Improving the Nutritive Value of Low Quality Roughage for Ruminants by Ensiling with Citrus Pulp and Poultry Litter

A thesis submitted to

The University of Adelaide

for the degree of

Master of Applied Science (Agriculture)

by

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UNIVERSITY OF ADELAIDE

AUSTRALIA

February 1997

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Abbreviations

ADF	Acid detergent fibre
ADL	Acid detergent lignin
AFRC	Agricultural and Food Research Council
°C	Degrees centigrade
cfu	colony forming unit
CP	crude protein
cm	centimetre
DE	digestible energy
DM	dry matter
FAO	Food and Agricultural Organisation of the United Nations
GE	gross energy
In sacco	in bag
In vitro	in glass
In vivo	in animal
IVOMD	*in vitro* organic matter digestibility
ME	Metabolisable energy
MJ	mega joule
ml	millilitre (10^{-3} L)
mm	millimetre

mM	millimole (10^{-3} Mole)
N	nitrogen
NDF	Neutral detergent fibre
NE	net energy
NPN	Non-protein nitrogen
OM	organic matter
OMD	organic matter digestibility
Pre-mix	homogenous mixture of various ingredients
rpm	rotations per minute
sem	standard error of means
µl	microlitre (10^{-6} L)
VFA	volatile fatty acid

Summary

Investigations were completed on the effect of ensiling wheat straw with different proportions of citrus pulp, in the presence of poultry litter as a non-protein nitrogen source with or without molasses. The study evaluated quality of fermentation of the silage and also its nutritive value to ruminants on the basis of *in vitro* and *in sacco* digestibility. Animal response to the silage was also evaluated in an *in vivo* digestibility and nitrogen balance trial with Australian Merino sheep.

The four treatments containing wheat straw, poultry litter and citrus pulp respectively on a DM basis were T1 (75:25:0); T2 (60:25:15); T3 (45:25:30) and T4 (30:25:45). For each treatment between 5-10 kg of the thoroughly mixed material was ensiled for a period of 60 days, in 20 L hard plastic container laboratory silos. Inclusion of 5% molasses did not have any significant effect on pH, NDF, ADF, ADL or *in vitro* OMD. However, the presence of molasses resulted in a significant decrease in volatile fatty acids including N-butyric acid and a complete elimination of coliforms. Although, there was a significant difference in silage titratable acidity levels between silages with 0 and 5% molasses, the magnitude of the difference was small except in the silage with 30% citrus pulp. Increase in citrus pulp from 0 to 45% resulted in a very highly significant increase in silage acidity. There was no significant difference in pH between silage with 30 or 45% citrus pulp.

Wheat straw, poultry litter and citrus pulp silage were prepared in commercial quantities for both *in sacco* and *in vivo* studies and evaluation of any changes in fermentation quality. The results on composition and biochemical characteristics of the ensiled material before and after 60 days fermentation (time effect) showed a significant increase in titratable acidity, soluble nitrogen, ADF, ADL and ash content. However, there was a significant decrease in pH, DM and OM, in addition to a complete elimination of coliforms in the silage. The increase in the level of citrus pulp in the silage from 0 to 45% resulted in a highly significant increase in potential degradability of DM from 470 to 581.4 g/kg and the fractional rate of degradation from 0.027 to 0.062 per hour. In an *in vivo* experiment, the organic matter intake and digestibility increased ($p<0.05$) with the level of citrus pulp in the diet. Silage with 30% citrus pulp included in the diet at 55.5% on DM basis resulted in the best performance for intake, digestibility and nitrogen retention.

It is was concluded from this study that ensiling wheat straw with about 25% poultry litter and 30 or 45% citrus pulp without molasses, could produce silage of relatively high fermentation quality and could provide ruminants with an inexpensive source of nutrients. However, the ration incorporating the silage needs to be well formulated and fortified with other non-ensiled ingredients, to correct any nutrient deficiencies in the diet and increase acceptability to the animals.

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Declaration

I hereby declare that this thesis contains no material that has been accepted for the award of another degree or diploma in any University and to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference is made in the text.

I therefore, consent to this thesis being deposited in the University Library and being made available for photocopying and loan.

Migwi, Perminus K.

28th February 1997

Acknowledgments

I would like to extend my sincere gratitude to the following people for their valuable assistance and support during the course of the work that resulted in the production of this thesis:

My supervisors Dr Jim Gallagher (Dept of Animal Science, University of Adelaide) and Dr R. J. van Barneveld (South Australia Research and Development Institute) who have guided and encouraged me during the supervision of this project and for their constructive criticism of this thesis. Dr van Barneveld also kindly completed the fistulation of the sheep for this project;

Dr John Brooker, for the use of his laboratory in the initial part of this project and for his helpful comments of this thesis;

Dr Brian Siebert, for his guidance and assistance during the analysis of the volatile fatty acids using gas liquid chromatography;

Dr Helena Ward, for her guidance in the microbiological studies, particularly the coliforms;

All the staff of the SARDI (Pig and Poultry Production Institute - Nutrition laboratory), particularly Ms Jeanine Baker and Mr Steve Szarvas for their valuable assistance and support during the various laboratory analyses;

Ms Michelle Lorimer for her biometrical support in data analysis and all the staff in Undergraduate Training Unit (UTU) in Roseworthy Campus for their assistance and use of their laboratory facility for microbiological studies;

All other staff and students particularly of the Animal Science Department who may have assisted me in one way or an other during the course of my project.

I would also like to acknowledge, with thanks, the financial support I received from Australian Agency for International Development (**AUSAID**) through the Government of the Republic of Kenya, which enabled me to undertake and complete this study and my employer, Egerton University for granting me the study leave.

Finally, I wish to thank my wife, Gladys and our three children Beatrice, Robinson and Brian for their love, encouragement and patience during the period of my study.

CHAPTER ONE

GENERAL INTRODUCTION

Low quality roughages which are primarily crop residues form an important feed resource for 70 to 80% of the 140 million cattle and other ruminants kept in tropical Africa. This applies particularly during the dry period when there is a scarcity of forage and few alternative feed sources are available (Mosi and Lambourne, 1982). In both developing and developed countries, it is estimated that crop residues account for about 25% of total feed energy suitable for ruminant livestock (Kossila, 1985). Crop residues are equally important feed resources in many densely populated countries of Asia, where food production for human consumption has priority over stockfeed (Bhattacharya and Pervez, 1973; Sundstol *et al.* 1993; Ward and Klopfenstein, 1993). Sundstol *et al.* (1993) noted that for centuries crop residues and perhaps some roadside grass have been used to feed draught animals in Asia, a situation that is also common in many other parts of the world particularly in the tropics.

It has been reported that in China un-treated crop residues mainly in the form of cereal straw and stover form a large proportion of ruminant diets (Sansoucy, 1995). Any means of increasing the energy yield of crop residues to ruminants by 10-20% would be a significant addition to world livestock feed resources (Chaudhry, 1996). Moreover, economics of production favour and even demand maximum inclusion of cheap and readily available agricultural and agro-industrial by-products in ruminant animal diets. This will release cereals and oil seed for human consumption and therefore reduce cost of production of animal products (Naga, 1986). Therefore, there is a need to re-examine the role of low quality roughage such as crop residues and agro-industrial by-products, with a view of utilising them for livestock production (Kossila, 1985).

Anderson (1978) reported that crop residues will form the main feed base for ruminants in most parts of the world as the population-food situation becomes more acute and competition between man and animals for available grain intensifies. Given the expected growth in human population from 5.3 billion people in 1990 to an estimated 8.5 billion by the year 2025 (Kennedy, 1993), there will be a large increase in food requirements to feed the additional human population thus limiting the availability of grain to feed livestock.

In most parts of tropical Africa, crop residues form an important and in some cases the only available feed resource to sustain animal production to meet the human requirements for meat, milk and animal traction power. Fibrous crop residues are often abundantly available in many parts of the tropics and generally contain a large proportion of lignocellulose. This consists mainly of structural cell wall components that generally have low digestibility. In Kenya an estimated 6.9 million tonnes of agricultural by-products were produced in 1978, which included mainly maize and sorghum stover, cereal straws, sugar cane tops and bagasse. The present production level could be higher given the rapid expansion in cereal production in the 1980's and early 1990's to meet the domestic food needs of the country's growing population (Said *et al.* 1982).

Crop residues have been recognised for many years as potential feed resources to support livestock production systems worldwide. Despite their abundant supply, these feed resources are generally under-utilised in ruminant feeding due to their low nutritive value arising from their low digestibility. Wheat straw, a crop residue, is produced in large quantities and is readily available in many parts of the world where ruminants are kept (Acock *et al.* 1979). It is also commonly used as a feed, particularly during the dry season in the tropics and in winter in temperate areas when quality forage is in short supply.

In the past, numerous attempts but with varying degree of success, have been made to improve the digestibility of crop residues such as cereal straw and stover using various methods that include chemical treatment. However, with few exceptions the practical application of these methods at the farm level has been hindered by various social and economic constraints (Owen and Jayasuriya, 1989; Devendra, 1991). The application of urea and ammonia treatments has been widely popularised at the farm level in some countries in Asia with limited success (Sundstol *et al.* 1993).

Developing an appropriate strategy of utilising crop residues through combination with animal wastes and some agro-industrial by-products, could provide ruminant livestock production systems with an inexpensive source of energy and nitrogen that is practical and economical for certain levels of production. Citrus pulp could be useful in enhancing the utilisation of fibrous crop residue such as wheat straw and in the presence of poultry litter as the source of non-protein nitrogen and minerals for ruminants (Hadjipanayiotou, 1993). However, a major limitation to the inclusion of poultry waste (excreta) as an ingredient in ruminant diets is the danger of potentially pathogenic organisms. Several strategies have been used successfully in overcoming this problem, among them being drying, deep stacking, ensiling (Bagley *et al.* 1996) and even the use of chemicals such as fumigants (Hadjipanayiotou, 1984). The ensiling of wheat straw with citrus pulp and in the presence of poultry litter as a non-protein nitrogen source may provide an inexpensive and practical option for improving digestibility and therefore the feeding value of the straw for ruminant animals.

The objective of this study was to investigate the opportunities for utilising citrus pulp, an agro-industrial by-product, and poultry litter, an animal waste product from poultry production, to improve the nutritive value of wheat straw, a fibrous crop residue of very low feeding value.

CHAPTER TWO

LITERATURE REVIEW

2.1. Nutritional value of wheat straw for ruminants.

Wheat straw like most fibrous crop residues has low nutrient digestibility as its primary nutritional limitation as a feed for ruminants (Klopfenstein, 1978). Neilson and Stone (1987) and Channa and Mackintosh (1990) reported that this low digestibility was due to plant cell wall polysaccharides being strongly associated with indigestible cell wall components such as lignin in secondary cell wall thickenings, thereby rendering cellulose inaccessible and therefore unavailable to rumen microbial enzyme systems. This observation was further supported by Vandervoorde *et al.* (1988) who in a study on bioconversion of lignocellulose products reported similar limitations to the use of low quality plant materials. However, some authors have reported that lignin was more closely associated with non-cellulose polysaccharides such as hemicellulose (Lowry and Kennedy, 1996) and the existence of direct covalent linkages between lignin and hemicellulose, is now already well known (Lam *et al.* 1992). This binding of lignin with hemicellulose molecules may be the main factor which ultimately determines the rate and extent of degradability of fibrous feed components in the rumen (Walli *et al.* 1988). It has often been reported that fibrous crop residues, which include cereal straws, have a low nutritive value as feed for ruminants due to their high fibre content, low crude protein and low level of readily available energy (Nour, 1986; Perdok, 1987).

Studies on cereal straws such as those of wheat and rice show that their low nutritive value is essentially due to low digestibility, which is variable but lower than 50% (Klopfenstein, 1978; Egan, 1990). The low digestibility in terms of both rate and extent of digestion results in low DE intakes, which results in animals feeding on cereal straw loosing weight. Intake and digestion are in most cases limited by physical and chemical properties of lignocellulose that restrict utilisation of cellulose by the animal (Tamminga and van Vuuren, 1988; Murphy, 1990). In a study of 12 wheat varieties, Acock *et al.* (1979) reported *in vitro* digestibility of the straw ranged from 33.9 to 48.1%. These values are comparable to those reported by Kiangi *et al.* (1981) who similarly reported on low *in vitro* digestibility for wheat and rice straw (**Table 1.1**).

Available literature indicates that the most important limitations to the use of crop residues as ruminant feed include bulkiness and therefore transport and storage problems (Urio and Kategile, 1986). Additional limitations include low nutrient content particularly nitrogen and minerals, high lignin content (Ely *et al.* 1953; Forbes *et al.* 1969; Klopfenstein *et al.* 1972; Slyter and Kamastra, 1974; Sundstol and Owen, 1984; Ward and Klopfenstein, 1993), low propionate fermentation in the rumen due to low level of available energy (Lindberg *et al.* 1984) and in some cases even low palatability. This results in low rates and extent of digestion, low intakes and in some cases even loss in body weight in animals feeding on crop residues (Silva *et al.* 1989).

As a result cereal straws, particularly those of wheat and rice, rank the lowest in digestibility and metabolisable energy content and therefore have the lowest nutritive value (**Table 1.1**).

Table 1.1 Nutritive value indicators of wheat and rice straw.

	Proportion/level in straw	**Reference**
Crude protein (%)	2-6 (wheat straw)	Khajarern and Khajarern, 1985
	3-5 (rice straw)	Jackson, 1977
In vitro digestibility (%)	44.9 (rice straw)	Kiangi *et al.* 1981
	48.0 (wheat straw)	"
	33.9-48.1 (wheat straw)	"
DE (MJ/kg DM)	6.9 (wheat straw)	Wilson *et al.* 1976
ME (MJ/kg DM)	5.9 (wheat straw)	"
	5.0-7.0 (wheat straw)	Khajarern and Khajarern, 1985
NE (MJ/kg DM)	3.5 (wheat straw)	Wilson *et al.* 1976
Crude fibre (%)	26-43 (rice straw)	Jackson, 1977
Calcium (%)	0.25-0.55 (rice straw)	"
Phosphorous (%)	0.02-0.16 (rice straw)	"

The utilisation of fibrous roughage such as straw by ruminants depends entirely on rumen microbial fermentation, which yields volatile fatty acids as the main source of energy (Dewhurst *et al.* 1986; Perdok, 1987). However, the high proportion of lignified fibre in roughages, in addition to their low nitrogen content generally results in low rates and extent of digestion in the rumen. This leads to a low voluntary intake of organic matter and therefore an inadequate intake of digestible energy to meet the maintenance requirements of the animal. This ultimately results in loss in body weight and poor animal performance (Lindberg *et al.* 1984). This observation is supported by many workers who have reported weight loss in animals fed on either un-treated or unsupplemented straw diets (e.g. Mathison *et al.* 1981; Silva *et al.* 1989). As a consequence, cereal straw and stover alone are normally considered inadequate as a ruminant animal feed unless they have been modified through treatment and/or supplemented with nitrogen or readily digestible energy sources. This improves their nutrient content and therefore increases digestibility and voluntary intake (Anderson, 1978; Nyarko-Badohu *et al.* 1992; Ward and Klopfenstein, 1993). Jackson (1978) noted that supplementation with a nitrogen source and minerals is an essential component in the enhancement of the utilisation of straws by ruminants. It has also been reported that when straw constituted more than 50% of a ruminant diet, any meaningful growth of the ruminant could only be realised when such straw was treated with either ammonia or NaOH and supplemented with small quantities of readily digested nutrients such as concentrates, by-pass protein and long chain fatty acids (Perdok, 1987).

2.2. Methods of improving digestibility and utilisation of crop residues.

Attempts to improve digestibility and/or intake of wheat straw and other crop residues for ruminants using various treatments are well documented (Wilson and Pigden 1964; Jackson, 1977; Chahil *et al.* 1985; Klopfenstein, 1978; Kategile *et al.* 1981; Leng and Preston, 1983; Sundstol *et al.* 1993).

Methods of improving the nutritive value of lignocellulose-rich fibrous crop residues include :

(i). Improving microbial fermentation activity in the rumen through the use of available readily digestible energy and protein or nitrogen supplements (Perdok, 1987). Increased microbial fermentation activity and growth in the rumen increases rate and extent of structural polysaccharides digestion, which releases more nutrients from the potentially degradable fraction of the low quality fibrous feed.

(ii). Increasing accessibility of potentially digestible plant cell wall components to rumen microbial enzymes through physical, chemical and in some cases biological or microbiological treatments.

(iii). Manipulation of rumen microbes, particularly anaerobic fungi (Gordon and Phillips, 1995) and cellulolytic bacteria, to create more powerful microbes that are more effective in digesting refractory lignocellulose plant cell wall constituents.

The ultimate aim of all these methods is to enhance the rate and extent of cell wall digestion in the rumen thereby releasing more nutrients from the fibrous feed resources for use by the ruminant animal. More often, a combination of two or more of these methods have been used in practical feeding situations where fibrous crop residues have been involved. Among these strategies, treatment of fibrous crop residues using various methods has received the most attention in recent years due to their potential for practical application and commercial use. Crop residue treatment increases digestibility and intake and therefore allows a higher intake of digestible energy by the ruminant animal (Jackson, 1978). The treated fibrous crop residue could then potentially meet both the animal's maintenance requirements and possibly a certain level of production.

2.2.1. Physical treatment

Several methods of treating low quality roughages to improve digestibility and/or intake have been tried with variable results. These include soaking, chopping and grinding, boiling or steam processing (pressure cooking), pelleting and even gamma irradiation. Most of the physical processing methods, with the exception of irradiation, generally improve intake and/or digestibility of low quality roughage through almost similar means. This is achieved through increase in surface area for microbial enzyme activity and also reduction of resistance to physical breakdown during chewing, rumination and microbial fermentation. This facilitates faster reduction of roughage to a particle size that is small enough to be acted on effectively by rumen microbial enzymes. This also enables the small-particle material to pass through the reticulo-omasal orifice to the lower parts of the gastro-intestinal tract. Here the digesta is subjected to intestinal enzymatic digestion and further microbial fermentation in the hindgut and finally egested, a process which may result in higher intake (Minson, 1990). Increase in intake, with even a modest improvement in digestion could increase the intake of digestible energy and therefore improve animal performance.

Chopping and grinding have been found to increase intake by as much as 30% (Jackson, 1978). The effectiveness of these treatments is improved even further by soaking. However, grinding of low quality roughage such as cereal straw has been known to reduce digestibility, by reducing particle size and therefore reducing retention time of digesta. This effect can be quite significant, particularly when the fibrous component of the feed constitutes over 50% of the diet (Jackson, 1978). However, the depression in digestibility appears to be compensated by the higher OM intake resulting in an improvement in DE intake (Ribeiro, 1989).

Steam processing involves prolonged boiling of the fibrous crop residue material at a high temperature (170 °C), at higher than normal atmospheric pressure. This is often followed by a sudden reduction in pressure. This treatment has been reported to have a beneficial effect on improving DM digestibility particularly in maize cobs (Abaza *et al.* 1981) and sugar cane bagasse (Rangnekar *et al.* 1986). However, Nour (1986) observed no improvement in DM digestibility when rice straw was subjected to steam processing. It has been suggested that steam processing increases digestibility and therefore intake through partial hydrolysis of lignin and also by softening of the straw (Guo peiyu *et al.* 1993). Despite the benefits associated with steam processing, the major limitations to the use of this method, particularly at the small scale farming level, include the high cost of investment in the plant and equipment necessary for steam generation.

2.2.2. Chemical treatment

Various chemical treatments have been applied to fibrous crop residues to reduce the content of resistant cell wall constituents such as lignin and therefore increase accessibility and susceptibility of lignocellulose substrate to degradation by rumen micro-organisms (Egan, 1990). These include alkali treatments (Klopfenstein, 1978; Cheeson *et al.* 1986; Sicilano-Jones and Murphy, 1989 and Murphy, 1990). Alkali hydrolyses the ester bonds linking structural polysaccharides such as hemicellulose and cellulose to lignin or the phenolic acids-carbohydrate complex in the plant cell walls. This improves the accessibility and availability of structural polysaccharides in the lignified and unlignified cell walls to rumen microbial enzymes thereby increasing both rate and extent of digestion (Sundstol, 1986; Egan, 1990). This is further supported by the recent findings of Lowry and Kennedy (1996) that the hydrolysis of lignin-polysaccharide ester linkages help in the solubilising of lignin in the cell walls, resulting in simpler and often soluble lignin carbohydrate complexes (LCC) which are more susceptible to hydrolysis by rumen microbial enzymes. The overall effect of this process is the release of soluble carbohydrates and other potentially available nutrients which favour increased rumen microbial activity (Silva and Ørskov, 1988). These nutrients become available to the host ruminant animal either directly or indirectly as metabolites of microbial fermentation and microbial biomass when they are absorbed across the epithelium of the reticulo-rumen and/or passage into the lower parts of the gut.

2.2.2.1. Alkaline Hydrogen Peroxide

The potentially degradable fraction of plant cell walls has been reported to be increased by alkaline hydrogen peroxide treatment (Murphy *et al.* 1989), although Egan (1990) observed no change in the rate of cell wall digestion following the treatment of straw with this chemical. Myung and Kennelly (1990) and Pond *et al.* (1990) also reported that the initial lag time of straw degradation was reduced by alkali hydrogen peroxide treatment. This suggests that alkaline hydrogen peroxide treatment could have increased the rate of colonisation of straw particles by cellulolytic bacteria consortia which is normally an important prerequisite for fibre digestion in the rumen (Cheng *et al.* 1991). Chaudhry (1996) reported that alkaline hydrogen peroxide was more effective than either CaO or NaOH in increasing both rumen and total tract digestibility of cell wall constituents in wheat straw. Other alkalis that have been reported to be useful, but slow in action, in increasing cell wall degradation include calcium hydroxide (Chaudhry, 1996).

2.2.2.2. Ammonia treatment

Ammonia treatment (Sundstol *et al.* 1978) in either aqueous or gaseous form and urea-ammonia (Ibrahim *et al.* 1989) have both been reported to increase the extent and rate of digestion of crop residues by ruminants. This has been attributed to mainly a combination of both alkali effects and improvement of the nitrogen status of the treated straw (Owens and Goetsch, 1988; Djajanegara and Doyle, 1989; Mgheni *et al.* 1993). The effectiveness of ammonia treatment on the improvement of digestibility and intake of low quality roughage, however, depends on optimal conditions of ammonia concentration, moisture content, temperature and appropriate treatment time (Oji *et al.* 1979). Sundstol *et al.* (1978) demonstrated a simple, low cost straw ammoniation technology that proved quite effective in increasing digestibility and was also easy to adapt at the farm level. Moreover, the use of urea compared to either NaOH or NH_3, the two most effective chemical treatments, has advantages including low cost and hence affordability. As well it is available in a familiar fertiliser grade that is easy to handle and is not hazardous to use (Said and Wanyoike, 1986; Sundstol *et al.* 1993).

2.2.2.3. Limitations to the use of chemical treatment in crop residues

Although the use of chemical treatments such as alkalis have been found to consistently increase digestibility and intake significantly, field application has been of limited scale (Owen and Jayasuriya, 1989; Devendra, 1991). This has been mainly due to socio-economic constraints and non-availability of most alkalis in many developing countries. This is in addition to practical problems and risks in the handling and application of corrosive chemicals like sodium hydroxide and ammonia. Sansoucy (1995) reported on the practical problems experienced by farmers in the application of anhydrous NH_3 in the treatment of crop residues. These problems are mainly due to the volatile and irritant nature of the ammonia.

Moreover, some of the methods of sodium hydroxide treatment such as soaking (wet method) involve use of large quantities of water which pose serious environmental problems during disposal (Jackson, 1978). In addition, availability of large quantities of water in some areas can be a problem. Some investigators have also reported that ingestion of large quantities of sodium from NaOH-treated straw could cause stress to the animal, as the increase in sodium load in the plasma could result in increased water consumption and an unusually high urine output (Jayasuria *et al.* 1982; Fahmey, 1985). For instance, an almost threefold increase in both water consumption and urine output was observed by Fahmey (1985) when sheep were fed on a basal diet consisting of NaOH-treated rice straw. Increased water consumption arising from higher urine output could be a constraining factor in animal production in localities where water is scarce or even during the dry period when availability of water is normally limiting.

With the exception of urea, which is currently in widespread use (particularly in parts of Asia) and to some extent ammonia in Europe (Sansoucy, 1995), there is still scope for further investigation on the practical application of various technologies related to the use of chemicals in improving digestibility and nutritive value of crop residues (Sundstol *et al.* 1993).

2.2.3. Biological (Microbiological) treatment

Biological treatments have received some attention in recent times particularly with the discovery that some species of white rot basidiomycetes fungi have the potential and capacity to de-lignify the lignocellulose material. Jung *et al.* (1992) reported that white rot fungi significantly increased *in vitro* dry matter digestibility of straw through de-lignification. De-lignification increases accessibility of cellulose and hemicellulose to microbial enzymes thereby increasing the digestibility of the straw. Despite white rot fungi improving *in vitro* digestibility of straw DM significantly, it is however, associated with unacceptably high DM loss of up to 42% (Sundstol *et al.* 1993).

Ensiling straw with low levels of molasses with or without monensin has been reported to increase straw digestibility and nutritive value (Mohsen *et al.* 1983). Monensin is a biologically active compound that alters the fermentation characteristics of straw silage. However, with very few exceptions most biological (microbiological) treatments have been confined to laboratory scale and successful application in the field has been limited by both technological and economic constraints.

2.3. Improving microbial fermentation through the use of supplements

2.3.1. Use of energy supplements with low quality roughage

Maximum digestion of lignocellulose components in fibrous crop residue cell walls is often limited by slow microbial growth and low fermentation activity in the rumen. The depression in microbial activity is mainly due to an inadequate supply of essential nutrients that are necessary for growth. This includes readily fermentable carbohydrate energy and nitrogen (Maeng *et al.* 1976), minerals such as phosphorus and sulphur, vitamin A (Tamminga and van Vuuren, 1988) and to some extent branched chain volatile fatty acids (Durand, 1989). The low microbial fermentation of lignocellulose materials in the rumen results in inadequate release of nutrients from the fibrous components of the feed to meet the requirements of the microbes, and by an extension, that of the host ruminant animal (Perdok, 1987). This leads to poor animal production performance when low quality roughage forms the main, and in some cases the only, source of feed. In some production systems supplementation of low quality roughage with readily fermentable energy, protein, minerals and vitamins results in improved utilisation of roughage by ruminants (Nour, 1986).

Available evidence shows that most of the plant primary cell walls in herbage are degraded first and usually to completion, while the secondary thickened highly-lignified cell wall components are only slowly hydrolysed and often not to completion

(Gordon *et al.* 1985; Chesson *et al.* 1986). Supplementation with small amounts of readily fermentable carbohydrates and protein sources have been known to increase both DM and OM digestibility, mainly through increased microbial fermentation activity and growth of cellulolytic bacteria which increases fibre digestion in the rumen. This in turn increases intake of herbage dry matter, particularly herbage high in cell wall constituents such as crop residues. Silva *et al.* (1989) reported that digestibility and intake of cereal straw increased when limited quantities of starch-rich concentrates or other readily digested carbohydrate supplements were included in straw based diets. When strategically organised, starch supplementation may provide an inexpensive, efficient and practical feeding management option that optimises utilisation of low quality roughage.

Supplements, however, particularly those rich in rapidly fermented carbohydrates have to be used very sparingly and only when other nutrients like nitrogen and minerals are not limiting, as the inclusion of such energy sources in the diet in large proportions can have a dramatic effect on the rumen fermentation pattern. This can depress digestibility of cell wall constituents such as structural polysaccharides (Church and Santos, 1981; Fahmey *et al.* 1984; Castro *et al.* 1991) and often results in a reduction in intake of cheap and readily available low quality roughage (McLennan *et al.* 1995). However, this substitution effect is more notable in high than in low quality forage (Favedin *et al.* 1991; Rowe *et al.* 1991).

The change in fermentation pattern is characterised by low rumen pH arising from rapid release of volatile fatty acids which significantly reduce the numbers and activity of fibre-digesting bacteria (Obara *et al.* 1991). The depression in pH is also partly facilitated by a reduction in ruminal fluid buffering capacity arising from lower salivary output. This arises from a decrease in mastication and rumination that is normally associated with diets high in concentrates (Balch, 1971).

It has generally been observed that maximum numbers of cellulolytic bacteria and peak fibrolytic activity normally occurs when the pH is between 5.9-7.0, and that when the pH falls to a level lower than 6.0, both number and activity of celluloytic bacteria are significantly reduced (Mould and Ørskov, 1983). The reduction in number and activity of cellulolytic bacteria results in depressed fibre digestion in the rumen and this has the consequential effect of reducing OM intake (Terry *et al.* 1969; Mould *et al.* 1983). This reduction in numbers and activity of cellulolytic microbes in the rumen (following supplementation of roughage with starch-rich concentrates) is due to low rumen pH arising from rapid release of organic acids from the readily fermentable supplements (Dixon, 1985; Robinson *et al.* 1987). However, since the use of buffers (Mould and Ørskov, 1983) to prevent decline in pH or the absence of decline in pH did not totally prevent decline in cell wall degradability (Henning *et al.* 1980), other factors may also be contributing to lower fibre digestibility. Among these factors could be the amylolytic bacteria, whose presence in large numbers may result in their successful competitive advantage for the scarce non-carbohydrate nutrients such as nitrogen and minerals that are essential for the growth of most rumen bacteria (Mould and Ørskov, 1983; Mould *et al.* 1983; Hoover, 1986). This may be responsible for the decline in the activity of most fibre-digesting bacteria in the rumen (El-Shazly *et al.* 1961).

Schiere and de Wit (1995) clearly demonstrated that supplementation of low quality straw with up to 20% concentrates increased both total OM and straw intake. However, when high quality straw was supplemented with a similar level of concentrate, a depression in intake of straw dry matter was observed although overall the total organic matter intake was increased indicating that a substitution effect of concentrates for straw had occurred. This substitution effect has been attributed to the sequential degradation where the most readily fermentable substrates such as those in concentrates are preferentially hydrolysed relative to the more refractory structural polysaccharides in the cell walls of low quality roughage (Mould and Ørskov, 1983). A moderate use of energy and protein, mineral and vitamin supplements has a beneficial role in promoting digestibility and intake of crop residues and therefore increasing their utilisation by ruminants.

2.3.2. Nitrogen and sulphur

Inadequate nitrogen and sulphur limit microbial growth and fermentation activity in the rumen, thereby resulting in poor utilisation of low quality roughage by the animal. When nitrogen is limiting in the diet as in the case of most crop residues, microbial fermentation can be enhanced through supplementation with nitrogen sources in the form of protein and / or non-protein nitrogen. This improves feed intake and live weight change in animals fed straw based diets (Weisenburger and Mathison, 1977). Rumen bacteria require ammonia nitrogen and for some species additional amino acid nitrogen for optimal growth (Maeng *et al.* 1976). It has been reported that rumen ammonia concentration of about 50-80 mg / L may be required for maximum microbial growth, which favours high fermentation activity (Satter and Slyter, 1974). However, to maximise voluntary intake of straw, a higher rumen ammonia level of 195 mg / L in sheep (Mehrez *et al.* 1977) or 200 mg / L in cattle (Perdok, 1987) would appear to be necessary. This suggests a higher ammonia nitrogen requirement for straw intake compared to the level required for optimal microbial synthesis. Leng and Nolan (1984) similarly reported on the ammonia nitrogen requirement in ruminants being in the range of 150-200 mg / L in the rumen depending on the type of diet. Supplementation of low quality feeds with adequate levels of nitrogen and other limiting minerals such as sulphur and phosphorus has been reported to increase intake of basal diets (Hennessy and Williamson, 1988; Leng, 1990), a factor that contributes to higher animal production performance. Hence in practice, it is beneficial to supplement low quality roughage with a grain source fortified with protein and non-protein nitrogen sources in addition to sufficient sulphur and phosphorous.

Naga (1986) reported that inclusion of concentrates at a level of up to 30% DM in roughage diets was necessary if feeding was to be for growth or reproduction. In addition, such a supplement has to be rich in protein, vitamin A and minerals such as phosphorous and sulphur. However, to reduce costs, economics of production of animal products demand maximum inclusion of cheaper and more readily available agricultural and agro-industrial by-products. As a consequence there will be potential savings in the use of cereals and oil seeds, which could be released for human consumption and to some extent feeding of monogastrics (Naga, 1986).

2.4. Citrus by-products in low quality roughage

Fresh citrus pulp is an agro-industrial by-product of the citrus juice industry and is normally a mixture of peels (60-65%), segment pulp and juice sacs (30-35%), seeds (0-10%) and culled fruit. The variation in composition being dependant mainly on fruit species and varieties (Hutton, 1987). It is highly succulent and rich in energy mainly due to the high content of soluble carbohydrates and non-starch polysaccharides but is generally low in protein and often contains high levels of pectin (Lanza, 1982; Cervera *et al.* 1985; Rihani *et al.* 1986). Despite the advantages citrus pulp has as a potential energy source, its high moisture content makes it a highly perishable product and creates storage problems (Carol *et al.* 1990). Moreover, due to the 75-85% moisture content that is present in wet citrus pulp, it is bulky and therefore uneconomical to transport for long distances (Shirley, 1986).

For a long time fresh and wet citrus pulp has been fed directly to ruminant livestock without any form of processing. However, in recent times, due to the need to overcome the perishability problem, there has been a trend towards either drying the citrus pulp or ensiling the by-product with other ingredients such as roughage (Hutton, 1987). When fresh citrus pulp is ensiled with a low quality roughage such as wheat straw and poultry litter as a non-protein nitrogen source, its high level of water soluble carbohydrates and organic acids such as citric acid (Cervera *et al.* 1985; Yang and Choung, 1986; Hutton, 1987) could enhance effective fermentation and therefore render the silage nutritious and free from potentially pathogenic organisms. Moreover, wheat straw being a high DM fibrous crop residue could absorb the excess moisture from the citrus pulp thereby reducing the effluent loss in silage which has been reported to be as high as 65-90% (Cervera *et al.* 1985).

Yang and Choung (1985, 1986) reported that pre-wilting of fresh citrus by-products before ensiling with straw reduced the high moisture content, enhanced fermentation which therefore resulted in production of high quality silage. Cervera *et al.* (1985) reported that high quality silage could be made by ensiling citrus pulp with urea as a NPN source. However, Yang and Choung (1985) noted that while inclusion of 8-10% untreated barley straw in citrus pulp pre-mix resulted in a well fermented silage with pH 3.4-4.7, the quality of silage declined when either urea or alkali-treated barley straw was included.

In an *in vivo* digestibility trial using sheep, Yang and Choung (1986) reported a higher dry matter intake (0.76-0.78 kg DM) for citrus pulp silage / hay ration (50:50 DM basis) compared to a control diet of hay (0.6 kg DM). In the same study, the dry matter digestibility of citrus pulp silage / hay ration was noted to be higher than that of hay. This suggests that, besides improving fermentative quality of silage, the high level of digestible energy in citrus pulp could also increase digestibility of low quality roughage such as straw (Silva and Ørskov, 1988). It has also been reported that the digestibility of organic matter in citrus pulp is quite high with values in the range of 80-90% being observed by most workers (Hadjipanayiotou and Louca, 1976; Lanza, 1982; Rehani *et al.* 1986). Although, citrus pulp is a pectin-rich, bulky concentrate that is high in energy and readily fermented in the rumen, its fermentation is characterised by high acetate / propionate ratio and a relatively high pH in the range of 5.9-7.0, a fermentation pattern that closely resembles that of roughage (Wing, 1975; Harris, 1991). This is in contrast to cereal concentrates whose fermentation pattern is characterised by a low acetate / propionate ratio and a substantially depressed rumen pH that tends to disrupt normal rumen fermentation processes. This indicates that, though citrus pulp is regarded as a concentrate due to its high digestibility, it nevertheless displays roughage-like characteristics that could favour normal rumen fermentation process in diets high in fibre (Schaibly and Wing, 1974).

2.5. Supplementing low quality roughage with poultry waste

There is a scope for exploring cheaper sources of nitrogen, including non-protein nitrogen, to supplement the low nitrogen levels of low quality roughage such as wheat straw and therefore improve on their nutritive value for feeding ruminants. Moreover, such a supplement has to be cheap, readily available and easy to use within the existing systems of livestock production. Poultry litter, a by-product of the rapidly expanding intensive system of poultry production can be used as a nitrogen supplement if it is of high quality and when properly processed or treated to minimise the effects of some of its undesirable characteristics. These include low palatability and high levels of contamination by potentially pathogenic micro-organisms like coliforms and salmonella and in some cases the presence of foreign materials that can lower the nutritive value of the litter (Bagley *et al.* 1996; Chaudhry *et al.* 1996). Ensiling wheat straw with citrus pulp and poultry waste as a non-protein nitrogen source may be considered as one such option due to the fact that it is simple and practical (Han and Garret, 1986, Hadjipanayiotou, 1993; Chaudhry *et al.* 1996).

Available literature indicates that high quality poultry waste is of relatively high nutritive value, being a potentially good source of nitrogen, minerals such as Ca and P, and to some extent energy (Hadjipanayiotou, 1984, 1993; Bagley *et al.* 1996). It could therefore be considered as a strategic nitrogen supplement that would allow better utilisation of low quality roughage high in lignocellulose (Preston, 1986) and by-products such as citrus pulp that are normally high in digestible carbohydrate energy but deficient in nitrogen. Poultry waste is also readily available and a cheap nitrogen source compared to the scarce and more expensive legume seeds and oil seed by-products (Channa and Mackintosh, 1990). This suggests there may be a great economic potential to incorporating poultry waste in low quality roughage such as wheat straw to improve their nutritive value (Bishop *et al.* 1971) particularly in the presence of a readily digestible energy source (Brosh *et al.* 1993). Mavimbela (1992) reported that when Boar goats were fed for 60 days on a diet containing either 71% citrus waste and 26% sunflower oil meal as the control or 53.4% citrus waste and 43.6% poultry litter, both groups had positive gains, although the animals on the control diet had a relatively higher gain.

In a review on feed from animal wastes, Muller (1980) noted that when poultry waste was used at a level of 35% or more in a ration high in readily fermentable energy, it could provide all the protein requirements of the sheep and also contribute substantially to the energy level of the ration. While poultry litter could be considered a potential source of nitrogen which could improve utilisation of low quality roughage through increased microbial fermentation activity, most of the nitrogen in the poultry litter is in the form of uric acid, a non-protein nitrogen which undergoes hydrolysis in the rumen producing ammonia. Therefore, for an efficient utilisation of this nitrogen, a complementary source of readily fermentable carbohydrate energy is required to match the available ammonia in the rumen and hence maximise microbial protein yield. However, cereal straws are normally degraded very slowly in the rumen and therefore cannot provide sufficient energy to match the ammonia nitrogen released from the NPN in the poultry litter.

Cereal grains such as maize could be suitable sources of DE in such poultry waste based diets (Brosh *et al.* 1993) but they are often unavailable and expensive particularly, to the small scale livestock producers. Hence there is a need to look for cheaper alternatives, particularly the ago-industrial by-products such as citrus pulp. Citrus pulp being high in readily digestible non-starch polysaccharides could provide the required carbohydrate energy source to enhance digestibility of wheat straw (Silva and Ørskov, 1988) and also to promote optimal microbial synthesis in diets based on roughage and poultry litter. Moreover, citrus pulp compared to cereal grain concentrates has the added benefit of promoting a fermentation pattern characterised by a high acetate / propionate ratio which could minimise incidents of depressed rumen pH and therefore promote optimal fibrolytic activity in the rumen (Schaibly and Wing, 1974).

2.6. Ensiling of low quality roughage with poultry waste

Proper fermentation of roughage / poultry waste based silages is very important for both safe use and maximising nutritional value of the silage. Kamra and Srivastava (1991) reported good fermentation characteristics when wheat straw was ensiled with

15 to 45% poultry droppings. These workers noted that poultry droppings could be used at levels of up to 25% on a dry matter basis to give good silage provided that 10% molasses was added in the pre-mix. In a further study, poultry droppings (25%), wheat straw (15%) and maize forage (60%) inoculated with lactic acid bacteria were ensiled successfully without the use of molasses (Kamra and Srivastava, 1992). Yoon *et al.* (1985) reported on the production of relatively high quality silage when poultry manure (20%) was ensiled with rice straw (50%) and de-fatted rice bran (30%) as the main source of fermentable substrate. When this silage was fed with concentrates (3:2 DM basis) to milking Holstein-Friesian cows, a significantly higher milk yield was reported on the silage diet compared to controls on a more expensive maize silage and concentrate diet.

These studies show that, while use of molasses in ensiling roughage with poultry waste has been popular, there is scope for utilising other readily available and possibly cheaper alternatives like citrus by-products. Moreover, the un-availability of molasses in areas far from sugar cane production normally results in high cost of this product. It has also been reported that citrus pulp compared to straw had a high capacity to absorb and retain ammonia nitrogen, mainly due to its high content of pectin which binds ammonia nitrogen forming stable poligalacturonic acid amides and ammonium salts (Kowalczyk, 1976). Similarly, Taiwo *et al.* (1995) reported on well fermented citrus pulp having a relatively high capacity to absorb ammonia, mainly due to the low pH of the silage. This could further enhance the preservation of ammonia nitrogen in silage, considering that following ensiling, poultry litter was likely to result in production of high levels of ammonia nitrogen due to hydrolysis of the uric acid that is present in the litter in high proportions.

2.7. Limitations to the use of poultry waste

Diets containing poultry litter can be eaten readily by ruminants and produce acceptable performance levels in gain particularly in beef cattle, provided that the animals have been allowed an adequate adaptation period to get used to the litter and the diet also was well fortified with a readily digestible energy source (Brosh *et al.* 1993). However, the incorporation of poultry waste in the diet, particularly at high levels presents a wide range of problems that include risk to the health of the animal due to potentially harmful pathogens such as Shigella bacteria, Proteus Salmonella and Coliforms. In addition, diets incorporating even the best quality poultry litter tend to have poor palatability and therefore low intake (Fontenot *et al.* 1971; Channa and Mackintosh, 1990; Bagley *et al.* 1996). It has been reported that when ensiling was well done, the ensiled material should attain a low and stable pH (pH 5.0 or lower) that eliminates most of the potentially harmful pathogens (Caswell *et al.* 1975; Hadjipanayiotou, 1993; Chaudhry *et al.* 1996). In addition, ensiling is simple and improves palatability and therefore acceptability of poultry waste based diets to the animals (Lober *et al.* 1992).

Enhancing favourable fermentation in silage containing poultry litter is necessary for the safe use of the silage as feed for ruminants (Gupta and Kamra, 1987; Kamra *et al.* 1987; and Kamra, 1989). In addition, this could be one way of ensuring safe use by people of animal products produced by animals fed on poultry waste based diets.

Favourable fermentation (pH 5.0 or lower) can be achieved if the ensiled material incorporating poultry litter also includes a readily fermentable carbohydrate energy source such as molasses. Unfavourable fermentation (pH higher than 5.0) was reported when poultry waste was ensiled without the use of molasses (Kamra and Srivastava, 1991, 1992).

Another major limitation associated with the use of poultry litter particularly when it is included at high levels in ruminant diets is its relatively high and variable ash content which is at least 15% (Bagley *et al.* 1996). Though poultry litter with ash levels in the range of 15-25% is normally acceptable as a feed ingredient (Bagley *et al.* 1996), other workers have suggested that poultry litter with ash levels higher than 28% should not be fed to cattle (Ruffin and McCaskey, 1991). High ash levels in poultry waste could be an indication of contamination with soil and other inorganic materials and this tends to reduce the organic matter content and hence nutritive value of poultry waste as an ingredient in ruminant diets (Channa and Mackintosh, 1990; Bagley *et al.* 1996; Downs *et al.* 1996).

2.8. Opportunities for ensiling wheat straw with citrus pulp and poultry litter

Citrus pulp is high in readily fermentable non-starch carbohydrates and also organic acids such as citric acid both of which could favour suitable fermentation in ensiled material. When this by-product is incorporated with wheat straw and poultry litter, a fermented product results that is relatively high in both fermentation quality and nutritive value. Despite the high potential of citrus pulp in enhancing favourable fermentation, some studies incorporating the use of citrus pulp as an ingredient in ensiled material have often included molasses, its high cost and non-availability in some localities notwithstanding. Molasses is a widely used ingredient both as a nutrient and a fermentation stimulant in ensiled material particularly those that are low in soluble carbohydrates (Gordon 1989). It has a relatively high water soluble carbohydrate content of about 650 g/kg DM (McDonald, 1981) of which sucrose forms the largest proportion (Thomas, 1978). The soluble sugars in molasses are readily metabolised by anaerobic bacteria to lactic acid, which lowers the pH of the ensiled material to a low and stable level resulting in well preserved silage.

Objectives of the study

The main objectives of this study were to investigate-:

1. The fermentation quality and the *in vitro* organic matter digestibility and therefore potential nutritive value for sheep, of wheat straw ensiled with poultry litter as an NPN source, and different proportions of citrus pulp with(5% w/w) and without molasses.

2. The changes in composition and fermentation quality following ensiling of wheat straw with poultry litter and citrus pulp without the use of molasses and the *in sacco* digestibility of the silage.

3. The response of sheep to the wheat straw, poultry litter and citrus pulp silage including intake, digestibility and nitrogen retention

CHAPTER THREE

THE EFFECT OF MOLASSES ADDITIVE ON THE FERMENTATION OF CITRUS PULP ENSILED WITH WHEAT STRAW AND POULTRY LITTER INCLUDING *IN VITRO* DIGESTIBILITY STUDIES

3.1. INTRODUCTION

Citrus pulp has been reported as having a high level of digestible carbohydrate energy, organic acids and also moisture content. The high moisture content and high level of readily fermentable carbohydrates make citrus pulp an ideal complementary ingredient for ensiling with dry, low quality roughage such as wheat straw. Moreover, poultry litter could be a potential source of nitrogen in such silage for use by ruminants. Despite this potential benefit, previous ensiling studies with citrus pulp have mainly concentrated on the inclusion of molasses as a fermentation stimulant even when it was apparent that citrus pulp on its own could provide adequate substrates to bring about suitable fermentation. Further, the use of molasses may not always be cost effective and may even be un-available in some localities far from sugar cane growing areas.

The objective of this research was to investigate the fermentation quality and nutritive value for ruminants of wheat straw ensiled with citrus pulp and poultry litter as a nitrogen source, with 5% molasses or without molasses.

3.2. MATERIALS AND METHODS.

3.2.1. Ingredients used in silage

New season wheat straw was obtained from the University of Adelaide, Roseworthy campus farm. Poultry litter was also sourced from the Roseworthy campus poultry unit where wood shavings had been used as bedding material in a broiler deep litter system. Fresh navel orange citrus pulp was obtained from Nippys Juice Pty Ltd in Adelaide.

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3.2.2. Preparation of pre-mix and the ensiling process

Four treatments containing combinations of wheat straw, poultry litter and citrus pulp with 5% or without molasses were prepared (**Table 3.1**).

Table 3.1. The physical composition of silage (%DM basis) prepared with and without molasses.

	Composition			
Ingredient	**T1**	**T2**	**T3**	**T4**
Wheat straw	75 (71)	60 (57)	45 (43)	30 (28)
Poultry litter	25 (24)	25 (24)	25 (24)	25 (24)
Citrus pulp	0 (0)	15 (14)	30 (28)	45 (43)
Molasses	0 (5)	0 (5)	0 (5)	0 (5)
Total	**100**	**100**	**100**	**100**

Figures in parentheses refer to adjusted DM levels for 5% molasses.

During the preparation of the pre-mix, a calculated quantity of water was added depending on the dry matter content of each of the ingredients so as to achieve a dry matter content of approximately 350 g kg $^{-1}$ (35%) in the silage, a level normally considered suitable for the optimal fermentation of silage. In the treatments where 5% molasses was included, it was added to water to dissolve before sprinkling the solution on the ingredients during the mixing process. In all treatments, thorough mixing of the ingredients was done to make the pre-mix as uniform as possible before ensiling. This was facilitated by the use of straw that had been chopped to 5-7 cm in length using a forage chopper. The material was ensiled in laboratory silos consisting of 20 litre hard plastic containers lined with a double layer of polyethylene bags. During the filling of the containers, thorough compaction was done to exclude as much air as possible. The pre-mix was left to ferment in the silo for a period of 60 days after which the silage was used for various chemical analyses and an *in vitro* digestibility study. There were three replicates for each of the eight treatments.

3.2.3. Preparation of the silage extract.

After 60 days of fermentation, twenty grams of the fermented product was taken from the centre of the ensiled material and added to 180 ml of distilled water and blended in a Waring Blender for 2-3 minutes. The slurry was sieved through 6 layers of cheese cloth and the filtrate centrifuged at 10,000 rpm for 10 minutes in a Sorval refrigerated centrifuge. The supernatant of the silage extract was used for estimation of pH, titratable acidity (Kamra *et al.* 1983), total volatile fatty acids (Kroman *et al.* 1967) and soluble nitrogen (AOAC, 1990).

3.2.4. Chemical Analyses

3.2.4.1. Determination of pH.

This was estimated on the supernatant using an electronic Beckman pH meter fitted with a glass electrode and previously calibrated with pH buffers (7.0 and 4.0) and adjusted for temperature.

3.2.4.2. Titratable acidity content of the silage

This was determined following the procedure described by Kamra *et al.* (1983). Five millilitres of supernatant were diluted with 50 ml of distilled water and titrated with 0.02N NaOH using phenolphthalein indicator. The end point was shown by the appearance of a faint pink colour. The dilution with distilled water was necessary to lower the acid concentration and reduce turbidity for better detection of the end point. The titratable acidity was expressed as equivalence of mM HCL per 100 g DM of silage. The same procedure was followed in analysing acidity in citrus pulp.

3.2.4.3. Proximate analysis of ingredients and the silage.

Dry matter, organic matter, ash and nitrogen content of ingredients and the silage were determined according to the standard methods of AOAC (1990). Soluble nitrogen in the silage extracts was determined also using the same standard methods. The silage for chemical analysis and the *in vitro* digestibility study was dried in a forced draught oven for 24 hours at 70 °C and then left to equilibrate with air for 1 hr. The silage samples were then ground with a Wiley mill to pass through a 1 mm screen and samples used for further analysis. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined on the dry samples following the procedure of Goering and Van Soest (1970).

3.2.4.4. Determination of volatile fatty acids.

Total volatile fatty acids (VFA) and proportion of individual VFAs were determined according to the procedure of Kroman *et al.* (1967) modified to fit a Markham still distillation apparatus. About 150 ml of the distillate was collected over a period of 30 minutes. To determine the total concentration of the VFA in the silage, three 25 ml aliquots (distillate) were titrated with 0.1N NaOH using phenolphthalein as an indicator. The results were expressed as VFA g/kg DM silage.

To determine the proportion of individual VFA, gas liquid chromatography (GLC) was used with reference to a specifically prepared standard with VFA proportions similar to that of test sample as recommended by Kroman *et al.* (1967). Due to the very low concentration of VFA in the distillate, it was found necessary to concentrate them further by reducing the moisture content by freeze drying. This was done by adding 1 ml of concentrated HCL to 10 ml of aliquot followed by freeze drying for 10-12 hr until approximately 1 ml of the sample remained. The 1 ml sample was used in the GLC to determine the proportion of individual VFA using helium gas as the elluting (carrier) agent.

3.2.5. Microbiological analysis

All silage samples for microbiological analysis were taken in sterilised plastic containers. The procedure for preparing the silage extract samples for microbiological analysis was similar to the one outlined in **3.2.3**. except that autoclaved physiological saline 0.85% NaCl (w/v) was used in the extraction in place of distilled water. MacConkey Agar was used as the nutrient media to grow the coliforms. Fifty grams of MacConkey Agar in 1000 ml of distilled water was sterilised at 121 °C for 15 minutes and poured into plates ready for inoculation with the silage extract. 100 µl (0.1 ml) of silage extract was added to 900 µl (0.9 ml) of sterilised physiological saline in eppendorf tubes and tenfold serial dilutions prepared as follows-: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. The Agar plates in duplicate were then inoculated with a 100 µl aliquot spread thoroughly on the surface of the media using a sterilised wire loop. The cultures were incubated at 37 °C for 36-48 hr after which growth of viable micro-organisms was assessed. Where growth was found to have occurred counting was done and expressed as number of colony forming units (cfu) per gram of fresh material.

3.2.6. *In vitro* dry matter and organic matter digestibility

The *in vitro* dry matter and OM digestibility of the silage was determined by the method of Tilley and Terry (1963). It involved a 48 hr incubation of samples with rumen fluid media containing rumen microbes and a buffer, followed by a 48 hr hydrochloric acid and pepsin digestion.

3.2.6.1. Animals, feeds and feeding management.

Four mature Australian Merino wether sheep weighing approximately 50 kg were used as the source of rumen fluid for *in vitro* digestibility studies. They were fed twice daily with 750 g of a roughage diet containing 50% oaten chaff and 50%

lucerne chaff. This diet was fed to sheep for a period of 10 days before rumen fluid was removed from the rumen of the sheep using a stomach tube. Water was available to the animals continuously during the experimental period. The experimental use of the animals was approved by the University Animal Ethics Committee (Approval No W/41/94).

3.2.6.2. Sample preparation

Duplicate samples weighing approximately 0.5 g for each silage treatment (T1, T2, T3 and T4) with either 0 or 5% molasses were used for *in vitro* organic matter digestibility (IVOMD) determination.

3.2.6.3. Rumen fluid and buffer

The rumen fluid was filtered through a funnel with 4 layers of muslin so as to exclude as much solid particles as possible in the strained rumen fluid. One volume of the strained rumen fluid was added to 4 volumes of McDougall's artificial saliva with pH adjusted to 6.9-7.0 and saturated with CO_2 at 39 °C. After addition of rumen fluid, the media was allowed to mix for 10 minutes by bubbling CO_2 through it.

3.2.6.4. Incubation

Incubation of samples in the rumen tubes was conducted in a water bath with temperature set at approximately 39 °C. Fifty millilitres of the media containing rumen fluid and buffer were dispensed into all the rumen tubes containing the samples and the blanks. Immediately after adding the 50 ml media, the air occupying the upper part of the tube was displaced by passing CO_2 into the tubes for about 15 seconds and then quickly stoppering the tubes to avoid any further entry of air. The rubber stopper [illegible] to allowed escape of gases produced during the fermentation process but preventing entry of air. The tubes were swirled gently three times each day. After a 48 hr incubation, acid digestion was initiated by adding to each tube 1 ml of 10% (v/v) HCl, followed by 1 ml of 20% HCl and another 4 ml of the same acid and then 2 ml of freshly prepared 5% pepsin. The samples were incubated for another 48 hr. After completing a total of 96 hours of incubation, the samples were neutralised with 5.5 ml of 1.2M Na_2CO_3 to prevent further acid hydrolysis. They were then stored at 4 °C to await filtration. The samples were suction-filtered into previously tared glass crucibles, washed with warm water to remove all the soluble and digested fraction and dried overnight at 105 °C after which they were cooled and weighed. The residue was further ignited in muffle furnace at 500 °C for 5 hr.

3.2.6.5. Dry matter and organic matter digestibility

In vitro DM and OM digestibility were determined by measuring the difference between their respective weights in the sample before and after incubation. The DM and OM digestibility were calculated using the following equation:-

$$\text{DM digestibility (\%)} = \left[100 - \left(\frac{\text{DM in residue x 100}}{\text{DM in sample}}\right)\right]$$

$$\text{OM digestibility (\%)} = \left[100 - \left(\frac{\text{OM in residue x 100}}{\text{OM in sample}}\right)\right]$$

3.2.7. Statistical analysis

The experimental design of this experiment was a 4 x 2 factorial randomised complete block design (RCB) corresponding to the 4 levels of citrus pulp 0, 15, 30 and 45% and at the two levels of molasses, 0 and 5%. Data was analysed by two-way analysis of variance and all statistical analysis was performed using Genstat 5 (Genstat 5 Committee, 1987). Where the F-value indicated significance, the significant difference among the treatments means was tested by Duncan's multiple range test (Duncan, 1955).

3.3. RESULTS.

3.3.1. Composition of the ingredients.

The results of the proximate analysis of the ingredients used in making of the silage are shown in **Table 3.2.** The citrus pulp had a very high moisture content of 796 g/kg (80%) which is typical of fresh and wet citrus pulp material. The organic matter in citrus pulp was quite high (967.7 g/kg). However, poultry litter had a low OM and a rather high ash content of 157.6 g/kg when compared to either wheat straw or citrus pulp. The nitrogen content of poultry litter was 34.3 g/kg (21.4% CP) and was higher than that of either citrus pulp or wheat straw which had 8.6 (5.4% CP) and 5.2 g N/kg (3.2% CP) respectively. The poultry litter used in this study had a high ADL content (165.0 g/kg) compared to wheat straw and citrus pulp. Citrus pulp generally had a low fibre content as indicated by NDF and ADF content which were 148.1 and 130.6 g/kg respectively when compared to the higher content in poultry litter and wheat straw.

Table 3.2. Chemical composition (g/kg DM) of the ingredients used in silage [1].

Description	Wheat straw	Poultry litter	Citrus pulp
	Ingredients		
Dry matter (DM)	890.7	431.7	204.2
Organic matter (OM)	936.8	842.4	967.7
Nitrogen	5.2	34.3	8.6
[2]NDF	795.7	619.5	148.1
[3]ADF	480.1	468.7	130.6
[4]ADL	31.4	165.0	8.0
Ash	63.2	157.6	32.3

[1]Each value represents a mean of three samples. [2]Neutral detergent fibre. [3]Acid detergent fibre. [4]Acid detergent lignin.

3.3.2. The fermentation characteristics and composition of wheat straw, poultry litter and citrus pulp silage with or without molasses.

pH

It can be seen from **Table 3.3** that there was no significant difference in pH between the treatments with either 0 or 5% molasses, however, there was a very highly significant difference in pH between treatments with various levels of citrus pulp. Treatment T4 with the highest level of citrus pulp (45%) had the lowest pH (4.50) which was significantly lower than that of treatments T1 and T2 which were 5.77 and 4.81 respectively. However, there was no significant difference in pH between treatments T3 with citrus pulp level of 30% and T4.

Table 3.3. The effect of ensiling wheat straw with citrus pulp and poultry litter on pH, titratable acidity and coliform levels.

[1]Treat.	pH		Titratable acidity (mM/100 g DM)		Coliform count (cfu)[2]	
Molasses	0	**5**	0	**5**	0	**5**
T1	5.87^{a}	**5.66^{a}**	28.5^{d}	**29.9^{d}**	3.6×10^{3}	**absent**
T2	4.77^{b}	**4.85^{b}**	67.5^{c}	**63.4^{c}**	absent	**absent**
T3	4.58^{bc}	**4.73^{bc}**	88.0^{b}	**75.5^{b}**	absent	**absent**
T4	4.50^{c}	**4.56^{c}**	102.8^{a}	**98.3^{a}**	absent	**absent**
Means	4.93	**4.95**	71.7	**66.8**	-	-

Standard error of means and statistical significance

Treatment	0.007	***	2.157	***	-	-
Molasses	0.054	NS	1.527	*	-	-
[3]Mol. x Treat.	0.109	NS	3.055	NS	-	-

[1]The ingredients wheat straw, poultry litter and citrus pulp are in the ratio-:
T1(75:25:0), T2(60:25:15), T3(45:25:30), T4(30:25:45) on DM basis.
[2]cfu -Colony forming units. [3]Mol = molasses

Significance of differences : NS= not significant; *($p<0.05$); ***($p<0.001$).
Means within columns with different superscripts differ significantly ($p<0.05$).
Means underlined within rows differ significantly ($p<0.05$).

Titratable acidity

There was a significantly higher titratable acidity in treatments with 0% molasses than those with 5% molasses, particularly in treatment T3 which had a relatively high level of citrus pulp. However, for treatment T1 which had no citrus pulp the results showed that the silage with 5% molasses had a higher titratable acidity and hence lower pH. Although the difference in titratable acidity between treatments with 0 and

5% molasses was significant, the absolute magnitude of these differences appeared small. For either 0 or 5% molasses, it was observed that the titratable acidity increased very highly significantly following increase of citrus pulp from 0% to 45%.

Level of coliforms in silage

The results on the determination of coliforms in the silage (**Table 3.3**) showed that no coliforms were present in the treatments with either 0 or 5% molasses, except in treatment T1 that was ensiled without either molasses or citrus pulp. This silage had a high level of contamination with coliforms in the range of 3.6 x 10^3 colony forming units per gram of fresh silage material. This treatment also had the lowest acidity level (28.5 mM/100 g DM) and the highest pH (5.87) of all the treatments that were investigated in this study.

NDF, ADF and ADL

The results on NDF ADF and ADL content of the silage (**Table 3.4**) showed that there was no significant ($p>0.05$) difference between treatments with either 0 or 5% molasses. However, citrus pulp had a very highly significant effect on the content of NDF, ADF and ADL. Treatment T4 which had the highest proportion of citrus pulp (45%) had a lower ($p<0.001$) NDF, ADF and ADL content than T1 with 0% citrus pulp. This indicated clearly that the fibre content decreased significantly with increase in the proportion of citrus pulp in the silage. Molasses had no significant effect on the fibre content of the silage.

***In vitro* organic matter digestibility (IVOMD)**

The results in **Table 3.4** show that there was no significant difference between the *in vitro* OM digestibility of silage with either 0 or 5% molasses. However, there was a very highly significant increase in the IVOMD following increase in the proportion of citrus pulp in the silage from 0 to 45%. Treatment T4 with the highest level of citrus pulp had an IVOMD of approximately 56% which was higher than the 34% for T1 without citrus pulp indicating an increase of 65% or 22 percent units.

Table 3.4. The effect of ensiling wheat straw with citrus pulp and poultry litter on fibre content and *in vitro* organic matter digestibility (IVOMD).

[1]Treatment	NDF (g/kg)		ADF (g/kg)		ADL (g/kg)		IVOMD (%)	
Molasses	0	5	0	5	0	5	0	5
T1	776.2^{a}	**778.4**^{a}	535.8^{a}	**539.1**^{a}	104.7^{a}	**102.5**^{a}	33.14^{d}	**34.10**^{d}
T2	735.1^{b}	**724.2**^{b}	536.9^{a}	**519.6**^{a}	103.8^{ab}	**90.5**^{ab}	40.58^{c}	**39.33**^{c}
T3	650.4^{c}	**630.3**^{c}	482.0^{b}	**491.6**^{b}	96.6^{b}	**94.0**^{b}	45.78^{b}	**41.97**^{b}
T4	566.9^{d}	**551.9**^{d}	460.3^{c}	**452.7**^{c}	86.3^{c}	**84.8**^{c}	55.69^{a}	**56.21**^{a}
Means	682.1	**671.2**	503.7	**500.7**	97.8	**93.0**	43.80	**42.90**
Standard error of means and statistical significance								
Treatment	7.66	***	3.56	***	2.41	***	0.82	***
Molasses	5.42	NS	2.52	NS	1.70	NS	0.58	NS
[2]Mol. x Treat.	10.83	NS	5.04	NS	3.41	NS	1.16	NS

[1] The ingredients wheat straw, poultry litter and citrus pulp are in the ratio-: T1(75:25:0), T2(60:25:15), T3(45:25:30), T4(30:25:45) on DM basis.
Significance of differences : NS= not significant; *(p<0.05); ***(p<0.001).
Means within columns with different superscripts differ significantly (p<0.05).
Means underlined within rows differ significantly (p<0.05).

[2] Mol = molasses

Soluble nitrogen

The level of soluble nitrogen in silage with 5% molasses was significantly higher when compared to that of silage without molasses (**Table 3.5**). In addition, there was a very highly significant increase in soluble nitrogen as the level of citrus pulp increased from 0% in treatment T1 to 45% in T4.

Table 3.5. The effect of ensiling wheat straw with citrus pulp and poultry litter on soluble nitrogen and volatile fatty acids content.

[1]Treatment	**Soluble nitrogen (%DM)**		**VFA (g/kg DM)**		**N-butyric acid (g/kg DM)**	
Molasses	0	5	0	5	0	5
T1	0.041^{d}	**0.047^{d}**	19.4^{b}	**2.1^{c}**	6.41	**4.54**
T2	0.045^{c}	**0.058^{c}**	37.4^{b}	**35.2^{b}**	6.19	**3.55**
T3	0.062^{b}	**0.065^{b}**	56.8^{a}	**22.4^{b}**	6.16	**2.57**
T4	0.071^{a}	**0.076^{a}**	54.8^{a}	**44.3^{a}**	3.54	**2.10**
Means	0.055	**0.062**	42.1	**26.0**	5.58	**3.19**
	Standard error of means and statistical significance					
Treatment	0.002	***	4.24	***	0.88	NS
Molasses	0.001	**	3.00	**	0.62	*
[2]Mol. x Treat.	0.003	NS	6.00	NS	1.24	NS

[1]The ingredients wheat straw, poultry litter and citrus pulp are in the ratio-: T1(75:25:0), T2(60:25:15), T3(45:25:30), T4(30:25:45) on DM basis.

[2]Mol = molasses

Significance of differences : NS= not significant; *(p<0.05); **(p<0.01), ***(p<0.001).
Means within columns with different superscripts differ significantly (p<0.05).
Means underlined within rows differ significantly (p<0.05).

Volatile fatty acids

Silage containing 5% molasses had a significantly lower level of VFA than that with 0% molasses (**Table 3.5**). However, there was a significant increase in VFA associated with increase in the level of citrus pulp. On the basis of individual VFAs, presence of molasses reduced the content of N-butyric acid significantly.

3.4. DISCUSSION AND CONCLUSIONS

3.4.1. Composition of the ingredients used in the silage

The citrus pulp used in this study had low DM content (204.2 g/kg) and this was consistent with values reported in the literature (Cervera *et al.* 1985; Yang and Choung 1985, 1986; Caro *et al.* 1990). Caro *et al.* (1990) for example reported on the high moisture content of citrus pulp that ranged from 76-89%. It has been reported that the water holding capacity of citrus pulp is normally very high and this is attributed to the high levels of pectin which are known for their hydrophilic properties (Martinez and Carmona, 1980; Hutton, 1987). The rather low DM content of poultry litter used in this study (431 g/kg) could have been due to the way the material had been stored before procurement. Poor storage conditions of poultry litter has been reported as a major factor that could influence its composition and therefore loss of nutrients particularly nitrogen (Bhattacharya and Taylor, 1975). The low DM content indicates that the poultry litter was quite moist, a situation that could have been caused by the weather considering that the material had been kept in the open and uncovered.

The ash content of the poultry litter **(Table 3.2)** was within the range of 15-25% reported by Bagley *et al.* (1996). However, the level was lower than that reported by Caswell *et al.* (1975); Flachowsky and Henning (1990) and Chaudhry *et al.* (1993). The high ash content in poultry litter was the main contributing factor to the low organic matter content observed in this material (842.4 g/kg). Poultry litter generally is high in ash content with levels of at least 15% (Bagley *et al.* 1996) and this is a major factor limiting its inclusion in ruminant diets in high proportion as this could reduce the organic matter content and hence affect the availability of nutrients (Channa and Mackintosh, 1990; Downs *et al.* 1996). The main source of the high ash content in poultry litter is contamination with soil and other inorganic materials in bedding (Bagley *et al.* 1996).

The poultry litter had a nitrogen content of 34.3 g/kg (21.4% CP) which was lower than that reported by Harmon *et al.* (1974); Bhattacharya and Taylor (1975); Caswell *et al.* (1975); El-Ashry *et al.* (1987) and Chaudhry *et al.* (1996) but was within the range reported by Muller (1980). It has been reported that the crude protein content of poultry litter is high and normally in the range of 20-30% (Bagley *et al.* 1996), with a significant proportion of the nitrogen (50-60%) being in the form of uric acid, a non-protein nitrogen [illegible]. Because of a high proportion of its nitrogen being in form of uric acid, poultry litter could be a good source of supplementary nitrogen for the synthesis of microbial protein in ruminants.

The poultry litter used in this study had a high lignin content measured as ADL (165.0 g/kg). This could have been due to the high proportion of wood shavings in the bedding that was present in the litter, given that this material is normally high in lignin. It has been reported by Chaudhry *et al.* (1996) that the type of base material used as bedding in poultry houses is a factor that has a significant influence on the quality of the final poultry litter. A high proportion of lignin could be expected to reduce the feeding value of the litter, as it lowers the proportion of useful nutrients, besides lignin itself being very poorly digested by ruminants.

3.4.2. Fermentation characteristics of the silage

The titratable acidity of the silage without molasses (0%) was consistently and significantly higher than that of silage with (5%) molasses, although the actual magnitude appeared small. This could have been the reason why there was no significant difference in the pH between the silages with or without molasses, despite a significant difference in titratable acidity. In the presence of citrus pulp, treatments without molasses generally had a slightly higher titratable acidity and hence lower pH than those with 5% molasses although this did not significantly affect the pH. However, in treatment T1 where wheat straw was ensiled with poultry litter but without any citrus pulp, the presence of molasses (5%) resulted in a higher acidity level and hence lower pH (5.66). This indicated that the presence of citrus pulp in the wheat straw and poultry litter silage masked the effect of molasses, possibly due to the level of readily fermentable substrates that have been reported as being quite high in citrus pulp (Cervera *et al.* 1985).

Citrus pulp had a highly significant effect on both pH and titratable acidity. As the proportion of citrus pulp in the silage increased from 0% in treatment T1 to 45% in T4, titratable acidity increased significantly from about 28.7 to 102.8 mM/100 g DM resulting in a decrease in pH from about 5.77 to 4.53. Citrus pulp is normally high in readily fermentable non-starch polysaccharides and organic acids such as citric acid. The level of glucose in particular, has been reported to be as high as 3.2 Meq/kg (Taiwo *et al.* 1995). These substrates are readily metabolised to lactic acid by the anaerobic homofermentative bacteria such as *Lactobacillus plantarum* and other micro-organisms in the ensiled material (Kamra and Srivastava, 1992). This could have contributed significantly to the observed increase in titratable acidity content in the silage with the resultant fall in pH.

The reduction in pH could have reached a level low enough to inhibit growth and activity of most silage spoilage micro-organisms such as clostridia, yeasts and coliforms. The presence of coliforms for instance, was not detected in the silage except in treatment T1 which had neither citrus pulp nor molasses, where the pH was exceedingly high. It has been reported that any silage incorporating poultry waste as an ingredient needs to have a pH 5.0 or lower, as at this level most of the potentially harmful micro-organisms and parasites are normally effectively eliminated (Gupta and Kamra, 1987; Kamra *et al.* 1987; Chaudhry *et al.* 1996).

When wheat straw and poultry litter were ensiled without any citrus pulp but with either 0 or 5% molasses, the effect of molasses was apparent resulting in an increase in silage acidity and subsequent decrease in pH compared to the silage without molasses. This suggests that, while there may not have been any advantage in the use of molasses when wheat straw and poultry litter were ensiled with relatively high levels of citrus pulp such as 30 and 45% used in this study, use of the additive could be necessary when ensiling the same ingredients at low levels of citrus pulp.

McDonald (1981) and Gordon (1989) noted that for effective silage preservation to occur the production of lactic acid needs to be high to reduce the pH to a level low and stable enough to inhibit activity of silage spoilage micro-organisms such as clostridia, coliforms and yeasts. However, Caro *et al.* (1990) in their work on *Listeria monocytogenes* (a bacterium that has frequently been associated with illness in both animals and humans), noted that this pathogenic organism could still survive even in apparently high quality and well preserved silage with pH 4.0 or less. In their study they also noted that the growth of *Listeria* bacteria in silage was inhibited by additives such as common salt in low concentrations. This suggests that while low and stable pH inhibited growth and activity of most silage spoilage and in some cases pathogenic micro-organisms, it could not by itself guarantee total elimination of all potentially pathogenic organisms. The relatively high pH (5.87) in treatment T1 silage that had neither molasses nor citrus pulp may have been the reason for the presence of high levels of coliforms which were over 3.6×10^3 colony forming units per gram of silage material, making such silage a potential health hazard to the animals.

The higher content of soluble nitrogen in treatments with 5% molasses compared to those with 0% suggests that there was consistently a slightly higher level of protein or

NPN hydrolysis in the presence of molasses. However, in absolute terms the magnitude of the soluble nitrogen levels in the silage with either 0% or 5% molasses appear to be too low to be of any major concern in the quality of the silage. Soluble nitrogen in silage, a high proportion of which is mainly ammonia, is normally associated with clostridia and coliform activity in poorly preserved silage particularly when the pH is high. These silage spoilage micro-organisms metabolise proteins and amino acids producing products such as ammonia, amines and higher VFAs like N-butyric acid (Ohshima and McDonald, 1978; McDonald, 1981) and may result in elevation of pH and therefore formation of poorly preserved silage of very low quality (Gordon, 1989). The poultry litter used in the silage had a high nitrogen content, and most of this nitrogen was mainly in the form of uric acid, a non-protein nitrogen that can easily be hydrolysed to ammonia and other forms of soluble nitrogen following ensiling (Muller, 1980). Moreover, even in citrus pulp only about a half of the nitrogen is in the form of true protein (Hutton, 1987), the rest being in the form of non-protein nitrogen that can also undergo hydrolysis resulting in the release of ammonia nitrogen. The increase in the proportion of citrus pulp in the silage from 0 to 45% may therefore have partly contributed to the observed increase in soluble nitrogen.

Citrus pulp had a low NDF and ADF content compared to either wheat straw or poultry litter **(Table 3.2).** It is therefore apparent that increases in the proportion of citrus pulp from 0% in treatment T1 to 45% in T4 could have contributed significantly to the observed decrease in both NDF and ADF in the silage. However, molasses by virtue of having low DM and virtually no fibre but a high level of readily metabolised carbohydrate substrates (Thomas, 1978), may have been the reason for it not having any significant effect on either NDF or ADF.

3.4.3. *In vitro* organic matter digestibility

It can be shown from the results in **Table 3.4** that increase in the proportion of citrus pulp from 0% in treatment T1 to 45% in treatment T4 resulted in a highly significant increase in IVOMD from about 33-34% to 55-56%. This was a substantial increase in the potential feeding value of silage for ruminants. This level of digestibility was comparable to that of medium to high quality hay and could support maintenance [illegible] There was no significant difference in the IVOMD between silage with either 0 or 5% molasses. This could partly be attributed to the fact that molasses, while being high in readily fermentable substrates, had no effect on the fibre content of the silage [illegible] **(Table 3.4)**.

3.4.4. Conclusions

Molasses is used widely and successfully when ensiling to stimulate favourable fermentation mainly through increased lactic acid production, which reduces the pH to a low and stable level. However, this study failed to show any clear advantage in the use of molasses when ensiling wheat straw and poultry litter with higher levels of citrus pulp. It was only in the treatment (T1) without any citrus pulp that the benefits of including molasses were apparent. This occurred in the form of increased acidity levels, which reduced the pH to a level that was enough to inhibit growth and activity of silage spoilage micro-organisms such as coliforms.

It was therefore apparent that, when citrus pulp at relatively high levels such as 30 or 45% used in this study, was ensiled with wheat straw and poultry litter, adequate acidity could be generated that reduced the pH to a low and stable level. This resulted in well preserved silage of relatively high fermentation quality irrespective of whether molasses was included at 5% or not at all. However, when ensiling the same materials with low levels of citrus pulp, there may be a need to include molasses. This could provide the required level of readily fermentable substrates to lower the pH to a level suitable for effective silage preservation.

The presence of citrus pulp in silage with 0 or 5% molasses increased the IVOMD significantly from a rather low level of 33% without citrus pulp, which could hardly be considered adequate even for maintenance to approximately 56% when citrus pulp was at 45% in the silage. This level of IVOMD was relatively high and is comparable to that of medium quality hay and could support maintenance [illegible] in ruminants under certain systems of production faced with feed resource constraints such as in the tropics.

It was concluded that the use of molasses in wheat straw, poultry litter and citrus pulp silage was not beneficial except in silage where no citrus pulp was included. However, ensiling 30-45% citrus pulp with wheat straw and poultry litter improved significantly both fermentation quality and potential feeding value of the silage for ruminants as measured by IVOMD. This could represent a significant saving to animal producers where large quantities of fresh citrus by-products are readily available and the cost or availability of molasses precludes its use.

CHAPTER FOUR

EFFECTS OF CITRUS PULP ON THE FERMENTATION CHARACTERISTICS OF WHEAT STRAW AND POULTRY LITTER INCLUDING *IN SACCO* STUDIES WITH SHEEP

4.1. INTRODUCTION

This study further investigated the changes in fermentation quality following ensiling of wheat straw and poultry litter with different levels of citrus pulp but without the use of molasses. Molasses was excluded in this study on the basis of the results of the previous study (**chapter three**) which investigated fermentation quality and *in vitro* organic matter digestibility (IVOMD) of wheat straw, poultry litter and citrus pulp silage with and without 5% molasses.

In addition to evaluation of the silage quality on the basis of fermentation characteristics, an *in sacco* degradability study was conducted on the silage to determine both the rate and extent of digestion. This aimed to determine the potential digestibility and feeding value of the silage for ruminants. In an attempt to assess the animal response to the silage, an *in vivo* digestibility trial was also planned using Australian Merino sheep (**chapter five).** The silage was therefore prepared in commercial quantities.

4.2. MATERIALS AND METHODS

4.2.1. Plant material and ingredients used in making the silage.

Wheat straw was supplied by the University of Adelaide farm at Roseworthy campus and was chopped to approximately 5-7 cm in length to facilitate proper mixing during ensiling and feeding in an *in vivo* digestibility trial. Poultry litter was obtained from a poultry farm at Reeves Plains in South Australia and this was a different source to that used in the previous study (**chapter three**). From the physical appearance, this batch of poultry litter appeared to be contaminated with substantial quantities of foreign materials that included soil, ballast and other inorganic debris and attempts were made to manually remove as much of the contaminants as possible. Fresh citrus pulp was obtained from Nippys Citrus juice Pty Ltd in Adelaide.

4.2.2. Preparation of the pre-mix and ensiling process.

Four treatments were prepared which contained wheat straw, poultry litter and citrus pulp respectively in the following proportions on a dry matter basis-:

T1(75:25:0)	T3(45:25:30)
T2(60:25:15)	T4(30:25:45).

For each of the four treatments approximately 175 kg of pre-mix was prepared which was considered enough for both laboratory analysis and *in vivo* studies (**chapter five**). For every treatment, a calculated quantity of water based on moisture content of the ingredients used was added so as to achieve an estimated 35% dry matter content in the silage. Using a watering can, the water was sprinkled on the ingredients spread on a polyethylene sheet and thoroughly mixed with a shovel to ensure a uniformly mixed pre-mix prior to ensiling in 200 litre PVC plastic bins lined with a double layer of polyethylene bags. Due to the high moisture content of citrus pulp, the quantity of water added to achieve about 35% DM decreased as the proportion of citrus pulp in the pre-mix increased from 0% in treatment T1 to 45% in T4. The material was left to ferment in the silos for a period of 60 days. Just before ensiling and after 60 days of fermentation, samples were taken for various chemical and microbiological analyses to determine the changes in composition and fermentation quality of the silage.

4.2.3. Animals and feeding management

Four rumen fistulated Australian Merino wether sheep were used in an *in sacco* degradability study of the silage samples. The sheep were kept in individual pens in the animal house and fed on a roughage diet containing 50% oaten chaff and 50% lucerne chaff at a rate of 1500 g per sheep per day. Water was available to the animals *ad libitum*. The use of the animals for the *in sacco* degradability study was approved by the University Animal Ethics Committee (Approval No W/137/95B).

4.2.4. Preparation of silage and pre-mix extracts for chemical analyses.

This was done following the same procedure as in **3.2.3**. The supernatant fluid material called silage extract was used for estimation of pH, titratable acidity (Kamra *et al.* 1983), total volatile fatty acid (Kroman *et al.* 1967) and soluble nitrogen (AOAC, 1990). The same procedure was followed in preparing extracts from the pre-mix. Dry matter, organic matter, total nitrogen and gross energy were determined following the standard methods of AOAC (1990). NDF, ADF and ADL were determined following the detergent procedure of Goering and Van Soest (1970).

4.2.5. Microbiological analysis.

This was to determine the level of coliform contamination and was done following the same procedure as that outlined in **3.2.5.** and was done on the ingredients and the pre-mix before ensiling (0 day) and the silage after 60 days of fermentation.

4.2.6. *In sacco* degradability studies on the silage

Four mature Merino wether sheep fitted with permanent rumen fistula were used for nylon bag degradation studies of the four silage treatments containing wheat straw, poultry litter and citrus pulp in the proportions shown in **4.2.2.** The four silage treatments were allocated randomly to the four sheep within the four periods in a 4x4 latin square design. This ensured that each silage treatment was separately incubated in each of the four sheep in a randomised sequence within the four periods. The *in sacco* degradability study was conducted in a time length of four weeks with each period taking about one week. Nylon bags with the pore size of approximately 40 microns and a dimension of 90 x 150 mm were used for the degradability study.

The fresh silage samples in triplicate were dried in a forced draught oven at 60 °C for 24 hours and ground to pass through a 2 mm screen. Approximately 2 g of the dried sample and a marble weighing about 5 g were placed in pre-dried and weighed nylon bags. The bags with the samples were then tied firmly with a nylon fishing line to prevent spillage of the sample both during incubation in the rumen and also when washing. The free end of the nylon line was secured firmly to a 15 cm long wooden handle suspended just outside the fistula, a modification that was necessary to ensure that the nylon bags did not disappear into the rumen if the fistula lid came off. The tied bags were wetted by placing them in a water bath for 1 minute and just before feeding the animals, the bags were lowered into the rumen through the fistula.

The degradation characteristics of the silage treatments (T1, T2, T3 & T4) were measured by incubating samples in nylon bags for 6, 12, 24, 48, 72 and 96 hours in the rumens of the four sheep fitted with rumen cannulae. One bag was used for each of the incubation times in each of the four treatments. The six bags containing the samples for the six incubation times per treatment were introduced into the rumen of the sheep sequentially in the following order-: 96, 72, 48, 24, 12 and 6 hours. At the end of the incubation period (96 hours), all the bags were withdrawn from the rumen at the same time (one sheep at a time) and washed thoroughly for 5 minutes until no more colour was visible in the water. The samples were then squeezed gently to remove excess water in readiness for oven drying overnight at 105 °C. The loss in weight was recorded and used in the calculation of degradability for each of the incubation times. For the zero hour degradation (control) for each of the four treatments, the bags containing the samples were moistened and washed thoroughly under tap water for 5 minutes to measure the small particle material and the readily soluble fraction. The samples were then dried overnight at 105 °C. The loss in weight was recorded and the zero (0) hour degradability determined.

The *in sacco* dry matter degradability was calculated as follows-:

$$\textit{In sacco}\ \text{DM degradability (\%)} = \left[\, 100 - \frac{\text{(DM wt. in sample after incubation x 100)}}{\text{(DM wt. in sample before incubation)}} \,\right]$$

The DM disappearance from the bags was described by the exponential model of Ørskov and McDonald (1979).

$PD = a+b\ (1-e^{-ct})$ where :-

PD	Potential degradability of dry matter at time t
a	the readily soluble fraction
b	the slowly degradable fraction and
c	fractional rate of degradation of the degradable fraction b.

The lag time (TL) was estimated as :-

$$TL = [(-1/c)\ \ln\ (1-(w-a)/b)]$$

where c, is the rate of degradation; w, is the washing loss at zero hour; a, is the readily soluble fraction and b is the potentially degradable fraction that is degraded in time t.

4.2.7. Statistical analysis.

Data were evaluated by analysis of variance for a 4 x 2 factorial randomised block design, with 4 levels of citrus pulp (0, 15, 30 and 45%) and 2 sampling times at days 0 and 60. The exponential model of the form $P = a+b\ (1-e^{-ct})$ was fitted to the degradability data of the silage as proposed by Ørskov and McDonald (1979), where a, b and c are constants and P is DM disappearance of substrate at time t. Non-linear regression analysis was used to obtain the values of the constants. All statistical analysis was performed using Genstat 5 (Genstat 5 Committee, 1987). Where F-values suggested a significant difference, the treatment means were separated using Duncan's multiple range test (Duncan, 1955) at 5% level.

4.3. RESULTS

4.3.1. Composition of the ingredients used in the silage.

The chemical composition of the ingredients used in the making of the silage are shown in **Table 4.1.**

Table 4.1. Chemical composition (g/kg DM basis) and some biochemical characteristics of ingredients used in making silage[1].

	Ingredients		
Description	**Wheat straw**	**Poultry litter**	**Citrus pulp**
Dry matter	911.5	520.1	242.4
Organic matter	919.0	478.4	963.0
Nitrogen	3.5	34.4	9.2
NDF	756.7	386.8	172.8
ADF	432.0	330.9	166.4
ADL	71.0	116.1	17.9
Ash	81.0	521.6	36.9
pH	[2]ND	6.78	4.16
Acidity (mM/100 g DM)	ND	ND	20.40
Coliforms ([3]cfu/g)	Absent	1.4×10^5	Absent

[1]Each value represents the mean value of three samples. [2]ND- not determined. [3]cfu - colony forming unit.

The citrus pulp used in this study had a low dry matter content (242.4 g/kg) compared to the other ingredients used in the silage. The fibre and lignin levels in the citrus pulp were consistently lower than those of either wheat straw or poultry litter. The poultry litter was quite high in lignin as indicated by the ADL content of 116.1 g/kg DM, which was higher than that of wheat straw. The organic matter content of the poultry litter was 478.4 g/kg DM which was lower than that of either wheat straw (919.0 g/kg) or citrus pulp (963.0 g/kg), indicating that the product had an exceedingly high ash content.

Citrus pulp had a relatively high titratable acidity content of 20.40 mM/100 g DM and low pH (4.16) compared to the almost neutral pH (6.78) of poultry litter (**Table 4.1**). The level of coliforms in the poultry litter, the only ingredient where they were detected was quite high at about 1.4 x 10^5 colony forming units per gram fresh material. No coliforms were present in either wheat straw or citrus pulp.

4.3.2. The chemical composition and biochemical characteristics of the pre-mix and silage.

The chemical composition and the biochemical characteristics of both pre-mix and the silage are shown in **Tables 4.2** and **4.3**. The proximate analysis results of the pre-mix and the silage show that the dry matter content decreased significantly ($p<0.001$) as the proportion of citrus pulp in the ensiled material increased from 0% to 45%. There was a significantly ($p<0.001$) lower DM and OM content in the silage than in the pre-mix. However, there was a significant ($p<0.001$) increase in ash content following ensiling. For both pre-mix and the silage, there was a highly significant decrease in NDF, ADF and ADL as the proportion of citrus pulp in the ensiled material increased from 0 to 45%.

Table 4.2. Chemical composition (g/kg DM) of pre-mix with wheat straw, poultry litter and citrus pulp before ensiling (P) and after fermentation for 60 days (S).

	Dry matter		Organic matter		Ash		NDF		ADF		ADL	
^{1}Treatment	P	S	P	S	P	S	P	S	P	S	P	S
T1	387.5^{a}	346.5^{a}	821.5^{b}	852.8^{b}	178.4^{b}	147.2^{b}	623.3^{a}	682.8^{a}	378.2^{a}	424.2^{a}	73.6^{a}	76.7^{a}
T2	349.2^{b}	316.9^{b}	857.7^{a}	788.1^{a}	142.4^{a}	211.9^{a}	617.6^{a}	607.7^{b}	368.3^{a}	392.2^{b}	73.0^{ab}	74.5^{a}
T3	346.2^{c}	288.0^{c}	854.0^{a}	792.7^{a}	146.1^{a}	207.3^{a}	577.8^{b}	538.6^{c}	374.9^{a}	358.6^{c}	68.9^{b}	69.4^{b}
T4	338.8^{d}	277.5^{d}	849.0^{a}	798.2^{a}	150.9^{a}	201.8^{a}	460.5^{c}	505.5^{d}	319.6^{b}	353.3^{c}	58.9^{c}	68.0^{b}
Means	355.4	**307.2**	845.5	**807.9**	154.4	**192.1**	569.8	**583.7**	360.2	**382.1**	68.6	**72.2**
	Standard error of means and statistical significance											
Treatment	3.66	***	4.56	***	4.56	***	7.29	***	4.11	***	1.41	***
Time	2.64	***	3.29	***	3.29	***	5.15	NS	2.91	***	0.99	*
Treat x time	5.29	NS	6.58	***	6.58	***	10.30	***	5.82	***	1.99	NS

[1] Treatment ingredients wheat straw, poultry litter and citrus pulp in the ratio-: T1(75:25:0), T2(60:25:15), T3(45:25:30), T4(30:25:45) on DM basis.
Significance of difference : NS= not significant, *(p<0.05); ***(p<0.001).

Means within columns with different superscripts differ significantly (p<0.05).
Means underlined within rows differ significantly (p<0.05).

Therefore, treatment T4 which had the highest proportion of citrus pulp (45%) had the lowest NDF, ADF and ADL content. It is also shown from the results in **Table 4.2** that ensiling was associated with a significant increase in the content of ADF and ADL in the silage (time effect).

pH and titratable acidity

The results **(Table 4.3)** on the fermentation characteristics of the ensiled material show that even before ensiling there was already a significant ($p<0.001$) decrease in pH of the pre-mix of the four treatments containing different levels of citrus pulp. However, treatments T3 and T4 with pH 5.54 and 5.32 were not significantly ($p>0.05$) different. The same trend in pH was repeated in the silage where there was a significant decrease in pH following increase in the level of citrus pulp. In both the pre-mix and the silage, there was no significant difference in pH between treatments with 30 or 45% citrus pulp. The ensiling of the material for 60 days was associated with a highly significant decrease in pH as shown by the time effect. However, the interaction effect between treatment and ensiling time period was not significant.

Table 4.3 Biochemical characteristics of pre-mix containing wheat straw, poultry litter and citrus pulp before ensiling (P) and after fermentation for 60 days (S).

	pH		Titratable acidity (mM 100 g DM)		Total nitrogen (g/kg DM)		Soluble nitrogen (%DM)		[1]Coliforms ($\times 10^4$) cfu / g fresh material	
[1]Treatment	**P**	**S**	**P**	**S**	**P**	**S**	**P**	**S**	**P**	**S**
T1	6.85^{a}	5.19^{a}	9.15^{b}	19.51^{d}	5.72^{c}	5.43^{c}	0.033	0.030	84.2	absent
T2	6.13^{b}	4.59^{b}	12.00^{b}	40.71^{a}	5.92^{c}	6.31^{c}	0.028	0.038	14.0	absent
T3	5.54^{c}	4.12^{c}	15.61^{a}	67.51^{b}	7.54^{b}	6.69^{b}	0.029	0.046	85.4	absent
T4	5.32^{c}	4.13^{c}	15.90^{a}	73.95^{a}	7.68^{a}	8.94^{a}	0.031	0.053	4.5	absent
Means	5.96	**4.51**	13.16	**50.42**	6.72	**6.84**	0.030	**0.042**	-	-

Standard error of means and statistical significance

Treatment	0.12	***	0.96	***	0.31	***	0.004	NS	-	-
Time	0.08	***	0.69	***	0.22	NS	0.003	**	-	-
Treat x Time	0.17	NS	1.39	***	0.43	NS	0.005	NS	-	-

[1] Treatment ingredients wheat straw, poultry litter and citrus pulp in the ratio-: T1(75:25:0), T2(60:25:15), T3(45:25:30), T4(30:25:45) on DM basis.

[1] cfu - colony forming unit.

Significance of difference : not significant; **($p<0.01$); ***($p<0.001$).
Means within columns with different superscript differ significantly ($p<0.05$).
Means underlined within rows differ significantly ($P<0.05$).

Total and soluble nitrogen content

The results in **Table 4.3** show that the total nitrogen content of the pre-mix and the silage increased significantly ($p<0.001$) as the proportion of citrus pulp increased. However, this did not significantly affect the content of soluble nitrogen. The results show that, while the ensiling period (time) did not significantly change the content of total nitrogen, the process resulted in an increase ($p<0.01$) in the level of soluble nitrogen in the silage.

Level of coliforms in the ensiled material

The pre-mixes of all the four treatments before ensiling were heavily contaminated with coliforms whose numbers ranged from 4.5×10^4 to 85×10^4 colony forming units (cfu) per gram of fresh pre-mix material. Following ensiling for 60 days and the associated fermentation process, which resulted in a decrease in pH, the coliforms were virtually eliminated, as they were not detected in the silage (**Table 4.3**). The microbiological studies on the ingredients used in the silage showed that while wheat straw and citrus pulp were negative for coliforms, poultry litter was heavily contaminated with a very high level of coliforms of about 1.4×10^5 colony forming units per gram of fresh material.

Volatile fatty acids (VFA)

The content of volatile fatty acids (VFAs) for treatment T1, T2, T3 and T4 silage were 13.1, 17.1, 17.4 and 20.9 g/kg DM respectively. The difference between the treatments was not statistically significant.

4.3.3. Dry matter degradability of the silage

The results of the dry matter degradability of the four treatments of the wheat straw, poultry litter and citrus pulp silage are shown in **Figure 4.1** and **Table 4.4**. There was a highly significant treatment effect, where increase in the proportion of citrus pulp from 0% in treatment T1 to 45% in T4 resulted in an increase in potential degradability (a+b) and also the fractional rate of degradation of the silage. For each incubation time up to 96 hours there was a very highly significant difference in potential degradability between the four silage treatments. The silage of treatment T4 had both the highest potential degradability and rate of degradation compared to the other three treatments. There was also a very highly significant treatment x time interaction effect.

Table 4.4. Effect of various levels of citrus pulp on the DM degradability of wheat straw, poultry litter and citrus pulp silage diets fed to Australian Merino sheep.

Parameter	**Treatments**				
Degradation constants	**T1**	**T2**	**T3**	**T4**	**SEM**[1]
a (g/kg)	132.7	171.5	241.1	242.3	
b (g/kg)	364.6	377.7	315.8	340.0	
PD = a+b (g/kg)	497.3	549.2	556.9	582.3	
c (/hr)	0.027	0.039	0.054	0.062	
TL(hr)	0.460	0.330	0.660	0.077	

[1] SEM = 10.96 for treatment x time interaction was very highly significant. ($P<0.001$)

PD, potential degradability; a, readily degradable fraction; b, slowly degradable fraction; c, fractional rate of degradation per hour; TL, time lag.

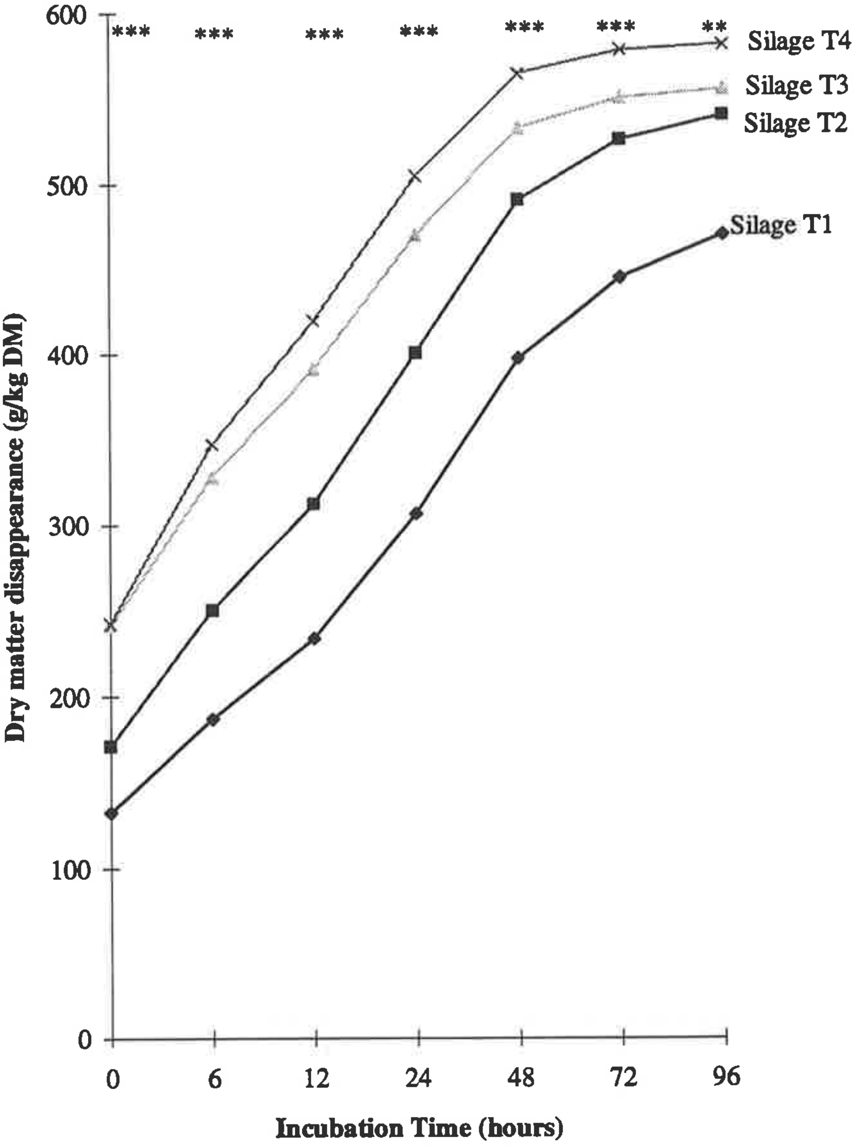

Figure 4.1 The effect of various levels of citrus pulp on the DM degradability of wheat straw, poultry litter and citrus pulp silage (T1, T2, T3 and T4) in Australian Merino sheep, (Statistics: **p<0.01, ***p<0.001)

4.5. DISCUSSION AND CONCLUSIONS

The citrus pulp used in this study had a low DM content and therefore high moisture level (75.7%). This is consistent with the findings of other workers who have reported on this product (Yang and Choung, 1985, 1986; Cervera *et al.* 1985; Caro *et al.* 1990) and is also in keeping with previous results (**chapter three**). The high moisture content of citrus pulp makes the product bulky to handle and also highly perishable. Yang and Choung (1985) reported, that pre-wilting citrus pulp to reduce moisture content before ensiling or ensiling with 8-16% barley straw, produced silage that was of comparable quality. This suggests that, ensiling of the succulent fresh citrus pulp with dry low quality roughage could be a practical and feasible solution to the problem of high moisture content that is associated with this product.

DM content is a critical factor that influences the fermentation pattern of silage. This occurs through its effect on the water activity which in turn determines the critical pH that needs to be achieved for proper fermentation and therefore preservation of the silage (Morgan *et al.* 1980). In addition, the extent of fermentation is progressively restricted by increase in dry matter content of the ensiled material which affects the quality of the final silage (McDonald and Whittenbury, 1967). Moisture content also affects the type and total bacteria that inhabit the silage, and determines the overall rate of fermentation which are critical factors in silage preservation (Gouet *et al.* 1965). The dry matter and moisture content are also among the most important factors that influence effluent loss from the silage (Miller and Clifton, 1965; Bastiman and Altman, 1985).

There was a notable reduction in the dry matter content following ensiling in respective treatments from pre-mix to the final silage product. The reduction in dry matter following ensiling, when expressed as a percentage of the initial ensiled material was 10.6, 9.2, 16.8 and 18.1% for treatments with 0, 15, 30 and 45% citrus pulp respectively. Loss in both DM and OM following ensiling is not unique to this study, as it has been observed by other workers (McDonald, 1983; Bastman and Altman, 1985; Kamra and Srivastava, 1992). This loss in DM could have been caused by many factors including aerobic and anaerobic oxidation of readily metabolised compound such as water soluble carbohydrates, by active plant tissue enzymes and micro-organisms. This normally occur before the pH of the ensiled material falls below 5.0, a level that is normally considered adequate to inhibit all respiration activity (Kamra *et al.* 1987; Gordon, 1989).

The relatively high DM loss in the silage containing 45% citrus pulp could have been partly attributed to effluent loss as this silage had the lowest DM content among the four treatments. In this study, it was noted that the silage with 45% citrus pulp had a small amount of effluent at the bottom of the silo. In addition, the procedure followed in dry matter determination involved drying the fresh silage in a forced draught oven at 70 °C for 24 hr. This has been found to under-estimate silage DM content compared to the Toluene distillation method (Haigh and Hopkins, 1977; Porter *et al.* 1984). This is mainly due to substantial losses of volatile components in the silage such as ammonia and VFAs during the drying process. Under estimation of silage DM leads to an over-estimation of fermentation loss in DM in silage (Bastman and

Altman, 1985). This could have been partly the reason for the higher dry matter loss observed in silage of treatments with 30 and 45% citrus pulp compared to those with 0 and 15%.

It is apparent that the poultry litter which had a low OM content of 478.4 g/kg DM and included at 25% DM basis was the main contributing factor to the low level of OM in the silage. The high ash content of both the pre-mix and the silage that ranged from 147.2 to 201.9 g/kg DM was attributed to poultry litter. The high ash content in the poultry litter was due to contamination with soil and other inorganic debris in the bedding.

The increase in the ash content in silage compared to the pre-mix may be attributed to the loss of OM from fermentation. The oxidation of substrates in the OM during silage fermentation increases the concentration of the remaining components particularly ash and fibre (McDonald *et al.* 1960; McDonald *et al.* 1964). However, in some cases as in very wet silage where effluent loss tends to be high, the final silage is likely to have a lower ash content than original material, as a result of the draining effluent being rich in soluble silage ingredients such as minerals, nitrogen and sugars (Saro and Sormunen, 1976).

The citrus pulp used in this study could be considered to have been moderately acidic with pH 4.16 and a titratable acidity level of 20.40 mM/100 g DM. This pH level is slightly higher but comparable to those reported by Cervera *et al.* (1985) and Caro *et al.* (1990) who reported on citrus pulp having a pH 3.7 or pH 2.5-3.1 respectively.

This difference could have been attributed to the variation in the materials from different sources and the procedures used in preparing the extracts for both pH and titratable acidity determination. Martinez and Carmona (1980) reported on the wide variation in the composition of citrus pulp due to differences in fruit variety and methods of processing citrus pulp.

The results (**Table 4.3**) showed that the pH of the pre-mix prior to ensiling decreased significantly ($p<0.05$) from 6.85 in treatment T1 with 0% citrus pulp to 5.32 in T4 with 45% citrus pulp. Given that citrus pulp had a high acidity level and low pH compared to the almost neutral pH (6.78) of poultry litter, it may therefore have been the main ingredient responsible for the observed increase in acidity and decrease in pH in the pre-mix before ensilage. Caro *et al.* (1990) reported that citrus pulp was characterised by high content of citric acid and other organic acids and this could have contributed to the observed increase in acidity and subsequent decrease in pH. However, it appeared from results of coliform determination that the pH before ensiling was either not low enough to inhibit activity of harmful micro-organisms or the acidity had not been allowed to act long enough. The results on coliforms showed that the pre-mix of all the four treatments generally had very high levels of coliforms that ranged from 4.5×10^4 to 85×10^4 colony forming units per gram of fresh material. The fact that no coliforms were detected in the silage after 60 days of fermentation (**Table 4.3**) was an indication that ensiling is an effective means of eliminating the potentially pathogenic micro-organisms from the contaminated material.

The influence of the ingredients and fermentation on the fibre content

The increase in the proportion of citrus pulp from 0 to 45% in the ensiled material may have been responsible for the observed decrease in fibre content as measured by NDF, ADF and ADL (**Table 4.2**). Citrus pulp had a low content of NDF, ADF and ADL compared to either wheat straw or poultry litter and therefore its proportional increase in the silage combined with a proportional decrease in the wheat straw could have contributed to the observed decrease in these components in both the pre-mix and the silage. The combination of wheat straw and poultry litter, both with relatively high cell wall constituents relative to citrus pulp, constituted 100% of all the DM in treatment T1 while the two ingredients combined constituted only 55% in T4. Hence as could have been expected the cell wall constituents (NDF, ADF & ADL) generally decreased with increase in citrus pulp. The decrease in OM content following ensiling of the pre-mix for 60 days may have been responsible for the observed increase in the ADF and ADL content in the silage.

pH and titratable acidity of the ensiled material

The pH of the silage ranged from 5.19 in treatment T1 with 0% citrus pulp to 4.13 in T4 with 45% citrus pulp (**Table 4.3**), suggesting that citrus pulp had a significant influence in the change of pH. This was strongly supported by results on titratable acidity which showed a highly significant increase following increase in the proportion of citrus pulp in the silage. This increase in acidity and the associated fall in pH appeared to have reached a level which inhibited growth and activity of coliforms, as they were not detected in any of the silage in all the four treatments.

This is in spite their presence in the pre-mix being in high numbers prior to ensiling (**Table 4.3**).

Citrus pulp, besides being rich in readily fermentable carbohydrates, also has been reported as having a high content of organic acids such as citric acid (Caro *et al.* 1990) and this was further confirmed by the results of titratable acidity of this material. The high level of organic acids may have contributed directly to the fall in pH in the pre-mix prior to ensiling. Following ensilage there was respectively more than twofold, threefold and fourfold increase in titratable acidity in the ensiled material with 0, 15 and 30 or 45% citrus pulp. It was concluded that, it is mainly the intense fermentation initiated by the presence of citrus pulp in the silage that contributed to most of the observed increase in titratable acidity and subsequent fall in pH. Given that silage with either 30 or 45% citrus pulp had pH levels that were not significantly different, the two proportions of citrus pulp may therefore have produced silage of almost similar fermentation quality.

Nitrogen levels present in the ensiled material

All the four treatments contained the same proportion of poultry litter on a dry matter basis (25%). Poultry litter, with 34.4 g N/kg DM (21.5% CP) had the highest nitrogen content among the three ingredients in the silage and therefore was the main source of nitrogen in the ensiled material. However, citrus pulp with a medium nitrogen content of 9.18 g/kg DM (5.74% CP) could be considered a better nitrogen source than wheat straw, which had a very low nitrogen content of only 3.5 g/kg DM (2.18% CP) a level that is typical of most low quality roughage such as straw. The significant increase in total nitrogen content in the ensiled material following increase in the proportion of citrus pulp from 0 to 45% may therefore be attributed to the medium level crude protein content in citrus pulp and also the reduction in the proportion of wheat straw. Hutton (1987) reported that, although citrus pulp was considered basically as a digestible energy source, it does contain a significant proportion of crude protein that could be useful in diets incorporating this product as an ingredient.

Following ensiling, [illegible] (Table 4.3). It was reported by Ohshima and McDonald (1978) that unless anti-proteolytic additives such as formaldehyde have been added to the herbage during ensiling, proteolysis of proteins and hydrolysis of NPN normally occurs through the activity of both plant and microbial proteases enzymes [illegible]. This results in an increase in soluble nitrogen mainly in the form of ammonia and amines, which can constitute about 40-70% of total nitrogen in the final silage (Chamberlain *et al.* 1989). Due to proteolysis, starting [illegible] before the material is ensiled, the protein nitrogen even in the very well preserved silage is normally less than 50% of total nitrogen (Wilkins, 1981).

Limitations to the inclusion of poultry litter in ensiled material

The poultry litter used in this study had a DM content of 520 g/kg which was lower than that reported by Muller (1980). A wide range of DM values has been reported for poultry litter, a factor that could be attributed to differences in handling of the product mainly during storage. In addition, poultry litter also had a low organic matter content, suggesting that it could have been contaminated with soil and other inorganic components which may have contributed to the observed high ash content. Generally, poultry litter has been known to have high ash content and this is widely reported in literature (Kayouli, 1993; Bagley *et al.* 1996).

The high ash content and other factors like the presence of potentially pathogenic micro-organisms such as salmonella tend to limit inclusion of poultry litter in ruminants' diet at high proportions (Channa and Mackintosh, 1990). The high ash content reduces the proportion of organic matter and hence availability of nutrients (Muller, 1980; Bagley *et al.* 1996; Downs *et al.* 1996). In some ways, however, the high ash content could be beneficial as a valuable source of minerals such as calcium and phosphorus both of which are comparably higher in poultry waste than in natural feedstuff (Bhattacharya and Taylor, 1965). Given that the poultry litter used in the present study had a rather high ash content (521.6 g/kg) and therefore low OM, its inclusion in the silage at a level of 25% on a DM basis could be considered to have been quite high. Ruffin and McCaskey (1991) suggested 280 g/kg as the upper limit in ash content for poultry litter destined for feeding cattle.

The poultry litter used in the study also had a high ADL content of 116.1 g/kg DM, suggesting that it [illegible] contained a high proportion of the original base material such as wood shavings and sawdust which are commonly used as bedding in the floor systems of poultry production. A high lignin content in the poultry litter is undesirable, as it could significantly reduce the proportion of otherwise useful components such as excreta and waste feed, thereby lowering its quality as an ingredient of ruminant feed (Harmon *et al.* 1975). Considerable variation in quality of poultry litter has been reported, a factor largely attributed to type and quantity of bedding material used, in addition to the ash content of the litter itself (Holzer and Levy, 1976).

The level of coliforms in the poultry litter was also very high. This high level of coliforms and other potentially pathogenic micro-organisms like salmonella in poultry waste has been reported before (Kayouli, 1993) and is a major concern in the safe use of poultry waste based diets in animals (Channa and Mackintosh, 1990; Bagley *et al.* 1996). Ensiling of poultry waste or poultry waste based materials prior to feeding them to ruminants has been suggested as one practical and economical way of eliminating harmful micro-organisms such as coliforms (Hadjipanayiotou, 1982; Lober *et al.* 1992). This was further confirmed by the results of this study that showed a complete elimination of coliforms after the heavily contaminated pre-mix material was ensiled for 60 days.

Volatile fatty acids in the ensiled material

The low levels of volatile fatty acid in the silage and lack of significant difference between the treatments showed that the silage was generally well preserved. VFA which include mainly acetic, propionic and butyric acids are formed in the silage mainly through metabolism of carbohydrates and in some cases lactic acid by clostridium and other micro-organisms. Since volatile fatty acids are generally weaker acids than lactic acid (McDonald, 1981), their presence in the silage in high levels could result in elevation of pH, a factor that could contribute to the formation of poorly preserved silage of low quality.

Effect of citrus pulp on the *in sacco* degradation of silage

The high potential degradability of silage with 45% citrus pulp could be attributed to several factors that include a higher proportion of both readily soluble and slowly degradable fractions, in addition to the higher rate of degradation. Moreover, this silage treatment also had a relatively shorter lag time indicating that the material could have been colonised faster by the rumen microbes compared to the other treatments. The increase in the proportion of citrus pulp from 0 to 45%, therefore could have improved the potential feeding value of the silage to ruminants through increase in both potential degradability and rate of DM degradation as evident from the *in sacco* results. While the *in sacco* degradability results may be useful in comparing the potential feeding value of different feeds, they do not provide information on intake (Ibrahim *et al.* 1989). However, an increase in the potential degradability and fractional rate of degradation as observed in this study may contribute to an increase in intake if the silage formed part of a ruminant diet.

It is apparent from **Figure 4.1,** that treatments T3 and T4 had almost attained maximum potential degradation (a+b) within 48-72 hours, mainly due to their higher rate of degradation. There was little change in potential degradation in these treatments from 72 to 96 hours. This was in contrast to treatments T1 and T2 where potential degradation was increasing at 72 hours, even at 96 hours the increase in degradability was still evident. This clearly shows that silage with 0 or 15% citrus pulp took longer time to reach their maximum potential degradation and which was still low when compared to that of silage with 30 or 45% citrus pulp.

The differences in rate of degradation and potential degradability between silage of treatments T1 & T2 and T3 & T4 within the 48 to 72 hour period appear linked to the various levels of citrus pulp and wheat straw contained in these treatments and could have an influence in their potential feeding value to ruminants. It has been reported that the normal mean retention time of fibrous feeds in the reticulo-rumen, where most of the fibre is digested in ruminants is about 36 to 60 hours (Ørskov, 1986). After this period normally any undigested material is passed on to the lower parts of the gut and finally voided as faeces. This suggests that, the potential degradation attained within 48-72 hours may be a more reliable indicator of the potential feeding value of fibrous feeds to ruminants, than the maximum potential degradation obtained at a longer period. Ørskov *et al.* (1988) reported that in straw based diets, the dry matter intake was more closely correlated with 48 hour degradation (r = 0.90) than the maximum potential degradation (r = 0.81).

On the basis of the results of the *in sacco* study, it was concluded that silage with 30 or 45% citrus pulp could potentially have higher feeding value to ruminants than one with 0 or 15%. The higher rate of DM degradation and potential degradability in 48-72 hours may result in a higher quantity of available nutrients to the rumen microbes, and, by extension, to the host ruminant animal, resulting in a higher feed intake and therefore better production performance.

Conclusions

The results of this study showed that ensiling wheat straw and poultry litter as a NPN source with different levels of citrus pulp without the use of molasses produced silage of variable fermentation quality. Increase in the level of citrus pulp in the silage from 0 to 45% resulted in a significant increase in titratable acidity and total nitrogen content but decrease in pH, DM, OM and fibre content as measured by NDF, ADF and ADL. These changes in the silage, particularly the acidity and pH levels, in addition to lack of significant increase in the level of soluble nitrogen, generally indicate an increase in silage fermentation quality. On the basis of the pH levels, 30 or 45% citrus pulp appeared to produce silage of almost similar fermentation quality.

The 60 days ensiling period resulted in some significant changes in both composition and biochemical characteristics of the ensiled material. This had beneficial effect on the final silage and also some negative effects. The increase in acidity, fall in pH and elimination of coliforms following ensiling, points to the beneficial effects of the silage fermentation. However, the loss in OM, which resulted in a significant increase in ash, ADF and ADL content in the silage, in addition to the increase in the level of soluble nitrogen, indicates clearly that not all effects of ensiling were beneficial.

Similarly, the *in sacco* digestibility studies on the silage indicated a progressive increase in digestibility with increase in the level of citrus pulp. The silage with 30 or 45% citrus pulp had a potential degradability of over 500 g/kg (50%) in 48-72 hours incubation period and a fractional degradation rate of more than 0.05 per hour. That, this silage also had a nitrogen content of 7.54-7.68 g/kg DM, compared to the rather low nitrogen content of wheat straw (3.5 g/kg DM) indicates a significant improvement in the nutritive value of wheat straw. This could have increased the feeding value of wheat straw to a level comparable to that of medium quality hay.

It is was concluded that ensiling of wheat straw and poultry litter with relatively high levels of citrus pulp such as 30 or 45%, could produce silage of relatively high fermentation quality and potential feeding value for ruminants. However, further studies are required for the rations based on this silage to be evaluated by ruminants in terms of their acceptability and nutritional value.

CHAPTER FIVE

THE EFFECTS OF CITRUS PULP ENSILED WITH WHEAT STRAW AND POULTRY LITTER ON *IN VIVO* DIGESTIBILITY AND NITROGEN BALANCE IN SHEEP

5.1. INTRODUCTION

Dietary manipulation through relative combination of low quality roughage with readily digestible carbohydrates is an important strategy that has long been recognised and used widely in improving utilisation of cheap and abundantly available fibrous crop residues such as cereal straw and stover by ruminants (Leng and Preston, 1983).

In chapter three, it was shown that ensiling of citrus pulp with wheat straw and poultry litter with and without the use of molasses produced silage of comparable quality. This conclusion was made on the basis of the observation that there was no significant difference in some important silage fermentation quality indicators like pH and also *in vitro* organic matter digestibility. In a further study (**chapter four**) involving ensiling of wheat straw and poultry litter with different levels of citrus pulp and without the use of molasses, it was also observed that the fermentation quality of silage generally improved with increasing proportion of citrus pulp from 0 to 45%.

An *in vitro* digestibility study on the silage (**chapter three**) showed a significant increase in digestibility from 33% to about 56% in silage with 0 to 45% citrus pulp, which represented an increase of about 65%. In addition, the *in sacco* digestibility studies on the silage also showed that both rate and extent of digestion increased significantly following increase in the proportion of citrus pulp. This indicated that wheat straw when ensiled with citrus pulp and poultry litter as an NPN source, even without the use of molasses, could produce a silage product of relatively high fermentation quality and digestibility, therefore making such a product a potential feed ingredient in ruminant diets. Gordon (1989) indicated that there was a need to link the improvement in preservation quality of silage with animal performance. This suggests that improvement in the fermentation quality of silage should not be an end by itself but should translate into improved animal performance, and, by an extension, higher production. This points to the need for a silage feeding trial with sheep.

In this study the nutritive value of silage prepared by ensiling wheat straw with citrus pulp and poultry litter without the use of molasses (**4.2.2**) was evaluated on the basis of intake, digestibility and nitrogen balance, in an *in vivo* digestibility experiment using Australian Merino wethers.

5.2. MATERIALS AND METHODS

5.2.1. Animals and housing

Twelve mature wether sheep (Australian Merino) weighing approximately 55 kg were assigned to 3 blocks of 4 animals each, based on weight. Sheep within each block were randomly allotted to the 4 diets such that digestibility *in vivo* was determined for each diet with 3 sheep. Prior to the experiment, the animals were also treated for internal parasites. Metabolic crates were used for the collection of faeces and urine and a preliminary feeding period of 10 days was followed by a 7 day period of total collection of faeces and urine.

Animal ethics approval for the experimental use of the animals in this experiment was given by the University Animal Ethics Committee (Approval No W/41/94).

5.2.2. Feeds and feeding management

Approximately 175 kg silage (fresh wt.) containing about 60 kg DM was prepared for each of the 4 treatments as outlined in **4.2.2.** in readiness for the *in vivo* digestibility and nitrogen balance study. The 4 treatments of silage contained wheat straw, poultry litter and citrus pulp respectively in the following proportions on a DM basis-:

T1(75:25:0)	T3(45:25:30)
T2(60:25:15)	T4(30:25:45).

The respective silage formed the basal part of the diets D1, D2, D3 and D4 in the proportions of 45.5%, 50.5%, 55.5% and 60.5% respectively on DM basis and balanced with other non-ensiled ingredients which included lucerne, wheat bran, molasses and minerals (**Table 5.1**). The addition of these other ingredients was necessary to ensure formulation of a balanced diet that met the maintenance requirements of the animals. The rations were formulated with the aid of the 'Rumnut Take Away' computer program developed by the Department of Primary Industries (DPI), South Australia. The ingredients and proportions used in the four diets are shown in **Table 5.1**. The four diets were offered to the animals to provide approximately 900 g DM/day in equal portions at 09.00 and 17.00 hr. This amount was designed to supply energy (8.30 MJ ME/day) and nitrogen (14.30 g/day) enough to meet the daily maintenance requirements of the animals. Every morning before feeding during the collection period, the diets were sampled for bulking and subsequent laboratory analysis. Similarly, any refusals were collected, weighed and kept for bulking and sampled for laboratory analysis at a later stage. Water was available to the animals continuously throughout the experiment.

5.2.3. Feed composition

The feed composition of the four diets is described in **Table 5.1** below.

Table 5.1. Composition of the diets by individual ingredients (% DM).

Description	**DIETS**			
Ingredients (%)	**D1(control)**	**D2**	**D3**	**D4**
Citrus pulp	0.00	7.60	16.60	27.20
Poultry litter	11.40	12.60	13.90	15.10
Wheat straw	34.10	30.30	25.00	18.20
Silage	**45.50**	**50.50**	**55.50**	**60.50**
Lucerne	27.50	25.00	22.50	20.00
Wheat bran	20.00	17.50	15.00	12.50
Molasses	5.00	5.00	5.00	5.00
Minerals	2.00	2.00	2.00	2.00
Total	**100.00**	**100.00**	**100.00**	**100.00**

The proportion of basal silage in the diets on DM basis ranged from the lowest 45.5% in Diet D1 to the highest 60.5% in Diet D4 and constituted the major proportion of each of the diets. Therefore, on individual ingredient basis the proportions of wheat straw, poultry litter and citrus pulp in the diets were slightly lower than in the basal silage. These proportions, in addition to those other non-ensiled ingredients used in the ration are shown in **Table 5.1**. The chemical composition of the diets is shown in **Table 5.2** while that of lucerne and wheat bran, the ingredients used in balancing the diets, is shown in **Table 5.3.**

5.2.4. Sample collection

Faeces were collected twice a day just before each feeding. The faecal output of each sheep was collected into a polyethylene bag attached to canvas bag held by the harness of each sheep as described by Fontenot and Hopkins (1965). The total daily faecal output from each sheep was weighed then mixed thoroughly and a 10% portion of the faeces dried at 65 °C for a minimum of 24 hr and bulked for each individual animal over the 7 days collection period. Later the dry bulked faecal samples were mixed thoroughly and sub-sampled for various chemical analysis. Urine from each sheep was collected into a 4 litre plastic container to which 50 ml of glacial (concentrated) acetic acid had been previously added to ensure that NH_3-nitrogen was not lost through volatilisation. For each sheep 10% of the daily urine output was retained and stored at -20 °C. After the 7 days collection period the urine was bulked, mixed thoroughly and subsampled for later nitrogen analysis.

Table 5.2. Chemical composition (g/kg DM) of diets used in the *in vivo* digestibility study.

Description constituents (g/kg)	Treatment Diets. D1(control)	D2	D3	D4	SEM
Dry matter	487.1	427.5	411.4	282.9	0.37
Organic matter	865.9	861.3	867.2	839.4	4.35
Nitrogen	17.0	16.7	15.3	14.8	1.20
NDF	513.0	513.4	501.7	459.8	3.27
ADF	291.4	300.8	315.4	305.2	4.33
Ash	134.1	138.7	132.8	160.6	4.15

Table. 5.3. Chemical composition (g/kg DM) of lucerne and wheat bran used to balance citrus pulp silage based diets.

Description	Lucerne	Wheat bran
composition(g/kg)		
Dry matter	921.1	931.6
Organic matter	891.3	948.0
Nitrogen	28.8	23.4
NDF	329.0	572.2
ADF	243.3	158.3

5.2 5. Chemicals analyses

5.2.5.1. Proximate analysis of the feed, faeces and refusals

Diets and refusals were dried at 80 °C for 24 hr and left at room temperature for 1 hr to equilibrate with the moisture content of the air. These dry samples were ground in a Wiley mill to pass through a 1 mm screen. The dry samples of feed, faeces and refusals were used for the determination of DM, OM, ash and nitrogen content following the standard procedures of AOAC (1990). Similarly, the nitrogen content of the urine was determined following the same standard methods. The NDF, ADF and ADL contents of diets, faecal and refusal samples were determined by the detergent procedure of Goering and Van Soest (1970).

5.2.5.2. Energy content

The gross energy of diets, faeces and refusals were determined following the standard procedures described by (AOAC, 1990). The gross energy (GE) value of the silage samples was used to estimate the metabolisable energy (ME) of the basal silage according to the formula proposed by Thomas (1990), using the *in vitro* digestibility values of the samples.

5.2.5.3. *In vivo* digestibility

The *in vivo* digestibility of DM, OM, NDF, ADF, energy and nitrogen for the four diets was determined using data collected from each of the 12 sheep that had been randomly allocated to each of the four diets. The *in vivo* apparent digestibility coefficients of DM, OM, NDF, ADF, nitrogen and energy were calculated by expressing the difference between the content of the nutrient (DM basis) in the daily food intake and its content in the total daily faecal output as a percentage of its daily intake.

5.2.5.4. Nitrogen balance

The nitrogen balance of each sheep fed with the four diets containing wheat straw, poultry litter and citrus pulp basal silage was determined by measuring differences between total nitrogen intake and nitrogen voided (excreted) through both faeces and urine. Nitrogen contents in diets, refusals, faeces and urine were determined by the standard methods of AOAC (1990).

5.2.5.5. Statistical analysis

The data for the *in vivo* digestibility of DM, OM, NDF, ADF, N and DE of the four groups of sheep were tested by one-way analysis of variance on mean values of individual sheep from the 7 day measurement period. All statistical analysis of the data was performed using Genstat 5 (Genstat 5 Committee, 1987). Where F-values suggested significance, Duncan's multiple range test (Duncan, 1955) was used to identify the means that were significantly different at 5% level.

5.3. RESULTS

5.3.1. Feed intake and *in vivo* digestibility

Intake and digestibility of the diets are shown in **Table 5.4.** It is shown from the animal data results that sheep on diet D3 which had 55.5% basal silage including 16.6% citrus pulp had the highest daily dry matter and organic matter intakes at 887.7

and 782.2 g/day respectively. Diet D4 with the highest proportion of both basal silage (60.5%) and citrus pulp (27.2%) had the lowest OM intake of all the four diets at 550.2 g /day. The OM intakes of diets D1 and D2 were 736.8 and 743.5 g/day respectively and were not significantly ($p>0.05$) different. A similar trend was observed in both NDF and ADF where intakes increased from a low level in diet D1 with 0% citrus pulp to the highest level in D3 with 16.6% citrus pulp. However, the intake finally decreased to a level lower than that of diet D1 when the proportion of citrus pulp in diet was 27.2%, thus resulting in diet D4 having the lowest intake of all the four diets. There was a significant increase in actual silage DM intake from 383.4 g/day in diet D1 to the highest level of 492.7 g/day in diet D3. However, the intake observed in diet D4 (377.5 g/day) was lower ($p<0.05$) than that of diet D2 and D3.

The OM digestibility increased significantly ($p<0.05$) from about 58% in both diets D1 and D2 to 65% in D3 and 64% in D4. The digestibility of both NDF and ADF showed a trend that was very similar to that of OM. Overall the results (**Table 5.4**) showed that diet D3 had the highest digestibility of OM, NDF and ADF for the sheep. These digestibility levels were only slightly higher but not significantly ($p>0.05$) different from those of sheep on diet D4 with 60.5% silage including 27.2% citrus pulp. The results in **Table 5.4** also show that sheep on diet D3 at 9.71 MJ/day had the highest digestible energy intake among the sheep on the four diets, while D4 at 6.80 MJ/day had the lowest.

Table. 5.4 Mean intake and digestibility of nutrients in the wheat straw, poultry litter and citrus pulp silage based diets for Merino sheep[1].

Description	**Treatment Diets**					
	D1 (control)	**D2**	**D3**	**D4**	**SEM**	**signif.**
Intake						
Silage DM(g/d)	383.4^{c}	430.2^{b}	492.7^{a}	377.5^{c}	9.26	***
Diet DM (g/d)	842.7^{b}	851.8^{b}	887.7^{a}	618.7^{c}	16.05	***
g DM/kg $W^{0.75}$/d	46.8^{a}	45.7^{a}	46.8^{a}	35.5^{b}	1.65	*
OM (g/d)	736.8^{a}	743.5^{a}	782.2^{b}	550.2^{c}	14.22	***
NDF (g/d)	426.6^{a}	445.2^{a}	443.9^{a}	298.2^{b}	16.45	**
ADF (g/d)	237.9^{b}	259.3^{a}	276.7^{a}	185.4^{c}	8.63	**
Nitrogen (g/d)	14.97^{a}	14.39^{a}	14.35^{a}	11.46^{b}	0.63	*
DE (MJ/day)	8.44^{b}	8.69^{b}	9.71^{a}	6.80^{c}	0.16	***
Total Diet Apparent Digestibility (%)						
Dry matter	53.76	53.74	58.20	57.68	1.17	NS
Organic matter	58.47^{b}	58.38^{b}	65.02^{a}	64.05^{a}	1.20	*
NDF	45.16^{b}	46.68^{b}	53.73^{a}	51.55^{a}	1.00	**
ADF	39.37^{b}	42.18^{b}	52.09^{a}	49.62^{a}	0.83	***
Nitrogen	68.33	70.51	68.95	68.27	1.99	NS

[1] Each value represents the means of three samples.

Significance of difference : NS = not significant, *($p<0.05$); **($p<0.01$); ***($p<0.001$).

Means within rows with different superscripts differ significantly ($p<0.05$).

5.3.2. Nitrogen balance

Table 5.5. Nitrogen (N) balance studies in Australian Merino wethers fed basal diet containing wheat straw, poultry litter and citrus pulp silage[1].

Description	Diet				statistics	
	D1	D2	D3	D4	SEM	sign.
N-value						
intake (g/d)	14.97^a	14.39^a	14.35^a	11.46^b	0.63	*
excretion (g/d)						
in urine	10.54^{ab}	10.80^a	9.34^{bc}	8.55^c	0.37	*
in faeces	4.68	4.24	4.01	3.64	0.28	NS
Balance (g/d)	-0.25^b	-0.65^b	0.96^a	-0.73^b	2.83	*

[1] Each value represents the means of three samples.
Significance of difference : NS = not significant, *(p<0.05).
Means within rows with different superscripts differ significantly (p<0.05).

The nitrogen intake of sheep on diet D4 was significantly lower than that of diets D1, D2 and D3 (**Table 5.5**). There was a significant difference between diets in urinary nitrogen output with diets D1 and D2 having a higher (P<0.05) level than D4. However, there was no significant (p>0.05) difference [illegible] in faecal nitrogen loss. Except sheep on diet D3 which had a significantly (p<0.05) higher and positive nitrogen balance, all the other three diets had negative nitrogen balances. There was no significant difference between the nitrogen balance levels of diets D1, D2 and D4. more meaningful results would have been achieved if faecal collection had exceeded seven days.

5.4 DISCUSSION AND CONCLUSIONS

5.4.1. Dry matter and its effect on intake

The low DM content of diet D4 appeared to be linked to the high moisture content of the silage (T4) which formed the basal part of that diet and this could have influenced its intake. Jackson and Forbes (1970) reported that the dry matter content is a factor that could influence the voluntary intake of silage diets and that for maximum voluntary intake, it was desirable that the DM content of silage or silage based diets be in the range of 300-350 g/kg. The results on the chemical composition of the diets in **Table 5.2** shows that it was only diet D4 which had a dry matter content that could be considered to be well below this limit and this may have affected the intake of this diet by the animals.

5.4.2. Digestibility and intake of the diets

The intake of the diets increased with digestibility and this appeared linked to the level of citrus pulp in the diets. However, increase in proportion of citrus pulp from 16.6% in diet D3 to 27.2% in D4, resulted in a substantial and significant decrease in both DM and OM intakes to a level that was surprisingly the lowest in all the four diets. This was in spite of only a slight reduction in organic matter digestibility from 65% to 64% which was not significant. It was possible that low rumen fermentation and therefore low digestibility could have restricted intakes of diets D1 and D2, both of which had relatively low levels of citrus pulp compared to D3 and D4 (**Table 5.4**). Low digestibility of fibre results in lower rate of clearance in the reticulo-rumen and this is likely to translate into lower intake, particularly if the rumen distension (gut fill) is the main factor limiting the intake (Mertens and Ely, 1979, 1982).

The results of this study are in agreement with the findings of Ayalwar *et al.* (1993) who also observed relatively high digestibility and intake in cattle fed on straw ensiled with orange pomace. The introduction of readily digestible energy source in the form of citrus pulp in diets high in fibre improves cellulose digestion, which results in higher dry matter intake (Silva *et al.* 1989).

5.4.3. Fermentation quality and its effect on the intake of silage based diets

An important factor that could have contributed to an increase in voluntary intake by sheep from diet D1 to D3, was the apparent increase in fermentation quality of the basal silage with increasing levels of citrus pulp. This was indicated by an increase in the level of titratable acidity and decrease in pH. It has been reported in literature that voluntary intake of silage generally increases with increase in its fermentation quality, although other factors such as palatability, chop length and animal eating behaviour may also play an equally important role (Wilkins, 1981; Chamberlain, 1987).

It was observed that sheep on diet D4 had a substantially lower intake of DM, OM, NDF and ADF than sheep on diet D3 in spite of both diets having digestibility coefficients that were not significantly different (**Table 5.4**). It has been reported that intake of silage or silage based diets is not always consistently and closely related to digestibility (Wilkins *et al.* 1971; Aston *et al.* 1994), which is in contrast to the case in fresh or dried forage where there is an established relationship between intake and digestibility (Blaxter and

Wilson, 1962; Taylor, 1971). The reason for this deviation in silage may partly be due to confounding of digestibility and fermentation quality of silage which could result in other factors such as products of fermentation influencing intake (Thomas *et al.* 1981; Wilkins, 1981; Gill *et al.* 1981). Although the pH of the diets was not determined, it was quite possible that diet sheep on D4 could have had a low pH compared to the other three diets having contained the highest proportion of basal silage (60.5%) which had a relatively low pH and a very high acidity level. Moreover, compared to the other three diets, D4 also had the lowest proportion of the other non-ensiled ingredients, such as lucerne and wheat bran that were used in the ration with the basal silage. This could have made the animals on this diet more exposed to the direct effects of the basal silage and this may have negatively affected its intake. Wilkins *et al.* (1971) and Gill *et al.* (1988) reported that low intake of silage diets was associated with extensive fermentation of silage with low pH, high concentration of organic acids and a high proportion of NH_3-nitrogen in total nitrogen.

[illegible] The intake levels of sheep on the other three diets were low. While sheep in the 55 kg live weight range used in the study could have been expected to consume over 900 g DM per day, only the animals on diet D3 had an intake that approached this level, having consumed approximately 887.7 g/day. This may have contributed to the better performance in nitrogen balance in animals on this diet compared to the poor performance of their contemporaries on the other three diets.

5.4.4. Digestible energy intake

The high digestible energy intake of diet D3 may be attributed to the relatively high OM intake and digestibility that was associated with this diet (**Table 5.4**). However, the low DE intake of diet D1 compared to D3 may have been due to the relatively high level of straw in the diet (34%) compared to 25% in diet D3. This may have depressed both digestibility and OM intake of that diet (D1). It has been reported that in mixed diets incorporating straw, the proportion of straw needs to be within the range of 20-30%, as levels above 30% tend to depress digestible energy intake through reduction of OM digestibility and intake (O'Donovan and Ghadaki, 1973; O' Donovan, 1983). In the present study, OM and digestible energy intake were positively correlated (r=0.90).

The low digestible energy intake of diet D4, in spite of its high digestibility illustrate clearly that digestibility is just one of the many factors involved in the complex issue of food intake in animals. This also shows that high digestibility alone, does not necessarily result in high OM intake (Taylor and Aston, 1976). The four diets were formulated to supply the animals with approximately 10 MJ DE/day (8.30 MJ ME) considered adequate to meet energy requirements (NRC, 1985). However, the DE intake results (**Table 5.4**) showed that only animals on diet D3 had a satisfactory DE intake while those on diets D1, D2 and D4 generally had lower than the recommended level. This may have contributed to the loss in body weight in animals on these three diets compared to the weight gain and therefore better performance for those on diet D3. This indicates that any dietary manipulations involving low quality roughage should aim at maximising digestible energy intake in ruminants through increase in both digestibility and intake of organic matter.

5.4.5. Nitrogen balance of the silage based diets

Diet D4 compared to the other three diets had a relatively low nitrogen intake and this may have been due to the low OM intake that was associated with this diet. Considering that the nitrogen intakes of diets D1, D2 and D3 were not significantly different, it is apparent that the relatively low total nitrogen excreted in diet D3 may have contributed to the positive nitrogen balance that was observed in this diet. Though diet D4 had the lowest level of total excreted nitrogen, it also had the lowest nitrogen intake of the four diets. The nitrogen intake of diets D1, D2 and D3 were all within the recommended maintenance level of 14.2 g/day for sheep in the 50-55 kg weight range (NRC, 1985). However, diet D4 had a daily nitrogen intake that was below the recommended level and this may have resulted in low and negative nitrogen balance observed in this diet (**Table 5.5**).

There was a significant difference between the diets in nitrogen excreted through the urine, with diets D1 and D2 having the highest and D4 the lowest. It was apparent that a relatively high proportion of nitrogen voided (70%) was urinary compared to only 30% via the faeces. This could have been due to the nature of the diets used in the study, particularly their nitrogen component. The diets were based on silage which included poultry litter as one of the ingredients and therefore had a high level of non-protein nitrogen due to the uric acid content in the poultry litter. Moreover, the results in **Table 4.3** showed that the 60 days ensiling period (time effect) had increased significantly the level of soluble nitrogen in silage compared to the same material prior to ensiling.

Intake of diets based on such silage with relatively high levels of degradable intake protein could result in rapid release of NH_3-nitrogen in the rumen. When excess ammonia is absorbed into the blood, it is then converted to urea and excreted in the urine, which results in wastage of nitrogen (Waldo, 1968). It has been reported that relatively high rumen ammonia levels are normally associated with consumption of silage based diets (Fantianoff *et al.* 1966; Durand *et al.* 1968). Some workers have also reported on the generally low efficiency of nitrogen utilisation in silage (Wilkins, 1981; Chamberlain and Choung, 1993), particularly those high in low quality roughage, characterised by low digestibility and containing high levels of non-protein nitrogen in readily soluble forms (Morgan *et al.* 1980).

The relatively high urinary nitrogen in the diets D1 and D2 both with relatively low levels of citrus pulp (0 and 7.6%) may have been due to low availability of digestible energy, leading to low capture of NH_3-nitrogen in the rumen. The capture of NH_3-nitrogen in the rumen by the rumen microbes tend to be limited by the OM fermented and therefore supply of energy (Sinclair *et al.* 1995). This could hamper the utilisation of nitrogen in silage based diets resulting in high urinary nitrogen loss and low nitrogen retention levels. It is apparent from the present study that diet D3 with 16.6% citrus pulp had a higher level of available energy for microbial protein synthesis, given its relatively high OM digestibility and intake. This may have resulted in better utilisation of released NH_3-nitrogen in the rumen and a therefore positive nitrogen balance. Pinzon and Wing (1976) observed lower rumen ammonia levels in steers fed pelleted or unpelleted diets containing 38 or 55% citrus pulp compared to those on a diet with 0 or 19%.

5.4.6. Energy supplements

To reduce the inefficiency in the use of nitrogen in the rumen and therefore improve the nitrogen balance, most workers have indicated that silage diets need to have adequate levels of readily digestible carbohydrate energy sources. This could be useful in matching the energy supply to the rapidly released NH_3-nitrogen from the silage in the rumen. This could increase efficiency in the utilisation of silage NPN for microbial protein synthesis (Conrad and Hibbs, 1968). Recently, Sinclair *et al.* (1995) reported that microbial nitrogen yield in the rumen was more efficient when dietary energy and nitrogen supply were synchronised on an hourly basis. Given that rumen microbial fermentation can supply up to 70-100% of ruminants amino acid requirements (AFRC, 1992), any improvement in the synchronisation of energy and nitrogen from silage diets in the rumen could result in a higher nitrogen retention.

5.4.7. Effect of poultry litter on the intake of the diet

The results on proximate analysis (**Table 5.2**) showed that diet D4 had a significantly higher ash content than the other three diets. This could have been due to the relatively higher proportion of poultry litter that was present in that diet (15%), a factor that could have affected the intake. Downs *et al.* (1996) in a study on dried poultry waste as a source of degradable intake protein reported that inclusion of this product at 14% DM basis in the finishing diets of yearling steers resulted in diminished performance in average daily gain. This was attributed to low intakes due the replacement of organic matter, the main source of dietary energy with minerals arising from the high ash content normally present in poultry waste.

Poultry litter even of the best quality has been reported as being generally unpalatable to ruminants particularly when present in the diet in relatively high proportions (Muller, 1980; Bagley *et al.* 1996). Low palatability can have a negative influence on the intake of a diet incorporating poultry litter as one of the ingredients. However, it was expected that somehow this could be alleviated by ensiling, as this process has been reported to improve palatability (Kamra and Srivastava, 1991; Lober *et al.* 1992). In addition, a 5% molasses was included during ration formulation to improve palatability. The combination of these two measures appear to have enhanced acceptability of diets, with the exception of diet D4, which had a very low intake compared to the other three diets.

5.4.8. Conclusions

The results of this study indicate that wheat straw, a fibrous crop residue of low quality could be ensiled successfully with citrus pulp, an agro-industrial by-product and poultry litter as a nitrogen and mineral source. This produced a silage product of high fermentation quality and an average feeding value for ruminants. The implication of these results are that up to 30-45% fresh citrus pulp, may be used as a source of readily fermentable carbohydrate source to promote favourable fermentation of silage incorporating about 45% wheat straw and approximately 25% poultry litter. In addition, citrus pulp as a good source of readily digestible energy and poultry litter as source of nitrogen and minerals could be useful ingredients to alleviate the nutrient deficiencies of wheat straw. This could substantially improve the utilisation of wheat straw by ruminants, particularly in areas with feed resource constraints.

Moreover, the utilisation of by-products like citrus pulp and animal wastes such as poultry litter in combination with low quality roughage, would not only provide the much needed nutrients for ruminants, but will also help to solve the disposal and pollution problems associated with these materials.

CHAPTER SIX

GENERAL DISCUSSIONS, CONCLUSIONS AND FUTURE STUDIES

6.1. GENERAL DISCUSSIONS AND CONCLUSIONS

Low quality roughage such as fibrous crop residues form an important feed resource for supporting ruminant livestock production. However, despite their abundant supply and representing a large reservoir of nutrients, particularly energy, low quality roughage are largely an under-utilised feed resource, mainly due to their low digestibility by ruminants.

Attempts to improve digestibility and intake of low quality roughage has attracted interest due to the potential benefit it promises to offer, in increasing the availability of nutrients to support ruminant livestock production. Different approaches have been tried that include the various forms of physical, chemical and biological treatments (Sundstol, 1978). These reduce the lignin content or modify the covalent linkages between lignin and structural polysaccharides such as hemicellulose and cellulose which increases accessibility and susceptibility of these cell wall polysaccharides to rumen microbial enzymes, thereby resulting in increased digestibility (Lam *et al.* 1992; Lowry and Kennedy, 1996). The supplementation of low quality roughage with readily digestible carbohydrate and nitrogen sources, minerals and some vitamins that are normally deficient in these roughage, also increases digestibility and intake of organic matter significantly, mainly through increased rumen microbial fermentation.

The presence of readily digestible carbohydrates in citrus pulp could improve fermentation quality, digestibility and therefore intake of silage, when this by-product is ensiled with wheat straw and poultry litter as a nitrogen and mineral source.

This study investigated the ensiling of wheat straw, a fibrous crop residue and poultry litter with various levels of citrus pulp without or with 5% molasses. The fermented product was evaluated for both fermentation quality and potential feeding value to ruminants in *in vitro*, *in sacco* and *in vivo* digestibility studies. The results showed that molasses at either 0 or 5% did not have any significant effect on the pH, however, there was a small but significant effect on the silage acidity. Similarly, molasses had no significant effect on *in vitro* OMD or fibre content as indicated by NDF, ADF and ADL. Molasses is low in DM and contains virtually no fibre and this may have been the reason why it had no significant effect on the silage fibre content and IVOMD. However, the 5% molasses reduced significantly the content of volatile fatty acids.

Molasses is widely used in ensiled materials both as a fermentation stimulant and a nutrient, due to its high content of readily fermentable substrates, which are mainly in the form of sucrose (Thomas, 1978; Gordon, 1989). These substrates are normally fermented rapidly to lactic acid by the homofermentative lactic acid bacteria such as *Lactobacillus plantarum*, resulting in a low and stable pH that preserves the silage effectively (Kamra and Srivastava, 1992). In ensiling materials such as crop residues and poultry litter that are naturally low in readily fermentable substrates, the use of molasses would appear appropriate. Moreover, presence of poultry litter as an ingredient could be expected to make the ensiled material resist a fall in pH due to the high buffering capacity.

It seems no advantage was gained by the addition of molasses to the ensiled material when 30-45% citrus pulp was used. However, it was apparent that when wheat straw and poultry litter were ensiled without or with very low level of citrus pulp such as 15%, it may prove advantageous to add molasses at a certain level to stimulate and promote favourable fermentation.

Citrus pulp had a highly significant effect on most factors that were considered in the evaluation of both the silage fermentation quality and potential feeding value. Increase in the level of citrus pulp from 0% to 45%, resulted in a significant increase in silage acidity from 30 to about 100 mM/100 gm DM irrespective of the silage having either 0 or 5% molasses. This lead to a significant fall in pH from 5.87 to 4.50 for silages with 0 to 45% citrus pulp respectively. Silage with 30 and 45% citrus pulp had pH 4.12 and 4.13 respectively and were not significantly different, therefore suggesting similar fermentation quality.

Prior to ensiling, it was observed that the presence of 0, 15, 30 and 45% citrus pulp in the pre-mix of wheat straw and poultry litter resulted in a significant fall in pH to 6.85, 6.13, 5.54 and 5.32 respectively. This decrease in pH even before any fermentation had taken place could be attributed to the direct effect of organic acids such as citric acid that are naturally present in high levels in citrus pulp (Caro *et al.* 1990). In consistent with findings of other workers (Cervera *et al.* 1985; Yang and

Choung, 1986 and Caro *et al.* 1990) this study also showed that citrus pulp had a high acidity level of 20 mM/100 g DM and a relatively low pH 4.16.

The ensiling of wheat straw and poultry litter with citrus pulp for 60 days and the associated fermentation process resulted in an increase in titratable acidity, decrease in pH and complete elimination of all coliforms. This indicated an increase in silage fermentation quality and therefore a positive effect of the ensiling process. However, the process was also associated with a significant loss of DM and OM, in addition to an increase in both soluble nitrogen and ash content. These changes tend reduce the fermentation quality and feeding value of the final silage compared to the same material prior to ensiling.

Besides improving the fermentation quality, citrus pulp also had a significant beneficial effect on the potential nutritive value of the wheat straw and poultry litter silage. This was evident from the results of both *in vitro* and *in sacco* digestibility studies. In the *in vitro* digestibility, the results showed that the OM digestibility of the silage increased significantly from a rather low level of about 33% to about 56% for silage with 0% and 45% citrus pulp respectively. The *in sacco* digestibility results showed a 96 hour DM degradability that increased by 24% from 470 g/kg (47%) to 581.4 g/kg (58%) for silage with 0% and 45% citrus pulp respectively. These results, show a significant improvement in the potential nutritive value for ruminants of the wheat straw, poultry litter and citrus pulp silage.

In the *in vivo* digestibility and nitrogen balance study in sheep it was observed that digestibility also increased with levels of citrus pulp in the diet. Although the presence of other non-ensiled ingredients in the diets may have variably contributed to the observed increase in OM digestibility, citrus pulp may have contributed a significant part in its double role of improving both silage fermentation quality and increasing digestibility. Citrus pulp is normally high in readily digestible non-starch polysaccharides and organic acids such as citric acid. These could provide substrates in adequate amounts that have the potential of promoting high silage fermentation quality and also enhancing rumen microbial fermentation, which may result in higher digestibility and therefore improved organic matter intake.

In the *in vivo* digestibility study, OM digestibility increased significantly from 58% in diets with 0 & 7.6% citrus pulp to 65 and 64% in diets with 16.6% and 27.2% citrus pulp respectively, suggesting an increase that was related to the level of citrus pulp. The OM intake also increased significantly with increase in the level of citrus pulp up to diet D3 which had an equivalent of 16.6% citrus pulp. However, the increase in citrus pulp above 16.6% in the diet appeared to reduce intake significantly. It was not known exactly why animals on the diet with the highest level of citrus pulp had such low intake, yet results on fermentation quality, *in vitro* and *in sacco* digestibility studies all suggested a relatively high potential feeding value for this silage or diet based on it. However, intake of silage based diets is often complex and variably influenced by a wide range of physical and biochemical factors (Wilkins *et al.* 1971). It was concluded that the low intake of the diet with the highest level of citrus pulp may have been due to a number of factors that could include a high proportion of poultry litter and moisture content and low levels of other non-ensiled ingredients such as lucerne and wheat bran. The low intake may have been an important factor contributing to the low and negative nitrogen retention in animals on this diet.

All the four diets were formulated to supply the animals with energy (10 MJ DE/d or 8.3 MJ ME) and nitrogen (14.30 g/d) for daily maintenance requirements (NRC, 1985). It was observed that only animals on diet D3 had a satisfactory intake of both energy (9.71 MJ DE) and nitrogen (14.35 g), while those on diet D4 had the lowest intake of both nutrients. This may have been the reason for the animals on diet D3 having a positive nitrogen retention compared to the poor performance of animals on diet D4. While the nitrogen intakes of animals on diets D1 and D2 were generally satisfactory, their DE intake were well below the recommended level of 10 MJ DE/day. This could have been the reason for their dismal performance in nitrogen retention.

It is therefore concluded from this study that when wheat straw is ensiled with poultry litter and relatively high levels of citrus pulp, use of molasses offers no significant benefit in fermentation quality, in spite of the cost associated with its use. When citrus pulp is included at relatively high levels of about 30-45%, it may stimulate favourable fermentation that reduces the pH of the ensiled material to a low and stable level. Such a level is normally adequate to effectively eliminate most of the potentially pathogenic micro-organisms, such as coliforms that are normally high in poultry waste based materials. However, when such silage forms the basal part of a ruminant diet in significant proportions, it may still be necessary to include some molasses in a moderate level such as 5% or some other suitable non-ensiled ingredients to improve on acceptability and therefore intake as a prerequisite for higher performance in ruminants.

6.2 FUTURE STUDIES

The present study established that ensiling wheat straw and poultry litter with 30% citrus pulp produced a basal silage that resulted in positive but low nitrogen retention. That it was possible to achieve such a performance partly from materials largely considered as by-products or 'waste' indicates the potential these materials have as feed resources for ruminants. However, more studies are needed to improve further on the performance of animals feeding on diets based on these materials, so as to exploit the full potential of these by-products.

The silage containing 45% citrus pulp compared to one with 30% had an almost similar fermentation quality and an even better potential feeding value, based on the results of pH determination, *in vitro* OMD and *in sacco* DM degradability. However, animals on the diet based on this silage had disappointingly very low intake of both organic matter and digestible energy and as a consequence, poor performance in nitrogen retention. The main cause of the low intake of a silage perceived to be of high fermentation quality needs further study. It is therefore suggested that further investigation be done on this aspect so as to provide more details on the depression of intake in cereal straw silage containing high content of citrus pulp, given its relatively high fermentation quality and potential nutritive value.

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