of alkaloid content (which is also a phenolic compound) among the four subspecies of *C. proliferus* (*C. palmensis, C. canariae. C. meridionalis, C. calderae*) between spring and autumn (2.9 vs. 1.6 g/kg DM, respectively). Muzquiz et al. (1996) also found variation in the alkaloid content among the sub species of *C. proliferus* with *C. palmensis* having the lowest alkaloid content (0.95 - 1.9 g/kg DM). Both of these studies identified sparteine as the major alkaloid found in the species. Contrary to the variation in TP (Edwards, 2000), the alkaloid contents of the forage were greater in spring than in autumn (2.3 vs. 1.3, 6.9 vs. 2.6 and 10.9 vs. 7.7, g/kg DM for *C. palmensis, C. canariae* and *C. meridionalis*, respectively). Importantly, for all seasons and for all *C. palmensis* subspecies, alkaloid concentrations were lowest in *C. palmensis*. Assefa et al. (2008b) reported a variability of HT, CT and alkaloids (in edible growing buds and leaves) concentrations among 65 accessions of *C. palmensis* during the growing period (up to 10 months) in different seasons (rainy or dry season). There was a greater variability in HT (mean 115, range 16 - 197, g/kg DM) and CT (mean 12.5, range 6.9 - 35.0 abs/g NDF) concentrations among the accessions. The edible, growing buds and

leaves had greater HT and CT concentrations (132.6 and 177.2 g/kg DM and 15.09 and 20.12 abs/g NDF, respectively) compared to those of non-edible branch, bark and stem fractions (65.2, 96.7 and 64.9 g/kg DM and 6.13, 6.21 and 6.71 abs/g NDF, respectively). Contrary, alkaloids concentrations were lower in growing buds and leaves (49.6 and 39.9 g/kg DM, respectively) than branch, bark and stem fractions (197.1, 235.4 and 137.0 g/kg DM, respectively). With progressing harvesting season, the HT content of leaves tended to decline while the alkaloid content increased from the 4th to 8th month and declined at the 10th month. Leaves harvested during the rainy season had higher HT concentrations compared to those harvested during the dry season. The CT concentration in leaves was low during the main rainy season, while the highest was during the short rainy and dry seasons. While HT and CT concentration were positively correlated, they were both negatively correlated with alkaloids concentrations.

2.3.2.3 Palatability and intake

The biological effect of *C. palmensis* forage reported in the literature where the nutritive value of the forage was studied is presented in Table 2.5. Reluctance of ruminant livestock to readily consume all of the *C. palmensis* forage offered has been reported in a number of studies. Hindrichsen et al. (2004) found that the intake of *C. palmensis* differed among individuals and 25% of the sheep in their experiment completely refused the forage. Species differences also exist and are impacted by feed form. Assefa et al. (2012) found that when fed *ad libitum*, sheep preferred fresh forage to wilted or dry. The restricted offering increased intake of the less preferred dried form. In contrast, steers and heifers preferred dried rather than the fresh or wilted forage. Animal preference and intake were affected by the preparation method of the forage, but not by regrowth age.

Seasonal productivity of cattle grazing on *C. palmensis* is suggested to be due to changes in feed intake throughout the year, mediated by the concentration of phenolic compounds in edible leaf and stem materials (Edwards et al., 1997b). However, tannin concentration in the forage is moderate (Table 2.4). Further, *Streptococcus caprinus*, which hydrolyses tannin-protein complexes failed to show a relationship with liveweight changes of steers in both the dry and wet season (McNeill et al., 1996) and thus provides evidence that tannins concentration might not be the reason for low palatability and intake of the forage. Instead, the negative correlation reported between alkaloid content and *C. palmensis* intake (Ventura et al., 2000) could explain the erratic intake of the forage (Varvikko and Khalili, 1993; Umunna et al., 1995; Kaitho et al., 1997) or low performance of animals fed the forage (Borens and Poppi, 1990). However, the anti-nutritive effect of alkaloid of *C. palmensis* could be the least among that of *C. proliferus* subspecies because sparteine (the major alkaloid in the species) is least in *C. palmensis* (Muzquiz et al., 1996). From field estimates, Oldham (1993) reported an intake of 1000 g DM/d for young, Merino wethers (35 kg) in summer-autumn. The value seems to be an extreme as from a pen feeding trial, Becholie et al. (2005) reported fresh forage intake of 139.6 g DM/d for young (3 - 4 months old) Ethiopian highland male lambs (14.74 kg). They also reported that the palatability was increased and thus intake increased (from 139.6 to 347.6 g DM/d) by drying of forage. Kaitho et al. (1997) reported increasing trend for palatability and intake, irrespective of the form offered (wilted, dried) to both sheep and goats; however, palatability was greater for goats compared to sheep.

2.3.2.4 Apparent digestibility

Studies investigating the apparent digestibility of nutrients in *C. palmensis* forage is lacking in the literature (Table 2.6). However, available data suggest that the digestibility/

degradability of the forage is relatively high. *In sacco* DMD of hand-harvested forage material was found to be seasonally variable but remained relatively high throughout the year (Edwards et al., 1997b). Similarly, *in vitro* OMD, ruminal degradability of OM and ruminal degradability of CP ranged at higher levels (604 - 676, 524 - 572 and 519 - 652 g/kg DM, respectively) among the seasons (Ventura et al., 2002). Borens and Poppi (1990) conducted a comprehensive study on *C. palmensis*. They reported that the *in vitro* DMD of leaves did not change after the first month. On average, the *in vitro* DMD of leaf and stem were 710 - 780 and 460 g/kg DM, respectively. They have further investigated the *in vivo* digestibility of DM, OM and NDF of *C. palmensis* using duodenal and ileal fistulated sheep lambs. The mean *in vivo* digestibility reported for DM, OM and NDF were 770, 780 and 650 g/kg DM, respectively. The proportion of *in vivo* DM and NDF digestion occurring in the reticulo-rumen was 550 and 800 g/kg DM, respectively.

2.3.2.5 Effect of supplementation on intake

The high CP and low fibre content of *C. palmensis* indicate the greater potential as a protein source for ruminants (Table 2.4). For this reason, many efforts have been made to explore the potential of the forage as a supplement to poor quality roughage and also to replace expensive concentrate mixtures in rations (Table 2.5). The substitution effect of *C. palmensis* supplementations on roughage intake is variable. Umunna et al. (1995) reported the intake by sheep of oats hay decreased with supplementation of *C. palmensis* leaves. Further, the supplementation increased the total intakes of DM (650 vs. 758 (g/d) and OM (573 vs. 678 g/d). As the level of supplementation of *C. palmensis* increased from 15 to 60% a significant decrease in the *Eragrostis tef* straw intake was observed in sheep (Kaitho et al., 1998a). Further, the substitution rate increased with increasing levels of supplementation from 10 to 134 g/kg supplement in *C. palmensis*. However, others

report absence of effect on intake due to supplementation of *C. palmensis* (Kaitho et al., 1998d; Becholie et al., 2005). The rate of substitution varies among browse species, depending on the rate of degradation of supplements in the rumen (Bonsi et al., 1994). Reduction in the intake of the basal diet could also be due to the high gut fill effect of *C. palmensis*.

Irrespective of the substitution effect, the increased of total nutrient (DM, OM, CP) intake has been a consistent finding. The total voluntary intake did not show diminishing returns to increasing supplementation levels of *C. palmensis* (Umunna et al., 1995; Kaitho et al., 1998a; 1998c; 1998d; Assefa et al., 2008a), possibly because the basal feed (native hay, oaten hay, *E. tef*) was offered *ad libitum* and hence animals could adjust their intake. Animals fed low quality forages improve performance due to supplementation of protein by means of stimulation of voluntary forage intake (Kartchner, 1980; DelCurto et al., 1990). The browse could have increased the total intake by supplying the deficient nutrients (largely N) in basal diets. The higher rate of degradation of *C. palmensis* increases the population of cellulolytic microbes, which rapidly utilise the digestible browse in the rumen, thereby allowing more microbes to colonise and degrade the basal diet leading to the increase in intake (Kaitho et al., 1998a).

2.3.2.6 Effect of supplementation on digestibility and nutrient availability

Chamaecytisus palmensis has a high available N content (22.3 g/kg DM; El hassan et al., 2000), therefore, in principle the forage should be able to provide adequate N for optimum ruminal microbial activity. This is likely to be reason for increased nutrient digestibilities evident due to supplementation of the forage in poor quality, basal diets (Kaitho et al., 1998a, 1998d; Bochelie and Tamir, 2006). Absence of such improvement (Umunna et

al., 1995; Kaitho et al., 1998d) could be due to either inadequacy of inclusion or imbalance of energy and N. Interpretation of low apparent *in vivo* digestibility of NDF (Kaitho et al., 1998d) in supplemented diets is complicated because CT in forage complex with other dietary components and therefore appear as faecal NDF which biases the estimation of true NDF digestibility (Reed, 1986; Makkar et al., 1995). Condensed tannins could also depress digestibility by inhibiting microbial enzymes and by forming indigestible complexes with protein and carbohydrates (Hagerman, 1989). Reduction of digestibility due to replacement of concentrate by *C. palmensis* could be due to: (1) inadequate ME for optimum function of rumen microbes; (2) presence of tannins which binds nutrients, especially proteins; and/or (3) fast outflow rate causing low effective degradability (Assefa et al., 2008a).

The level of CP in *C. palmensis* published in the literature is in the ranges from 144 to 240 g/kg DM (Table 2.4). This level of CP is well above the threshold CP requirement (69 g/kg DM) for adequate rumen microbial activity (ARC, 1980). Although, the occurrence of CT in many browse species could impair their nutritive value, low CT concentration has been shown to increase N retention (Barry and Duncan, 1984). Most grasses grown in warm and hot climates are typically poor in protein and rich in fibre thus inclusion of CT from browse further reduces protein availability for absorption. They limit ruminal microbial growth and absorption of amino acids. Thus, in these environments' intakes of CT from browse, in combination with a medium–poor quality diet, are detrimental to performance. However, studies have shown inclusion of as little as 10 g/d of polyethylene glycol (PEG) in diets for sheep and goats grazing scrub and woodland can markedly improve performance (Waghorn, 2008). As the level of CT in *C. palmensis* is moderate (Table 2.4; Borens and Poppi, 1990) supplementation would

likely increase N retention. Another reason for higher N retention may be due to the relatively low rate of rumen degradation compared to other browse species such as *Sesbania* (Umunna et al., 1995). *Sesbania* is degraded faster than *C. palmensis*, producing higher concentrations of ammonia in the rumen and consequently diverting energy to the excretion of excess nitrogenous products such as urea. Supplementation of *C. palmensis* leads to greater liveweight gain (Table 2.5) through increased digestible OM intake and supply of microbial protein. Kaitho et al. (1998b) found a strong relationship ($P < 0.0001$;) between the level of *C. palmensis* supplementation and liveweight gain and proposed the following quadratic relationship (RSD = 8.42, $R^2 = 0.87$) to predict the liveweight gain from *C. palmensis* supplemented diets:

$$Y = -22.48\ (3.25) + 0.43\ (0.06)X - 0.001\ (0.0002)X^2$$

Where;

Y = Liveweight gain (g/d)

X = Level of supplementation (g/d)

Based on the tannin concentrations of the studies on browse, it seems that low tannin-containing browse species like *C. palmensis* increase animal performance by enhancing DM intake. Bochelie and Tamir (2006) found supplementation of grass hay with *C. palmensis* at 300 - 400 g/kg DM improved digestibility and nutrient intake of the total ration, thereby giving better returns from sheep. Mengesha et al. (2017) concluded that supplementation of dried *C. palmensis* leaves up to 50% of the diet DM, resulting in NDF: CP ratio of 5.3, produced no deleterious effects on the performance of sheep, and inclusion to this level could be applied for superior growth performance and carcass yield

in sheep fed crop residue-based diets. Declining digestibility of the feed DM and nutrients of *C. palmensis* beyond this level could be due to the greater effect of anti-nutritional substances, presumably the alkaloids. The explanation for low milk protein and yield when more than 66% of a concentrate supplement was replaced with *C. palmensis* was low intake of NDF and CP (Varvikko and Khalili, 1993). *Chamaecytisus palmensis* could not be used to replace the entire concentrate supplement in dairy cow (Varvikko and Khalili, 1993) and steer (Assefa et al., 2008a) rations.

2.3.3 Atriplex amnicola and Atriplex nummularia

2.3.3.1 Proximate composition

The chemical composition of *A. nummularia* and *A. amnicola* reported in the literature is presented in Table 2.7 and Table 2.8, respectively. Being halophyte species, the ash content of both *A. amnicola* (170 - 281 g/kg DM) and *A. nummularia* (228 - 344 g/kg DM) is high. However, the level of salt is less while that of fibre (NDF, ADF) is greater in *A. amnicola* compared to that of *A. nummularia* (Norman et al., 2004a, 2004b; Tiong et al., 2004). According to Norman et al. (2004b), the N content was less in *A. amnicola*; however, El-Hyatemy et al. (1993) found that both the CP and crude fibre (CF) contents were less while the level of ash was higher in *A. amnicola* compared to *A. nummularia*. Norman et al. (2004b) reported that sheep preferred *A. nummularia* bushes with significantly greater N content (24.6 vs. 20.3 g/kg DM), although the bushes did not differ in NDF (305.4 vs. 312.2 g/kg DM) and ADF (171.1 vs. 178.5 g/kg DM) contents. However, there were no significant differences in N, NDF and ADF contents (16.2 vs. 15.5, 422.7 vs. 394.5 and 660.6 vs. 719.1 g/kg DM, respectively) between most and least preferred *A. amnicola* bushes. The research did not clearly justify the reasons for the differences in voluntary intake between species. However, it was observed that ash and

oxalate were at levels likely to depress the voluntary intake of the saltbush species. Ben Salem et al. (2002a) reported 659 g/kg N of soluble N in consumable parts (i.e., leaves and small branches) of *A. nummularia* harvested during the spring months in Central Tunisia.

Islam and Adams, (2000) reported seasonal variation of N fractions for both *A. amnicola* and *A. nummularia* forage grown under differing environments in WA. Total N content of the leaves was greatest in winter and least in the summer months. Soluble N content of leaves was also greater in winter than in other seasons. Overall, *A. amnicola* had a greater total N content compared to that of *A. nummularia* throughout the year. Atiq-ur-Rehman et al. (1999) also observed a significant variation in the N concentration in different plant parts of *A. amnicola* from January to June in WA. The N content in the leaf fraction started to increase in January (14 g/kg DM), was about 14% higher in April and 100% higher in June (30 g/kg DM). However, the N content of stem varied within a narrow range from 6 to 8 g/kg DM within the period. Leaves consistently had greater N concentrations than the stems, being two times higher in January and five times higher in June. It is clear that the variation in the chemical composition particularly that of N can be attributed to the growth stage which coincided with the growing season.

The nutritive value of *A. amnicola* may vary among genotypes and could be influenced by the NaCl concentration in the surrounding water and soil. Masters et al. (2010) found the average ME across all genotypes grown at the highest concentration of NaCl (400 mM) was 6.2 MJ/kg DM. The ME of these plants was lower because of both a reduction in OMD and because of the decrease in the proportion of OM in the plant (due to increased ash as a proportion of the DM).

Table 2.7: Chemical composition (g/kg DM) of *Atriplex nummularia* reported in past research.

DM[a]	Ash	NDF	ADF	ADL	CP	TP[b]	TT[c]	T[d]	CT[e]	TSP	TO	SA	Nature and origin of forage samples analysed	Reference
	227.7				175							56	Plant samples from Nubaria in Egypt	El-Hyatemy et al. (1993)
386	249	594	368	93	127								Fresh leaves and lush stems, Southern Sinai, Egypt	Abou El Nasr et al. (1996)
	308	348	152	80	137			0.07					Leaves and tender twigs from plantations, Morocco	Chriyaa et al. (1997a)
	313	342	144	76	134			0.1					Harvested from Forest Service in Morocco	Chriyaa et al. (1997b)
	344	379	171	103	175	0				2			Leaves from ILRI, Seed multiplication Centre, Zwai, Rift valley, Ethiopia	Kaitho et al. (1998b)
	344	379	171	103	175	0				2			Leaves from ILRI, seed multiplication Centre, Zwai, Rift valley, Ethiopia	Kaitho et al. (1998e)
270	276	442			169	3	<1		<1		37		Leaves, small branches, Central Tunisia	Ben Salem et al. (2002a)
	291	252	134		156								Leaf material collected from five sites, Western Australia	Tiong et al. (2004)
	280	220	81										Forage from Perth, Western Australia	Norman et al. (2004a)
286	255	445	225	102	178								Consumable parts (leaves, twigs), Central Tunisia	Ben Salem et al. (2004)

Table 2.7: *Continued*

DM[a]	Ash	NDF	ADF	ADL	CP	TP[b]	TT[c]	T[d]	CT[e]	TSP	TO	SA	Nature and origin of forage samples analysed	Reference
289	228	521			171								Consumable parts of the forage from Tunisia	Ben Salem et al. (2005a)
		299			107								Edible leaf and twigs (<1.5 mm) from South Australia	Franklin-McEvoy et al. (2007)

DM[a], Dry matter (g/kg Fresh foliage); NDF, Neutral detergent fibre; ADF, Acid detergent fibre; ADL, Acid detergent lignin; CP, Crude protein; TP[b], Total phenolics (in tannic acid equivalents); TT[c], Total tannins (in tannic acid equivalents); T[d], Tannins (in catechin equivalents); CT[e], Condensed tannins (in leucocyanidin equivalents); TSP, Total soluble proanthocyanidins; TO, Total oxalates; SA, Saponin.

Table 2.8: Chemical composition (g/kg DM) of *Atriplex amnicola* reported in past research.

Ash	NDF	ADF	CP[a]	SA	Nature and origin of forage samples analysed	Reference
247.9			168.5	9	Plant samples from Nubaria, Egypt	El-Hyatemy et al. (1993)
281	322	180	131[a]		Leaf material collected from five sites in Western Australia	Tiong et al. (2004)
170	467	164			Forage from Perth, Western Australia	Norman et al. (2004a)

NDF, Neutral detergent fibre; ADF, Acid detergent fibre; CP, Crude protein; SA, Saponin.
[a], CP content, calculated from N content (CP=6.25 N).

2.3.3.2 Anti-nutritive compounds

Negligible levels of phenolics and tannins in Atriplex species is a consistent finding in many studies (Table 2.7, Table 2.8). Norman et al. (2004b) also measured negligible levels of crude tannin in *A. amnicola* and *A. nummularia* species. Their findings on high levels of oxalates for *A. amnicola* (12.5 - 32.9 g/kg DM) and *A. nummularia* (25.6 - 26.9 g/kg DM) agrees with Ben Salem et al. (2002a). The presence of saponins has also been reported for many Atriplex species. Of the species, *A. nummularia* had the highest saponins concentration while *A. amnicola* was one of the species that had a low saponins concentration (El-Hyatemy et al., 1993).

Atriplex nummularia accessions introduced from Australia have been shown to contain moderate levels of oxalates (47 - 88 g/kg DM) when grown in semi-arid regions in the western United States. Abu-Zanat et al. (2006) found *A. nummularia* grown in the arid regions of Jordan contained high levels of oxalate in spring compared to fall season (76.2 vs. 47.8 g/kg DM). The oxalate content is likely to be at toxic concentrations in young, succulent, rapidly growing tissues but the concentration subsequently decreases with plant maturity (Davis, 1981). When oxalate concentrations exceed 30 g/kg DM, sheep

have been shown to decrease their voluntary feed intake and at 70 g/kg DM unadapted sheep can be poisoned (Burritt and Provenza, 2000).

2.3.4 Mineral composition of browse species

The limited information published on the mineral composition of the browse species investigated in this research is summarised in Table 2.9. Acacia species are poor in P (0.8 – 1.3 g/kg DM), moderate in Ca (6.4 – 11.3 g/kg DM), Mg (1.4 – 4.0 g/kg DM) and S (1.0 – 5.3 g/kg DM). They are rich in most microminerals such as Mn (18 – 37 mg/kg DM), Mo (16 – 42 mg/kg DM), Zn (13 – 21 mg/kg DM), Co (3.1 – 4.8 mg/kg DM) and Cu (28 – 66 mg/kg DM). The Fe (198 – 459 mg/kg DM) and Se (12.5 - >100 mg/kg DM) contents vary widely among the species (Abduleazak et al., 2000). As George et al. (2007) reported, there is variation in the mineral content within and between naturally occurring *A. saligna* populations in WA. With the exception of P, the variation in mineral content was not closely associated with genotype but was significantly associated with the climatic conditions and soil types. The variation in B and S contents was significantly correlated with rainfall and temperature variation. The populations with the highest Ca content (32, 33, 22 and 17 g/kg DM) were from coastal areas of calcareous soils, and populations with the highest Mg content (18, 11, 13 and 12 g/kg DM) were from the soils with higher Mg cation exchange capacity of clay subsoil.

The Na content of *A. nummularia* is greater than that of *Acacia cyanophylla* (Ben Salem et al., 2002a). Borens and Poppi (1990) found that the leaves of *C. palmensis* had greater P, K, Na, Mg, Ca, Cu and Mn concentrations but lower Fe and Zn concentrations compared to those of the edible bark fraction of the forage. Although S, Mg, Ca and P is also present in relatively high concentrations, most of the salt in *A. nummularia* is present as sodium chloride and potassium chloride (Ben Salem et al., 2010). They reported a

wide variability in Ca (0.30-1.44% DM), P (0.07-0.25% DM), Mg (0.30-0.84% DM), K (1.9-5.6% DM), Na (3.95-7.90% DM), Cl (11.76-12.3%) and S (0.45-9.3% DM) concentrations in *A. nummularia* leaves/ leaves and twigs under rainfed/irrigated with saline water conditions. The Fe, Zn, Cu, Mn and Se concentrations were 101.5-391, 14-54, 3.5-24, 44-146.68 and 0.4-1.39% DM, respectively. The analysis done in Chile using *A. nummularia* leaves harvested in January reported 4.78% Cl and 6.47% Na (Meneses et al., 2012). Most minerals present *A. nummularia* exceed the concentrations recommended for daily intake for ruminants as per the feeding standards for Australian livestock species (SCA, 1990).

2.4 Predicting dietary attributes

The dominant land use in the arid and semi-arid climatic zones (over 70%) of Australia is extensive grazing by sheep and cattle, which is characterised by wide variation in stocking rates from 1-4/ha to 1-40/ha in dry sheep equivalents. This variation in carrying capacity reflects the wide variation in vegetation type (Lefroy et al., 1992) and availability. The rangelands are composed of diverse plant communities (Holechek et al., 1982b) thus livestock grazing on them are often subject to variation in their nutrition. Determination of the nutritive value of the diet of grazing ruminants remains one of the main problems faced by grazing scientists and managers.

Holechek et al. (1982b) summarised the various methods for determining the nutritive value of the diet(s) of grazing ruminants. The most accurate representation of diets for research purposes could be achieved using fistulated animals. Due to less labour requirement and potential to collect more representative forage samples, oesophageal fistulated animals are preferred by researchers despite potential salivary contamination of

samples. The samples can be subsequently analysed for chemical composition using established procedures (AOAC, 2004-5). *In sacco* nylon bag (De Boer et al., 1987) or *in vitro* gas fermentation (Menke and Steingas, 1988) methods can be employed to evaluate the nutritive value of collected samples. For digestibility studies the *in vitro* digestion method is the preferred method. The *in vitro* procedure takes much less time compared to *in vivo* digestibility methods and the results are highly correlated to those from *in vivo* studies (Holechek et al., 1982b). As most of the browse species contain phytochemicals such as tannin (Makkar, 2003a) the *in vitro* gas fermentation method gives more precise information of the nutritive value of diets containing browse (Makkar, 2005). The method is versatile as it can be used to determine OMD, ME, biological effect of tannins, microbial protein synthesis and volatile fatty acid (VFA) production of diet samples.

Faecal NIRS and faecal chemical composition also have shown potential for predicting the nutritive value of grazing ruminant diets (Holechek et al., 1982a). Kneebone and Dryden (2015) established significant predictive models for nutrient intake and digestibility from faecal chemical properties along with excretion rates and *f*NIRS.

Table 2.9: Mineral composition of forage of *Acacia saligna*, *Chamaecytisus palmensis*, *Atriplex amnicola* and *Atriplex nummularia*.

Species	Macro minerals (g/kg DM)							Microminerals (mg/kg DM)					Nature and origin of forage samples analysed	Reference
	Na	Ca	P	Mg	K	S	Cl	Cu	Mn	Zn	Fe	B		
C. palmensis	0.76	4.83	1.71	2.52	7.19			6.43	130.0	57.6	62.0		Leaves from Bank Peninsula in New Zealand	Borens and Poppi (1990)
A. nummularia	42	31	1	5									Consumable parts (leaves, small branches) from Central Tunisia	Ben Salem et al. (2002a)
A. nummularia	72.5	7.7		7.7	36.3	4.5	11.76		146.68	18.84	189.50	113.77	Most preferred bushes from Tammin, Western Australia	Norman et al. (2004b)
A. nummularia	68.9	7.3		7.8	38.3	4.8	11.64		170.17	19.61	178.40	109.77	Least preferred bushes from Tammin	Norman et al. (2004b)
A. amnicola	59.3	8.2		10.0	26.7	3.8	10.30		142.15	18.76	186.11	70.09	Most preferred bushes from Tammin	Norman et al. (2004b)
A. amnicola	70.4	8.5		11.7	26.6	4.1	12.35		288.46	21.67	200.56	81.97	Least preferred bushes from Tammin	Norman et al. (2004b)
A. saligna	1.2	15.6	0.8	4.7	6.9	6.5		0.04		0.4	1.0	0.5	Phyllode (<4 mm) from natural populations in Western Australia	George et al. (2007)

DM, Dry matter.

2.4.1 Predicting dietary attributes from faecal chemical composition

There has been number of studies conducted to investigate the relationship between faecal chemical composition and nutritional attributes of ruminant diets such as nutrient intake, composition, energy content, and digestibility. A summary of the predictive regression models for dietary attributes resulting from these studies are presented in Table 2.10. Close correlation between faecal N (*f*N) and dietary N concentrations have been reported by Mubanga et al. (1985) and Kamler and Homolka (2005). Although *f*N and CP contents have been shown to have a close relationship with dietary OMD (Boval et al., 2003) and ME content (Kamler and Homolka, 2005), respectively, Vera (1973) observed a poor predictability of dietary OMD from *f*N content. In their experiment *f*N (model 6, Table 2.10) and ADF (model 2, Table 2.10) were associated with the largest error among the regression equations for dietary OMD. In addition, the relationship of faecal organic matter N and dietary OMD varied with the level of N in the diet (greater than or less than 10%). Therefore, *f*N was considered a poor predictor of dietary N and OMD. Instead, the best predictions of digestibility were obtained using faecal fibre fractions (ADL as a percentage faecal ADF and cell wall contents, models 4 and 5, Table 2.10). Kneebone and Dryden (2015) also proposed significant predictive models for both DMD (R^2=0.55) and OMD (R^2=0.55) from *f*N measured as a percentage of faecal OM for sheep (model 27 and 28, Table 2.10). According to Holloway et al. (1981), *f*N alone does not have broad application to predict dietary properties. Faecal N alone explained 45% of the variability of DMD (R^2=0.45) and additional 20% of the variation in DMD was explained by the addition of faecal EE, DM and Na contents (R^2=0.65, model 8, Table 2.10). Holecheck et al. (1982a) showed that the prediction model for fistula sample *in vitro* OMD of grassland and forest forage could be improved by inclusion of *f*N as an independent variable with faecal *in vitro* OMD (R^2=0.83, model 16, Table 2.10).

Results from several studies confirm the usefulness of faecal properties in predicting nutrient intake in ruminants. Holloway et al. (1981) reported that although the *f*N alone explained only 32% of the variation in DM intake (R^2=0.32), the inclusion of faecal ether extract (EE), DM and cell wall content (CWC) improved the predictive model substantially (R^2=0.69, 0.46 RSD, model 7, Table 2.10). In agreement with the potential of multiple variables in predicting dietary attributes in sheep, Kneebone and Dryden (2015) proposed more strong predictive models for intake of DM (R^2=0.91, RPD=3.3, model 24, Table 2.10), OM (R^2=0.91, RPD=3.3, model 25, Table 2.10) and CP (R^2=0.90, RPD=2.5, model 26, Table 2.10) from *f*N (in faecal OM) alone with faecal CP, DM, OM, NDF and ADF excretion rate.

The best single variable that predicts digestible DM intake was *f*N content (R^2=0.44) and the addition of faecal EE, CWC and DM increased the R^2 by 30% while decreasing the residual standard deviation (RSD) by 0.17 (R^2=0.74, model 9, Table 2.10 (Holloway et al., 1981). However, faecal CP excretion rate alone was able to make better predictions of digestible DM intake (R^2=0.82, RPD=2.4, model 30, Table 2.10) and digestible OM intake (R^2=0.82, RPD=2.4, model 31, Table 2.10) in sheep (Kneebone and Dryden, 2015). Further, these predictive models will have a broader application as they were generated from feeding experiments conducted with sheep fed on 25 different diets consisting of different hays (*Chloris gayana, Heteropogon contortus, Medico sativa, Pennisetum glaucum, Sorghum bicolor, Hordeum vulgare*) with or without four different supplements (urea, molasses plus urea, sorghum grain, cottonseed meal).

Table 2.10: Summary of the regression models predicts dietary attributes (Y/ dependant variable) from faecal composition (independent variable) reported in past research

Model	Animal species	Regression model	Variables	R^2	Error	Reference
1	Cattle[a]	Y = 125.23 – 1.04 *f*CWC	Y = Dry matter digestibility (DMD; % DM) *f*CWC; Faecal cell wall content (CWC, % DM)	-0.78	3.84^{RE}	Vera (1973)
2	Cattle[a]	Y = 148.23 – 2.19 *f*ADF	Y = DMD (% DM) *f*ADF; Faecal acid detergent fibre (ADF, % DM)	-0.61	4.90^{RE}	Vera (1973)
3	Cattle[a]	Y = 11.54 + 3.87 *f*ADL	Y; DMD (% DM) *f*ADL; Faecal acid detergent lignin (ADL, % DM)	0.77	3.94^{RE}	Vera (1973)
4	Cattle[a]	Y = 16.25 + 144 (*f*ADL/*f*ADF)	Y; DMD (% DM)	0.83	3.41^{RE}	Vera (1973)
5	Cattle[a]	Y = 27.54 + 163 (*f*ADL/*f*CWC)	Y = DMD (% DM) *f*ADL; Faecal ADL (% DM) *f*CWC; Faecal CWCt (% DM)	0.81	3.64^{RE}	Vera (1973)
6	Cattle[a]	Y = 41.16 + 7.78*f*X + 2.3041fN^2	Y = DMD (% DM) *f*N; Faecal nitrogen (N, % DM)	0.68	4.59^{RE}	Vera (1973)
7	Steers[b]	Y = -10.36 + 1.92*f*N + 0.36*f*EE + 0.13*f*CWC	Y = Dry matter intake (DMI) *f*N; Faecal N (% DM) *f*EE; Faecal ether extract (EE, % DM) *f*CWC; Faecal CWC (% DM)	0.69	0.46^{RSD}	Holloway et al. (1981)

Table 2.10: *Continued*

Model	Animal species	Regression model	Variables	R^2	Error	Reference
8	Steers[b]	Y = 31.22 + 15.88*f*N + 4.35*f*EE – 1.65*f*DM + 5.52*f*Na	Y = DMD (%) *f*N; Faecal N (% DM) *f*EE; Faecal EE (% DM) *f*DM; Faecal dry matter (DM, %) *f*Na; Faecal sodium (% DM)	0.65	7.07^{RSD}	Holloway et al. (1981)
9	Steers[b]	Y = -6.66 + 1.65*f*N + 0.37*f*EE + 0.08*f*CWC – 0.08*f*DM	Y = Digestible DMI *f*N; Faecal N (% DM) *f*EE; Faecal EE (% DM) *f*CWC; Faecal CWC (% DM) *f*DM; Faecal DM (%)	0.74	0.41^{RSD}	Holloway et al. (1981)
10	Fistulated cows, steers[c]	Y = -0.276 + 0.855*f*N	Y = Dietary N (%) *f*N; Faecal N (%)	0.78	0.29^{RE}	Holechek et al. (1982a)
11	Fistulated cows, steers[d]	Y = -0.262 + 0.815*f*N	Y = Dietary N (%) *f*N; Faecal N (%)	0.88	0.23^{RE}	Holechek et al. (1982a)
12	Fistulated cows, steers[c,d]	Y = -0.269 + 0.835*f*N	Y = Dietary N (%) *f*N; Faecal N (%)	0.83	0.26^{RE}	Holechek et al. (1982a)
13	Fistulated cows, steers[c]	Y = 28.7 + 1.41*f*OMD	Y = *In vitro* organic matter (OM) digestibility (OMD) of diet, %) *f*OMD; *In vitro* OMD of faeces (%)	0.67	3.13^{RE}	Holechek et al. (1982a)
14	Fistulated cows, steers[d]	Y = 26.9 + 1.47*f*OMD	Y = *In vitro* OMD of diet (%) *f*OMD; *In vitro* OMD of faeces (%)	0.75	2.21^{RE}	Holechek et al. (1982a)

Table 2.10: *Continued*

Model	Animal species	Regression model	Variables	R^2	Model	Animal species
15	Fistulated cows, steers[c,d]	Y = 27.8 + 1.44*f*OMD	Y = *In vitro* OMD of diet (%) *f*OMD; *In vitro* OMD of faeces (%)	0.71	2.68^{RE}	Holechek et al. (1982a)
16	Fistulated cows, steers[c,d]	Y = 28.48 + 0.659*f*ID - 5.948*f*N	Y = *In vitro* OMD of diet (%) *f*ID; *In vitro* OMD of faeces (%) *f*N; Faecal N (%)	0.83	2.43^{RE}	Holechek et al. (1982a)
17	Mule deer[e]	Y = -2.99 + 2.70*f*N	Y = Dietary N (% OM) *f*N; Faecal N (% OM)	0.79	0.10^{RE}	Mubanga et al. (1985)
18	Mule deer[e]	Y = 0.19 + 0.19 *f*DIG	Y = Dietary N (% OM) *f*DIG; *In vitro* digestibility of faeces (%)	0.92	0.08^{RE}	Mubanga et al. (1985)
19	Mule deer[e]	Y = 31.38 + 1.71 *f*DIG	Y; *In vitro* digestibility of feed (%) *f*DIG; *In vitro* digestibility of faeces (%)	0.88	3.68^{RE}	Mubanga et al. (1985)
20	Bucks[fg]	Y = 0.816 - 19.864/*f*CP	Y = OMD (g/kg OM) *f*CP; Faecal crude protein (CP, g/kg OM)	0.79	0.0265^{RSD}	Boval et al. (2003)
21	Bucks[fg]	Y = 0.475 + 0.143*f*CP	Y = OMD (g/kg OM) *f*CP; Faecal CP (g/kg OM)	0.79	0.0269^{RSD}	Boval et al. (2003)
22	Rams[fg]	Y = 0.866 - 26.623/*f*CP	Y = OMD (g/kg OM) *f*CP; Faecal CP (g/kg OM)	0.74	0.0295^{RSD}	Boval et al. (2003)
23	Rams[fg]	Y = 0.441 + 0.164*f*CP	Y = OMD (g/kg OM) *f*CP; Faecal CP (g/kg OM)	0.68	0.33^{RSD}	Boval et al. (2003)

Table 2.10: *Continued*

Model	Animal species	Regression model	Variables	R^2	Model	Animal species
24	Sheep[h]	Y = -8.782 + 6.14*f*CPex + 1.65*f*DMex + 6.8*f*N$_{\%}$om	Y = DMI (g/kg BW$^{0.75}$/d) *f*CPex; Faecal CP excretion (g/d) *f*DMex; Faecal DM excretion (g/d) *f*N$_{\%}$om; Faecal N in faecal OM (%)	0.91	3.3^{RPD}	Kneebone and Dryden (2015)
25	Sheep[h]	Y = -6.340 + 5.41*f*CPex + 1.69*f*OMex + 5.6*f*N$_{\%}$om	Y = OM intake (g/kg BW$^{0.75}$/d) *f*CPex; Faecal CP excretion (g/d) *f*OMex; Faecal OM excretion (g/d) *f*N$_{\%}$om; Faecal N in faecal OM (%)	0.91	3.3^{RPD}	Kneebone and Dryden (2015)
26	Sheep[h]	Y = 0.9128 + 4.30*f*CPex – 0.414*f*NDFex + 1.59*f*ADFex – 0.49*f*DMex	Y = CP intake (g/kg BW$^{0.75}$/d) *f*CPex; Faecal CP excretion (g/d) *f*NDFex; Faecal neutral detergent fibre excretion (g/d) *f*ADFex; Faecal ADF excretion (g/d) *f*NDFex; Faecal DM excretion (g/d)	0.90	2.5^{RPD}	Kneebone and Dryden (2015)
27	Sheep[h]	Y = 0.3340 + 0.133*f*N$_{\%}$om	Y = DMD (decimal) *f*N$_{\%}$om; Faecal N (% of faecal OM)	0.55	1.5^{RPD}	Kneebone and Dryden (2015)
28	Sheep[h]	Y = 0.3718 + 0.140*f*N$_{\%}$om	Y = OMD (decimal) *f*N$_{\%}$om; Faecal N (% of faecal OMr)	0.53	1.5^{RPD}	Kneebone and Dryden (2015)
29	Sheep[h]	Y = 0.3468 + 0.106*f*N$_{\%}$om	Y = Digestible OM (% DM) *f*N$_{\%}$om; Faecal N (% OM)	0.50	1.5^{RPD}	Kneebone and Dryden (2015)

Table 2.10: *Continued*

Model	Animal species	Regression model	Variables	R^2	Model	Animal species
30	Sheep[h]	Y = 4.835 + 11.90*f*CPex	Y = Digestible DMI (g/kg $BW^{0.75}$/d) *f*CPex; Faecal CP excretion (g/d)	0.82	2.4^{RPD}	Kneebone and Dryden (2015)
31	Sheep[h]	Y = 5.262 + 10.77*f*CPex	Y; Digestible OM intake (g/kg $BW^{0.75}$/d) *f*CPex; Faecal CP excretion (g/d)	0.82	2.4^{RPD}	Kneebone and Dryden (2015)

[a], Fed on *Eragrostis curvula;* [b], fed on 39 diets with *Festuca arundinacea* and three legumes (*Trifolium incarnatum, T. pratense*, *Lespedeza stipulacea*); [c], Fed on forest pasture; [d], Fed on grassland pasture; [e], fed four different forage diets; [f], Fed on *Digitaria decumbens;* [g], Estimated from established regression equations; [h], Fed on diets consisting of six hays (*Chloris gayana, Heteropogon contortus, Medico sativa*, *Pennisetum glaucum*, *Sorghum bicolor*, *Hordeum vulgare*) with or without four supplements (urea, molasses plus urea, sorghum grain, cottonseed meal). RE, Residual error; RSD, Residual standard deviation; RPD, Residual prediction deviation.

The potential of faecal fibre fractions to predict digestible energy content also have been reported (Short and Remmenga, 1965; Hodgman et al., 1996).

The use of faecal N as an indicator of diet quality may be influenced by: (1) the bias introduced by differences in intake between grazing and housed animals; (2) the bacterial origin faecal N which does not necessarily hold a causal relationship to digestibility; and (3) the differences in the type of relationship between N and digestibility introduced by selective grazing (Vera, 1973). Dietary N/ME ratio and the level of anti-nutritive compounds are the most important factors influencing the accuracy of faecal N as a quality indicator of diets, in addition to the differences in N digestibility (Kamler and Homolka, 2005). Hobbs (1987) re-examined the data of Sinclair et al. (1982) and Leslie Jr and Starkey (1985) on herbivore diets and respective faecal matter. The slopes of the regression between faecal and dietary N were influenced by variability of diets, animals and presence of tannins in the forage and it was concluded that faecal N does not offer reliable quantitative predictions of diet quality in herbivorous. Faecal N was considered only useful to qualitatively illustrate large differences in diet quality (winter vs. summer) and even then, may be biased by confounding effects of protein-precipitating metabolites in plants.

It appears that protein binding capacity of tannins often occur in most of the herbaceous species (Makkar, 2003a) and limits the use of faecal N as an indicator of diet quality. It is also evident that the models which include other faecal indices substantially overcome the associated shortcomings. Therefore, multiple regression models containing faecal N along with other indices such as fibre fractions may be useful to derive predictive regression models for properties of grazing ruminant diets. As reported by Holloway et al. (1981) and Kneebone and Dryden (2015), the stepwise multiple regression procedure is useful tool to derive best-fit models for dietary variables from an array of independent variables. Similarity in the regression

model for rams and bucks (models 20 and 23, Table 2.10) is an encouraging indication that the regressions models established for one ruminant species could be used to derive predictive models for another ruminant species under similar conditions of feeding (Boval et al., 2003).

Chapter 3: General Materials and Methods

3.1 Introduction

The research program consisted of several *in vivo* digestibility and *in vitro* gas fermentation studies on browse-containing diets fed to sheep. The nutritive quality of the diets was also predicted from faecal chemical indices and fNIRS calibration equations. The procedures common for many of the experiments in terms of animal management (selection, housing, feeding), chemical and biological assays (including sampling and sample preparation) and statistical analyses are described in this Chapter. Specific procedures, experimental diets and experimental designs of each experiment are described under the materials and methods section of the respective experimental Chapter.

Approval from the Animal Ethics Committee of Curtin University was obtained prior to the commencement of each *in vivo* digestibility trial and also to permit fistulation of the sheep to be used for the *in vitro* digestibility studies. Sheep were maintained in accordance with the Animal Welfare Act (2002) of WA.

3.2 Experimental site and analytical laboratories

The sheep used in the *in vivo* digestibility and as ruminal fluid donors for the *in vitro* gas fermentation studies were housed in the animal house facilities at the (previous) Northam campus of Curtin University.

Wet chemistry, fNIRS and *in vitro* gas fermentation studies were carried out in the laboratories of either the university or the ChemCentre (WA). The ChemCentre is an

accredited laboratory for chemical testing of the National Association of Testing Authorities of Australia.

3.3 Forages for digestibility studies

The two leguminous (*A. saligna, C. palmensis*) and three halophytic (*A. amnicola, A. nummularia, R. eremaea*) browse species used in the experiments (Table 3.1) were grown on the university farm at Northam (31° 45' S, 116° 40' E), approximately 100 km east of Perth, WA. The climate of the area is described as Mediterranean with an average annual rainfall of 430 mm. The soil type at the site is classified as non-saline (6.45 mS/m; Moore, 1998) sandy duplex soil (Sharma et al., 2006). The browse plants were irrigated during summer as necessary. Oaten (*Avena sativa*) chaff was also included in the rations used in the digestibility studies. This was locally purchased in bulk to minimise potential variation in nutritive value during the experiment periods.

The proximity of the browse plots to the animal house enabled the time between harvesting and feeding of the browse forages to be minimised, thereby limiting the potential denaturation of phenolic compounds of the harvested browse before feeding.

Table 3.1: Browse species used in digestibility studies.

Scientific name	Common name	Season of seedling planting
Acacia saligna	Saligna	Winter, 2005
Chamaecytisus palmensis	Tagasaste	Winter 2005
Atriplex amnicola	River saltbush	Winter 2005
Atriplex nummularia	Old man saltbush	Spring 2006
Rhagodia eremaea	Tall saltbush	Spring 2006

3.4 Selection and housing of sheep for in vivo apparent digestibility studies

For the *in vivo* apparent digestibility studies Merino wethers of the same age were sourced from the university farm's commercial flock. The same sheep were not used for multiple experiments. The sheep were weighed prior to each trial. Depending on the experimental design for each feeding trial, the sheep were selected so as the standard deviation (SD) of body weights (BW) was minimised. Each sheep was then randomly allocated to the dietary treatments according to the experimental design of the respective feeding trial. During the trials the sheep were individually penned in raised, timber-slatted, metabolism cages (Figure 3.1). The floor space of each cage was 1.1 m^2. Each cage was fitted with a feed trough, water trough and tray to enable collection of total faecal output without contaminating with urine.

Figure 3.1: Housing of rumen-fistulated sheep in individual metabolism pens

3.5 Feeding and sheep management for in vivo apparent digestibility studies

Experimental periods comprising 7-days adaptation followed by 5-days collection were used in all feeding experiments. During each period of each experiment, the amount of fresh forage browse required to make the daily rations was hand-harvested each morning at around 9.30 am. The forage harvested for each browse species under investigation included leaves and edible twigs (<5 mm diameter). Immediately after harvest and prior to wilt, the forage was chopped (about 2 cm) using a mechanical shredder (Rover Chip'N Shred, Patent No: 12795, Western Australia). Soon after chopping, the experimental rations were mixed and the sheep were fed their respective diets once a day, around 11.30 am. Chopping and mixing of the forage was to avoid selective feeding of ingredients with the experimental diets by the sheep. Clean, fresh water was provided *ad libitum* at all times.

During each day of the collection periods, a representative sample (500 g) of the experimental diets was taken immediately after mixing and stored frozen (Makkar, 2003b) at -18°C in clearly labelled and sealed polythene bags. During the collection period, total faecal matter voided and feed refusals (from the previous day's feeding) by each sheep was carefully collected and the weight was recorded. Representative sub samples (15%) of these feed refusals and faeces were also taken (from each sheep) and stored frozen at -18°C in clearly labelled and sealed polythene bags.

3.6 Feeding and sheep management for in vitro gas fermentation studies

Three Merino wethers were ruminally-fistulated (as shown in Figure 3.1) and used as ruminal fluid donors for the *in vitro* gas fermentation studies. The sheep were individually housed in raised, timber-slatted pens to facilitate easy collection of ruminal

fluid. Each pen was fitted with a feed trough and water trough. The floor space of each pen was 2.5 m^2.

For at least 10 d (adaptation period) prior to and during the period of ruminal fluid collection for the *in vitro* gas fermentation studies, the sheep were fed a maintenance, roughage-based diet (Makkar, 2003b) consisting of 800 g oaten chaff (air-dry), 200 g air-dry lucerne (*Medicago sativa*) chaff and 150 g air-dry lupins (*Lupinus angustifolius*). They were fed once a day in the morning. The sheep had *ad libitum* access to clean, fresh water.

During the periods when the sheep were not being used in experiments, they were managed in small paddocks adjoining the animal house where they had access to variable amounts of pasture. They were supplementary fed where appropriate to ensure feeding of at least a maintenance diet, based on the maintenance of body condition score of 3 (out of 5). In the paddock the sheep had free access to clean water from an automatic trough.

The sheep were monitored daily, regardless of whether they were housed in the pens or free grazing in the paddock. This included monitoring of daily feed and water intakes, consistency of faecal output and general alertness of the animals. Particular attention was paid to the area surrounding the rumen cannula to ensure it was free of infection, fly eggs and/or maggots, excessive wool and that it was not leaking. The wool around the fistula was removed every month or so, as necessary. The sheep were shorn every 6 months.

3.7 Preparation of samples

Feed samples were freeze-dried (Heto Laboratory Equipment) without thawing (Makkar, 2003b), under 188 microns pressure (vacuum pressure) at -48°C. Half of the freeze-dried

sample was milled using a power mill (Retsch SK 100, Retsch GmbH, Germany) to pass through a 1 mm sieve. Composite samples (to represent the dietary treatment of the respective collection period) were prepared from this milled material for subsequent analysis of composition (including oven-dried DM content) and *in vitro* gas fermentation. The other half of the feed sample was milled to pass through a 0.5 mm sieve and stored frozen (-18°C) for subsequent analysis of TP and TT concentrations and PPC of tannins (Makkar, 2003b).

Frozen feed refusals and faecal samples were thawed overnight at room temperature. Composite samples (to represent dietary treatment of respective collection period) were made by mixing 15% of either the feed refusals or faeces void per day per sheep and then drying to a constant weight at 60°C (AFIA, 2006) in a conventional forced-air oven (Contherm Thermotec, 2000). The samples were then milled using the power mill fitted with a 1 mm sieve. Sub samples of about 100 g of composite samples of either the feed refusals or faeces were stored in clearly labelled, plastic storage containers below -18°C pending chemical analyses and NIRS scanning (faeces only).

3.8 Determination of proximate composition

Established analytical protocols were followed in the determination of proximate composition of feed, feed refusals and faeces samples.

3.8.1 Dry matter and ash contents

The DM content of feed was determined by freeze-drying while the DM contents of the feed refusals and faeces were determined by oven drying at 60°C to reach a constant weight (AFIA, 2006). Analytical DM content of ground samples was determined by overnight drying of a known weight (about 2 g) of sample in a porcelain crucible using a

forced-air oven at 100°C (AOAC, 2004-5; M934.01). Oven dried samples were ignited in the crucible at 600°C until all C was removed to determine the ash content (AOAC, 2004-5; M942.05). Ash content was expressed on a DM basis.

The formulae involved were:

$$DM = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 10^3$$

$$Ash = \frac{(W_4 - W_1)}{(W_3 - W_1)} \times 10^3$$

Where;

DM = Dry matter (g/kg)

Ash = Ash (g/kg DM)

W_1 = Weight of the crucible (g)

W_2 = Weight of the crucible and wet sample (g)

W_3 = Weight of the crucible and dried sample (g)

W4 = Weight of the crucible and residue ash (g)

3.8.2 Neutral detergent fibre (ash free) content

Neutral detergent fibre is the residue of a sample after extraction with boiling neutral detergent solution (sodium lauryl sulphate and ethylenediamine tetraacetic acid) and consists mainly of lignin, cellulose and hemicellulose (Van Soest, 1967). It is regarded as a measure of plant cell wall constituents. Procedures described by Van Soest et al. (1991) were used to determine the NDF content (without using α-amylase and sodium sulphite). A known amount (about 1 g) of sample was boiled for 1 h with neutral detergent

solution and then filtered through a sintered glass pyrex crucible. The weight of the crucible with filtrate was recorded after overnight drying at 100°C in a forced-air oven. The filtrate was then completely ignited to ash at 520°C in a muffle furnace and the weight of the crucible with ash was recorded. The ash-free NDF content was determined as the loss in weight of filtrate by ignition and expressed on a DM basis.

The formula involved was:

$$NDF(ash\ free) = \frac{(W_2 - W_3)}{(W_1 - DM)} \times 10^3$$

Where;

NDF	=	Neutral detergent fibre (g/kg DM)
W_1	=	Weight of the sample (g)
W_2	=	Dried (overnight) weight of the crucible and filtrate after treating with neutral detergent solution (g)
W_3	=	Weight of the crucible and ash (g)
DM	=	Dry matter content of the sample (g/kg)

3.8.3 Acid detergent fibre (ash free) and acid detergent lignin (ash free) contents

Acid detergent fibre is the residue of a dried sample after refluxing with acid detergent solution (0.5 M sulphuric acid and cetyltrimethyl ammonium bromide). This represents essentially the crude lignin and cellulose fractions of plant material but also includes silica (Van Soest, 1967). Principles and procedures described by Van Soest et al. (1991) were used to determine the ADF and ADL contents. A known amount (about 1 g) of sample was boiled for 1 h with acid detergent solution, filtered through a sintered glass Pyrex

crucible and washed initially with hot water and then acetone. The weight of the crucible with filtrate was recorded after overnight drying at 100°C in a forced-air oven. The filtrate in the crucible was then immersed in 72% (g/g) sulphuric acid for 3 h, stirred hourly with a glass rod, breaking all lumps. The filtrate was then washed with hot water until free from acid. The weight of the crucible with filtrate was recorded after drying overnight in a forced-air oven at 100°C. Finally, the filtrate was completely ignited to ash at 520°C in a muffle furnace and the weight of the crucible with ash was recorded. Ash-free ADF and ADL contents were determined as the loss in weight of filtrate treated with acid detergent solution and sulphuric acid, respectively after ignition. The contents were expressed on a DM basis.

The formulae involved were:

$$ADF(ash\ free) = \frac{(W_2 - W_4)}{(W_1 - DM)} \times 10^3$$

$$ADL(ash\ free) = \frac{(W_3 - W_4)}{(W_1 - DM)} \times 10^3$$

Where; ADF = Acid detergent fibre (g/kg DM)

ADL = Acid detergent lignin (g/kg DM)

W_1 = Weight of the sample (g)

W_2 = Dried (overnight) weight of the crucible and filtrate after treating with acid detergent solution (g)

W_3 = Dried (overnight) weight of the crucible and filtrate after treating with acid detergent solution and then with 72% (g/g) H_2SO_4 (g)

W_4 = Weight of the crucible and ash (g)

DM = Dry matter content of the sample (g/kg)

3.8.4 Crude protein content

The sample was first prepared by drying to a constant weight at 65°C in a fan-forced oven and then crushing the dried sample in a cutter mill (Christy and Norris, UK). To determine the N content, a representative portion (approximately 0.2 g) of the sample was accurately weighed to four decimal places on a tared electronic balance (Mettler-Toledo, AE200, USA) into a 25 x 200 mm glass test tube (Corning, USA). The sample was then digested with a sulphuric acid/salicylate solution (0.05% salicylic acid in concentrated sulphuric acid, both analytical grade reagents from BDH, Univar USA) at room temperature for 4 h prior to heating for 20 min at 120°C. A portion of 0.5 mL of hydrogen peroxide (AR Grade 30% w/w, ex BDH Australia) was added to the solution which had been allowed to cool to room temperature. Additions of 0.5 mL aliquots of hydrogen peroxide and heat were repeated until the solution was clear or a light straw colour (or until a total of 2 mL of hydrogen peroxide had been added). The final solution was allowed to cool and was made to a total volume of 50 mL with deionised water (MilliQ water, > 18M Ohm, Australia). The digestion was carried out in a laboratory fume cupboard. The digest solution was presented to a segmented flow analysis instrument (Technicon Autoanalyzer II, USA) using the Berthelot colorimetric determination (after Searle, 1984). The Berthelot reaction reacts N in the digest solution with buffered sodium nitroprusside solution to produce a coloured solution. The intensity of this colour (measured on the instrument at 660 nm) is directly proportional to the N content of the sample. The intensity of the colour of the sample solution was compared to a calibration made from several known concentrations of N solution. The methodology was validated against certified reference samples of similar matrix (NIST 1547, Peach Leaves, US Department of Commerce, USA) and in-house controls that have been analysed many

times by various laboratories around Australia. Nitrogen content in the sample was converted to protein content, using a factor of 6.25 (Benedict et al., 1987).

3.8.5 Mineral content

Mineral contents of the dried, ground samples were determined using Varian VistaPro (Varian, Melbourne, Victoria, Australia) inductively coupled plasma atomic emission spectrophotometer (ICP-AES), according to the protocol of McQuaker et al. (1979). Following this procedure, 0.2 - 0.3 g of each sample was digested with 5 mL of a 1:1 mixture of concentrated nitric and perchloric acids. During the digestion organic material is oxidised and the nitric acid not consumed in the digestion is evaporated, leaving a portion of perchloric acid. The digest is taken to a final temperature of 250°C for 15 min, but not to dryness. The solution is then made up to a volume of 20 mL with deionised water and presented to the ICP-AES. The Na, Ca, P, Mg, K, S, Cu, Mn, Zn and Fe contents were determined by comparison with known matrix matched standards.

3.9 Determination of total phenolics and total tannin contents

The TP and TT contents of the experimental diets were determined according to the protocols establish by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (Makkar, 2003b). They were determined colorimetrically by the Folin-Ciocalteu method and results were expressed as tannic acid equivalents on a DM basis. The Folin-Ciocalteu assay has been particularly recommended for the quantification of phenolics and tannins in trees and shrubs foliage (Makkar, 2003b).

3.9.1 Preparation of phenolics extract

A known weight (about 200 mg) of the freeze-dried and milled (0.5 mm sieve) feed sample was taken into a scintillation vial. Phenolic compounds of the feed sample were extracted into 70% (v/v) aqueous acetone in two steps. In each step, 10 mL of acetone was added to the sample, which was then subjected to ultrasonic treatment for 20 min (two 10 min periods with 5 min break in between) in an ultrasonic water bath. Thereafter, the contents were centrifuged for 10 min at 3,000 g at 4°C and the supernatant (extract) was collected. In the same manner, the extraction was repeated with another 10 mL of 70% (v/v) aqueous acetone added to the pellet and the resultant supernatant was collected and added to the first extract. The extract was kept in a refrigerator until TP and TT contents were determined later that same day.

3.9.2 Preparation of calibration curve

Tannic acid obtained from Merck and Co. (Germany) was used to make the calibration curve. From a standard tannic acid solution (0.1 mg/mL), aliquots (0.0, 0.2, 0.4, 0.6, 0.8, 1.0 mL) were taken into test tubes and the volumes were made to 5 mL with distilled water. Into the diluted aliquots containing tubes, 2.5 mL of 1N Folin-Ciocalteu reagent and 12.5 mL of 20% (g/v) sodium carbonate solution were added. The tube was vortexed, and the absorbance was recorded at 725 nm after 40 min. Colour development and recording of absorbance for the calibration curve were done using the same reagents used for the samples and simultaneously with the determination of TP and TT contents of the samples. The calibration curve was established by plotting the amount (μg) of tannic acid in the aliquot against the absorbance. The gradient and intercept of the curve were determined.

3.9.3 Total phenolics content

The assay is based on the reducing property of phenolics. An aliquot of 0.2 mL was taken from the phenolics extract (extracted into 70% v/v aqueous acetone) into a test tube and the volume was made up to 5 mL with distilled water. To this solution 2.5 mL of 1N Folin-Ciocalteu reagent and 12.5 mL of 20% (g/v) sodium carbonate solution was added. The test tube was vortexed, and absorbance was recorded at 725 nm after 40 min. The amount of TP in the aliquot was calculated using the gradient and intercept of the calibration curve. Thereafter, the concentration of TP of the sample was calculated by taking into account the total volume of the acetone used to extract the phenolics, the volume of the aliquot and the DM weight of the sample and the DM content of the sample. The results were expressed as tannic acid equivalents, on a DM basis.

The formulae involved were:

$$TPa = (m \times Absorbance) + c$$

$$TP = \frac{TPa \times V_1}{V_2 \times W \times DM}$$

Where; TPa = Amount of total phenolics in the aliquot (µg)

TP = Total phenolics (g/kg DM)

m = Gradient of the calibration curve

c = Intercept of the calibration curve

V_1 = Total volume of acetone used to extract phenolics (mL)

V_2 = Volume of the aliquot (mL)

W = Weight of the sample (g)

DM = Dry matter content of the sample (g/kg DM)

3.9.4 Total tannins content

The method is partially chemical, based on the reducing property of tannins, and partially physical because tannins are measured as the reduction in phenolics that occur when the tannin binding agent (polyvinyl polypyrrolidone, PVPP) is added to the extract. To a test tube containing 100 mg of PVPP, 1.0 mL of distilled water and 1.0 mL of tannin-containing extract (extracted into 70% v/v aqueous acetone) was added. The tube was vortexed, kept at 4°C for 15 min, vortexed again, centrifuged at 3,000 g for 10 min and the supernatant was collected. This supernatant has only simple phenolics (SIP) other than tannins. Tannins in the extract would have been precipitated along with the PVPP.

A 0.6 mL aliquot was taken from the supernatant into a test tube, made up to 5.0 mL with distilled water, after which was added 2.5 mL of 1N Folin-Ciocalteu reagent and then 12.5 mL of 20% (g/v) sodium carbonate solution. The tube was vortexed, and absorbance was recorded at 725 nm after 40 min. The amount of simple phenolics in the aliquot was calculated using the gradient and intercept of the calibration curve. Thereafter, the concentration of simple phenolics was calculated, taking total volume of the acetone used to extract phenolics, dilution factor (2) of the extract, volume of the aliquot and the weight and DM content of the sample into account.

Total tannin content of the sample was determined by subtracting simple phenolics content from the TP content of the sample. The results were expressed as tannic acid equivalents, on a DM basis.

The formulae involved were:

$$\text{SPa} = (\text{m} \times \text{Absorbance}) + \text{c}$$

$$SIP = \frac{SPa \times DF \times V_1}{V_2 \times W \times DM}$$

$$TT = TP - SIP$$

Where;

SPa	=	Amount of total phenolics in the aliquot (µg)
m	=	Gradient of the calibration curve
c	=	Intercept of the calibration curve
SIP	=	Simple phenolics content (g/kg DM)
TP	=	Total phenolics (g/kg DM)
DF	=	Dilution factor (2)
V_1	=	Total volume of acetone used to extract phenolics (mL)
V_2	=	Volume of the aliquot (mL)
W		Weight of the sample (g)
DM		Dry matter content of the sample (mg/kg DM)
TT		Total tannin (g/kg DM)

3.10 Determination of protein precipitation capacity of tannin

The biological effect of phenolics contained in the experimental diets on the precipitation of proteins (PPC) was determined using bovine serum albumins (BSA, fraction v)

according to the protocol established by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (Makkar, 2003b). The assay is based on the formation of tannin-protein complexes (tannins in the plant extract and protein) by BSA, fraction v. Tannins present in the complex were determined using ferric chloride.

3.10.1 Preparation of phenolics extract

A known weight (about 200 mg) of the freeze-dried and milled (0.5 mm sieve) feed sample was taken into a scintillation vial. Phenolics compounds of the sample were extracted into 10 mL of 50% (v/v) aqueous methanol. After addition of methanol, the sample was subjected to ultrasonic treatment for 20 min (two 10 min periods with 5 min break in between) in an ultrasonic water bath. The content in the vial was then centrifuged for 10 min at 3,000 g at 4°C and the resultant supernatant (extract) was collected into another vial. Another 10 mL of methanol was added to the pellet, the extraction was repeated in the same manner and the resultant supernatant of this second extraction was collected and added to the first extract. The extract was kept in a refrigerator until the determination of PPC began on the following day.

3.10.2 Formation of the tannin-protein complex

From the tannin containing extract (extracted into 50% v/v aqueous methanol), aliquots of 0.1, 0.2, 0.3, 0.4 and 0.5 mL were taken into test tubes. The volumes were made up to 1.0 mL with 50% (v/v) methanol after which was added 2.0 mL of BSA solution (containing 1.0 mg BSA/mL acetate buffer). The content of the test tube was vortexed and then allowed to stand overnight at 4°C in a refrigerator to precipitate the tannin-protein complex. The tubes were then centrifuged at 3,000 g for 10 min and the resultant supernatant was carefully removed without disturbing the precipitate. The precipitate

was then dissolved by adding 1.5 mL of 1% sodium dodecyl sulfate (SDS) solution and vortexing the contents.

3.10.3 Preparation of calibration curve

Standard tannic acid solution (0.5 mg/mL in 1% SDS) was prepared using tannic acid obtained from Merck and used to make the calibration curve. Aliquots (0.0, 0.1, 0.2, 0.3, 0.4, 0.5 mL) of standard tannic acid were taken into test tubes and the volumes were made to 1.0 mL with 1% SDS solution. To each tube, 3.0 mL of SDS-TEA solution (1% sodium dodecyl sulfate and 7% triethanolamine in distilled water) and then 1.0 mL of ferric chloride reagent were added. Precipitation of proteins was achieved by addition of SDS-TEA solution (1% sodium dodecyl sulfate and 7% triethanolamine in distilled water) while colour development was achieved by addition of ferric chloride reagent. The tubes were vortexed, and absorbance was recorded at 510 nm after 15 min. Colour development for the calibration curve and recording of absorbance were done using the same reagents used for the samples and simultaneously with testing of the samples.

The calibration curve was established by plotting the amount of tannic acid (μg) in the aliquot against the absorbance. The gradient and intercept of the curve were determined.

3.10.4 Protein precipitation capacity of tannin

A 1.0 mL aliquot was taken from the dissolved tannin-protein complex into a test tube. To this was added 3.0 mL of SDS-TEA solution and then 1.0 mL of ferric chloride reagent. The tube was vortexed, and absorbance was recorded at 510 nm after 15 min. The concentration of tannins in the tannin-protein complex was calculated, using the gradient and intercept of the calibration curve.

The formula involved was:

$$T = [(m \times Absorbance) + c] \times 1.5$$

Where;

T = Amount of tannin in the aliquot (μg)

m = Gradient of the calibration curve

c = Intercept of the calibration curve

A linear regression was performed between μg of experimental diet (in aliquot taken for the assay) and tannins precipitated as tannic acid equivalent. The gradient of the regression curve (μg of tannic acid precipitated per μg of diet) was considered as the PPC of the experimental diet.

3.11 Determination of in vivo apparent digestibility

The DM of food is conveniently divided into organic and inorganic material. The OM content in feed and faeces is determined by subtracting the inorganic matter (ash) content from the DM content. The apparent digestibility of a food is most accurately defined as the proportion, which is not excreted in faeces and, therefore, assumed to be absorbed by the animal. It is commonly expressed on a DM basis and as a coefficient, a percentage or g/ kg (McDonald et al., 2002).

The amount of each constituent (DM, OM, Ash, NDF, ADF, ADL, CP) was determined for the feed offered, feed refusals and faeces. The HCEL and CEL contents were calculated from the difference between NDF and ADF contents and between ADF and ADL contents, respectively. By subtracting the amount of each constituent in the feed

refusals from that in the offered feed, nutrient intakes were calculated. The apparent digestibility of the nutrients (DM, OM, NDF, ADF, HCEL, CEL, CP) were then determined and expressed on a DM basis.

The formula involved was:

$$D = \left(\frac{(W_1 - W_2) - W_3}{(W_1 - W_2)} \right) \times 10^3$$

Where;

D = Apparent digestibility of nutrient (g/kg DM)

W_1 = Amount of nutrient in offered (g/d)

W_2 = Amount of nutrient in feed refusals (g/d)

W_3 = Amount of nutrient in faeces (g/d)

3.12 In vitro gas fermentation technique (tannic bioassay)

In vitro gas fermentation assay was undertaken according to the procedure of Makkar (2003b) to determine the OMD, ME, production of short chain fatty acids (SCFA) and biological activity of tannins in the feed samples. The *in vitro* GP technique, based on gas-tight syringes, enables large numbers of samples to be analysed at one time and is more efficient than alternative digestion techniques in evaluating the effects of tannins (Makkar, 2005). Polyethylene glycol is a synthetic polymer that has a very high affinity for tannins across a wide range of pH and its inclusion in the syringe (1 g of PEG 6000) results in the formation of PEG-tannin complexes which inactivate the tannins in the sample. When samples are evaluated with and without PEG using the gas method, the change in GP represents the biological effect of the tannins (Makkar, 2003b).

3.12.1 Preparation of syringes and in vitro *rumen fermentation buffer solution*

A known weight (about 500 mg) of the experimental feed sample was weighed into a glass weighing boat and carefully transferred into a 100 mL calibrated, gas-tight glass syringe (Hamilton Company, USA). A second syringe was prepared in the same manner to which was added approximately 1 g of PEG (6000 molecular weight; Sigma-Aldrich). Syringes (Figure 3.2) were prepared (feedstuffs with and without PEG) in triplicate for every experimental diet. With every batch of fermentation three 'standard' syringes were prepared with the hay standard (*Lolium perenne*) provided by Hohenheim University as well as three 'blank" syringes (without the substrate). The prepared syringes were stored in the oven at 39°C overnight until the rumen fermentation buffer solution was added to the syringes the following morning.

Figure 3.2: Gas-tight, glass syringe used for the *in vitro* gas fermentation assay (courtesy F. Brayshaw)

Three rumen-fistulated Merino wethers, fed on a roughage diet, as described in Section 3.6, were used as ruminal fluid donors for the *in vitro* gas fermentation studies. The time between last meal and sampling of ruminal fluid was set to about 23 h to minimise residual feed in the sampled ruminal fluid. Ruminal fluid was collected from each of the donor animals about an hour before the morning feed and transferred into a pre-warmed (39°C) thermos flask that had been flushed with carbon dioxide (CO_2). The ruminal fluid was obtained by suction using a probe which consisted of a metal frame (5 cm x 1 cm x 1 cm) covered with a double layer of nylon stocking material to ensure filtering of the ruminal fluid. The probe was attached to a curved, stainless steel metal tube (about 250 mm long). It was placed in a caudal position in the ventral sac of the rumen and held in this position tight fit through the rubber stopper in the cannula. Ruminal fluid obtained from the three sheep was combined and homogenised as recommended by Makkar (2003b) to avoid any effect of the donor animal on the assay.

Fermentation buffer solution was prepared by mixing respective volumes of reagents to be adequate to fill 40 syringes per fermentation batch (Table 3.2). Bicarbonate buffer solution, macro mineral solution, micromineral solution, resazurin and distilled water were mixed in that order in a glass container and placed in a water bath maintained at 39°C. The solution was bubbled and flushed with CO_2. After about 5 min, reducing solution was added and CO_2 bubbling was continued for another 15 - 20 min, until the solution was completely reduced (blue colour changed to pink and then to colourless). Strained, homogenised ruminal fluid (including some particulate matter) was added to the reduced buffer solution and CO_2 bubbling was continued. After another 10 min, 40 mL of rumen fermentation buffer solution was transferred into each glass syringe using a bottle top dispenser (Figure 3.3) and immediately placed in the water bath in an upright

position (Figure 3.4) where the temperature was maintained at 39°C. Once all the syringes had been filled, they were carefully shaken, and this was repeated every hour for the first 4 h and then every 2 h until 12 h of the fermentation. After shaking the syringes were immediately returned to the water bath. The gas volumes collected in the syringes were recorded at 2, 4, 6, 8, 10, 12 and 24 h during the fermentation.

Table 3.2: Preparation on *in vitro* rumen fermentation buffer solution.

Reagent		Volume (mL)
Bicarbonate buffer solution: Dissolved 35 g of sodium bicarbonate ($NaHCO_3$) and 4 g of ammonium carbonate (NH_4HCO_3) in about 500 mL of distilled water and then made up the volume to 1 L with distilled water.	-	420.00
Macro mineral solution: Dissolved 6.2 g of potassium dihydrogen phosphate (KH_2PO_4), 5.7 g of disodium hydrogen phosphate (Na_2HPO_4), and 0.6 g of magnesium sulphate ($MgSO_4.7H_2O$) in about 500 mL of distilled water and then made up the volume to 1 L with distilled water.	-	210.00
Micro mineral solution: Dissolved 10 g of manganese chloride ($MnCl_2.4H_2O$), 13.2 g of calcium chloride ($CaCl_2.2H_2O$), 1 g of cobalt chloride ($CoCl_2.6H_2O$), 8 g of ferric chloride ($FeCl_3.6H_2O$) in about 50 mL of distilled water and then made up the volume to 100 mL with distilled water.	-	0.11
Resazurin: Dissolved 0.1 g of resazurin in 100 mL of distilled water.	-	1.07
Reducing solution (freshly prepared): Dissolved 996 mg of sodium sulphide ($Na_2S.9H_2O$) in 94 mL of distilled water and then added 6 mL of 1N sodium hydroxide solution.	-	40.00
Distilled water	-	630.00
Ruminal fluid (filtered, homogenised)	-	440.00

The volume was adequate to fill up 40 syringes plus 10% extra.

Figure 3.3: Filling the glass syringes with buffer solution using the bottle top dispenser (courtesy C. von Pirch)

Figure 3.4: Incubation of gas syringes in the water bath at 39ºC

3.12.2 In vitro net gas production, organic matter digestibility, metabolisable energy, short chain fatty acid production and biological activity of tannins

The net gas produced (GP) by the fermentation of the 200 mg sample (in the absence and presence of approximately 1 g of PEG 6000) was calculated from gas produced from 500 mg sample after 24 h, taking into consideration the gas produced by the hay standard and that of the blank. Differences in gas production due to variations in ruminal fluid collected on different days were accounted by using triplicate blanks and Hohenheim hay standards with every batch of incubation. *In vitro* OMD and ME were estimated from net GP of the samples fermented in the absence and presence of PEG, together with CP and ash contents of the respective experimental diet (Menke and Steingass, 1988). Production of SCFA was estimated (Getechew et al., 2002). The nutritional properties estimated in the absence of PEG were deemed the *in vitro* nutritive vales of the experimental diet. The difference of the estimates in the presence and absence of PEG was considered as a measure of the biological activity of tannin in relation to rumen fermentation (Makkar, 2003b).

The formulae involved in the estimations were:

$$In\ vitro\ GP = (GS - GB)\ \left(\frac{200}{W \times DM}\right)\ \left(\frac{49.61}{GH - GB}\right)$$

$$In\ vitro\ OMD = 14.88 + 0.889\ GP + 0.45\ CP + 0.0651\ Ash$$

$$In\ vitro\ ME = 2.20 + 0.136\ GP + 0.057\ CP$$

$$In\ vitro\ SCFA(-PEG) = -0.0601 + 0.0239\ GP$$

$$In\ vitro\ SCFA(+PEG) = -0.0521 + 0.0207\ GP$$

Where;

GP	=	Net gas production by 200 mg of the sample (mL)
OMD	=	Organic matter digestibility (g/100 g DM)
ME	=	Metabolisable energy (MJ/kg DM)
SCFA (-PEG)	=	Short chain fatty acid production without PEG (mmol/40 mL)
SCFA (+PEG)	=	Short chain fatty acid production with PEG (mmol/40 mL)
GS	=	Gas produced by the sample (mL)
GB	=	Gas produced by the blank (mL)
GH	=	Gas produced by 200 mg of hay standard (mL)
CP	=	Crude protein content of the sample (g/100 g DM)
Ash	=	Ash content of the sample (g/100 g DM)
W	=	Weight of the sample (mg)
DM	=	Dry matter content of the sample (g/100 g)

3.13 Experimental designs and statistical analyses

Experimental design(s) are described under the materials and methods section of each of the experimental Chapters. Statistical analyses were performed using version 9.1 of SAS software (2002-3). The effect of treatments (forage species, PEG, supplementation) was tested by analysis of variance procedure (ANOVA). An appropriate mean separation procedure [t test, least significant difference (LSD) test, Duncan's new multiple range test (DNMRT)] was employed to compare means of the treatments. Limitations of forages were assessed with the help of Pearson pair wise correlation coefficient (r). The stepwise regression procedure was used to develop multiple regression models (best-fit predictive model) to predict nutritional characteristics of diets from faecal chemical composition. Simple linear regression was used to validate the best-fit predictive models. The statistical models (Samita, 2006) used are described below.

Completely randomised design

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Where;

Y_{ij} = The j^{th} observation on the i^{th} treatment

μ = Grand mean

τ_i = Effect of i^{th} treatment

ε_{ij} = Random error

Completely randomised design in 2 factor factorial arrangement

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where;

Y_{ijk}	=	The k^{th} observation on i^{th} level of the first factor and j^{th} level of the second factor
μ	=	Grand mean
α_i	=	Effect of i^{th} level of first factor
β_j	=	Effect of j^{th} level of second factor
$(\alpha\beta)_{ij}$	=	Interaction effect of i^{th} level of the first factor and j^{th} level of the second factor
ε_{ijk}	=	Random error

Latin square design

$$Y_{ijk} = \mu + \rho_i + \delta_j + \tau_k + \varepsilon_{ijk}$$

Where;

Y_{ijk}	=	The observation on k^{th} treatment in i^{th} row and j^{th} column
μ	=	Grand mean
ρ_i	=	Effect of i^{th} row
δ_j	=	Effect of j^{th} column
τ_k	=	Effect of k^{th} treatment
ε_{ijk}	=	Random error

Balance incomplete block design

$$Y_{ij} = \mu + \rho_i + \tau_j + \varepsilon_{ij}$$

Where;

Y_{ij} = The value of the j^{th} treatment from the i^{th} block

μ = Grand mean

ρ_i = Effect of i^{th} block

τ_j = Effect of j^{th} treatment

ε_{ijk} = Random error

Critical value for the least significant different (LSD) test

$$t_{\alpha/2,dfError} = \sqrt{\frac{2RMS}{r}}$$

Where;

$t_{\alpha/2,df}$ = t table value corresponding to error degrees of freedom (df)

Error and at α probability

RMS = Residual mean square from ANOVA table

r = Number of replicates

Critical value for the Duncan's new multiple range test (DNMRT)

$$q_{\alpha,p,dfError} = \sqrt{\frac{RMS}{r}}$$

Where;

$q_{\alpha,p,df}$	=	DMRT table value corresponding to error degrees of freedom
Error		(df) with range p and at α probability
RMS	=	Residual mean square from ANOVA table
r	=	Number of replicates

Pearson correlation coefficient ($\rho_{x,y}$ /r)

$$\rho_{x,y} = \frac{E\left((X - \mu x)\ (Y - \mu y)\right)}{\sigma_x, \sigma_y}$$

Where;

$\rho_{x,y}$	=	Correlation coefficient between X and Y variables
X	=	Expected value of X variable
Y	=	Expected value of Y variable
μx	=	Mean of X variable
μy	=	Mean of Y variable
ρ_x	=	Standard deviation of variable X
ρ_y	=	Standard deviation of variable Y

Predicted equation for the simple linear regression

$$\hat{Y}_i = \beta_0 + \beta_i X_i$$

Where;

$\hat{Y}_i$ = Predicted response value for the i^{th} observation

X_i = Independent variable value for the i^{th} observation

β_0 = Estimated intercept of the regression

β_i = Estimated slope of the regression

Predicted equation for the multiple linear regression

$$Y_i = \beta_0 + \beta_{1X_1 i} + \beta_2 X_{2i} + \ldots\ldots\ldots\ldots\ldots\ldots\ldots + \beta_k X_{ki}$$

Where;

Y_i = Predicted response value for the i^{th} observation

X_{1i} = Value for i^{th} observation o f the 1^{st} independent variable

X_{2i} = Value for i^{th} observation o f the 2^{nd} independent variable

X_{ki} = Value for i^{th} observation o f the k^{th} independent variable

β_0 = Estimated intercept of the regression

β_1 = Estimated coefficient of the 1^{st} independent variable

β_2 = Estimated coefficient of the 2^{nd} independent variable

β_k = Estimated coefficient of the k^{th} independent variable

Chapter 4: Chemical composition, biological effects of tannin and in vitro nutritive value of selected browse species

4.1 Introduction

Trees and shrub foliage play an important role in ruminant feeding systems in many tropical and Mediterranean environments around the world. *Acacia saligna* is indigenous to WA while *C. palmensis* was introduced (1879), but both have become naturalised tree legumes throughout the country (Lefroy et al., 1992). *Atriplex amnicola* and *A. nummularia* are halophyte species indigenous to WA. These four species are major cultivated browse species used for grazing and land rehabilitation throughout Australia. *Rhagodia eremaea* is also a native browse species, known to be palatable to livestock grazing on the southern rangelands of WA (Mitchell and Wilcox, 1994), and it is currently being investigated as a potential forage species for sheep.

The chemical and mineral composition of *A. saligna* (George et al., 2007) as well as *A. amnicola* and *A. nummularia* (Norman et al., 2004b) grown in WA have been reported previously. *Acacia saligna* contains high levels of phenolics and CT (Krebs et al., 2007) and the PPC of the tannin (George et al., 2007) is also high. Both Atriplex species contain high amounts of ash (predominantly sodium chloride) while the tannin content is low (Norman et al., 2004b). Low to moderate levels of phenolics and tannins have been reported for *C. palmensis* grown in Ethiopia (Tolera et al., 1997; Kaitho et al., 1998d). However, the concentrations of tannins contained in trees and shrubs varies widely, is largely unpredictable, and their effects on animals vary from beneficial to toxicity and death (Makkar, 2003a). Therefore, the amount of phenolics and tannins, and their effect in forages of *C. palmensis* grown in WA, could differ from that of the same species grown

in other parts of the world. Published information on the nutritive value (chemical and mineral composition, digestibility, ME) of *R. eremaea* is lacking.

Chemical analysis alone is of limited use to evaluate the nutritive value of plants, especially those containing secondary compounds (El hassan et al., 2000). The *in vitro* gas fermentation technique, which provides empirical equations to estimate digestibility and the ME content of animal feeds (Menke and Steingas, 1988), has gained wide acceptance in research on the nutritional evaluation of animal feeds (Getachew et al., 2005), especially those containing tannins. Incorporation of PEG in the technique (tannin bioassay) provides the opportunity to assess the biological effect of tannin in forage (Makkar et al., 2003b).

The objective of this study was to investigate the nutritive value of selected fresh browse species (*C. palmensis, A. saligna, A. amnicola, A. nummularia, R. eremaea*) grown in the Mediterranean environment of WA. Oaten chaff was also included as it formed the basal diet used in all the feeding trials reported in this thesis.

4.2 Materials and methods

Two leguminous browse species (*A. saligna, C. palmensis*) and three halophyte browse species (*A. amnicola, A. nummularia, R. eremaea*), grown on adjoining fodder paddocks were used for the study. All the species were hand-sown (examples in Figures 4.1 and 4.2) and the plots were fenced with netting to prevent destruction of the plants by rabbits. The forage oats from which the oaten chaff used was derived was also grown locally. Methods of sample preparation, chemical analysis, *in vitro* gas fermentation assay and data analysis were as described in Chapter 3.

Figure 4.1: Hand-sown *Acacia saligna* – six months growth

Figure 4.2: Hand-sown *Atriplex amnicola*

4.2.1 Experimental designs and sampling

The experimental design for the study of chemical composition was a completely randomised design (CRD). Fresh forage samples (400 g, comprising of leaves and stems < 5 mm diameter) of each browse were harvested from six randomly selected plants in late spring (3rd week of November). Six oaten chaff samples (400 g) were also randomly collected from the bulk supply available, to create six replicates of each forage species. Samples were stored in clearly labelled, sealed polythene bags at -18ºC within 30 min of the collection.

The effects of forage species and inclusion of PEG were studied in the *in vitro* gas fermentation assay where treatment combinations were arranged as a two-factor factorial experiment (6 x 2). Factor 1 had six levels (forage species) and the factor 2 had two levels (without and with PEG). Each treatment combination had six replicates.

4.2.2 Chemical and in vitro gas fermentation assays and statistical analysis

Chemical (DM, ash, NDF, ADF, ADL, CP, TP, TT) and mineral (Na, Ca, P, Mg, K, S, Cu, Mn, Zn, Fe) composition of the forage samples were determined, as previously described in Chapter 3. *In vitro* gas production (Chapter 3.12) in the presence and absence of PEG was measured up until 24 h of incubation. The *in vitro* OMD and ME content were estimated from net 24 h GP, without and with PEG to assess the biological effect of tannins. The difference in the *in vitro* nutritive properties without and with PEG was considered the biological effect of tannin.

Data were subjected to ANOVA procedures. The LSD test was used for mean comparison among forage species within PEG levels. The t test was employed to test the difference of the means between with and without PEG within forage species.

Differences between means with P<0.05 were accepted as being statistically significant. As there were great differences in the chemical composition (ash, TP, TT) and *in vitro* characteristics among the leguminous species and the halophyte species, Pearson correlation coefficients (*r*) between chemical composition and nutritive properties were estimated separately within leguminous (n=12) and halophyte species (n=18).

4.3 Results

4.3.1 Proximate composition and phenolics contents

The chemical composition and phenolic content of the forage species are presented in Table 4.1. As expected, the DM content of oaten chaff was higher (P<0.05) than those of the fresh browse forages. Among the browse species, DM content was highest (P<0.05) in *C. palmensis* and lowest (P<0.05) in *R. eremaea*. The ash content was lowest in *C. palmensis* and highest in *A. nummularia* and *R. eremaea* (P<0.05). Oaten chaff had the highest (P<0.05) NDF and the lowest (P<0.05) CP contents. Among the browse species, NDF content was highest (P<0.05) in *A. amnicola* followed by *A. saligna, C. palmensis* and *A. nummularia*, which did not differ. *Acacia saligna* and *A. amnicola* had higher (P<0.05) ADF contents than the other browse species. *Acacia saligna* had the highest (P<0.05) ADL content while *R. eremaea* and oaten chaff had the lowest (P<0.05). *Rhagodia eremaea* had the lowest (P<0.05) NDF and ADF contents but the highest (P<0.05) CP content. *Atriplex amnicola* had the lowest (*P*<0.05) CP content. The CP contents of *C. palmensis, A. saligna* and *A. nummularia* did not differ, while that of *A. amnicola* was lower (P<0.05) than them all. *Chamaecytisus palmensis* and *A. saligna* had the highest (P<0.05) TP and TT contents, respectively. Neither the TP nor the TT contents differed among oaten chaff, *A. amnicola, A. nummularia* and *R. eremaea*.

Table 4.1: Chemical composition and phenolics contents (mean ± SE) of oaten chaff, *Chamaecytisus palmensis*, *Acacia saligna*, *Atriplex amnicola*, *Atriplex nummularia* and *Rhagodia eremaea*.

	DM (g/kg fresh foliage)	**Ash (g/kg DM)**	**NDF (g/kg DM)**	**ADF (g/kg DM)**	**ADL (g/kg DM)**	**CP (g/kg DM)**	**TP (g/kg DM)**	**TT (g/kg DM)**
Oaten chaff	884.8±3.25	51.0±3.10	611.6±5.17	353.5±5.35	48.0±2.44	52.5±1.72	3.4±0.17	1.3±0.10
C. palmensis	454.2±4.37	29.5±0.85	410.9±15.07	297.1±10.75	87.2±4.14	121.3±10.89	44.3±3.82	8.9±1.04
A. saligna	356.1±7.93	65.2±1.66	412.8±6.13	360.4±9.92	123.2±2.98	134.5±2.69	36.9±2.69	28.9±3.70
A. amnicola	329.1±12.80	156.9±7.63	487.8±8.94	335.0±6.94	110.5±4.69	96.7±4.63	5.2±0.30	0.9±0.13
A. nummularia	313.8±10.66	178.8±7.11	388.4±14.54	246.5±12.14	85.2±4.08	133.8±5.05	3.4±0.17	1.5±0.11
R. eremaea	174.9±12.49	177.5±7.48	233.0±11.94	160.2±8.30	41.7±1.40	216.7±11.47	6.2±0.44	2.0±0.22
LSD value (P<0.05)	27.0	15.7	31.8	26.6	10.0	20.7	5.6	4.5

DM, dry matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; TP, total phenolics (in tannin acid equivalents); TT, total tannins (in tannin acid equivalents).
Six replicates from each browse species were analysed for every parameter.

Table 4.2: Mineral composition (mean ± SE) of oaten chaff, *Chamaecytisus palmensis*, *Acacia saligna*, *Atriplex amnicola*, *Atriplex nummularia* and *Rhagodia eremaea*.

	Macro minerals (g/kg DM)						**Microminerals (mg/kg DM)**				**N/S ratio**
	Na[†]	**Ca**	**P**	**Mg**	**K**	**S**	**Cu**	**Mn**	**Zn**	**Fe**	
Oaten chaff	0.26±0.022	1.47±0.040	1.12±0.042	0.85±0.017	8.39±0.632	0.91±0.017	2.6±0.09	56.0±2.47	6.9±0.37	101.2±9.16	9.20±0.289
C. palmensis	0.20±0.044	4.75±0.175	0.67±0.064	3.29±0.280	6.98±0.462	0.91±0.070	4.9±0.41	245.9±52.22	45.8±7.54	303.1±18.33	21.33±0.371
A. saligna	0.81±0.114	11.64±0.766	1.12±0.042	2.76±0.093	18.58±0.974	5.87±0.356	3.2±0.44	42.4±6.30	22.6±2.40	65.5±2.86	3.73±0.245
A. amnicola	42.46±3.843	7.11±0.570	1.61±0.233	7.86±0.537	12.39±2.101	4.76±0.417	2.8±0.31	141.0±28.93	28.1±4.43	137.5±12.37	3.39±0.350
A. nummularia	48.04±2.690	6.86±0.706	2.49±0.139	6.54±0.822	18.96±2.274	4.31±0.201	3.5±0.46	50.8±7.27	24.0±1.18	101.5±6.17	5.02±0.262
R. eremaea	27.32±3.298	7.92±0.491	5.69±0.718	10.41±0.671	38.99±2.491	2.69±0.104	10.4±1.11	173.4±37.15	201.4±37.52	83.4±12.40	12.97±0.798
LSD value	6.76	1.53	0.91	1.45	4.91	0.70	1.6	83.7	45.5	32.8	1.2

DM, dry matter; N/S, ratio of N and S.

Table 4.3: *In vitro* gas production (GP), organic matter digestibility (OMD) and metabolisable energy (ME) of forage of oaten chaff, *Chamaecytisus palmensis*, *Acacia saligna*, *Atriplex amnicola*, *Atriplex nummularia* and *Rhagodia eremaea*, measured with and without polyethylene glycol (PEG).

	GP at 4 h† (mL/0.2 g DM)		**GP at 24 h** (mL/0.2 g DM)		**Organic matter digestibility** (g/kg DM)		**Metabolisable energy** (MJ/kg DM)	
	PEG −	**PEG +**	**PEG −**	**PEG +**	**PEG −**	**PEG +**	**PEG −**	**PEG +**
Oaten chaff	13.5±1.21[a]	13.3±0.98[a]	45.7±2.19[a]	45.6±1.87[a]	582.2±19.37[a]	581.1±16.77[a]	8.7±0.30[a]	8.7±0.26[a]
C. palmensis	15.3±0.34[a]	15.0±0.47[a]	51.6±0.99[a]	49.4±1.20[a]	667.5±10.21[a]	648.4±9.44[a]	10.0±0.15[a]	9.7±0.14[a]
A. saligna	7.4±2.03[a]	14.1±0.93[b]	26.7±3.11[a]	37.8±1.63[b]	452.5±28.15[a]	551.3±15.65[b]	6.6±0.43[a]	8.1±0.23[b]
A. amnicola	8.8±0.69[a]	8.1±0.86[a]	32.5±1.48[a]	32.1±1.88[a]	494.9±12.55[a]	491.6±15.90[a]	7.2±0.19[a]	7.2±0.24[a]
A. nummularia	9.6±0.54[a]	8.1±0.45[a]	33.9±0.92[a]	32.8±0.95[a]	526.6±10.64[a]	517.0±10.90[a]	7.6±0.15[a]	7.5±0.16[a]
R. eremaea	9.8±0.64[a]	9.4±0.58[a]	25.6±2.60[a]	25.2±2.37[a]	492.9±18.26[a]	488.7±16.83[a]	7.0±0.30[a]	7.0±0.27[a]
LSD value	3.1	2.1	5.9	5.0	51.1	42.0	0.8	0.6
Significance of effects								
Forage species	***		***		***		***	
PEG	ns		ns		ns		ns	
Forage x PEG	**		*		*		*	

Level of significance of main effects: *, P<0.05; **, P<0.001; ***, P<0.0001; ns, not significant (P>0.05).

Values without (−) and with (+) 1 g of PEG with different superscripts within browse and response parameter differ (P<0.05).

†, Mean±SE.

Table 4.4: Pearson correlation coefficient (*r*) matrix of chemical composition, *in vitro* gas production and nutritive value of leguminous browse species (*Acacia saligna, Chamaecytisus palmensis*).

	NDF	**ADF**	**ADL**	**CP**	**TP**	**TT**	**GP**	**OMD**	**ME**
Ash	-0.01	0.75*	0.86**	0.40	-0.45	0.82**	-0.88***	-0.87***	-0.87***
NDF		0.49	0.17	-0.21	-0.71*	-0.12	-0.04	-0.05	-0.05
ADF			0.84**	0.00	-0.60*	0.55	-0.78*	-0.79*	-0.79*
ADL				0.01	-0.34	0.84**	-0.87**	-0.88**	-0.88**
CP					-0.37	0.29	-0.29	-0.21	-0.23
TP						-0.11	0.34	0.32	0.32
TT							-0.87**	-0.86**	-0.87**

NDF, neutral detergent fibre (g/kg DM); ADF, acid detergent fibre (g/kg DM); ADL, acid detergent lignin (g/kg DM); CP, crude protein (g/kg DM); TP, total phenolics (g/kg DM); TT, total tannin (g/kg DM); GP, cumulative net gas production at 24 h (mL/0.2 g DM); OMD, OM digestibility (g/kg DM); ME, metabolisable energy (MJ/kg DM).

Six replicates from each browse species were analysed for every parameter.

Level of significance: *, $P<0.05$; **, $P<0.001$; ***, $P<0.0001$.

Table 4.5: Pearson correlation coefficient (*r*) matrix of chemical composition, *in vitro* gas production and nutritive value of forage of halophyte browse species (*Atriplex amnicola, Atriplex nummularia, Rhagodia eremaea*).

	NDF	**ADF**	**ADL**	**CP**	**TP**	**TT**	**GP**	**OMD**	**ME**
Ash	-0.54*	-0.61*	-0.43	0.48*	-0.11	0.49*	-0.18	0.13	0.03
NDF		0.98***	0.97***	-0.96***	-0.41	-0.80***	0.55*	0.04	0.17
ADF			0.96***	-0.92***	-0.31	-0.81***	0.51*	0.00	0.13
ADL				-0.96***	-0.44	-0.77**	0.58*	0.10	0.22
CP					0.49*	0.82***	-0.71**	-0.23	-0.36
TP						0.44	-0.53*	-0.38	-0.42
TT							-0.54*	-0.13	-0.24

NDF, neutral detergent fibre (g/kg DM); ADF, acid detergent fibre (g/kg DM); ADL, acid detergent lignin (g/kg DM); CP, crude protein (g/kg DM); TP, total phenolics (g/kg DM); TT, total tannin (g/kg DM); GP, cumulative net gas production at 24 h (mL/0.2 g DM); OMD, organic matter digestibility (g/kg DM); ME, Metabolisable energy (MJ/kg DM).
Six replicates from each browse species were analysed for every parameter.
Level of significance: *, $P<0.05$; **, $P<0.001$; ***, $P<0.0001$.

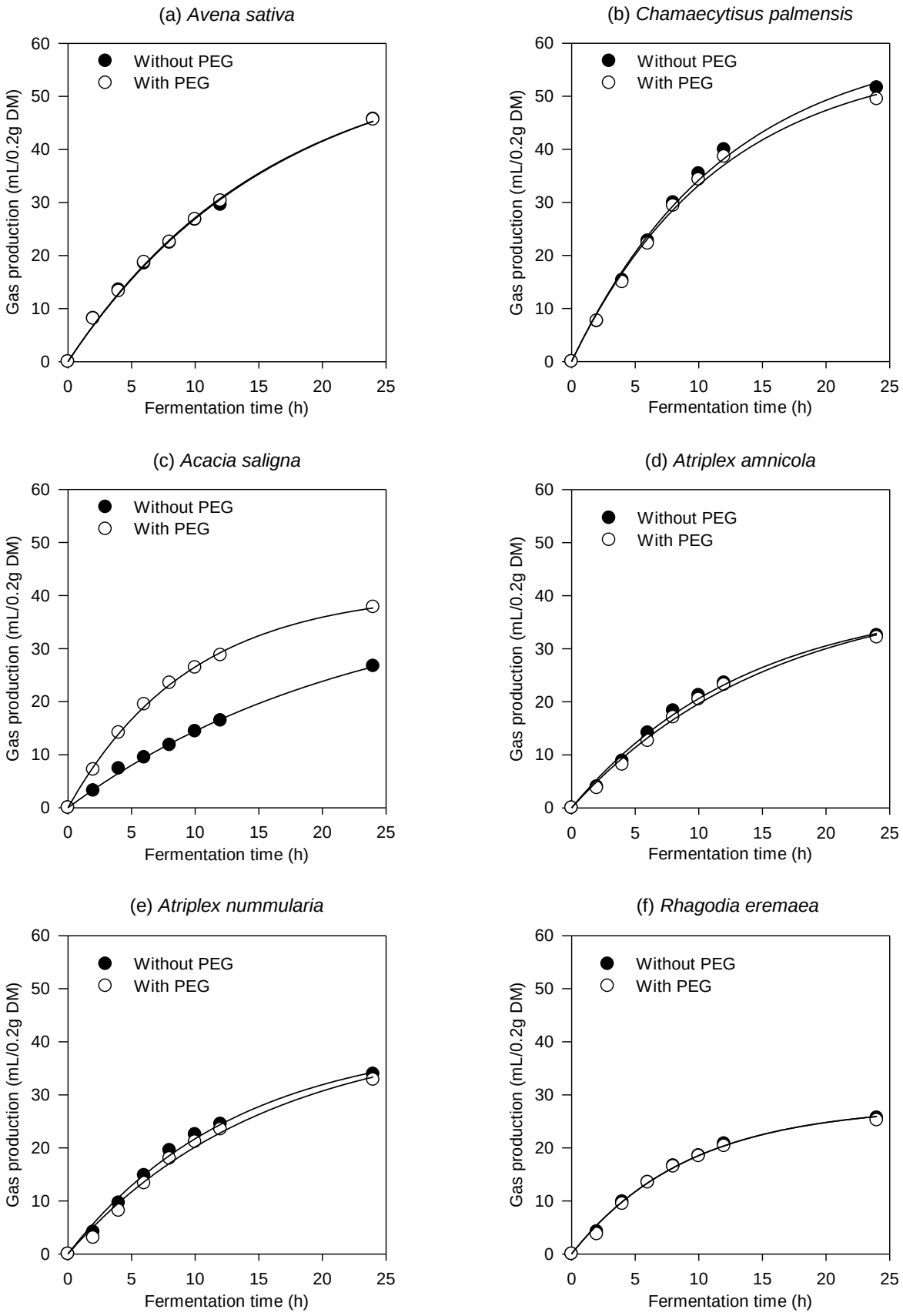

Figure 4.3: *In-vitro* gas production (24 h) of *Avena sativa* hay, *Chamaecytisus palmensis*, *Acacia saligna*, *Atriplex amnicola*, *Atriplex nummularia* and *Rhagodia eremaea* without and with polyethylene glycol

4.3.2 Mineral composition

As shown in Table 4.2, there was considerable variation in the mineral composition of the browse species and well as between the browse species and oaten chaff. *Atriplex amnicola* and *A. nummularia* had the highest (P<0.05) Na content *C. palmensis, A. saligna* and oaten chaff had the lowest (P<0.05). While *C. palmensis* had the highest (P<0.05) Mn and Fe contents, *A. saligna* had the highest (P<0.05) Ca and S contents and the lowest Fe content (P<0.05). *Rhagodia eremaea* had the highest (P<0.05) contents of P, Mg, K, Cu and Zn. Oaten chaff contained the lowest (P<0.05) levels of Ca and Mg. The low P content was similar for oaten chaff, *A. saligna* and *A. amnicola*. Both oaten chaff and *C. palmensis* (P>0.05) contained relatively lower (P<0.05) K and S contents. The Cu and Zn contents of oaten chaff, *A. saligna, A. amnicola* and *A. nummularia* were similar. The N to S ratio was highest (P<0.05) in *C. palmensis* and lowest (P<0.05) in *A. saligna* and *A. amnicola*.

4.3.3 In vitro gas fermentation and effect of PEG

There was an effect of species (P<0.05) on *in vitro* GP, OMD and ME (Table 4.3, Figure 4.3). Gas production is presented at 4 h and 24 h to assess the fermentation characteristics of the browse species. Comparison with and without PEG enabled assessment of the biological effect of tannins. In the absence of PEG, oaten chaff and *C. palmensis* had higher (P<0.05) GP at 4 h and 24 h compared to the other forage species. *Chamaecytisus. palmensis* had the highest (P<0.05) OMD and ME. Only *A. saligna* had an increased (P<0.05) GP, OMD and ME due to addition of PEG. In the presence of PEG, *A. saligna* had higher (P<0.05) GP, OMD and ME than those of the halophyte species (P<0.05).

4.3.4 Correlations among chemical composition and nutritional properties

Data of leguminous (*A. saligna, C. palmensis*) and halophyte (*A. amnicola, A. nummularia, R. eremaea*) browse species were pooled into two groups for correlation analysis. The correlation coefficients among chemical composition and *in vitro* nutritional properties of the leguminous browse species are presented in Table 4.4. Ash, ADF and ADL contents were positively correlated ($P<0.05$), and NDF content was negatively correlated ($P<0.05$) with TP content. As expected, there were strong positive correlations among *in vitro* GP, OMD and ME (data not presented). They were also negatively correlated ($P<0.05$) with ash, ADF, ADL and TT contents. Correlation coefficients among chemical composition and *in vitro* nutritional properties of the halophyte browse species are shown in Table 4.5. Positively correlated ($P<0.0001$) NDF, ADF and ADL contents were negatively correlated with CP ($P<0.0001$) and TT ($P<0.001$) contents. The CP content was positively correlated ($P<0.0001$) with TT contents while negatively correlated ($P<0.001$) with *in vitro* GP. Weak negative ($r>-0.53$, $P<0.05$) correlations were evident between TP and TT contents and *in vitro* GP.

4.4 Discussion

4.4.1 Composition

Results for DM, OM and CP contents were comparable to those reported by Krebs et al. (2007) for *A. saligna* grown in WA (350, 927 and 114 g/kg DM, respectively). The CP content (134 g/kg DM) was also similar to that found by George et al. (2007) who studied the nutritive value of the browse in natural populations across the southern regions of WA. However, their results for NDF and ADF contents (368 and 291 g/kg DM, respectively) were lower than those in this study.

Norman et al. (2004b) analysed edible materials from the most and least preferred bushes of saltbush species grown in WA and reported higher ash, comparable N and lower NDF and ADF contents for *A. amnicola*, as well as for *A. nummularia*, compared to the present study. Atiq-ur-Rehman et al. (1999) also reported a comparable N content for *A. amnicola* grown in WA. The chemical composition of *C. palmensis* and *R. eremaea* grown in a similar environment has not been previously published.

Overall, the browse species were high in CP content while low in NDF content compared to oaten chaff. Multipurpose tree species are rich in N (Osuji and Odenyo, 1997) and none of them are particularly high in ash (El hassan et al., 2000). Both *C. palmensis* and *A. saligna* are MPT species. Kaitho et al. (1998b), in a study with 40 plant species, including *A. saligna*, *C. palmensis* and *A. nummularia*, found that leguminous browse species typically contained high N contents. A higher level of ADL was also found in browse species (except for *R. eremaea*) compared to oaten chaff in the present study. This agrees with Hummel et al. (2006) who found that while NDF contents were lower, ADL contents were higher in browse species compared to grass species.

The halophyte species (*A. amnicola*, *A. nummularia*, *R. eremaea*) were very high in ash contents. Their CP contents increased (*A. amnicola* < *A. nummularia* < *R. eremaea*) while NDF, ADF and ADL contents decreased (*A. amnicola* > *A. nummularia* > *R. eremaea*). Similar inverse relationships have been reported for CP (*A. amnicola* < *A. nummularia*) and fibre fractions (*A. amnicola* > *A. nummularia*) in previous studies (El-Hyatemy et al., 1993; Norman et al., 2004b; Tiong et al., 2004). However, in contrast, Islam and Adams (2000) reported higher total N content in *A. amnicola* than in *A. nummularia* grown in WA.

The threshold CP requirement for adequate ruminal microbial activity is 69 g/kg DM (ARC, 1980). *Rhagodia eremaea* was superior in terms of CP content to all the forage species studied and the value was higher than that of *Medicago sativa* (NRC, 2007). Although *A. amnicola* had a lower CP content compared to the other browse species, it still met this threshold CP requirement. The browse species studied can be considered potential sources of N to supplement poor quality basal diets. In contrast, oaten chaff was a poor source of CP, not meeting the threshold CP requirement for adequate ruminal microbial activity.

4.4.2 Total phenolics and tannin contents

Despite higher TP content, *C. palmensis* had lower TT content compared to *A. saligna*. Edwards et al. (2000) reviewed TP contents in *C. palmensis* and found that they increased from 5 to 50 g/kg DM in the cool, wet winter-spring growth up to 100 to 120 g/kg DM in the hot, dry late summer-autumn period in Australia. Thus, the TP content of *C. palmensis* harvested in the late spring approached the upper margin proposed for winter/spring growth. The TT content of the forage also agreed with the reported contents (<15 g/kg DM) in the same study. Alkaloid content of the species is higher in spring compared to autumn (Ventura et al., 2000). Assefa et al. (2008b) reported that phenolic contents (HT, CT, alkaloids) of *C. palmensis* are influenced by maturity, rainfall and temperature. Further, the shikimic pathway by which phenolic compounds are synthesised in plants facilitates production of HT during favourable environments while stress stimulates production of CT. In the present study, the environmental conditions were favourable several months prior to harvest. Therefore, based on the present data, *C. palmensis* grown in the southern region of WA may have a higher proportion of non-

tannin phenolics of which alkaloid may be considerably higher in spring. Further research on the alkaloid content of *C. palmensis* grown in WA is needed.

Krebs et al. (2007) reported higher TP content (94.5 g/kg DM) of *A. saligna* grown in the same environment as in the present study but harvested from 3 years old trees. Forage for the present study was harvested from younger trees (2 years) in late spring. According to Degen et al. (1997), TP and TT contents of this species are higher in mature trees than in young trees. *Acacia saligna* is reported to contain lower levels of CT in winter compared to other seasons (Salem, 2005). Therefore, the relatively low levels of TP and TT in *A. saligna* compared to other studies was likely due to harvesting the forage from young plants grown in a favourable Mediterranean environment during late spring.

The levels of both TP and TT were extremely low in the halophyte species. The low levels of phenolic compounds in *A. amnicola* and *A. nummularia* were consistent with those reported by Norman et al. (2004b).

4.4.3 Mineral composition

The Na, Ca, P, Mg, S, Cu and Zn contents of *A. saligna* agreed with those reported by George et al. (2007). However, they reported lower K content (6.9 g/kg DM) and higher Fe content (0.1 g/kg DM) for the species. According to them, variation in mineral content (except P) within and between populations of *A. saligna* tended to be more associated with climate and soil characteristics than genetic groups. The site for the current study was classified as a non-saline soil (6.45 mS/m). *Atriplex amnicola* and *A. nummularia*, grown on moderately saline soil (2300 mS/m) in WA had greater Na and K contents (Norman et al., 2004b) than found in this study. Therefore, high soil salinity appears to increase Na and K contents of Atriplex species.

Minimum requirements for sheep of Na, Ca, P, Mg, K and S are 0.9, 2.0, 1.6, 1.2, 5.0 and 1.4 g/kg DM, respectively and for Cu, Mn and Zn are 7, 20 and 20 mg/kg DM, respectively (NRC, 2007). All the browse species studied were rich in Ca, Mg, K, Mn, Zn and Fe. While *A. saligna* and *C. palmensis* were deficient in Na and P, *A. amnicola, A. nummularia* and *R. eremaea* were abundant in these minerals. Except for *C. palmensis*, all browse species were rich in S. Whilst an adequate amount of Cu was in *R. eremaea*, the other forage species were deficient in this element. Osuji and Odenyo (1997) reported in a review that MPT species (including *A. saligna, C. palmensis*) are generally rich in minerals. *Acacia saligna* contains essential minerals (except P) in adequate amounts to meet the requirements of sheep (George et al., 2007). The recommended N:S ratio in sheep diet is 10:1 and excess S in diets could lead to a reduction in rumen motility (NRC, 2007). Low N:S ratio may lead to reduced feed intake and rumen motility (Norman et al., 2004b). However, the maximum tolerable level of dietary S as sodium sulphate for sheep is 4 g/kg for sheep (NRC, 2007), thus the S content of none of the forages studied exceeded the tolerable level. Sulphur functions in the synthesis of S-containing amino acids (methionine, cysteine) and B-vitamins (biotin, thiamine) during microbial digestion in the rumen, thus ruminal microorganisms that are deficient in S do not function normally. Low S diets decrease DMI, DMD and milk production and cause the S balance to be negative in lactating dairy cows (Bouchard and Conrad, 1973). Supplementation of rumen protected (rumen undegradable) methionine has been shown to significantly increase both body weight gain (by 27%) and clean wool growth in Merino wethers (Rodehutscord et al, 1999). The low level of S in *C. palmensis* may limit the nutritive value of the browse and, therefore, supplementation of S may be needed when the forage is fed as a sole diet to sheep. Oaten chaff was only rich in K, Mn and Fe and thus, in comparison with the browse species, was a poor source of most minerals.

4.4.4 Nutritive value, biological effect of tannin and limitations

In vitro GP can be used to determine the nutritive value and identify differences in the potential digestibility and energy contents of forages, including legumes, halophytes and grasses (Sallam, 2005). There were differences in the *in vitro* nutritive characteristics of the forage species in this study. During the initial hours of incubation, a major part of the protein is degraded together with fermentation of the soluble carbohydrate components of animal feeds (Cone et al., 1997). Therefore, the low level of GP recorded in halophyte species at 4 h could be due to their low content of soluble carbohydrates. However, cumulated GP and rate of GP were lowest during 0 to 4 h in grass with greater CP content (Cone and van Gelder, 1999). The *in vitro* OMD (derived from 24 h GP) for both Atriplex species was slightly lower than those previously reported for the species grown in WA (Norman et al., 2004b; Tiong et al., 2004).

In the absence of PEG, *A. saligna* had the lowest GP; however, GP increased in the presence of PEG. This response was typical of browses high in tannin that undergo slow degradation of DM and CP in the rumen (Kaitho et al., 1998e) resulting in lower GP (Kaitho et al., 1998b). Trees and shrub foliage are generally rich in secondary compounds, particularly tannins (Makkar, 2003a). As PEG binds with CT to inactivate them, and thus the addition of PEG to tannin-containing browse samples tends to increase *in vitro* GP (Tolera et al., 1997; Getachew et al., 2002). The increase in the *in vitro* nutritional attributes of *A. saligna* in the presence of PEG supports the biological effect of CT demonstrated in past research involving this species. Administration of PEG has been shown to increase *in vitro* ME yield and *in vivo* digestibility of *A. saligna* in sheep and goats (Degen et al., 2000). Degen et al. (1995) suggested that the presence of a tannin-protein complex in substantial amounts in the NDF and ADF fractions of *A.*

saligna was the cause for the negative digestibility of these fractions and the unsuitability of the forage as the sole energy source for small ruminants. Krebs et al. (2007) demonstrated an increase of *in vivo* DMD in response to administering PEG to sheep fed *A. saligna*.

In their study involving six Acacia species, Rubanza et al. (2005) also found an increase in *in vitro* GP, OMD and ME in the presence of PEG. The difference in the volume of gas produced in the absence and presence of PEG measured by tannin bioassay is an indication of the biological effects of CT (Makker, 2003b). In the present study, the negative biological effect of CT in *A. saligna* was 30.0%, 18.1% and 18.8% in terms of *in vitro* GP, OMD and ME, respectively. Most of the CT in Acacia species binds to protein (Rubanza et al., 2005), limiting N availability for rumen microbes. Sallam (2005) found that high levels of ADL limited *in vitro* GP from leucaena and rice straw. In the present study, strong negative correlations ($r>-0.78$, $P<0.0.05$) occurred between ADF, ADL and TT contents and nutritive values (OMD, ME) of the leguminous browse species. Similar relationships have been reported between *in vitro* GP with cell wall fibre (ADF, ADL) fractions (Kaitho et al., 1998b; Ammar et al., 2004; Sallam, 2005) and phenolics (Longland et al., 1995) of browse species. Therefore, despite its higher CP content, *A. saligna* was inferior to *C. palmensis* owing to higher negative effects of ADL and TT.

Browse species containing less than 45 and 20 g/kg DM of phenolics and tannin, respectively, are generally considered not likely to have substantive adverse effects on ruminants (Getachew et al., 2002). Except for *A. saligna*, the addition of PEG had no effect on *in vitro* GP from the other browse species, suggesting that any tannin (in particular CT) they may contain has little biological activity. Tolera et al. (1997) found that even though *C. palmensis* contained high amounts of total extractable phenolics and

total extractable tannins, it maintained high *in vitro* GP and did not respond to inclusion of PEG in a tannin bioassay. They observed an improvement of *in vitro* GP in other browse species due to the presence of high amounts of CT, thus suggesting that the depression of *in vitro* GP was mainly due to CT rather than TP and TT. The principle phenolic metabolites in *C. palmensis* belong to the flavones group (aglycone apigenin, letolin) which do not have a detrimental effect on rumen fermentation (Edwards, 2000). Therefore, although the content of TP in *C. palmensis* used in the current study approached the level that could have an adverse effect, the content of TT was inadequate to have a substantive negative effect on rumen fermentation. The presence of alkaloid in the species does not affect rumen microbial activity (Ventura et al., 2000) and, therefore, would not affect *in vitro* GP. However, other toxins such as nitrates and hydrolysable tannins present in browse species may create detrimental effects in ruminants. The halophyte browses species and oaten chaff all had very low phenolic and tannin contents, which explains the lack of response to the addition of PEG.

Halophyte species were inferior in terms of *in vitro* nutritive characteristics. The ash content of these species was extremely high, as previously reported by Kaitho et al. (1998b). Islam and Adams (2000) reported that soluble N content in both *A. amnicola* and *A. nummularia* is high throughout the year. According to Ben Salem et al. (2002a), as much as 2/3 of the total N (659 g/kg N) in *A. nummularia* is in a soluble form. Cone and van Gelder (1999) found that protein was not extensively degraded, and the ammonia produced reduced cumulative GP in the *in vitro* assay. Although Kaitho et al. (1998b) reported a strong positive correlation between soluble N with *in sacco* effective degradability ($r = 0.71$) and 24 h degradability ($r = 0.71$), the correlation with asymptotic GP was low ($r = 0.44$). Due to the high salt content, sheep fed saltbush decrease ruminal

fermentation of OM resulting low dietary energy availability (Hemsley et al., 1975). In the present study, correlation coefficients of CP content of halophyte species with *in vitro* GP were strongly negative suggesting that the inferior GP and *in vitro* nutritive characteristics (OMD, ME) in halophytes could be attributed to their high ash and soluble N fractions. This finding is further supported by low absorption of SCFA associated with consumption of high salt diets in sheep (Hemsley et al., 1975) because SCFA is the result of rumen fermentation. Improvements have been made to digestibility (Ben Salem et al., 2004; 2005a) and microbial CP synthesis (Ben Salem et al., 2002a) by supplementing Atriplex forage diets with barley grain, thus providing further evidence for the low ME value of Atriplex forage.

The estimated ME content of oaten chaff was sufficient to meet the maintenance ME requirement of the dry ewe (8.37 MJ/kg DM) while that of *C. palmensis* was able to meet the ME requirements of a number of classes of sheep (NRC, 2007). This could be the reason why sheep fed unsupplemented oaten hay were able to sustain weight gain but those fed oaten hay supplemented with *C. palmensis* had higher weight gain (Umunna et al., 1995). However, the other browse species studied were low in ME, and thus would not be able to support animal production if fed as the sole diet.

4.5 Conclusions and suggestions

The browse species studied (*A. saligna, C. palmensis, A. amnicola, A. nummularia, R. eremaea*) can all be considered as potential sources of N and most minerals to supplement poor quality basal diets. However, except for *C. palmensis* these browse species are poor in digestibility and ME content and therefore would not be suitable as a sole diet for sheep. The nutritive value of *C. palmensis* could be further improved by supplementation

with adequate amounts of S. Conducting of *in vivo* feeding trials would enable determination of the optimum levels of inclusion of these browse species in sheep diets.

Chapter 5: Effects and limitations of inclusion of Acacia saligna in sheep diets

5.1 Introduction

Many researchers have identified the potential of *A. saligna* as a supplementary fodder (Degen et al., 2000; Asefa and Tamir, 2006; Krebs et al., 2007). However, the effects of level of inclusion have not been adequately investigated. Administration of PEG has not been able to sufficiently improve *A. saligna* as a sole source of nutrients for sheep (Degen et al., 2000; Krebs et al., 2007) and, therefore, the limitation (DMI, digestibility) of the species is not merely the high tannin content. The present study was conducted to investigate the effects of the level of inclusion of *A. saligna* on nutritive value and associated limitations in sheep diets.

5.2 Materials and methods

Acacia saligna used in the feeding study was grown on the fodder paddocks of Curtin University's farm and harvested during May-July. The methods of conducting *in vivo* feeding trial, sampling, sample preparation, chemical analyses, *in vitro* gas fermentation assay and data analysis were as previously described in Chapter 3.

5.2.1 Dietary treatments and experimental design

Six experimental diets were formulated to contain varying proportions of *A. saligna*. The amount of fresh forage to be offered was determined to ensure a total DMI per day equivalent to 2% of the mean BW of the sheep. Oaten chaff was used to balance the required amount and DM content of the experimental diets. The experimental diets, the level of *A. saligna* inclusion in the ration and respective DM intakes are presented in Table 5.1. The experiment was based on a Latin Square design. Each diet was fed to six

(replicate) mature, Merino wethers (mean ± SE; 43.5 ±2.22 kg), during the 7-day adaptation period followed by the 5-day collection period.

5.2.2 Chemical analysis, calculations and statistical analysis

Proximate composition of the experimental diets and faeces were analysed. The HCEL and CEL contents were calculated from the NDF, ADF and ADL contents. Apparent *in vivo* digestibility of nutrients and digestible nutrient contents were calculated. Total tannin contents and 24 h *in vitro* GP of the experimental diets were measured. Metabolisable energy contents of the rations were computed. Data were subjected to ANOVA using the General Linear Model procedure. Duncan's new multiple range test was used to separate means.

5.3 Results

5.3.1 Proximate composition and tannins

The proximate composition and total tannin content of the experimental diets are presented in Table 5.2. *Acacia saligna* had greater (P<0.05) ADF, ADL, CP and TT contents and lower (P<0.05) OM and HCEL contents compared to oaten chaff. The NDF, CEL and ash contents of the two forages did not differ (P>0.05). The contents of ADF, ADL, CP and TT increased (P<0.05) while the contents of OM and HCEL decreased (P<0.05) with increasing inclusion level of *A. saligna*. The CEL and ash contents did not differ (P>0.05) among the experimental diets.

5.3.2 Apparent digestibility and digestible nutrient availability

The effect of inclusion level of *A. saligna* on apparent digestibility of the experimental diets is presented in Table 5.3. *Acacia saligna* had lower (P<0.05) digestibility of OM,

NDF (NDFD) and ADF (ADFD) compared to oaten chaff and thus digestibility of these nutrients decreased ($P<0.05$) with increasing level of inclusion of *A. saligna* in the diet. Inclusion level had no effect ($P>0.05$) on DMD. Neither the digestibility of HCEL (HCELD) nor CEL (CELD) differed ($P>0.05$) between the experimental diets. Presented in Table 5.4 is a summary of the digestible nutrient contents of the diets used in the experiment. *Acacia saligna* had a greater digestible CP (DCP) content ($P<0.05$) but lower digestible DM (DDM), OM (DOM), NDF (DNDF), ADF (DADF) and CEL (DCEL) contents ($P<0.05$) compared to oaten chaff. Accordingly, there were decreases in DDM, DOM, DNDF, DADF and DCEL contents ($P<0.05$) and an increase in the DCP content ($P<0.05$) with increasing *A. saligna* inclusion level. The diets did not differ ($P>0.05$) in digestible HCEL content. As shown in Table 5.5, *in vitro* 24 h GP and ME were less ($P<0.05$) in *A. saligna* compared to oaten chaff. In addition, a decline in ME was observed with increasing browse supplementation.

Table 5.1: Experimental diets, *Acacia saligna* inclusion levels and dry matter intake.

	Ration (g FM/d)		**Ration (g DM/d)**		***Acacia saligna* inclusion level (g/kg DM)**	**Dry matter intake***	
	Acacia saligna	**Oaten chaff**	***Acacia saligna***	**Oaten chaff**		**(g DM/d)**	**(g DM/kg BW)**
1.	0	870	0	770	0	941[a]	21.6[a]
2.	544	696	210	616	254	940[a]	21.6[a]
3.	1088	522	426	462	479	920[a]	21.1[a]
4.	1631	348	631	308	686	882[ab]	20.3[ab]
5.	2175	174	864	155	845	826[ab]	19.0[bc]
6.	2719	0	1037	0	1000	770[b]	17.7[c]

FM, fresh matter; DM, dry matter; BW, body weight.
*Means within a column followed by different superscripts are significantly different ($P<0.05$).

Table 5.2: Proximate composition and total tannin content (mean ± SE) of *Acacia saliga* included in experimental diets of sheep.

***A. saligna* inclusion level (g/kg DM)**	**DM*†** **(g/kg FM)**	**OM (g/kg DM)**	**NDF (g/kg DM)**	**ADF (g/kg DM)**	**ADL (g/kg DM)**	**CP (g/kg DM)**	**Ash (g/kg DM)**	**HCEL (g/kg DM)**	**CEL (g/kg DM)**	**TT (g/kg DM)**
0	885±3.3^{a}	915±3.9^{a}	612±5.2^{a}	353±5.4^{d}	48±2.4^{d}	52±1.7^{e}	51±3.1^{a}	258±4.6^{a}	306±3.9^{a}	2.4±0.20^{e}
254	666±6.4^{b}	903±3.1^{ab}	602±2.3^{ab}	381±6.1^{cd}	75±4.5^{cd}	67±2.6^{d}	54±3.0^{a}	222±5.6^{b}	305±3.8^{a}	10.6±1.14^{d}
479	548±9.3^{c}	898±4.2^{bc}	602±3.4^{ab}	400±8.8^{bc}	94±7.4^{bc}	79±3.2^{c}	51±4.2^{a}	201±11.1^{bc}	306±5.1^{a}	20.3±1.44^{c}
686	474±11.2^{d}	887±4.7^{cd}	601±3.8^{ab}	423±6.4^{ab}	124±9.8^{ab}	89±4.4^{c}	56±3.8^{a}	178±5.7^{cd}	299±8.5^{a}	27.1±1.98^{b}
845	424±12.5^{e}	879±5.5^{d}	586±6.5^{b}	432±10.8^{a}	140±12.6^{a}	101±4.5^{b}	59±2.8^{a}	154±7.0^{d}	292±7.7^{a}	34.9±2.62^{a}
1000	387±13.5^{f}	873±5.8^{d}	599±9.3^{ab}	439±17.4^{a}	143±18.8^{a}	113±5.1^{a}	61±3.7^{a}	160±15.8^{d}	296±14.2^{a}	36.2±3.38^{a}

DM*, dry matter (g/kg fresh matter); FM, fresh matter; OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; HCEL, hemicellulose; CEL, cellulose; TT, total tannins (in tannin acid equivalents).
Means within a column followed by different superscripts are significantly different ($P<0.05$).

Table 5.3: Effect of level of inclusion of *Acacia saligna* on apparent digestibility (mean ± SE) in sheep.

***A. saligna* inclusion level (g/kg DM)**	**DMD (g/kg DM)**	**OMD (g/kg DM)**	**NDFD (g/kg DM)**	**ADFD (g/kg DM)**	**CPD (g/kg DM)**
0	505±14.6^{a}	547±12.1^{a}	383±23.1^{a}	361±23.8^{a}	361±25.7^{a}
254	515±13.1^{a}	542±13.8^{ab}	336±20.7^{ab}	288±21.7^{ab}	356±31.8^{a}
479	497±27.7^{a}	520±27.3^{abc}	305±43.2^{abc}	276±43.3^{ab}	361±17.7^{a}
686	488±17.8^{a}	501±16.6^{abc}	282±26.3^{abc}	221±23.5^{bc}	285±31.1^{ab}
845	463±32.8^{a}	471±30.3^{bc}	211±41.6^{c}	182±24.5^{c}	259±33.9^{b}
1000	446±31.2^{a}	449±30.3^{c}	239±50.8^{bc}	182±41.3^{c}	290±12.0^{ab}

DMD, dry matter digestibility; OMD, organic matter digestibility, NDFD, neutral detergent fibre digestibility; ADFD, acid detergent fibre digestibility; CPD, crude protein digestibility.
Means within a column followed by different superscripts are significantly different ($P<0.05$).

Table 5.4: Effect of level of inclusion of *Acacia saligna* on digestible nutrient contents (mean ± SE) of the experimental diets fed to sheep.

***A. saligna* inclusion level (g/kg DM)**	**DDM (g/kg FM)**	**DOM (g/kg DM)**	**DNDF (g/kg DM)**	**DADF (g/kg DM)**	**DHCEL (g/kg DM)**	**DCEL (g/kg DM)**	**DCP (g/kg DM)**
0	447±13.7^{a}	501±11.8^{a}	234±14.9^{a}	128±9.5^{a}	129±8.6^{a}	106±7.3^{a}	19±1.7^{b}
254	343±8.9^{b}	489±12.9^{a}	202±12.5^{ab}	110±8.7^{ab}	136±6.9^{a}	92±9.0^{ab}	24±2.9^{ab}
479	273±15.4^{c}	467±23.7^{ab}	183±25.5^{abc}	111±18.6^{ab}	143±10.4^{a}	90±9.5^{ab}	28±2.3^{ab}
686	232±10.2^{d}	445±14.3^{abc}	170±16.8^{abc}	94±10.3^{ab}	136±12.5^{a}	76±8.8^{ab}	26±3.6^{ab}
845	196±14.7^{de}	413±25.0^{bc}	123±24.6^{c}	79±10.9^{b}	127±20.1^{a}	60±14.9^{b}	26±4.2^{ab}
1000	172±13.4^{e}	392±25.9^{c}	144±31.3^{bc}	83±19.8^{b}	126±17.4^{a}	64±12.2^{b}	33±2.3^{a}

DM, dry matter; FM, fresh matter; DDM, digestible dry matter; DOM, digestible organic matter; DNDF, digestible neutral detergent fibre; DADF, digestible acid detergent fibre; DHCEL, digestible hemicellulose; DCEL, digestible cellulose, DCP, digestible crude protein.
Means within a column followed by different superscripts are significantly different ($P<0.05$).

Table 5.5: Effect of level of inclusion of *Acacia saligna* on *in vitro* gas production and estimated metabolisable energy intake (mean ± SE) in sheep.

***A. saligna* inclusion level (g/kg DM)**	**Gas production (mL/0.2 g DM)**	**ME content (MJ/kg DM)**	**Estimated ME intake (MJ/d)**
0	45.7±2.19[a]	8.7±0.30[a]	8.2±0.29[a]
254	39.5±1.52[b]	8.0±0.21[b]	7.5±0.19[ab]
479	32.9±1.11[c]	7.1±0.15[c]	6.5±0.25[ab]
686	28.3±1.89[c]	6.6±0.25[c]	5.8±0.19[ab]
845	22.7±0.96[d]	5.9±0.14[d]	4.9±0.32[ab]
1000	17.8±1.77[e]	5.3±0.24[d]	4.1±0.72[b]

DM, dry matter; ME, metabolisable energy.
Means within a column followed by different superscripts are significantly different ($P<0.05$).

5.4 Discussion

5.4.1 Chemical composition

The DM content of the *A. saligna* foliage used in the present study was greater than that reported by Asefa and Tamir (2006) and Krebs et al. (2007). The OM content was similar to that reported by Degen et al. (1997) and Asefa and Tamir (2006) for mature *A. saligna* trees but slightly lower than reported by Krebs et al. (2007) for forage grown in the same environment. Degen et al. (1995) and Salem (2005) reported a comparable ash content of the browse. While the CP content was in agreement with many other studies (Getachew et al., 2002; George et al., 2007; Krebs et al., 2007), the browse had much greater NDF and ADF contents than previously reported (Makkar et al., 1995; Asefa and Tamir, 2006; George et al., 2007). The ADL content was in agreement with that of most past studies (Degen et al., 1995; Salem, 2005; Asefa and Tamir, 2006). The HCEL and CEL contents were calculated from the difference between NDF and ADF contents and between ADF and ADL contents, respectively. Thus, greater CEL content of *A. saligna* used in the study could be attributed to the higher ADF content. The content of HCEL was in agreement with Degen et al. (1995), greater than that of Salem (2005) and lower

than that of Degen et al. (1997). The TT content was much lower than reported by Degen et al. (1997; 2000) and Getachew et al. (2002).

The differences in chemical composition may be attributed to the genotype, composition of forage harvested, maturity of the plant, and season of the year. The nutrient and tannin contents of *A. saligna* vary between leaves and stems (Degan et al., 2000) and with plant maturity (Degen et al., 1997). The present study was conducted in winter when *A. saligna* is reported to have lower CT contents compared to other seasons (Salem, 2005). Plants allocate more soluble carbohydrates to growth and reproduction in winter rather than producing tannins with high metabolic costs (Skogsmyr and Fagerstrom, 1992). Contents of CP, NDF and HCEL have been previously found to vary between genotypes of *A. saligna* in WA (George et al., 2007), which may account for the differences in the chemical composition of the *A. saligna* used in this study compared to that previously reported by Krebs et al. (2007) for forage grown in the same environment, but from a different plantation.

The variations in the nutrient compositions of the experimental diets followed the ratio of *A. saligna* and oaten chaff (in the diet). In terms of CP content, *A. saligna* was superior to oaten chaff but inferior in terms of OM, fibre and TT contents.

5.4.2 Effect of inclusion on digestibility of nutrients

Previous research has confirmed the negative influence of higher tannin levels of *A. saligna* on its DMD and OMD in sheep (Degen et al., 1995; 1997; Krebs et al., 2007). The presence of tannin-protein complexes in the NDF and ADF fractions account for the adverse effect of tannin on the digestibility of fibre fractions and CP in the browse (Degen et al., 1995; Makkar et al., 1995). However, DM, OM, NDF, ADF and CP digestibilities

found in the present study were greater than reported by other researchers (Degen et al., 1995; Makkar et al., 1995; Degan et al., 1997). It appears that the effect of CT on digestibility was low due to the low level of CT as well as the high level of HCEL and CEL in the *A. saligna*.

Published information comparing the digestibility of diets containing varying levels of *A. saligna* and oaten chaff (or any other roughage) is lacking. Although the DM, OM and ADF digestibilities of the experimental diets had a declining trend with increasing *A. saligna* inclusion, the effect was significant only when the *A. saligna* inclusion level exceeded 686 g/kg DM of the diet.

Ben Salem et al. (2005a; 2005c) reported that low level of *Acacia cyanophylla* increased the proportion of rumen undegradable protein, and consequently benefited the growth performance of Barbarine lamb. Min et al. (2003) reviewed that CT ranging from 20 to 45 g/kg DM improves efficiency of N use in ruminant diets. The highest level of TT measured in the present study was 36.2 g/kg DM; and thus, the concentration of CT would be well below the level that can have an adverse impact on CP digestibility. It was likely the high level of ADL caused the reduction in digestibility of the diet with higher inclusion levels of *A. saligna* (Gasmi-Boubaker et al., 2005).

5.4.3 Availability of digestible nutrients and limitations to the inclusion level

Nutritive value of forage is a function of intake, nutrient content and digestibility of the forage. Therefore, a reliable assessment of the experimental diets can be made by taking the availability of digestible nutrients into account. Despite the decline in CP digestibility with increasing inclusion of *A. saligna* in the diet, there was a steady increase in DCP content. Contrary, the DOM, DNDF, DADF and DCEL contents were reduced once the

inclusion level of *A. saligna* exceeded 686 g/kg DM. Hummel et al. (2006), in a study involving 62 plant species, found that *in vitro* (24 h) GP and ME of grass species was greater than that of browse species. Out of all the nutritional properties studied, GP was the most adversely affected parameter with increasing inclusion of *A. saligna* in the diet. The level of GP of the pure *A. saligna* diet was only 39% of that of the oaten chaff diet. Getachew et al. (2000), Rubanza et al. (2005) and Osuga et al. (2007) all reported that tannins of Acacia species restricted *invitro* GP. The amount of gas produced by the pure *A. saligna* diet was in agreement with Getachew et al. (2002) but only half of that reported by Salem (2005) for leaves harvested in winter. The ME of *A. saligna* calculated in the present study was slightly greater than that reported by Degen et al. (1995; 1997; 2000). These researchers suggested the greater tannin content of the browse as a cause for low energy intake of sheep. However, the TT content of the browse used in the present study was lower than those reports and thus would account for the differences in ME content. The digestible hemicellulose (DHCEL) content was not affected while that of DCP increased with increasing inclusion level of the browse. Therefore, the decline in DDM, DOM, DNDF and DADF contents was surely due to the decline in DCEL content. The decline in *in vitro* GP and poor ME intake was attributable to the decline in DCEL content with increasing inclusion of *A. saligna*.

Relatively high level of CP in *A. saligna* was also associated with high levels of ADL and TT. Browse species high in ADL are generally high in CT as well (Kaitho et al., 1998). Proteins and CT bind with fibre fractions in *A. saligna* leaves (Degen et al., 1995; Makkar et al., 1995). However, the TT content of *A. saligna* used in the present study was not sufficiently high enough (Min et al., 2003) to make a significant negative effect on the availability of digestible nutrients, in particular DCP. At higher inclusion levels of *A.*

saligna, greater ADL and lower HCEL and DCEL reduced the nutritive value of the diet, in particular the value of ME for sheep.

Restricted feeding was practiced in the feeding trial and almost all the feed offered was eaten by sheep except in the pure *A. saligna* treatment. Average live weight of the sheep used in the present feeding trial was 43.5 ± 2.22 kg. According to NRC (2007), the ME requirement for maintenance of 40 kg yearling range ewes is 6.36 MJ/d; and thus, diets containing up to 479 g/kg DM *A. saligna* ensure the ME intake of 6.5 ± 0.25 MJ/d would safely meet the energy requirement of sheep.

5.5 Conclusions

Inclusion level of *A. salina* with oaten chaff in sheep diets should be limited to 479 g/kg on a DM basis. However, supplementation of an energy supplement such as molasses may enable the inclusion level of *A. saligna* to be increased beyond 479 g/kg DM to supply more protein in the diet, potentially resulting in greater animal performance.

Chapter 6: Effects of supplementation with Chamaecytisus palmensis in sheep diets

6.1 Introduction

In Australia, *C. palmensis* is one of the two cultivated leguminous browse species being commercially accepted for supplementary feeding for ruminants (Lefroy et al., 1992). The species has been established as a profitable and sustainable addition to annual pasture systems in areas with deep sandy soils (Lefroy et al., 1997). Forage of *C. palmensis* is high in both CP and digestibility (Borens and Poppi, 1990; El hassan et al., 2000; Ventura et al., 2000) while being low in phenolics (Longland et al., 1995), particularly the CT that many other browse species (for example, *Acacia cyanophylla, Leucaena leucocephala, L. pallida, Sesbania sesban, S. goetzei*) contain (Longland et al., 1995; Kaitho et al., 1998a, 1998d). However, researchers have reported the presence of alkaloids, particularly sparteine in *C. palmensis* (Muzquiz et al., 1996; Ventura et al., 2000), which does not generally occur in other browse species.

Inclusion of wilted (Varvikko and Khalili, 1993), shade-dried (El hassan et al., 2000), sun-dried (Becholie et al., 2005; Bochelie and Tamir, 2006) and air-dried (Kaitho et al., 1998a) *C. palmensis* forage at relatively low levels as a protein supplement for ruminants fed poor quality pasture (native grass hay, *E. tef* straw) has been previously reported. Supplementing 250 g/d of air-dried *C. palmensis* to sheep fed oaten hay *ad libitum* improved the growth rate compared to unsupplemented sheep (25.0 vs. 15.0 g/d; Umunna et al., (1995)). The effect of inclusion of higher levels of fresh *C. palmensis* has not been adequately investigated; although, the importance of determining the optimum level of inclusion of *C. palmensis* to grass hay has been previously highlighted (Becholie et al., 2005).

The present study was conducted to investigate the effects of the level of inclusion of *C. palmensis* on total diet nutritive value and thus any associated limitations in sheep diets.

6.2 Materials and methods

Chamaecytisus palmensis used in the study was grown on the fodder paddocks of Curtin University's farm at Northam. The experiment was conducted during the period from August to October. Methods of conducting the feeding trial, sampling, sample preparation, chemical analyses, *in vitro* gas fermentation assay and data analysis were as previously described in Chapter 3.

6.2.1 Experimental design and dietary treatments

Six mature Merino wethers weighing 50.7±1.14 kg (mean±SE) were selected for the digestibility study. The experiment was based on a 6 x 6 Latin square design. Six experimental diets were formulated to contain increasing DM levels of *C. palmensis* including the two extreme levels: 0 and 1000 g/kg DM. The amounts of fresh forage to be fed was determined to ensure a total DMI per day equivalent to 2% of the mean BW of the sheep. Oaten chaff was added to *C. palmensis* to balance the DM content of the experimental rations. The experimental rations, the levels of inclusion of *C. palmensis* and respective DM intakes are presented in Table 6.1. The feeding trial consisted of six periods, each having 7-days for adaptation followed by 5-days for collection of samples.

Table 6.1: Experimental diets, *Chamaecytisus palmensis* inclusion levels and dry matter intakes.

	Ration (g FM/d)		**Ration (g DM/d)**		***Chamaecytisus palmensis*** **inclusion level (g/kg DM)**	**Dry matter intake***	
	Chamaecytisus palmensis	**Oaten chaff**	***Chamaecytisus palmensis***	**Oaten chaff**		**(g DM/d)**	**(g DM/kg BW)**
1.	0	912	0	829	0	829^{f}	16.4^{f}
2.	456	766	149	696	176	845^{e}	16.7^{e}
3.	912	620	306	563	352	870^{d}	17.2^{d}
4.	1368	474	475	431	524	906^{c}	17.9^{c}
5.	1824	328	785	302	722	1087^{a}	21.4^{a}
6.	2850	0	1080	0	1000	1080^{b}	21.3^{b}

FM, fresh matter; DM, dry matter; BW, body weight.
*, Means within a column followed by different superscripts are significantly different ($P<0.05$).

6.2.2 Chemical analysis

Proximate analysis was undertaken for the experimental diets and faecal samples. The HCEL and CEL contents were calculated from NDF, ADF and ADL contents. Feed intake was calculated by the difference between ration offered and the refusals. The apparent digestibility of nutrients and digestible nutrient contents were calculated. Experimental diets were assessed for TP content and *in vitro* GP at 24 h fermentation. The *in vitro* ME content of diets were estimated from the net 24 h GP.

6.2.3 Statistical analysis

Data were subjected to ANOVA procedures. Duncan's new multiple range test was used to separate means. Effect of supplement level of *C. palmensis* on nutritive value of experimental diets was further assessed by polynomial regression approach. Observed nutritive properties (digestibility, digestible nutrient content, GP, ME content) were regressed against inclusion level of *C. palmensis*. Linear, quadratic and cubic effects were tested, and the best-fit regression models were selected. The selected regression equations were solved to find the optimum level of inclusion that resulted in the highest level of the respective nutritive property. Pearson correlation coefficients (r) between chemical composition and nutritive properties of experimental diets were also estimated.

6.3 Results

6.3.1 Proximate composition and phenolics

The proximate composition and TP content of the experimental diets are presented in Table 6.2. *Chamaecytisus palmensis* had a much lower DM content and greater ADL, CP and TP contents than the oaten chaff. The contents of NDF, HCEL and CEL were slightly lower while that of OM and ADF were slightly higher in *C. palmensis* than in

oaten chaff. Accordingly, there were marked decreases in DM content and increases in ADL, CP and TP contents with increasing inclusion of *C. palmensis* in the diet.

6.3.2 Feed intake, apparent digestibility and digestible nutrient availability

Dry matter intake of the experimental diets is presented in Table 6.1. Intake increased ($P<0.05$) with increasing inclusion level of *C. palmensis* and decreased ($P<0.05$) when the forage was fed as a sole ration. The effect of *C. palmensis* inclusion level on apparent digestibility of nutrients is presented in Table 6.3. *Chamaecytisus palmensis* had higher ($P<0.05$) DMD and CPD compared to oaten chaff. However, the species did not differ ($P>0.05$) in OMD, NDFD, ADFD, HCELD and CELD. The level of *C. palmensis* supplementation affected ($P<0.05$) apparent digestibility. The summary of the regression analysis of digestibility against *C. palmensis* inclusion level is also presented in Table 6.3. The highly significant and quadratic models provided maximum values for digestibility and respective *C. palmensis* inclusion level.

A summary of the effect of *C. palmensis* inclusion level on digestible nutrient contents is presented in Table 6.4. *Chamaecytisus palmensis* had lower ($P<0.05$) DDM content but greater DCP content compared to oaten chaff. Accordingly, there were decreases ($P<0.05$) in the DDM content and an increase in the DCP content of the total diet with increasing *C. palmensis* inclusion levels. Although pure diets of *C. palmensis* and oaten chaff did not differ ($P<0.05$) in DOM, DNDF, DADF and DHCEL contents, increased inclusion of *C. palmensis* affected ($P<0.05$) these variables.

The effects of *C. palmensis* supplementation on 24 h *in vitro* GP and ME of the experimental rations are presented in Table 6.4. Although *in vitro* 24 h GP and ME contents did not differ ($P>0.05$) between the pure *C. palmensis* and oaten chaff diets,

these parameters varied (P<0.05) when the diets contained different inclusion levels of *C. palmensis* (Table 6.4).

Regression models of nutrient contents, *in vitro* GP and ME against level of inclusion of *C. palmensis* were also highly significant (P<0.05; Table 6.4). The DDM and DHCEL models were linear with negative slopes while DCP was a linear model with positive slope. The rest of the regression models (quadratic/ cubic) provided maximum values for nutrient contents and respective *C. palmensis* inclusion levels.

Table 6.2: Proximate composition and total phenolics content of diets containing *Chamaecytisus palmensis*.

***Chamaecytisus palmensis* inclusion level (g/kg DM)**	**DM (g/kg DM)**	**OM (g/kg DM)**	**NDF (g/kg DM)**	**ADF (g/kg DM)**	**ADL (g/kg DM)**	**CP (g/kg DM)**	**HCEL (g/kg DM)**	**CEL (g/kg DM)**	**TP (g/kg DM)**
0	909	878	638	380	54	52	258	326	10.3
176	692	865	651	391	73	74	260	317	11.9
352	568	870	642	405	71	85	237	334	14.0
524	492	898	615	395	70	95	220	325	-
722	505	900	608	383	83	99	225	301	23.3
1000	379	902	632	407	104	130	225	303	24.2

DM, dry matter; OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; HCEL, hemicellulose; CEL, cellulose, TP, total phenolics.

Table 6.3: Effect of supplementation of *Chamaecytisus palmensis* on apparent digestibility (mean ± SE) and associated regression analysis.

	DMD (g/kg DM)	**OMD (g/kg DM)**	**NDFD (g/kg DM)**	**ADFD (g/kg DM)**	**HCELD (g/kg DM)**	**CELD (g/kg DM)**	**CPD (g/kg DM)**
C. palmensis supplementation level (g/kg DM)							
0	533±9.2^{d}	559±8.9^{b}	460±11.7^{b}	442±11.9^{bc}	485±12.6^{c}	478±12.1^{bc}	292±23.9^{c}
176	576±7.3^{bc}	583±6.5^{b}	507±7.2^{a}	489±8.2^{ab}	533±7.4^{ab}	535±11.1^{a}	542±14.7^{b}
352	571±10.2^{c}	570±11.1^{b}	486±16.6^{ab}	484±12.8^{ab}	490±23.5^{bc}	528±15.1^{ab}	582±23.3^{b}
524	615±7.6^{a}	627±8.0^{a}	522±11.8^{a}	512±12.8^{a}	540±10.3^{a}	561±17.7^{a}	648±13.4^{a}
722	605±11.1^{ab}	621±11.3^{a}	515±15.0^{a}	486±17.2^{ab}	565±11.7^{a}	543±16.8^{a}	653±11.2^{a}
1000	567±15.8^{c}	563±16.1^{b}	451±22.1^{b}	413±30.5^{c}	519±17.7^{abc}	450±31.5^{c}	699±8.4^{a}
Regression analysis							
Model	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic	Quadratic
RSD	26.98	30.76	37.16	41	41.09	45.17	55.34
R^2	48	34	31	40	18	42	85
Probability (P)	<0.0001	0.001	0.0023	0.0002	0.0364	0.0001	<0.0001
Y″ (g/kg DM)	604	611	516	504	541	555	698
X″ (g/kg DM)	592	554	490	454	679	468	808

DM, dry matter; DMD, dry matter digestibility; OMD, organic matter digestibility; NDFD, neutral detergent fibre digestibility; ADFD, acid detergent fibre digestibility; HCELD, hemicellulose digestibility; CELD, cellulose digestibility; CPD, crude protein digestibility; RSD, residual standard deviation; Y″, maximum digestibility (dependant variable), X″, level of *C. palmensis* supplementation (independent variable) for maximum digestibility (Y″).
Means within a column followed by different superscripts are significantly different (P<0.05).

Table 6.4: Effect of supplementation of *Chamaecytisus palmensis* on nutrient availability, *in vitro* gas production and metabolisable energy (mean ± SE) and associated regression analysis.

	DDM (g/kg DM)	**DOM (g/kg DM)**	**DNDF (g/kg DM)**	**DADF (g/kg DM)**	**DHCEL (g/kg DM)**	**DCEL (g/kg DM)**	**DCP (g/kg DM)**	***In vitro* 24 h GP (mL/0.2g DM)**	***In vitro* ME (MJ/kg DM)**
C. palmensis inclusion level (g/kg DM)									
0	485±8.3^{a}	491±7.8^{b}	293±7.5^{bc}	168±4.5^{b}	125±3.2^{bc}	156±3.9^{c}	15±1.2^{f}	45±0.8^{ab}	8.7±0.10^{bc}
176	398±5.0^{b}	504±5.6^{b}	330±4.7^{a}	191±3.2^{a}	139±1.9^{a}	170±3.5^{abc}	40±1.1^{e}	44±1.5^{bc}	8.5±0.19^{c}
352	325±5.8^{c}	497±9.6^{b}	312±10.6^{ab}	196±5.2^{a}	116±5.6^{c}	176±5.1^{ab}	49±2.0^{d}	41±0.9^{c}	8.3±0.12^{c}
524	303±3.7^{d}	563±7.2^{a}	321±7.2^{ab}	202±5.1^{a}	119±2.3^{bc}	182±5.7^{a}	61±1.3^{c}	48±0.6^{a}	9.2±0.08^{a}
722	306±5.6^{d}	559±10.1^{a}	314±9.1^{ab}	186±6.6^{ab}	127±2.6^{b}	163±5.1^{bc}	65±1.1^{b}	46±0.9^{ab}	9.1±0.12^{ab}
1000	215±6.0^{e}	508±14.6^{b}	285±14.0^{c}	168±12.4^{b}	116±4.0b^{c}	137±9.6^{d}	91±1.1^{a}	44±1.2^{bc}	9.0±0.16^{ab}
Regression analysis									
Model	Linear	Quadratic	Quadratic	Quadratic	Linear	Quadratic	Linear	Cubic	Cubic
RSD	30.74	30.34	23.23	16.33	10.79	13.89	5.93	2.66	0.36
R^2	88	36	26	40	11	55	94	26	40
Probability (P)	<0.0001	0.0006	0.0063	0.0002	0.0524	<0.0001	<0.0001	0.0202	0.0008
Y″ (g/kg DM)	449	543	323	199	129	179	90	48	9.3
X″ (g/kg DM)	0	617	436	482	0	423	1000	788	831

DM, dry matter; DDM, digestible dry matter; DOM, digestible organic matter; DNDF, digestible neutral detergent fibre; DADF, digestible acid detergent fibre; DHCEL, digestible hemicellulose; DCEL, digestible cellulose; DCP, digestible crude protein; GP, gas production; ME, metabolisable energy; RSD, residual standard deviation; Y″, maximum nutrient content (dependant variable), X″, level of *C. palmensis* supplementation (independent variable) for maximum nutrient content (Y″).
Means within a column followed by different superscripts are significantly different (P<0.05).

Table 6.5: Pearson correlation coefficient (*r*) matrix of chemical composition and nutritive value of experimental diets.

	HCEL	**CEL**	**ADL**	**Ash**	**TP**	**DDM**	**DOM**	**DHCEL**	**DCEL**	**DCP**	***In vitro* 24 h GP**	***In vitro* ME**
CP	-0.79***	-0.63**	0.94***	-0.61***	0.91***	-0.97***	0.30	-0.33*	-0.31	0.99***	-0.09	0.52*
HCEL		0.35*	-0.57**	0.77***	-0.92***	0.84***	-0.58**	0.47*	-0.01	-0.81***	-0.42*	-0.71***
CEL			-0.75***	0.66**	-0.84***	0.49**	-0.30	-0.14	0.47*	-0.62***	-0.31	-0.55**
ADL				-0.49*	0.88***	-0.87***	0.14	-0.17	-0.43*	0.93***	-0.07	0.36*
Ash					-0.85***	0.55**	-0.54**	0.29	0.29	-0.61***	-0.69***	-0.87***
TP						-0.87***	0.51**	-0.26	-0.39*	0.91***	0.37*	0.81***
DDM							-0.25	0.45*	0.25	-0.95***	-0.04	-0.47*
DOM								0.25	0.57**	0.39*	0.62***	0.66***
DHCEL									0.33*	-0.27	0..01	-0.16
DCEL										-0.22	0.05	-0.10
DCP											0.12	0.53**
In vitro 24 h GP												0.90***

CP, crude protein content; HCEL, hemicellulose content; CEL, cellulose content; ADL, acid detergent lignin content; Ash, ash content; TP, total phenolics content; DDM, digestible dry matter content; DOM, digestible organic matter content; DHCEL, digestible hemicellulose content; DCEL, digestible cellulose content; DCP, digestible crude protein content; GP, gas production; ME, metabolisable energy content.
Significance at: *P<0.05, **P<0.001, ***P<0.0001.

6.3.3 Correlations among nutritional properties

Pearson correlation coefficients (*r*) among chemical composition and nutritive value of the experimental diets are presented in Table 6.5. Correlations among dietary CP, ADL and TP contents were strongly positive (P<0.0001). They were negatively correlated (P<0.0001) with DDM content but positively correlated (P<0.0001) with DCP content. The correlation coefficients between TP content with *in vitro* ME were positive (P<0.0001).

6.4 Discussion

6.4.1 Proximate composition and phenolics

The DM and OM contents of *C. palmensis* foliage harvested in spring (Table 6.2) was comparable with those of hand-plucked foliage harvested in the same season by Ventura et al. (2002). However, foliage used in their study had higher CP and lower fibre contents. The ADL content was also in agreement with that reported by Ventura et al. (2002). The greater HCEL and CEL contents of *C. palmensis* were attributed to the greater NDF and ADF contents. The moderate level of TP agrees with Edwards (2000) who reviewed that TP contents in edible *C. palmensis* forage vared from 5-50 g/kg DM in the cool, wet winter-spring growth.

Chamaecytisus palmensis had lower DM content but higher OM, ADF, ADL, CP and TP contents compared to oaten chaff. The persistent trends observed in DM, OM, ADL, CP and TP contents across the experimental diets follow their contents in *C. palmensis* and oaten chaff and the ratio of the species in the respective diets. Browse species have greater lignin content compared to grass species (Hummel et al., 2006), and thus the greater ADF content of *C. palmensis* was due to its greater ADL content. Higher CP

levels in *C. palmensis* compared to oaten (Umunna et al., 1995) and grass (Becholie et al., 2005; Bochelie and Tamir, 2006) hay was again evident in this study.

6.4.2 Effect of supplementation on apparent digestibility of nutrients

The lower DMD of the *C. palmensis* pure diet compared to that reported by Borens and Poppi (1990) was probably due to the greater lignin content, as ADL has been reported to lower the DMD of other browse species such as *Quercus suber* and *Erica arborea* (Gasmi-Boubaker et al., 2005).

In previous studies, the highest DMD, OMD, and NDFD were reached when natural grass hay basal diet (CP=47 - 48 g/kg DM) was supplemented with sun-dried *C. palmensis* at 280 g/kg DM (Becholie et al., 2005) and 300 g/kg DM (Bochelie and Tamir, 2006). Digestibility of ADF were also highest at 300 g/kg DM (Bochelie and Tamir, 2006) supplement level. Umunna et al. (1995) found supplementation with 265 g/kg DM of *C. palmensis* air-dried leaves resulted in a significant increase in the digestibility of N compared to the unsupplemented oaten hay basal diet (CP = 49 g/kg DM) in sheep. The oaten chaff basal diet used in the present study had comparable CP level (52 g/kg DM) to that reported for these studies. Therefore, the highest digestibility reached at 524 g/kg DM supplement level was due to the better balance of nutrients (energy and N) provided by *C. palmensis* (Becholie et al., 2005; Bochelie and Tamir, 2006). Further, the lack of effect on apparent digestibility at low levels of supplementation could be due to the unsupplemented oaten chaff basal diet already being able to maintain ruminal ammonia concentrations at the optimal 50 mg/L (Satter and Slyter, 1974) for roughage digestion (Umunna et al., 1995). Regression analysis confirmed that supplementation with *C. palmensis* provided a better balance of nutrients, thereby significantly increasing apparent

digestibility. Maximum DMD, OMD, NDFD, ADFD and CPD were achieved at 592, 554, 490, 454 and 808 g/kg DM inclusion levels, respectively.

6.4.3 Effect of supplementation on availability of nutrients and energy

Nutrient content and digestibility were considered and the quality of the experimental diets was assessed in terms of nutrient availability (Table 6.4). The DDM content declined linearly with increasing *C. palmensis* supplementation despite the quadratic variation of DMD, which was largely due to the low DM content of *C. palmensis* compared to oaten chaff (Table 6.2; 379 vs. 909 g/kg). The DHCEL content remained unchanged (within a narrow range of 23 g/kg DM) except the inexplicable greater content recorded at 176 g/kg DM inclusion level. However, both DCEL and ADF contents were highest at 524 g/kg DM inclusion level and showed a quadratic relationship with inclusion level. Therefore, the quadratic relationships of DADF and DOM observed with increasing *C. palmensis* inclusion were largely attributed to the variation in DCEL content of the total diet. Both the greater content and digestibility of CP of *C. palmensis* attributed to the linear, positive relationship of DCP with level of inclusion and thus the highest DCP was reached in the pure browse diet.

Chamaecytisus palmensis was the highest *in vitro* gas producer of the 11 tropical browse species tested by Longland et al. (1995). In the present study, foliage of *C. palmensis* and oaten chaff produced similar amounts of gas, which disagrees with Hummel et al. (2006) who reported lower GP from legumes compared to grass species. The present results agreed with the increase in *in vitro* GP (45.9 mL/0.2 g DM) observed when *E. tef* straw was supplemented with 600 g/kg DM of *C. palmensis* (El hassan et al., 2000). Regression analysis showed that the highest GP (48 mL/0.2 g DM) and ME content (9.3 MJ/kg DM) would be achieved at 788 and 831 inclusion levels, respectively. This ME content is well

beyond the ME requirement for maintenance of a dry ewe (8.37 MJ/kg DM) and many other classes of sheep (NRC, 2007). This could be the reason that sheep fed oaten hay supplemented with *C. palmensis* had significantly greater BW gain while those fed unsupplemented hay experienced BW losses (Umunna et al., 1995). The ability of *C. palmensis* supplementation to support animal production is highlighted by Assefa et al. (2008a) who found that complete substitution of a concentrate supplement (wheat bran and *Guizotia abyssinica* cake) by fresh *C. palmensis* in a poor-quality, roughage-based diet resulted in similar daily weight gain in sheep.

The main objective of the use of legume species (*C. palmensis*) in supplementary feeding programmes is to provide adequate N for ruminal fermentation while deriving maximum energy from the total ration. The present data showed that *C. palmensis* could be supplemented to oaten chaff at a greater level than reported previously (Umunna et al., 1995) in order to achieve the objective of supplementary feeding programmes.

6.4.4 Limitations to supplementation

Both tannins and lignin are polyphenolics, and their monomers are synthesised through the shikimic pathway in plants (Wong, 1973) thus shrubs high in ADL are also high in CT (Gasmi-Boubakker et al., 2005), as shown by the strongly positive correlation between TP and ADL contents. Most CT is bound to CP in browse species (Rubanza et al., 2005) and thus the positive correlations observed among CP, ADL and TP were largely due to their presence in greater amounts in *C. palmensis* compared to oaten chaff. Similar positive association of lignin and tannins has been reported by Kaitho et al. (1998c; 1998d) in browse (including *C. palmensis*) supplemented sheep diets. The negative correlation coefficient between CP and DDM content was due to the low DM content of *C. palmensis* compared to oaten chaff. One should not misinterpret this result

as “increasing CP causes reduction of DDM of roughage diets”. It was obvious that greater CP content of *C. palmensis* lead to greater DCP content.

A comprehensive study conducted with 11 tropical legume species (*A. cyanophylla, C. palmensis, Calliandra species, Dioclea guianensis, Flemingia macrophylla, Leucaena leucocephala, Sesbania sesban, S. goetzei,* three accessions of *Tadehagi triquetrum*) reported the lowest level of TP, with no detectable total CT, bound CT or extractable CT was in *C. palmensis* (Longland et al., 1995). Due to very low level of total proanthocyanidins (4.66 mg/g DM), total extractable CT (8.00 mg/g DM) and total extractable proanthocyanidins (1.74 mg/g DM), no response has been shown to the addition of PEG (Tolera et al., 1997). Condensed tannin at levels over 55 g/kg DM reduce digestibility in grazing ruminants (Min et al., 2003). Thus, even the pure *C. palmensis* diet which contained only 24.2 g TP /kg DM would not have an adverse impact on digestibility. These findings, together with the positive correlations observed between TP with *in vitro* ME and DCP contents, show that the low level of phenolics in *C. palmensis* does not limit ruminal fermentation and availability of N in oaten chaff supplemented with *C. palmensis* diets fed to sheep. Lignin shows high resistance to microbial fermentation (McDonald et al., 2002) and thus the greater ADL content of *C. palmensis* may be a factor that limits the availability of cell wall components (DHCEL, DCEL) and, therefore, DDM as was apparent in the negative correlations and their reduction at higher levels of inclusion of the browse in the diet.

Chamaecytisus palmensis contains alkaloid within the range capable of reducing the digestibility of ruminant feed, although the concentration is not adequate to cause toxicity (Assefa et al., 2008b). Sparteine is the major alkaloid present in *C. palmensis* (Muzquiz et al., 1996; Edwards et al. 1997a; Edwards, 2000; Ventura et al., 2000), and alkaloid has

been suggested as the cause of the decline in digestibility at higher levels (> 280 g/kg DM) of *C. palmensis* supplementation (Becholie et al., 2005). Unfortunately, alkaloid content was not measured in the present study. Yet, it can be suggested that the greater content of alkaloid in *C. palmensis* could be another cause of the decline in digestibility of the fibre fractions, resulting in the lower availability of energy at higher inclusion levels.

6.5 Conclusions

A greater level of *C. palmensis* than previously reported by other researchers could be added to oaten chaff in supplementary feeding programmes. The low level of TP of *C. palmensis* does not limit ruminal fermentation; however, greater lignin content limits its inclusion level in sheep diets.

Chapter 7: Effects of increasing the inclusion level of Atriplex amnicola on feeding value for sheep

7.1 Introduction

Establishment of halophytic plant species such as Atriplex remains one of the few feasible opportunities to revegetate salinity affected grazing landscapes in Australia (Masters et al., 2001), in addition to their role as an alternative source of feed for grazing animals. *Atriplex amnicola* (river saltbush), *A. cinerea* (grey saltbush) and *A. nummularia* (oldman saltbush) are indigenous to Australia (Lefroy et al., 1992). Much of the research in the past has focused on feeding *A. nummularia*. When fed as a supplement it has been shown to improve carcass quality characters without having adverse effects on the eating quality characteristics of lamb (Hopkins and Nicholson, 1999; Pearce et al., 2008). Furthermore, when pregnant ewes were fed with *A. nummularia* there were no adverse effects on reproductive performance, milk production or lamb growth (Abu-Zanat and Tabbaa, 2006).

Atriplex amnicola contains high concentration of N (Islam and Adams, 2000). The forage is a rich source of minerals and the digestibility remains moderate (500-600 g/kg DM) during the dry summer-autumn period (Atiq-ur-Rehman et al., 1999). Sheep have been shown to prefer *A. amnicola* over *A. nummularia* (Norman et al., 2004b). However, the potential of *A. amnicola* as a supplementary feed for sheep has not been adequately investigated, particularly with regards to the impact of level of inclusion on the nutritional characteristics of the total diet. With knowledge of its optimal level of inclusion in the diet, grazing systems could then be designed to encourage sheep to select *A. amnicola* at this level.

7.2 Materials and methods

The feeding trial was conducted from August to October. *Atriplex amnicola* grown on the fodder paddocks of Curtin University's farm at Northam was used to feed the sheep. Methods of conducting the feeding trial, sampling, sample preparation, chemical analyses and *in vitro* gas fermentation assays and data analysis were as previously described in Chapter 3.

7.2.1 Experimental design and dietary treatments

Six Merino wethers weighing 42.8 ± 0.98 kg (mean ± SE) were used for the feeding trial. The experiment was based on a Latin square design. Six experimental diets were formulated to contain increasing DM levels of *A. amnicola*. The amount of fresh forage to be fed was determined to ensure a total daily DMI equivalent to 2% of the mean BW of the sheep. Oaten chaff was used to balance the DM content of the experimental diets. As preliminary investigations revealed a significant reduction in intake with a sole *A. amnicola* diet, the highest level of browse inclusion was set at 2741 g FM/d (836 g/kg DM). The six experiment diets were set to ensure intake between 0 and 2741 g FM/d. The experimental diets, the inclusion levels of the *A. amnicola* and respective dry matter intakes are presented in Table 7.1. The feeding trial consisted of six periods, each having a 7-day adaptation period followed by 5-day for the collection of samples.

Table 7.1: Experimental diets, *Atriplex amnicola* inclusion levels and dry matter intakes.

	Ration (g FM/day)		**Ration (g DM/d)**		***Atriplex amnicola* inclusion level (g/kg DM)**	**Dry matter intake***	
	Atriplex amnicola	**Oaten chaff**	***Atriplex amnicola***	**Oaten chaff**		**(g DM/d)**	**(g DM/kg BW)**
1.	0	857	0	779	0	779^{d}	18.2^{d}
2.	548	720	125	654	161	779^{d}	18.2^{d}
3.	1096	582	300	529	362	829^{b}	19.4^{b}
4.	1645	445	398	405	496	803^{c}	18.8^{c}
5.	2193	308	527	284	650	811^{bc}	18.9^{bc}
6.	2741	171	788	155	836	885^{a}	20.7^{a}

FM, fresh matter; DM, dry matter; BW, body weight.
*, Means within a column followed by different superscripts are significantly different ($P<0.05$).

7.2.2 Chemical assays and analysis

Proximate composition and mineral contents of experimental diets were analysed. Respective faecal samples were also analysed for proximate composition. Apparent *in vivo* digestibility of nutrients was calculated. The ME content of the diets was estimated in two ways: (i) based on 24 h *in vitro* GP; and (ii) based on *in vivo* DMD according to SRC (1990).

The formulae involved was:

$$ME = (0.17 \times DMD) - 2.00$$

Where;

ME = Metabolisable energy content based on *in vivo* DMD (MJ/kg DM)

DMD = Dry matter digestibility of the diet (g/100 g DM)

7.2.3 Statistical analysis

Data were subjected to ANOVA procedures to test the effect of inclusion level of *A. amnicola* on the nutritive value of experimental diets. Mean comparison was performed using Duncan's new multiple range test.

7.3 Results

7.3.1 Feed intake

The amount of feed offered daily to the sheep was restricted to the level of 2% of mean BW. However, due to variations in the DM contents of *A. amnicola* (229 - 288 g/kg DM) and oaten chaff (906 - 922 g/kg DM) over the study period, the total amount of DM

offered varied (Table 7.1), resulting in differences (P<0.05) in DMI between the experimental diets. The only feed refusals occurred when sheep were offered the diet containing the highest level of inclusion (836 g/kg DM) of *A. amnicola*.

7.3.2 Proximate composition and mineral contents

The proximate composition of the experimental diets is presented in Table 7.2. With increasing inclusion of *A. amnicola* in the diet OM, NDF and ADF contents decreased, and CP content increased. Mineral contents of the diets (Table 7.3) increased with increasing inclusion of *A. amnicola*.

Table 7.2: Proximate composition (g/kg DM) of experimental diets and pure *Atriplex amnicola*.

***Atriplex amnicola* inclusion level (g/kg DM)**	**OM**	**CP**	**NDF**	**ADF**	**ADL**
0	878	46	650	378	58
161	839	62	624	375	76
362	818	79	608	373	71
496	831	88	600	331	55
650	782	96	546	323	67
836	758	119	541	334	87
1000[†]	741	129	516	311	79

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, ccid detergent fibre; ADL, acid detergent lignin.
[†], *A. amnicola* only (not included in the *in vivo* feeding trial).

7.3.3 In vitro gas production and metabolisable energy content

As shown in Table 7.4, both 24 h *in vitro* GP and ME content were highest when the diet contained no *A. amnicola* and declined (P<0.05) with increasing inclusion of *A. amnicola* in the diet. The lowest values obtained were from pure *A. amnicola* (which was not included in the feeding trial).

A comparison of the ME values of the experimental diets, predicted from both GP technique (*in vitro* ME) or *in vivo* DMD, is presented in Table 7.4. There was considerable variation in the results and the differences between the values were inconsistent. When based on *in vivo* DMD, the low ME content in the pure oaten chaff diet increased (P<0.05) with inclusion of *A. amnicola* and decreased (P<0.05) when *A. amnicola* was included at 836 g/kg DM level. The *in vitro* ME predicted from GP decreased (P<0.05) with increasing inclusion of *A. amnicola* in the diet.

Table 7.3: Mineral composition of the experimental diets and pure *Atriplex. amnicola*.

	***Atriplex amnicola* inclusion level (g/kg DM)**						
	0†	**161**	**362**	**496**	**650**	**836**	**1000**††
Macro minerals (g/kg DM)							
Na	0.75	7.15	12.37	20.38	30.17	38.47	44.87
Ca	2.36	2.84	3.7	5.62	6.18	8.74	9.32
P	1.18	1.68	2.01	2.44	2.99	3.52	3.91
Mg	1.39	2.63	3.7	5.52	7.36	9.06	10.39
K	6.75	12.62	14.06	11.78	16.84	20.14	21.54
S	1.07	1.47	2.01	2.76	3.84	4.80	5.31
Microminerals (mg/kg DM)							
Cu	2.2	3.8	4.1	3.4	4.3	5.1	5.4
Mn	58	52	80	138	117	192	199
Zn	8	14	19	24	28	38	42

†, Oaten chaff only.
††, *A. amnicola* only (not included in the feeding trial).
DM, dry matter.

Table 7.4: In vitro 24 h gas production and ME (mean ± SE) of the experimental diets and pure Atriplex amnicola

Atriplex amnicola inclusion level (g/kg DM)	*In vitro* 24 h GP (mL/0.2g DM)	*In vitro* ME (MJ/kg DM)	ME (MJ/kg DM)*
0†	45.3±2.29[a]	8.6±0.31[a]	6.9±0.18[b]
161	40.5±0.73[b]	8.1±0.10[b]	7.7±0.13[a]
362	37.1±0.39[bc]	7.7±0.05[bc]	8.0±0.15[a]
496	39.9±0.73[b]	8.1±0.10[b]	8.1±0.21[a]
650	35.2±0.15[c]	7.5±0.02[c]	7.6±0.15[a]
836	26.1±0.98[d]	6.4±0.13[c]	6.7±0.25[b]
1000††	24.4±1.70[d]	6.3±0.23[d]	-

Means within a column followed by different superscripts are significantly different ($P<0.05$).
*, Calculated using *in vivo* DM digestibility percentage.
†, Oaten chaff only.
††, *A. amnicola* only (not included in the feeding trial).

7.3.4 Apparent nutrient digestibility

The apparent digestibility of nutrients of the experimental diets is presented in Table 7.5. The apparent digestibilities of DM, OM, CP, NDF and ADF of oaten chaff were 522, 544, 288, 462 and 434 g/kg DM, respectively. I. With increasing inclusion of *A. amnicola*, the apparent digestibilities of DM, OM, and NDF increased (P<0.05) and reached the highest when the inclusion level was 496 g/kg DM of the total diet. However, further increase in the level of inclusion reduced (P<0.05) the apparent digestibility of these nutrients. Apparent digestibility of ADF also initially increased (P<0.05), reaching the highest at the 362 g/kg DM inclusion level, but declined thereafter. However, the apparent digestibility of CP steadily increased (P<0.05) up until the highest inclusion level of *A. amnicola* studied (836 g/kg DM). The highest apparent digestibilities for DM, OM, CP, NDF and ADF were achieved at inclusion levels of 593, 599, 677, 519 and 471 g/kg DM, respectively.

7.4 Discussion

7.4.1 Feed intake

Restricted feeding was practiced in the experiment. For five of the experimental diets, all of the offered feed was consumed; however, this was not the case when the sheep were offered the 836 g/kg DM *A. amnicola* diet. The average DMI of this ration was 885 g/d which equated to DM and OM intakes of 15.7 g/kg $W^{0.75}$ and 40.2 g/kg $W^{0.75}$, respectively. This DMI was lower than the recommended DMI for sheep (NRC, 2007). On this basis, it is recommended that the feeding of *A. amnicola* be restricted to less than 840 g/kg DM of the total diet to overcome any negative effects on DMI.

7.4.2 Nutrient and mineral contents

Grass species such as perennial ryegrass, grown in the warm, temperate climate of Australia have a relatively high ME (10.6 MJ/kg DM) (Fulkerson et al. (2007), and in comparison, *A. amnicola* was high in terms of CP (Table 7.2), but limited in terms of its ME (Table 7.4). The poor energy value of *A. amnicola* was evidenced by the reduction in GP (and thus *in vitro* ME contents) with increasing inclusion (of *A. amnicola*) in the diet. The decrease in ME with increasing level of *A. amnicola* could be due to the poor digestibility of available fibre because of low retention time, poor absorption of SCFA (Hemsley et al., 1975) and low energy utilisation of the browse due to an increase in energy expenditure related to mineral metabolism in the rumen (Arieli et al., 1989).

Table 7.5: Effect of increasing inclusion level of *Atriplex amnicola* on apparent digestibility (mean ± SE) of nutrients.

***Atriplex amnicola* inclusion level (g/kg DM)**	**DMD (g/kg DM)**	**OMD (g/kg DM)**	**CPD (g/kg DM)**	**NDFD (g/kg DM)**	**ADFD (g/kg DM)**
0	522±10.8^{b*†}	544±11.4^{c}	288±18.9^{d}	462±14.5^{bc}	434±16.4^{ab}
161	573±7.6^{a}	587±6.7^{ab}	519±16.6^{c}	502±8.3^{ab}	468±9.6^{a}
362	588±8.8^{a}	586±9.6^{ab}	617±6.3^{b}	508±13.4^{a}	471±15.0^{a}
496	593±12.4^{a}	599±11.9^{a}	642±10.7^{ab}	519±17.6^{a}	441±18.9^{a}
650	567±8.6a	561±10.5^{bc}	653±6.5^{ab}	448±15.1^{c}	387±16.9^{b}
836	510±14.8^{b}	489±12.2^{d}	677±11.3^{a}	383±13.5^{d}	314±21.3^{c}

DM, dry matter; DMD, dry matter digestibility; OMD, organic matter digestibility; CPD, crude protein digestibility; NDFD, neutral detergent fibre digestibility; ADFD, acid detergent fibre digestibility.
Means within a column followed by different superscripts are significantly different ($P<0.05$).

The method used to predict ME content impacted on results, raising the issue as to the most appropriate means of determining the ME content of halophyte species. *In vitro* ME was calculated based on the amount of GP in 24 h from feed samples incubated in the gas system, which has been shown to be closely correlated with digestibility of OM *in vivo* (Getachew et al., 1998). The gas produced consists of CO_2 and methane (CH_4) produced directly as a result of the fermentation of carbohydrates and also CO_2 produced indirectly as a result of the buffering of SCFA (CO_2 released from the bicarbonate buffer as a result of the production of SCFA). When ME was predicted on the basis of percentage DMD, increasing the inclusion of *A. amnicola* in the total ration generally resulted in an increase in apparent ME content, an illogical result in light of the decreased GP. Furthermore, previous studies (Franklin-McEvoy et al., 2007; Pearce et al., 2008) have demonstrated an improvement of sheep BW by supplementing *A. nummularia* with barley grain indicating insufficient energy in diets containing high levels of Atriplex species.

Increasing the level of inclusion of *A. amnicola* in the ration increased CP content. The CP content of *A. amnicola* tends to vary seasonally and depending on location. In this study the CP content of new-growth forage was intermediate to that (107 - 168 g/kg DM) reported by other researchers (El-Hyatemy et al., 1993; Atiq-ur-Rehman et al., 1999; Tiong et al., 2004; Franklin-McEnvoy et al., 2007), whilst the NDF and ADF contents were considerably higher than values (209 - 477 g/kg DM and 164 - 180 g/kg DM for NDF and ADF, respectively) reported by other researchers (Norman et al., 2004a; Tiong et al., 2004; Franklin-McEvoy et al., 2007). The higher NDF and ADF values may be due to the inclusion of small twigs in the forage material in this study, whereas leaves only were analysed in the studies by the other researchers. The ADL content was similar to that reported by Chriyaa et al. (1997a; 1997b) for *A. nummularia*. The differences in

the nutrient content of *A. amnicola* between the present and past studies were likely to be due to variations in plant genotype and climatic conditions.

High content of ash is a feature of halophytic shrub species grown in saline environments (Tiong et al., 2004) and the salt content of Atriplex leaves may be as high as 300 g/kg DM (Masters et al., 2001). The ash content of the *A. amnicola* forage (leaves and small twigs) used in this study was 259 g/kg DM, which is similar to that (242 - 281 g/kg DM) reported in other studies (El-Hyatemy et al., 1993; Norman et al., 2004b; Tiong et al., 2004).

The mineral composition of *A. amnicola* is of concern among researchers, because of the capability of halophytic species to accumulate salts in large amounts in vegetative parts (Norman et al., 2004a). The contents of Ca, Mg, K, S and Mn recorded in this study were comparable with the range of values reported (7.0 - 8.5, 10.0 - 13.1, 24.6 - 26.7, 5.6 and 142.2 - 288.5 g/kg DM, respectively) for the species in spring/winter samples (El-Hyatemy et al., 1993; Atiq-ur-Rehman et al., 1999; Islam and Adams, 2000; Norman et al., 2004b). The content of Na was lower, P was intermediate, and Zn was higher than the range of reported values in previous studies (49.0 - 70.40, 1.3 - 11.9, and 18.76 - 21.67 g/kg DM, respectively) (El-Hyatemy et al., 1993; Atiq-ur-Rehman et al., 1999; Islam and Adams, 2000; Norman et al., 2004b). For sheep, the minimum requirements of Na, Ca, P, Mg, K and S are 0.9, 2.0, 1.6, 1.2, 5.0 and 1.4 g/kg DM and Cu, Mn and Zn are 7, 20 and 20 mg/kg DM, respectively (NRC, 1985). Even at the lowest *A. amnicola* inclusion level (161 g/kg DM) Na, P and S were adequate to meet their minimum requirements. At the 496 g/kg DM inclusion level the diet was adequate in all minerals studied, except for Cu.

7.4.3 Effect on digestibility of increasing inclusion level of A. amnicola

Increasing the inclusion level of *A. amnicola* in the diet resulted in a sharp increase in CPD. Including *A. amnicola* in the diet up to 496 g/kg DM resulted in a 13.6% increase in DMD and a 10.1% increase in OMD. Riaz et al. (2003) also improved the DMD and OMD (in Teddy goats) of poor quality roughage diet (60% *Triticum aestivum* straw and 40% *Trifolium alexandrinum*) by 50% substitution with *A. amnicola*. The reduction in digestibility observed when the inclusion level of *A. amnicola* exceeded 496 g/kg DM could be due to a number of reasons. Although the browse was relatively high in CP, its energy content was low. Much of the N in *A. amnicola* (Masters et al., 2001) as well as in *Atriplex dimorphostegia* (Riasi et al., 2008) is non proteinous N. Al-Masri et al. (2010) reported high content of buffer-soluble non proteinous N in another saltbush forage species (*Kochia indica*). Some of the N would be lost without being effectively used for microbial protein synthesis due to the lack of available energy. Mayberry et al. (2006) reported Merino sheep fed saltbush produced significantly more CH_4 per unit DOM intake compared to those fed pellets consisting of wheaten chaff, barley, oats and lupins or fed pellets with salt. Further, the OMD of the saltbush was low (48%) compared to other diets (58-66%). Thus, energy lost as CH_4 may have further reduced energy available for ruminal microbes at high *A. amnicola* inclusion levels. The retention time of digesta in the rumen would be low due to the high ash content, particularly Na. Although water intake was not measured, it was noticed that sheep drunk more water when fed on the rations with high inclusion levels of *A. amnicola*. Atriplex consumption by free-grazing, stall-feeding or supplementary feeding is generally associated with high consumption of water (Abou El Nasr et al., 1996; Alicata et al., 2002; Pearce et al., 2008). The recommended S level and N:S ratio for sheep diets are 1.4-2.6 g/kg DM and 10:1, respectively while the maximum tolerable level of S is 4 g/kg DM (NRC, 1985). The

amount of S recorded at 836 and 1000 g/kg DM *A. amnicola* inclusion rates (Table 7.3) were slightly greater than the maximum tolerable level while the N: S ratio of these diets (3.97: 1 and 3.91, respectively) were below the recommended ratio. Therefore, the higher S content of the experimental diet containing 836 g/kg DM *A. amnicola* may have led to lower the N:S ratio resulting in low rumen motility and ultimately low digestibility (NRC, 1985) of the diet.

7.5 Conclusions

Including *A. amnicola* at a level of 496 g/kg DM of the total diet resulted in the best N to energy ratio for fermentation of cell wall fibre in sheep. Using this knowledge, the next logical progression is the establishment of appropriate grazing systems to encourage sheep to select *A. amnicola* at this level.

Chapter 8: Faecal indices predict organic matter digestibility, short chain fatty acid production and metabolisable energy content of browse-containing sheep diets

8.1 Introduction

Determination of the nutritive value of the diet of grazing ruminants is one of the basic problems still confronting grazing scientists and managers. The nutritive value of browse-containing diets is largely affected by the PPC of tannins that are commonly found in browse species. The concentrations of tannins vary widely and is largely unpredictable (Makkar, 2003a). Condensed tannins bind to fibre and protein in the ruminant digestive tract (Degan et al., 1995; Makkar et al., 1995), increasing the excretion of faecal N and fibre (Kaitho et al., 1998a; Ben Salem et al., 2005; Krebs et al., 2007). Browse species high in tannins also tend to be high in lignin (Gasmi-Boubakker et al., 2005). Digestibility of lignin in the ruminant digestive tract is extremely low (Degen et al., 1995; McDonald et al., 2002). Therefore, faecal fibre, lignin and N may have the potential to predict the level of phenolics and tannins in ruminant diets. In early studies, Vera (1973) and Hodgman et al. (1996) showed the potential of faecal fibre fractions to predict digestibility and energy contents of ruminant diets. Faecal N has been found to be closely associated with dietary N (Holecheck et al., 1982a; Mubanga et al., 1985), OMD (Boval et al., 2003) and ME content (Kamler and Homolka, 2005) of typical (grass/legume) ruminant diets. These associations were based on pair-wise correlation and simple linear regression analysis with faecal N content as the independent variable. However, when using such analyses, faecal N was suggested to be less useful for predicting dietary N in forages high in soluble phenolics and tannins (Nastis and Malecheck, 1981; Mould and Robins, 1981). Also, faecal N may be a poor predictor of digestibility when diets are high in browse, which can have both high lignin and N contents (Holecheck et al., 1982a). However, Holloway et al. (1981) reported a substantial

improvement of the predictability of forage digestibility (in steers) when other faecal components were also included as independent variables by use of a stepwise multiple regression procedure.

The objective of this study was to investigate the potential of faecal NDF, ADF, ADL, ash and N contents to predict CP, TP, TT and ME contents, OMD and SCFA production of browse containing diets fed to sheep.

8.2 Materials and methods

Data derived from the feeding trials reported in Chapters 5, 6 and 7 (and hereafter referred to as Experiments 1, 2 and 3) were used to develop predictive regression models. A fourth feeding trial was conducted to derive data to validate the predictive models.

8.2.1 Experimental diets and chemical analysis

Six levels of *A. saligna* (0, 254, 479, 686, 845, 1000 g/kg DM), six levels of *C. palmensis* (0, 176, 352, 524, 722, 1000 g/kg DM) and six levels of *A. amnicola* (0, 161, 362, 496, 650, 836 g/kg DM) were fed to sheep, as reported in Table 5.1, Table 6.1 and Table 7.1 in Chapter, 5, Chapter 6 and Chapter 7, respectively.

The validation feeding trial involved feeding 11 rations, consisting of six levels of *A. saligna* (359, 445, 532, 659, 742, 843 g/kg DM) and five levels of *C. palmensis* (391, 524, 597, 670, 777 g/kg DM). *Atriplex amnicola* was not included due to insufficient supply. Oaten chaff was used to balance the DM contents of all experimental diets. The experimental diets were fed to six replicate sheep for a 7-day adaptation period followed by 5-day collection period.

Representative samples (500 g) from the experimental diets were collected prior to feeding the daily ration on every day of the faecal collection period. A representative sample (15%) of faeces void by each sheep was also collected every day during the faecal collection period. These samples were stored frozen at -18^{o}C. Upon thawing, composite samples of feed and faeces were made up to represent each sheep and collection period. Proximate composition of the composite feed and faecal samples was analysed. The feed samples were assessed for TP and TT contents as well as 24 h *in vitro* GP. The *in vitro* OMD, SCFA production and ME contents were estimated. Procedures pertaining to conducting of feeding trials, sampling, sample preparation, analysis of chemical composition and the *in vitro* gas fermentation technique were as previously described in Chapter 3.

8.2.2 Predictive regression models and validation

A summary of the chemical composition and digestibility of the diets used for each of the feeding experiments is present in Table 8.1. As indicated in Table 8.1, a total of 108 paired feed and faecal samples from feeding Experiments 1 (n=36), 2 (n=36) and 3 (n=36) were used to establish the predictive regression models. Total phenolics and TT contents and *in vitro* OMD, SCFA production and ME content of feed and proximate composition of faecal matter derived from the analysis of feed and respective faecal samples collected from experiments 1, 2 and 3 were pooled. Pearson correlation coefficients (*r*) among feed and faecal variables were estimated as described in Chapter 3. The predictive regression models of dietary CP, TP, TT, OMD, SCFA, and ME were developed from faecal indices by forward stepwise regression procedure. Faecal N, ash, ADL/NDF and ADL/ADF were the specified independent variables. The stepwise regression procedure adds independent variables one by one to the model and proceeds towards increasing of the precision. The model was specified to retain the coefficient of independent variables significant at $P<0.05$ level.

Therefore, the final best-fit multiple regression model was the most precise (best fit) model with the highest R^2 and the lowest residual standard deviation (RSD).

A summary of chemical composition and digestibility of the diets used for the fourth feeding trial is presented in Table 8.2. Accuracy (validation) of the best-fit predictive regression models was examined using the data obtained from the same experiment. The nutritional attributes of the diets included in the fourth feeding trial were predicted from the respective faecal composition using the best-fit predictive model. The predicted dietary qualities were then regressed against measured dietary qualities, and the statistics of the regression were considered to validate the best-fit regression model. The intercept, slope and R^2 of validation regression will be equals to zero, 1 and 1, respectively in case of perfect predictive models.

8.3 Results

The correlation coefficients between TT content and 24 h *in vitro* GP, SCFA production, OMD, ME content (of the diet) were negative ($r>-0.72$, $P<0.0001$). There were strong positive correlations between dietary TP and TT contents with faecal N, NDF, ADF and ADL contents ($r>0.82$, $P<0.0001$). *In vitro* 24 h GP, OMD, SCFA and ME content of the diets were negatively correlated to faecal N, ADF and ADL contents ($r>-0.69$, $P<0.0001$).

Multiple regression models predicting dietary attributes from faecal indices on a DM basis are presented in Table 8.3. The only significant model that predicted dietary CP content also had a very low R^2. The best-fit regression models predicting dietary TT, TP and ME contents, OMD and SCFA from faecal N, ash and ADL/NDF reported higher R^2 and low RSD. Coefficients of independent variables of these models were also significant at $P<0.05$. Similar results were found in the regression models when predicting feed properties from faecal indices on an OM basis (data not presented).

Table 8.1: Chemical composition and digestibility of the diets from in the feeding experiments (1, 2, 3) used to develop the predictive regression models.

	Feed				Faecal			
	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Experiment 1								
NDF (g/kg DM)[a]	600	14.7	555	635	830	43.2	734	950
ADF (g/kg DM)[a]	405	38.2	341	482	603	82.1	443	717
ADL (g/kg DM)[a]	104	42.9	39	191	276	102.7	90	425
CP (g/kg DM)[a]	83	22.1	48	123	113	31.2	63	172
Ash (g/kg DM)[a]	55	8.7	36	73	79	13.8	60	118
TP (g/kg DM)[a]	30.1	14.12	8.2	53.6				
TT (g/kg DM)[a]	21.9	13.35	1.8	44.7				
OMD (g/kg DM)[a]	467	83.3	301	628				
ME (MJ/kg DM)	6.91	1.30	9.41	4.36				
SCFA (mmol/40 mL)	0.68	0.25	1.15	0.23				
Experiment 2								
NDF (g/kg DM)[a]	631	15.2	608	651	763	25.3	726	841
ADF (g/kg DM)[a]	394	10.2	380	407	494	32.6	446	583
ADL (g/kg DM)[a]	76	15.2	54	104	130	27.3	83	185
CP (g/kg DM)[a]	89	24.3	52	130	84	7.0	74	103
Ash (g/kg DM)[a]	53	21.9	25	85	82	24.9	34	122
TP (g/kg DM)[b]	16.7	5.95	10.3	24.2				
TT (g/kg DM)[b]	4.8	1.99	2.9	7.9				
OMD (g/kg DM)[a]	590	21.3	560	619				
ME (MJ/kg DM)	8.77	0.31	9.24	8.32				
SCFA (mmol/40 mL)	1.00	0.05	1.08	0.93				

Table 8.1: *Continued*

	Feed				Faecal			
	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Experiment 3								
NDF (g/kg DM)[a]	595	39.9	541	650	715	21.2	671	742
ADF (g/kg DM)[a]	352	23.5	323	378	463	15.3	432	490
ADL (g/kg DM)[a]	69	10.9	55	87	138	17.2	92	155
CP (g/kg DM)[a]	82	23.9	46	119	74	4.9	63	81
Ash (g/kg DM)[a]	124	39.6	56	180	131	17.8	103	177
TP (g/kg DM)[a]	7.4	2.16	4.3	10.4				
TT (g/kg DM)[a]	1.7	0.39	1.0	2.3				
OMD (g/kg DM)[a]	526	41.2	446	576				
ME (MJ/kg DM)	7.75	0.69	8.63	6.43				
SCFA (mmol/40 mL)	0.83	0.14	1.02	0.56				

NDF, Neutral detergent fibre; ADF, Acid detergent fibre; ADL, Acid detergent lignin; CP, Crude protein; TP, Total phenolics (in tannin acid equivalent); TT, Total tannin (in tannin acid equivalent) ; OMD, Organic matter digestibility; SD, Standard deviation; Max, Maximum; Min, Minimum.

[a], n=36; [b], n=30.

Table 8.2: Chemical composition and digestibility results from the feeding trial used to validate the predictive regression models (n=66).

Variable	**Feed**				**Faecal**			
	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
NDF (g/kg DM)	559	20.9	512	586	709	28.2	640	760
ADF (g/kg DM)	391	28.6	343	458	562	77.0	458	704
ADL (g/kg DM)	100	25.2	71	166	251	83.8	138	411
CP (g/kg DM)	77	7.2	64	92	107	23.9	72	164
Ash (g/kg DM)	41	11.2	23	61	67	11.7	45	92
TP (g/kg DM)	33.4	14.79	13.8	75.0				
TT (g/kg DM)	14.9	13.98	1.5	54.4				
OMD (g/kg DM)	526	81.1	351	639				
ME (MJ/kg DM)	7.81	1.25	9.54	5.14				
SCFA (mmol/40 mL)	0.83	0.22	1.12	0.36				

NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; TP, total phenolics (in tannin acid equivalent); TT, total tannin (in tannin acid equivalent); OMD, organic matter digestibility; SD, standard deviation; Max, maximum; Min, minimum.

Table 8.3: Multiple regression models of feed properties on faecal composition.

Feed property	Model No.	Predictive regression model (P<0.05)	Adjusted R^2	RSD
CP	1	CP = 58.82729 + 107.83681 (*f*ADL/*f*NDF)	0.21	2.15
TP	2	TP = – 23.90381 + 2.89754 (*f*N)	0.81	0.57
	3	TP = – 2.92108 + 2.34357 (*f*N) – 0.13105 (*f*Ash)	0.88	0.46
	4[a]	TP = 2.2036 + 1.55408 (*f*N) – 0.14331 (*f*Ash) + 32.22348 (*f*ADL/*f*NDF)	0.89	0.45
TT	5	TT = – 29.54565 + 2.70745 (*f*N)	0.85	0.48
	6	TT = – 25.38584 + 1.73693 (*f*N) + 42.39481 (*f*ADL/*f*NDF)	0.86	0.45
	7[a]	TT = – 17.43746 + 1.36504 (*f*N) + 50.37654 (*f*ADL/*f*NDF) – 0.04475 (*f*Ash)	0.87	0.44
OMD	8	OMD = 661.8729 – 596.24096 (*f*ADL/*f*NDF)	0.68	4.15
	9	OMD = 765.41012 – 702.81681 (*f*ADL/*f*NDF) – 0.7957 (*f*Ash)	0.77	3.51
	10[a]	OMD = 814.55657 – 514.4869 (*f*ADL/*f*NDF) – 0.93196 (*f*Ash) – 5.4971 (*f*N)	0.78	3.45
SCFA	11	SCFA = 1.23177 – 1.72276 (*f*ADL/*f*NDF)	0.68	0.01
	12	SCFA = 1.52063 – 0.00222 (*f*Ash) – 2.0201 (*f*ADL/*f*NDF)	0.77	0.01
	13[a]	SCFA = 1.64152 – 0.00256 (*f*Ash) – 1.55683 (*f*ADL/*f*NDF) – 0.01352 (*f*N)	0.78	0.01
ME	14	ME = 9.88937 – 9.19426 (*f*ADL /*f*NDF)	0.66	0.07
	15	ME = 11.61401 – 10.96951 (*f*ADL/*f*NDF) – 0.01325 (*f*Ash)	0.77	0.05
	16[a]	ME = 12.32323 – 8.25181 (*f*ADL/*f*NDF) – 0.01522 (*f*Ash) – 0.07933 (*f*N)	0.78	0.05

CP, crude protein (g/kg DM); TP, total phenolics (g/kg DM, in tannin acid equivalent); TT, total tannin (g/kg DM, in tannin acid equivalent); OMD, organic matter digestibility (g/kg DM); SCFA, short chain fatty acid production (mmol/40 mL); ME, metabolisable energy (MJ/kg DM); *f*Ash, Faecal ash (g/kg DM); *f*N, faecal N (g/kg DM); *f*ADL, faecal acid detergent lignin (g/kg DM); *f*NDF, faecal neutral detergent fibre (g/kg DM); RSD, residual standard deviation.
[a], Selected best-fit regression model for validation (Highest R^2, lowest RSD, coefficients of estimates significant at $P<0.05$).

The statistics of the regression between predicted and measured dietary qualities of the validation experiment (experiment 4) are summarised in Table 8.4. All regressions were highly significant (P<0.0001). The regressions between measured and predicted TP and TT contents had positive intercepts (P<0.05) and low R^2. Slopes of these regressions were much lower than 1.0. Regressions between measured and predicted OMD, SCFA and ME contents did not have significant intercepts. Both slope and R^2 of these regressions were close to 1.0.

Table 8.4: Statistics of regression between observed (X) and predicted[a] (Y) feed properties of the validation experiment.

	Intercept	**Slope**	**Adjusted R^2**	**RSD**	**CV**
TP (g/kg DM)	13.90*	0.50*	0.62	5.74	18.75
TT (g/kg DM)	11.62*	0.61*	0.65	6.18	29.84
OMD (g/kg DM)	12.64	0.89*	0.82	32.86	6.89
SCFA (mmol/ 40 mL)	-0.08	0.94*	0.84	0.09	12.99
ME (MJ/ kg DM)	0.03	0.90*	0.83	0.51	7.23

TP, total phenolics (in tannin acid equivalent); TT, total tannin (in tannin acid equivalent); OMD, organic matter digestibility; SCFA, short chain fatty acid production; ME, metabolisable energy; RSD, residual standard deviation; CV, coefficient of variation. All regressions were significant at $P<0.0001$.
[a], Feed properties were predicted from the respective best-fit regression model (Table 8.3) derived in stepwise regression procedure.
*, Estimates were significant at $P<0.0001$.

8.4 Discussion

8.4.1 Total phenolics and tannin contents

Feeding sheep *Acacia cyanophylla*, which is high in phenolics and tannins, increased excretion of faecal N (Ben Salem et al., 2005d). Similar results occurred with increasing supplementation of *Leucaena leucocephala* and *L. pallida*, which are high in soluble CT (Kaitho et al., 1998a). Krebs et al. (2007) reported a negative N balance due to the presence of CT in sheep fed *A. saligna*. Extractable tannins in *A. saligna* bind to dietary or endogenous proteins in the digestive tract and these tannin protein complexes are excreted in the fibre and lignin fractions of sheep faecal matter (Degen et al., 1995;

Makkar et al., 1995). Sheep fed non-conventional feed containing greater concentrations of phenolics and tannins produced higher volumes of faeces which contained significantly higher concentration of N but they produced urine containing low concentration of N (Mahgoub et al., 2008). More recently, Caprarulo et al. (2020) reported that faecal matter contained greater content of N when the diets included chestnut and quebracho tannins. Higher level of N in the faeces is a characteristic of feeding tannins as they bind to proteins in the digestive system (Longstaff and McNab, 1991; Walton et al., 2001; Robins and Brooker, 2005). Tannins also form complexes with carbohydrates and minerals (Robins and Brooker, 2005). These findings agree with the positive correlations found between feed TP and TT with faecal N, NDF, ADF and ADL contents. As presented in Table 8.3, faecal N content alone explained a large proportion of the variation of TP (model 2, R^2=0.81) and TT (model 5, R^2=0.85) contents of the diet. An additional 7% of the variation of TP content was explained by addition of faecal ash content to the model (model 3, R^2=0.88). However, inclusion of variables that are related to faecal fibre fractions brought only a marginal improvement to the regression model, suggesting that most of the phenolics make complexes with protein fractions in the digestive tract and are then excreted in faeces. Thus, faecal N alone can be used to predict the TP and especially the TT contents of browse-containing sheep diets.

8.4.2 Crude protein content

None of the investigated faecal properties had a strong correlation ($r<0.43$) with dietary CP content. Further, the only significant regression model explained only 21% of the variation of the dietary CP content (model 1, R^2=0.21). Mubanga et al. (1985) also reported poor correlations of dietary CP with faecal NDF, ADF and ADL contents. Close association of faecal N and dietary CP contents has been reported in wild ruminants

(Mould and Robbins, 1981; Mubanga et al., 1985; Kamler and Homolka, 2005) and domesticated, grazing cattle (Holechek et al., 1982). However, such association prevailed only when the diets had low levels of soluble phenolics or tannins, as they had protein complexing capacity. Data reported by Mould and Robins (1981) indicated that faecal N content was considerably elevated when species high in soluble phenolics comprised over 25% of the diet. *Acacia saligna* (Degen et al., 2000; Getachew et al., 2002; Krebs et al., 2007) and *C. palmensis* (Kaitho et al., 1998a; El hassan et al., 2000) are known to have high and moderate levels of phenolics, respectively. These browse species were included at levels of at least 250 g/kg DM in half of the experimental diets used to develop the predictive regression models, which could be a reason for the absence of the casual positive correlation between dietary and faecal CP contents in the present study. Additionally, a large proportion of faecal protein is of bacterial (Virtanen, 1966) and metabolic (Blaxter and Mitchell, 1948) origins. Excretion of metabolic protein is a constant per unit OM intake in sheep (Jarrige, 1965; Mason, 1969). Therefore, poor predictability of dietary CP from faecal N might be attributed to the presence of both the phenolics and tannin of the browse species used, as well as the metabolic and bacterial proteins excreted with faeces. Hobbs (1987), who re-examined the data of Sinclair et al. (1982) and Lesli and Starkey (1985), concluded that faecal N does not accurately provide reliable quantitative prediction of the small differences of dietary CP in herbivores and results of the present study confirmed this.

8.4.3 Organic matter digestibility, short chain fatty acid production and metabolisable energy contents

Variability in the chemical composition among the experimental diets (Table 8.1) was achieved by changing the level of inclusion of browse in the diets. The negative

correlation between faecal N and OMD (r=-0.72, P<0.0001) was contrary to the correlation (r=+0.74, P=0.01) reported by Holechek et al. (1982a) in cattle. The present result was presumably due to the adverse antinutritive effects of tannins and lignin, irrespective of the beneficial effect of higher CP content on digestibility with increasing level of browse in the diet. As Vera (1973) and Holloway et al. (1981) also observed, negative relationships between digestibility and faecal fibre fractions were possibly due to higher concentrations of these fractions in forages of lower digestibility, resulting in increased concentration in the corresponding faeces. Predictive regression models of OMD, SCFA and ME all show similar trends. Faecal ADL/NDF explained a large proportion (R^2=0.66 to 0.68) of the variation of OMD, SCFA and ME of diets. There is neither any endogenous secretion of fibre fractions (NDF, ADF, ADL) into the digestive tract nor any synthesis of them by microbes in the gastrointestinal tract. Van Soest (1992) also pointed out that plant cell wall contents are not exposed to microbial and metabolic contamination. Thus, the total amounts of fibre fractions present in faecal matter can only originate from the diet. The potential of faecal fibre fractions to predict digestibility (Vera, 1973) and digestible energy contents (Short and Remmenga, 1965; Hodgman et al., 1996) has been reported previously. The predictive regression models used by these researchers had only one independent variable (simple linear regression). Inclusion of faecal ash content as an independent variable resulted in substantial improvement of the precision (R^2=0.77) of OMD (model 9), SCFA (model 12) and ME (model 15) predictive regression models. The potential of faecal N to predict digestibility (Mubanga et al., 1985; Boval et al., 2003; Kneebone and Dryden, 2015) and ME (Kamler and Homolka, 2005) have been reported. The presence of a substantial proportion of faecal N with faecal fibre fractions of sheep fed high tannin diets (Degen et al., 1995; Makkar et al.,

1995) could be the reason for the slight improvement (R^2=0.01) of the OMD, SCFA and ME predictive models due to inclusion of faecal N as an independent variable.

8.4.4 Validity of the predictive regression models

Statistics of the regression between measured and predicted variables (Table 8.4) of the validation regression were used to test the validity of the predictive regression models when the sheep were fed different diets. The intercept, slope and R^2 of the validation regression will be equal to zero, 1 and 1, respectively, in case of perfect predictive models. The positive ($P<0.05$) intercept for the validation regression for TP and TT indicated overestimation of the variables by the respective best-fit predictive models (model 4 and 7). However, considerably lower slopes (compared to a perfect prediction) of the predictive models indicated underestimation of the predicted TP and TT values. Such association is undesirable in a predictive equation (Draper and Smith, 1981). Therefore, the predictability of the best-fit models of TP and TT is lower. Data from the *A. amnicola* containing sheep diets were included in the predictive model although the browse was not included in the validation experiment. Reanalysing the predictive models without inclusion of data derived from the *A. amnicola* diets did not affect the regression between measured and predicted values.

8.5 Conclusion

The present data is not adequate to explain the reason for low validity of TP and TT predictive models. An insignificant intercept, and slope close to 1.0, with very high R^2 of validation regressions for OMD, SCFA and ME imply that the predictive models approached perfect prediction. Therefore, the best-fit predictive regression models proposed for OMD (model 10), SCFA production (model 13) and ME (model 16) will

have broader application. Faecal composition (*f*NDF, *f*ADL, *f*Ash, *f*N) provides a means to predict dietary OMD, SCFA production and ME of browse-containing diets fed to sheep.

Chapter 9: Predicting the quality of browse-containing diets fed to sheep using faecal near-infrared reflectance spectroscopy

9.1 Introduction

Predicting nutritive attributes of small ruminant's diets with the help of faecal NIRS (*f*NIRS) is one of the unusual though interesting applications of chemometrics as the spectral measurements are made not on the material of interest but on a derived material (Dixon and Coates, 2009). Faecal NIRS equations that predict CP, *in vitro* DMD and PEG binding tannin of diets containing lucerne hay, concentrate and browse species have been derived for Mediterranean goats in Israel (Landau et al., 2004). Faecal NIRS calibrations developed for cattle (Coates, 2004) have been subsequently used to predict dietary CP and DMD of subtropical grass-legume pastures to establish key determinants of liveweight changes of grazed steers in Queensland (Hill et al., 2009). Each livestock species is unique in its digestive physiology (Huston et al., 1986) and, therefore, *f*NIRS equations derived for one livestock species may not be applicable for another species, essentially due to spectral differences. Separate *f*NIRS equations have been derived to predict CP and *in vivo* DOM of sheep diets containing grass hay, forbs and browse species grown in North America (Li et al., 2007). A *f*NIRS calibration was derived to predict *in vivo* OMD of sheep diets containing a wide range of temperate forages by merging the database of feeding trials performed at the Walloon Agricultural Research Centre in Belgium and at the National Institute of Agricultural Research in France (Decruyenaere et al., 2009). Data mining of past digestibility trials conducted in France, Italy and Israel has resulted in the development of *f*NIRS equations to predict an array of nutritive attributes including CP, OMD and CPD of diets fed to goats (Landau et al., 2008). There are no *f*NIRS calibrations developed to predict chemical or functional nutritive attributes

of browse-containing sheep diets in Australia, and particularly in reference to browse species grown in WA.

Five sheep feeding trials were undertaken involving different inclusion levels of *A. saligna* (Chapter 5), *C. palmensis* (Chapter 6), *A. amnicola* (Chapter 7), *A. nummularia* and *R. eremaea*. The present study examined the potential of using the data and faecal samples collected from these trials to derive *f*NIRS calibrations to predict chemical and functional nutritive attributes of browse-containing sheep diets.

9.2 Materials and methods

Data and faecal samples collected from five digestibility trials were used for the study. Methods of conducting *in vivo* feeding trials, sampling, sample preparation and chemical and *in vitro* gas fermentation assay were as previously described in detail in Chapter 3.

9.2.1 Experimental diets, chemical analysis, in vivo digestibility and in vitro gas fermentation assay

In total, 40 experimental diets consisting of varying levels of fresh *A. saligna*, *C. palmensis*, *A. amnicola*, *A. nummularia* and *R. eremaea* and oaten chaff were fed to individually penned sheep. Details of the experimental diets and experimental designs are presented in Table 9.1. Each diet was fed to six sheep for 7-days for adaptation, followed by a 5-days collection period. During the collection period, feed and faeces samples were collected daily from each sheep and stored in clearly labelled, sealed polythene bags at -18ºC, pending preparation of composite samples. Freeze-dried feed samples were first ground to pass a 1-mm sieve to determine chemical composition and *in vitro* GP and then half of the sample was reground to pass through a 0.5-mm sieve in order to determine TP, TT and PPC of tannin (Makkar, 2003). Faecal samples were dried

to a constant weight at 60ºC in a conventional forced-air oven (AFIA, 2006) and ground to pass through a 1-mm sieve for subsequent chemical and NIRS analyses. Composite samples were made by combining either the feed or faecal samples collected from each sheep during the 5-days collection period. After oven-drying, the composite samples were stored in sealed air-tight containers until chemical analyses were performed.

Dry matter and ash constants of the diet and faecal samples were determined. The total N and total P content of the diet samples was also determined. Diet samples of trials 1 (Chapter 5), 2 (Chapter 6), 3 (Chapter 7) and 4 (Chapter 8) were analysed for TP and TT while those of trials 1 (Chapter 5), 2 (Chapter 6) and 3 (Chapter 7) were analysed for PPC content of tannin. The *in vivo* DMD, OMD and CPD of the diets were calculated from the difference between nutrients consumed and voided in the faeces. The *in vitro* OMD, ME content and SCFA were estimated from the 24 h GP, and CP and ash contents.

Table 9.1: Forages fed and details of experiments designs.

Feeding trial No.	**Forages mixed to formulate experimental diets**	**Experimental design**	**No. of experimental diets**	**N**
1	*Acacia saligna*, oaten chaff	Latin square design	6	36
2	*Chamaecytisus palmensis*, oaten chaff	Latin square design	6	36
3	*Atriplex amnicola*, oaten chaff	Latin square Design	6	36
4	*Acacia saligna, Chamaecytisus palmensis*, oaten chaff	Balanced incomplete block design	11	66
5	*Acacia saligna, Chamaecytisus. palmensis, Atriplex amnicola, Atriplex nummularia, Rhagodia eremaea*, oaten chaff	Balanced incomplete block design	11	66
Total			40	240

N, number of feed and faecal pairs derived.

9.2.2 Calibration and validation of fNIRS equations

Ground faecal samples were re-dried at 60°C for 1 h and left to equilibrate in a desiccator at ambient temperature for 1 h before scanning. Samples were packed into sample cells with a near infrared (NIR) transparent quartz cover glass and scanned between 1100 and 2498 nm in 2 nm increments using a Foss 6500 NIR system (FOSS Pacific Pty Ltd, Sydney, NSW, Australia) with spinning sample compartment attachment. Results of chemical analysis (CP, TP, TT, PPC, P), *in vivo* digestibility studies (DMD, OMD, CPD) and *in vitro* digestibility studies (OMD, ME) were used as reference values. Calibration equations were developed using version 1.04a of WinISI (II) software. The software was provided with the purchase of the NIR instrument, from FOSS Pacific Pty Ltd, North Ryde, New South Wales, Australia. The calibration relies on modified partial least squares (MPLS) procedures (Martens and Naes, 1987). First order derivatives were used in the calibrations, with scatter correction. A global H (GH) factor of 3 was applied to eliminate outliers. GH, the standardised Mahalanobis distance, ascertains the degree of difference of the result in the data set. Those with a GH of 3 or greater are eliminated, to retain the rigor and accuracy of the calibration data set. The precision of calibrations was evaluated by the coefficient of determination (R^2_c) and standard error of calibration (SEC). The predictive ability of calibrations was internally evaluated by standard error of cross-validation (SECV) and standard error of prediction (SEP; Stuth et al., 2003; Landau et al., 2006). The slope of the validation regression (Landau et al., 2006) and the ratio of the SD of the original data to the SECV (RPD; Williams and Sobering, 1993) were used to evaluate accuracy of calibrations. The *f*NIRS equations with R^2_c and RPD greater than 0.80 and 3, respectively were considered as acceptable predictive equations (Williams, 2004).

9.3 Results

Statistics of reference data, calibration and validation of *f*NIRS prediction equations for chemical (CP, TP, TT, PPC, P) and functional (DMD, CPD, OMD, ME) nutritive attributes of browse-containing sheep diets are presented in Table 9.2. The number of reference data and faecal spectra (n) pairs used to derive *f*NIRS calibrations differed among nutritive attributes. The least number of pairs was for PPC calibration (94) and those for TP (159) and TT (153) were less than those used for the other (CP, P, DMD, OMD, ME, SCFA) calibrations (219-226). The R^2_c was greater than 0.80 for all *f*NIRS calibrations. The SEC was close to the respective SECV. Slope of the validation regressions (predicted values against respective reference data) of chemical attributes did not deviate from 1 (0.85-1.07) while that of functional attributes (except for SCFA) deviated from 1 (0.51-0.80). The RPD of *in vivo* DMD and *in vivo* OMD was less than 3 but was greater than 3 for other (CP, TP, TT, PPC, P, *in vivo* CPD, *in vitro* OMD, *in vitro* ME, *in vitro* SCFA) calibrations.

Table 9.2: The *f*NIRS calibration performance of chemical and functional nutritive attributes of browse containing sheep diets.

Attribute	**n**	**Reference data statistics**				**Calibration statistics**		**Validation statistics**					**RPD**
(g/kg DM)		**Mean**	**SD**	**Min**	**Max**	**R^2_C**	**SEC**	**R^2_{CV}**	**SECV**	**SEP**	**Slope**	**Bias**	
Chemical attributes													
CP†	226	89.0	20.53	45.5	130.3	0.96	3.97	0.94	4.91	6.69	0.95	0.87	4.19
TP†	159	24.2	14.96	4.3	75.0	0.96	3.09	0.92	4.34	9.13	0.85	1.28	3.45
TT†	153	11.8	10.77	1.0	54.4	0.93	2.89	0.90	3.44	5.83	1.04	0.44	3.13
PPC†	94	4.9	6.84	0.0	38.7	0.93	1.78	0.90	2.20	4.19	1.07	0.61	3.10
P†	233	1.51	0.679	0.56	3.52	0.98	0.102	0.97	0.115	0.112	1.00	0.01	5.90
Functional attributes													
In vivo DMD†	223	529	53.1	332	638	0.83	21.6	0.79	24.3	40.7	0.74	3.23	2.18
In vivo CPD†	219	455	168.5	65	728	0.95	38.5	0.94	42.7	175.9	0.51	17.32	3.95
In vivo OMD†	223	536	55.3	350	655	0.85	21.6	0.80	25.0	46.6	0.65	5.11	2.21
In vitro OMD†	223	511	72.7	301	639	0.95	15.7	0.93	19.3	67.5	0.57	6.09	3.77
In vitro ME††	223	7.6	1.13	4.4	9.5	0.95	0.24	0.93	0.30	0.98	0.62	0.09	3.80
SCFA†††	224	0.8	0.20	0.2	1.2	0.94	0.05	0.92	0.06	0.12	0.80	-0.02	3.49

*f*NIRS, Faecal near infrared reflectance spectroscopy; CP, Crude protein; TP, Total phenolics (in tannin acid equivalents); TT, Total tannin (in tannin acid equivalents); PPC, Protein precipitation capacity of tannin (in tannin acid equivalents); P, Phosphorus; DMD, Dry matter digestibility; OMD, Organic matter digestibility; CPD, Crude protein digestibility; ME, Metabolisable energy; SCFA, Short chain fatty acid production.
n, Number of reference data and faecal spectra pairs used to derive *f*NIRS calibration; SD, Sanded deviation; Min, Minimum; Max, Maximum; R^2_c, R^2 of calibration; SEC, Stranded error of calibration; R^2_{cv}, R^2 of cross validation; SECV, Stranded error of cross validation; SEP, Stranded error of prediction; RPD, SD/SECV.
†, g/kg DM; ††, MJ/kg DM; †††, mL/0.2g DM.

9.4 Discussion

9.4.1 Quality of reference data for calibrations

Typically, a wide variety of species are fed to sheep over 10 d and faecal samples are collected on the last 2 d in order to derive *f*NIRS calibrations for sheep (Stuth et al., 2003). However, Li et al. (2007: 5 d adaptation followed by 2 d feed and faecal collection period), Landau et al. (2004: 7 d adaptation followed by 3 d feed and faecal collection period), Landau et al. (2005: 5 d adaptation followed by 5 d feed and 2 d faecal collection period) and Landau et al. (2008: 14 d and 15 d adaptation followed by 5 d and 8 d feed and faecal collection period, respectively) have derived successful *f*NIRS calibrations for chemical and functions properties of small ruminant diets. In this study feeding of each experimental diet to six replicate sheep, with 7 d for adaptation followed by 5 d for collection of diet and faecal samples ensured better representation of the nutritive attributes of diets in the reference data and respective *f*NIRS spectra. The diet samples from feeding trial 5 were not assessed for PPC while those of feeding trials 4 and 5 were not assessed for TP and TT contents. Therefore, *f*NIRS calibration for PPC was derived from merged data of feeding trials 1, 2 and 3 while those for TP and TT were derived from merged data of feeding trials 1, 2, 3 and 4. Data gathered from all feeding trials were merged to derive other calibrations (CP, P, DMD, OMD, ME, SCFA). The NIRS calibration data set should be well distributed, representing the range of expected variation in the constituent of interest (Stuth et al., 2003). The statistics of reference data (mean, SD, minimum, maximum) indicates that the present data set was well distributed covering a wide range in the nutritive attributes investigated (Table 9.2). Such variation was accomplished by merging data originating from 40 different experimental diets formulated with two legume (*A. saligna, C. palmensis*), three halophyte (*A. amnicola, A. nummularia, R. eremaea*) and a grass (oaten chaff) species (Table 9.1).

9.4.2 Acceptability of fNIRS calibrations

The R^2_c indicates the linearity (proportion of variability in the reference data accounted for by the regression equation) while the SEC indicates the variability in the difference between predicted and reference values. Acceptable NIRS equations must have R^2_c and RPD greater than 0.80 and 3, respectively (Williams, 2004). The SEC and SEP should be as small as possible and similar (Stuth et al., 2003), the SECV should close to the SEC (Williams, 2004) and the slope of validation regression should not deviate from 1 (Landau et al., 2006).

9.4.3 Calibrations for dietary chemical attributes

Faecal NIRS calibrations developed to estimate dietary chemical attributes had excellent performance. The R^2_c (0.93 - 0.98) and RPD ratio (3.10 - 5.90) were well above the minimum acceptable levels for NIR calibrations (Stuth et al., 2003; Williams, 2004). The slopes of validation regressions close to 1 (0.85 - 1.07) confirmed that the calibrations derived for dietary chemical attributes do not underestimate or overestimate the true values. In addition, the estimates of SEC, SECV and SEP were small, and the difference between SEC and SECV was marginal. A summary of the statistics of *f*NIRS calibrations reported in some previous studies is presented in Table 9.3. From a similar stall-feeding study conducted with sheep, Li et al. (2007) also developed efficient *f*NIRS calibrations to predict the CP content of diets consisting of grass hay, forbs and browse species. Although, the present calibrations for CP had comparable linearity (R^2_c) the calibration had higher variability (SEC) and lower predictive ability (SECV) than the calibration developed by Li et al. (2007). Calibrations with acceptable linearity, variability and predictive ability of CP content have been reported for stall-fed goats (Landau et al., 2004; Landau et al., 2005) and steers (Boval et al., 2004) as well as for grazing goats (Leite and

Stuth, 1995) and steers (Lyons and Stuth, 1992). Landau et al. (2008) also published efficient calibrations in terms of linearity (R^2_c), variability (SEC) and predictability (SECV) for dietary CP content using merged data of the feeding trials conducted in France, Italy and Israel (n=134). In another study, Landau et al. (2005) used an external data set to validate the predictive ability of the *f*NIRS calibration developed to predict dietary CP content of stall-fed goats. Low SEP and slope that did not deviate from 1, confirmed the potential of *f*NIRS calibration to predict CP content in goats' diets. The error and robustness of *f*NIRS calibrations to predict CP concentration of herbivore diets are comparable with those of the calibrations to predict the property directly from the forage NIR spectra (Dixon and Coates, 2009). Faecal NIRS calibrations predicting dietary TP, TT, PPC and P are lacking in the literature. However, an acceptable calibration has been derived for PEG binding tannin of stall-fed Mediterranean goat diets consisting of concentrates and browse species (Landau et al., 2004). Tolleson et al. (2000) also successfully distinguished three levels of tannin content in white-tailed deer (*Odocoileus virginianus*) diets using *f*NIRS. A good prediction for P (R^2_c=0.91, SEP=0.02) in white-tailed deer diets was obtained using representative diets of their natural habitats (Showers, 1997).

Table 9.3: An overview of selected *f*NIRS calibrations reported from past research.

Dietary attribute	Data source	Faecal matter source	R^2_c	SEC	R^2_{cv}	SECV	SEP	Slope	Reference
Chemical attributes									
CP†	Fistulated grazing steers	Non-fistulated grazing cows	0.63, 0.63	0.87, 0.87	0.45, 0.57	1..21, 1.09	-	1.14, 1.23	Lyons and Stuth (1992)
CP†	Fistulated grazing goats	Non-fistulated grazing goats	0.94	1.12	0.94	1.28	-	1.18	Leite and Stuth (1995)
CP†	Stall-fed goats	Stall-fed goats	0.98	0.40	-	0.53	-	-	Landau et al. (2004)
Tannin†*	Stall-fed goats	Stall-fed goats	0.96	0.85	-	1.07	-	-	Landau et al. (2004)
CP††	Stall-fed steers	Stall-fed steers	0.98	0.33	0.95	0.50	-	-	Boval et al. (2004)
CP†	Stall-fed goats	Stall-fed goats	0.98	0.42	-	0.50	1.6, 1.7, 2.2	1.05, 0.98, 0.98	Landau et al. (2005)
CP†	Stall-fed sheep	Stall-fed sheep	0.95, 0.93	1.08, 1.27	-	1.51, 1.35	2.17, 1.65	0.94, 1.01	Li et al. (2007)
CP†	Goat feeding trials	Goat feeding trials	0.94	0.80	0.92	0.90	-	-	Landau et al. (2008)
CP††	Goat feeding trials	Goat feeding trials	0.97	0.80	0.95	1.0	-	-	Landau et al. (2008)

Table 9.3: *Continued*

Dietary attribute	Data source	Faecal matter source	R^2_c	SEC	R^2_{cv}	SECV	SEP	Slope	Reference
Functional attributes									
In vivo DOM[†a]	Fistulated grazing steers	Non-fistulated grazing cows	0.70, 0.70	1.70, 1.70	0.70, 0.71	1.93, 1.88	-	1.03, 0.92	Lyons and Stuth (1992)
In vitro DOM[†b]	Fistulated grazing goats	Non-fistulated grazing goats	0.93	2.02	0.92	2.12	-	0.91	Leite and Stuth (1995)
In vivo OMD[†††c]	Stall-fed steers	Stall-fed steers	0.72	0.02	0.69	0.02	-	-	Boval et al. (2004)
In vitro DDM[†b]	Stall-fed goats	Stall-fed goats	0.98	1.65	-	1.98	-	-	Landau et al. (2004)
In vitro DMD[†b]	Stall-fed goats	Stall-fed goats	0.97	1.71	-	2.14	7.5, 6.4, 6.5	1.25, 1.1, 0.95	Landau et al. (2005)
In vivo DOM[†d]	Stall-fed sheep	Stall-fed sheep	0.80, 0.78	1.51, 1.58	-	2.06, 1.65	2.09, 1.98	0.86, 0.84	Li et al. (2007)
In vivo OMD[†]	Goat feeding trials	Goat feeding trials	0.88	2.1	0.81	2.7	-	-	Landau et al. (2008)
In vivo CPD[†]	Goat feeding trials	Goat feeding trials	0.75	4.2	0.52	5.8	-	-	Landau et al. (2008)

*f*NIRS, faecal near infrared reflectance spectroscopy; CP, C=crude protein; *, polyethylene glycol (PEG) binding tannin; DOM, digestible organic matter; OMD, organic matter digestibility; DDM, digestible dry matter; DMD, dry matter digestibility; DOM, digestible organic matter; [a], *in vitro* OMD corrected to *in vivo* DOM; [b], Tilley and Terry method; [c], Total collection method; [d], *in sacco* DOM corrected to *in vivo* DOM.
[†], g/ 100g DM; [††], g/ 100g OM, [†††], digestible coefficient.

9.4.4 Calibrations for dietary functional attributes

The low RPD (<3) of *in vivo* DMD and OMD calibrations indicated low accuracy due to greater variability in predicted data compared to reference data (Williams, 2004). Low slopes (0.51 - 0.65) of *in vivo* CPD, *in vivo* OMD, *in vitro* OMD and *in vitro* ME calibrations would seriously underestimate the respective predictions. Therefore, *f*NIRS calibrations for these dietary functional attributes would not be accepted. However, the *in vitro* calibrations (OMD, ME, SCFA) were superior in terms of precision (higher R^2_C, lower SEC) and accuracy (RPD>3) to *in vivo* DMD and OMD calibrations. Reference data for *f*NIRS calibrations for DDM and DMD of stall-fed goat diets (Landau et al., 2004 and Landau et al., 2005, respectively), DOM of stall-fed sheep diets (Li et al., 2007) and DOM of grazing cattle and goat diets (Lyons and Stuth, 1992 and Leite and Stuth, 1995, respectively) have also been derived from *in vitro* or *in sacco* digestibility estimates. Indirect methods of digestibility determination either provide conditions of the rumen (*invitro*) or digest the feed samples in the rumen (*in sacco*). Therefore, the digestibility estimated from *in vitro* or *in sacco* methods does not necessarily capture the post-ruminal digestion of the feed. In addition, ruminal fluid collected from fistulated sheep is homogenised before inclusion in the buffer solution for *in vitro* gas fermentation (Makkar, 2003b). Therefore, the digestibility estimates derived from *invitro* and *in sacco* estimates are not subject to variation due to animal or post-ruminal digestion effects. Boval *et al.* (2004) suggested that *in vivo* estimates of digestibility derived from *in vitro* analysis were subjected to appreciable error due to the imperfect correlation between *in vitro* and *in vivo* digestibilities. Further they reported a considerable variation between animals (12%) which contributed to reduce linearity (R^2_c) and increase variability (SEC) of *f*NIRS calibration on *in vivo* OMD of stall-fed steer diets. In the present study, the correlation between *in vivo* and *in vitro* OMD was not perfect (r=0.76, P>0.0001).

Therefore, imperfect correlation between *in vivo* and *in vitro* digestibility measurements and absence of animal effect in *in vitro* estimates could be the reasons for having better statistics of NIRS calibrations developed from reference data originated from *in vitro* methods compared to those originated from *in vivo* methods.

From the study conducted using merged data (n=134) of the goat feeding trials conducted in France, Italy and Israel, Landau et al. (2008) derived *f*NIRS calibrations to predict *in vivo* digestibilities. As was the case in the present study, neither Boval et al. (2004) nor Landau et al. (2008) were able to develop acceptable *f*NIRS calibrations to predict functional properties of diets using reference values derived by *in vivo* methods. The RPD for *in vivo* OMD (2.00, Boval et al., 2004; 2.30, Landau et al., 2008) and *in vivo* CPD (1.43, Landau et al., 2008) were well below the acceptable levels suggested by Williams (2004). Therefore, poor predictability of the present *in vivo* calibrations could be due to *in vivo* estimates incorporated animal variation in this limited dataset, was in agreement with them. The present calibration derived to predict *in vitro* SCFA met the requirements for NIRS calibrations (Stuth et al., 2003; Williams, 2004) and was therefore acceptable. The calibration showed high linearity (R^2_c=0.94), low variability (SEC=0.12) and moderate predictability (SECV=0.06; SEP=0.12, slope=0.80). The reason for considerable deviation of the slope from 1, observed in *in vitro* OMD and *in vitro* ME in the present study is difficult to explain.

Faecal NIRS calibrations predicting *in vitro* (Leite and Stuth, 1995; Landau et al., 2004; Landau et al., 2005) as well as *in vivo* (Lyons and Stuth, 1992; Lyons et al., 1995; Boval et al., 2004; Li et al., 2007) functional properties had poor predictability compared to calibrations predicting the chemical properties of ruminant diets. Statistics of the calibrations developed in the present study confirmed these findings.

9.5 Conclusions

Data derived from past feeding trials could be used to develop robust *f*NIRS calibrations to predict chemical attributes of browse-based sheep diets. However, calibrations predicting dietary functional properties were not so robust. Statistics of *f*NIRS calibrations developed using reference data originated from *in vitro* based methods needs to be interpreted carefully.

Chapter 10: General discussion

Trees and shrubs have long been considered important for the nutrition of grazing and browsing animals in Australia, particularly where the quantity and quality (CP content and digestibility) of pastures is poor for long periods of time (Dynes and Schlink, 2002). The CP content of the browse species investigated in the research ranged between 96.7 and 216.7 g/kg DM (Table 4.1). The threshold CP requirement for adequate rumen microbial activity is 69 g/kg DM (ARC, 1980). Therefore, all the browse species investigated have the potential to be used as N supplements in ruminant feeding systems. However, the CP of browses alone does not fully justify their value as protein supplements. The nutritive value of browse species is determined by various factors such as tannin, NDF and lignin concentrations (Kaitho et al., 1998e). The TT content of *A. saligna* was the highest and that of *C. palmensis* was moderate (28.9 and 8.9 g/kg DM, respectively). The halophyte species (*A. amnicola, A. nummularia, R. eremaea*) had very low TT contents (0.9, 1.5 and 2.0, respectively). Condensed tannins ranging from 20 to 45 g/kg DM improves efficiency of N use while that over 55 g/kg DM reduces digestibility of grazing ruminant diets (Min et al., 2003). Therefore, the levels of CT of the studied forage browse species may be below the level that could potentially have a negative effect on nutritive value, in particular the CP value, when they are grown in the Mediterranean environment of WA. However, the concentrations of tannins and their effects in browse species varies widely (Makkar, 2003a), thus the predictability of the effect of tannin on the nutritive value of forage may be limited to the investigated forages and environments.

While the halophytes were extremely high in ash (156.9 to 178.8 g/kg DM), all the browse species were rich sources of minerals (Ca, Mg, K, Mn, Zn, Fe; Table 4.2) and sufficient

to meet the requirements recommended by NRC (1985). The abundance of Ca, Mg and Zn in browse is particularly important because these elements are deficient in oaten chaff basal diet, and potentially other cereal crop hays and straws. However, low levels of S in some of the browse species may be of concern in situations where they are used as protein supplements in sheep diets.

The *in vitro* ME contents of *A. saligna*, *C. palmensis*, *A. amnicola*, *A. nummularia* and *R. eremaea* were 6.6, 10.0, 7.2, 7.6 and 7.0 MJ/kg DM, respectively (Table 4.3). The maintenance ME requirement for 40 kg weighing yearling ewe is 6.36 MJ/d (NRC, 2007). If the potential DMI of an ewe is assumed to be 2% BW per day (800 g/d), the calculated ME intake would be 5.3, 8.0, 5.8, 6.1 and 5.6 MJ/kg DM, for *A. saligna*, *C. palmensis*, *A. amnicola, A. nummularia and R. eremaea*, respectively. The maintenance ME requirement of a dry ewe is 8.37 MJ/kg DM (NRC, 1985); therefore, with the exception of *C. palmensis*, these browse species, do not provide adequate energy for maintenance and thus are not recommended as sole diets for sheep.

10.1 Level of supplementation

The level of inclusion of the browse species had significant effects on the digestibility and nutritive value of the total diet. Although the level of DCP increased with increasing inclusion of browse in the diet the ME content was affected at higher levels of inclusion. Therefore, the optimum inclusion level of *A. saligna*, *C. palmensis* and *A. amnicola*, in an oaten chaff basal diet fed to sheep is 479, 831 and 496 g/kg DM, respectively. The level of inclusion of browse should not be further increased due to their low levels of energy. The low energy levels of *A. saligna* and *C. palmensis* diets were associated with the low concentration of fibre and the high concentration of lignin in these browses. A

low N: S ratio and high ash and presumably high soluble N in Atriplex were the cause for the poor energy value of *A. amnicola* supplemented diets.

The levels of inclusion recommended from the present study are greater than those recommended for trees and shrubs by previous authors (300-500 g/ kg DM, Devendra 1988; 300-450 g/kg DM, Kaitho et al., 1998a). Findings of Makkar and Becker (1998) and Salem (2005) provide evidence that browse grown in harsh environments contains increased levels of phenolics and tannins. Although rainfall data was not monitored in the present study, the region in which the fodder was grown received above average rainfall during the period of the study. In addition, the forages used in the sheep feeding experiments were irrigated during the peak summer months. Therefore, favourable environmental conditions likely resulted in the low level of phenolics in the investigated forages, which enabled recommending them at higher inclusion levels in sheep diets.

10.2 Predictability of dietary properties from faecal indices

The stepwise multiple regression procedure used to derive satisfactory predictive models for OM digestibility, SCFA production and ME content of browse-containing diets from faecal composition. The predictive models derived for *in vitro* OMD, SCFA production and *in vitro* ME from faecal ash and fibre (*f*ADL/*f*NDF) contents had acceptable R^2 (0.77) and RSD (3.51, 0.01 and 0.05 for OMD, SCFA and ME, respectively) values (model 9, 12 and 15, respectively, Table 8.3). Inclusion of faecal N as an independent variable brought only minor improvements of the R^2 (0.78) and RSD (3.45, 0.01 and 0.05 for OMD, SCFA and ME, respectively) of the best-fit model (model 10, 13 and 16, respectively, Table 8.3). Therefore, the user (livestock producer/ researcher) has the opportunity to decide upon the most appropriate predictive model (model including or

excluding faecal N) depending on the accuracy expected in the predicted property of the diet and the cost for analysis of N in faecal samples.

Contrary to faecal chemical indices, *f*NIRS analysis derived excellent calibrations to predict chemical composition of browse-containing diets (Table 9.2). Faecal NIRS calibrations derived for dietary CP, TP, TT, PPC and P had calibration statistics (R^2_c = 0.93-0.98; RPD ratio = 3 10-5.90; Table 9.2) well above the minimum levels acceptable for *f*NIRS calibrations (Stuth et al., 2003; Williams, 2004). Validation statistics of these calibrations were also at an acceptable level (R^2_{cv}= 0.92-0.97; SECV = 0.115-4.91; SEP = 0.112-9.13; Slope = 0.85-1.07; Bias = 0.01-1.28).

Highly commendable work has been carried out in the area of predicting and monitoring the quality of the diet and associated productive performance of grazing cattle in Australia (Coates and Dixon, 2007; 2008; Dixon and Coates, 2005; 2008). However, the present study would be the first such attempt to develop *f*NIRS calibrations to predict diet quality in sheep fed browse species available in Australia. Further, the present study confirmed the finding (Landau et al., 2008) that data mining of past digestibility trials could result in valuable *f*NIRS calibrations for sheep.

10.3 Conclusions

Acacia saligna, *C. palmensis* and *A. amnicola* can be included at levels of 479, 831 and 496 g/kg DM, respectively in oaten chaff basal diets. Calibrations based on faecal chemical composition would give better predictions of dietary functions attributes whereas *f*NIRS calibrations would give better predictions of dietary chemical attributes of browse-containing diets fed to sheep. With better understanding of nutrient contents

and anti-nutritive effects, browse species could become invaluable sources of protein for strategic supplementation.

10.4 Research limitations

- Representing leguminous (*A. saligna, C. palmensis*) and halophytes (*A. amnicola, A. nummularia, R. eremaea*), only five browse species were included in the present study. However, sheep grazing in diverse ecosystems such as the rangelands derive nutrients from a large number of herbaceous species.
- In relation to anti-nutritive compounds, the interest was on phenolics, in particular the tannins, of the experimental diets. However, occurrence of oxalates, saponins and alkaloids (sparteine) in the selected species may have impacted on the nutritive value of some experimental diets.
- To ensure adequate supply of forage materials for the feeding trials, browse species were grown on the university farm. The agronomic practices used potentially may have created more favourable microclimates for the browse plants than would be associated with practical sheep feeding systems in WA (or elsewhere). This may have led to changes in the chemical composition, in particular the composition of anti-nutritive compounds, of the investigated forages.
- The nutritive and anti-nutritive value of browse-containing diets was investigated using chemical methods and *in vitro* and *in vivo* digestibility trials. However, the

palatability (intake) and production aspects (liveweight gain, wool growth) were not investigated.

– *In vitro* data were used to derive predictive models.
– Due to the limited forage availability, it was compelled to limit the adaptation and collection periods of feeding trials to 7 and 5 days, respectively. The periods are just above the minimum period adapted in similar experiments reported in the literature.

10.5 Future research suggestions

Protein quality is important in evaluating responses to supplementation in forage-based ruminant diets. Ruminal microbial protein synthesis is influenced by the proportion of soluble and ruminal degradable N of forage, in addition to its digestible energy content. On the other hand, protein available for post-ruminal absorption is influenced by the amount of ruminal non-degradable protein plus microbial protein (Brown and Pitman, 1991). The high levels of CP, low levels of ME and variable levels of TP, TT and PPC are important findings of the browse investigated in the present study. As reviewed by Min et al. (2003), low levels of CT (20-45 g/ kg DM) reduce degradation of forage protein in the rumen because of the reversible binding of CT to proteins, which reduce the populations of proteolytic ruminal bacteria. Therefore, the feed N (as true protein) which escapes rumen degradation but is digested in the lower tract is one of the major factors that determine the feed N value of the browse species. Low energy level of Atriplex species was suspected to be due to the potentially high level of soluble N. The mobile nylon bag method is useful for assessing the digestibility of rumen undegradable feed protein in the intestine (De Boer et al., 1987). The *in vitro* tannic bioassay provides means to estimate ME content, effect of biological activity of tannin and microbial biomass production (Makkar et al., 2003a). Therefore, it is suggested future research should focus

on investigation of rumen degradable and undegradable proteins (bypass proteins) and microbial protein synthesis in sheep fed browse-containing diets.

Numerous *in vivo* feeding experiments conducted across the continent continue to generate thousands of faecal samples that are often stored for years. The feeding trials are typically conducted using individually penned animals under conditions allowing for full or partial collection of faeces without urine contamination. Well controlled digestibility trials yield high quality data for DMI, nutrient intake and digestibility. Conducting digestibility trials for *f*NIRS calibrations are expensive and time consuming because an acceptable period of diet adaptation is unavoidable, which reduces the number of forage combinations that can be included in *f*NIRS calibrations. As was shown in the present study, past digestibility trials can readily yield such datasets by simply scanning the faecal samples. Therefore, it is suggested to derive robust *f*NIRS calibrations using the already collected data and faecal samples which will have broader application.

10.6 Suggestions for industrial application

The research conducted enabled development of successful fNIRS calibrations for predicting important nutritive attributes of sheep diets containing C3 grass and browse species. The data used for the fNIRS calibrations were derived from a limited number of diet-faecal pairs from 40 diets and 240 animals. For practical application of fNIRS calibrations for predicting important nutritive attributes of the large variety of browse (and other plant species) found in the rangelands in Australia, as well as seasonal changes in that vegetation, a very large database of reference samples is required. Amalgamating the data generated from the research presented in this thesis with the reference data of other existing fNIRS calibrations that do not include browse species would assist in the

development of more robust and more generic fNIRS calibrations for application in the field.

Chapter 11: References

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