UTILISATION OF ROSEELLE (HIBISCUS SABDARIFFA, L.)
BY-PRODUCTS AS ROUGHAGE FEED FOR SHEEP

TRI HESTI WAHYUNI

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA
2000
UTILISATION OF ROSELE (HIBISCUS SABDARIFFA, L.)
BY-PRODUCTS AS ROUGHAGE FEED FOR SHEEP

By

TRI HESTI WAHYUNI

Thesis Submitted in Fulfilment of the Requirements for the Degree
of Master of Science in the Faculty of Agriculture
Universiti Putra Malaysia

September 2000
DEDICATION

Dedicated especially to my beloved husband, Ahmad Armijn Nasution, SH whose sacrifice and support has enabled me to complete this study successfully and to my lovely daughters and son, Windha Arresti Hartanty, Thia Ayu Armindasari and Ahmad Gunawan Muttaqien, whom I love most.
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Agriculture

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Chairman: Associate Professor Abdul Razak Alimon, Ph.D.

Faculty: Agriculture

Roselle (Hibiscus sabdariffa, L.) is cultivated for the calyces, which are
used for making roselle juice, a drink known to be high in vitamin C. The leaves
are also used as a pot herb and some varieties are grown for their fibre. Roselle
pods and seeds are by-products obtained after the calyces are removed from the
fruits. The objective of the study was utilised the roselle by-products for small
ruminant especially as sheep feed. Roselle by-products were obtained from
farmers in Terengganu. In the first part of the study, chemical analysis was
performed on the whole pods, seeds and empty pods. The roselle by-products
were found to be high in protein, fat and fibre. The nutrient content of roselle by-
products was higher than other agricultural by-products, such as sago waste,
straw, cocoa pods, stalks and sugar cane bagasse.

In the second part of the study, in situ degradability of roselle empty pods
and seeds was investigated. The result indicated that degradation of dry matter
and organic matter of roselle seeds was higher than roselle empty pods,
significantly different (P< 0.01) in 48 hours, percentage of DM and OM of roselle seeds and empty pods were 36.38%, 28.36% and 23.01%, 17.66%, respectively; and also in 72 hours by 39.87%, 31% and 26.30%, 20.85%, respectively. Even though the seeds were more degradable than the empty pods, both were still low in degradability (< 50%). Degradability of roselle by-products can be improved by using chemical treatment, physical treatment or microbial treatment.

In the third part of the study, roselle by-products were treated with soaked alkali such as NaOH and Ca(OH)₂ at various levels (2%, 4% and 6%), and the nutrient contents analysed. It was found that the chemical composition of roselle treated with NaOH and Ca(OH)₂ decreased, except for ash.

In the fourth part of the study, feed intake, ADG and digestibility of untreated whole pod roselle and treated whole pod roselle with soaked NaOH 2% and Ca(OH)₂ 4% were used. It was found that total dry matter intake (TDMI) of untreated whole pods roselle and those treated with NaOH 2% and Ca(OH)₂ 4% were 25.22 ± 5.62%, 31.03 ± 4.67%, 36.11 ± 6.88% and total organic matter intake (TOMI) of untreated whole pods roselle and those treated with NaOH 2% and Ca(OH)₂ 4% were 22.64 ± 5.02%, 26.32 ± 3.95%, 30.53 ± 5.93%, respectively; and ADG (g/day) was 10.95 ± 2.56, 18.57 ± 2.88 and 15.95 ± 3.24, respectively. OM and DM digestibility of treated whole pods roselle was increased by 35.65% and 56.71% by 2% NaOH, and 14.04% and 14.43% by 4% Ca(OH)₂. NDFD was increased by 23.18% and 71.18%, ADFD 24.74% and 88.10%, and also DE 26.12% and 27.47%, respectively.
Based on this study, it can be concluded that treated roselle by-products are potential alternative roughage for small ruminants, but these by-products cannot be used as a sole feed in the diet.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGGUNAAN HASIL SAMPINGAN ROSELLE (HIBISCUS SABDARIFFA, L.) SEBAGAI PEMAKANAN SERAT UNTUK BEBIRI

Oleh

TRI HESTI WAHYUNI

September 2000

Pengerusi: Profesor Madya Abdul Razak Alimon, Ph.D.
Fakulti: Pertanian


Analisis kimia telah dilakukan untuk mendapatkan kandungan proksimat. Hasil sampingan roselle didapati mengandungi protein, lemak dan serat yang tinggi berbanding dari kebanyakan hasil sampingan pertanian yang lain, seperti hasil sampingan sago, batang padi, kulit coklat, batang jagung dan ampas tebu. Dalam bahagian kedua kajian ini pula penghadaman secara in situ telah dilakukan

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pada kulit dan biji roselle. Keputusan ujian tersebut mendapatkan penghadaman bahan organik (OM) dan bahan kering (DM) biji roselle lebih tinggi daripada kulit perbezaanya adalah berarti. (P < 0.01) pada penghadaman selama 48 jam masing-masing 36.38%, 28.36%; 23.01%, 17.66% dan 72 jam, 39.87%, 31%; 26.30%, 20.85%. Walaupun penghadaman biji lebih tinggi daripada kulit, penghadaman kedua ini masih rendah (< 50%). Penghadaman hasil sampingan roselle dapat ditingkatkan dengan menggunakan uji kaji kimia, fisik atau mikrobi.

Dalam bahagian ketiga kajian ini, sisa hasil sampingan roselle telah diuji dengan menggunakan peraturan yang berbeza (2%, 4% dan 6%) NaOH dan begitu juga dengan Ca(OH)₂. Analisa kimia telah dibuat untuk mendapatkan kandungan proksimat. Didapat kandungan proksimat ujian hasil samping roselle dengan NaOH dan Ca(OH)₂ mengalami penurunan, kecuali abu.

Dalam bahagian keempat kajian ini, kadar pengambilan makanan dan penghadaman hasil sampingan roselle diuji dengan NaOH 2% dan Ca(OH)₂ 4% dan juga tidak diuji (sebagai kontrol). Keputusan ujian mendapatkan jumlah bahan kering dan jumlah bahan organik yang dimakan dari hasil sampingan roselle yang tidak diuji, yang diuji dengan NaOH 2% dan Ca(OH)₂ 4% masing-masing 25.22 ± 5.62%, 31.03 ± 4.67%, 36.11 ± 6.88% dan 22.64 ± 5.02%, 26.32 ± 3.95%, 30.53 ± 5.93%. Pertambahan berat badan per hari masing-masing 10.95 ±2.56, 18.57 ± 2.88 dan 15.95 ± 3.24. Penghadaman bahan organik dan bahan kering menunjukkan ujian hasil sampingan roselle dengan NaOH 2% dapat
ditingkatkan menjadi (35.65% dan 56.71%) dan Ca(OH)2 4% dapat ditingkatkan menjadi (14.04% dan 14.43%). Penghadaman NDF pula dapat ditingkatkan menjadi (23.18% dan 71.18%), ADF menjadi (24.74% dan 88.10%) dan juga tenaga penghadaman meningkat kepada (26.12% dan 27.47%).

Berdasarkan atas kajian ini, boleh disimpulkan bahawa hasil sampingan roselle merupakan salah satu sumber serat bagi pemakanan ternakan. Tetapi disebabkan oleh nilai penghadamannya yang rendah, maka pemakanan tersebut tidak boleh digunakan sebagai makanan tunggal didalam ransum.
ACKNOWLEDGEMENTS

In the name of Allah The Beneficent and The Compassionate.

First of all, I would like to convey thanks and praises to The Almighty Allah for blessing and guiding me in completing this thesis. I would also like to express my appreciation and sincere gratitude to Associate Professor Dr. Abdul Razak Alimon, Chairman of Supervising Committee, Professor Dr. Hasanah Mohamad Ghazali and Dr. Che Roos Saad for their valuable guidance and advice throughout this study and in the preparation of this thesis. Also thanks are due to Professor Dr. Dahlan for his comments and recommendation.

Deep appreciation is also due to Dean of Agriculture Faculty, Professor Dr. Yusof Hussein and Head of Department of Animal Science, Associate Professor Dr. Zainal Aznam Jelani, Universiti Putra Malaysia and also Dean of Agriculture Faculty, Universitas Sumatra Utara, Medan and Rector of Universitas Sumatra Utara, Medan for allowing me to pursue my Master of Science degree in Universiti Putra Malaysia.

I would also like to thank Mr. Ibrahim bin Mohsin, Mr. Saparin bin Demin, Mr. Bakari bin Abd. Rahman and Mr. Baharum bin Utar for their technical assistance.
I wish to thank all postgraduate students particularly in the Department of Animal Science for their generous assistance, encouragement, support and humour.

Special thanks to my beloved father who was called by God, my mother, my sisters, my brothers and brothers in-law for their support and encouragement.

Finally, I wish to express my deepest gratitude to my beloved husband Ahmad Armijn Nasution, SH and lovely daughters and son, Windha Arresti Hartanty, Thia Ayu Armindasari and Ahmad Gunawan Muttakien for their support, understanding and encouragement.
I certify that an Examination Committee met on 8\textsuperscript{th} September 2000 to conduct the final examination of Tri Hesti Wahyuni on her Master thesis entitled “Utilisation of Roselle (\textit{Hibiscus sabdariffa}, L.) By-products as Roughage Feed for Sheep” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Dahlan Ismail, Ph. D.
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Abdul Razak Alimon, Ph. D.
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Hasanah Mohamad Ghazali, Ph. D.
Professor
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Che Roos Saad, Ph. D.
Faculty of Bioscience
Universiti Putra Malaysia
(Member)

\[signature\]

MOHD. GHAZALI MOHAYIDIN, Ph. D.
Professor/Deputy Dean of Graduate School
Universiti Putra Malaysia

Date: 07 NOV 2000
This thesis submitted to the senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science.

KAMIS AWANG, Ph. D.
Associate Professor
Dean of Graduate School
Universiti Putra Malaysia

Date:
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

Signature:

(Tri Hesti Wahyuni)
Date: 6 Nov. 2000
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<td>DF</td>
<td>Acid Detergent Fibre</td>
</tr>
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<td>ADFD</td>
<td>Acid detergent Fibre Digestibility</td>
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<tr>
<td>ADG</td>
<td>Average Daily Gain</td>
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<td>ADL</td>
<td>Acid Detergent Lignin</td>
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<tr>
<td>ADLD</td>
<td>Acid Detergent Lignin Digestibility</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>ARC</td>
<td>Agricultural Research Council</td>
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<tr>
<td>dm</td>
<td>Decimetre</td>
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<tr>
<td>CF</td>
<td>Crude Fibre</td>
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<tr>
<td>CFD</td>
<td>Crude Fibre Digestibility</td>
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<tr>
<td>cm</td>
<td>Centimetre</td>
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<tr>
<td>CP</td>
<td>Crude Protein</td>
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<tr>
<td>CPD</td>
<td>Crude Protein Digestibility</td>
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<tr>
<td>°C</td>
<td>Celsius Degree</td>
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<td>DE</td>
<td>Digestible Energy</td>
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<td>ha</td>
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<tr>
<td>h</td>
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<td><strong>in sacco</strong></td>
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**in vitro**

**in vivo**

Kg  
Kilogram

ME  
Metabolism Energy

ml  
Millilitre

mm  
Millimetre

NDF  
Neutral Detergent Fibre

NRC  
National Research Council

um  
Manometer

S.D.W  
Sample Dry Weight

SAS  
Statistical Analysis System

TADFI  
Total Acid Detergent Fibre Intake

TADLI  
Total Acid Detergent Lignin Intake

TCPI  
Total Crude Protein Intake

TDMI  
Total Dry Matter Intake

TNDFI  
Total Neutral Detergent Intake

VFA  
Volatile Fatty Acids
CHAPTER I

GENERAL INTRODUCTION

One factor limiting the development of animal production in tropical countries is the poor quality of forage. The low energy of forages is the main constraint, although protein supplementation is needed to obtain high performance of livestock production. From an economic point of view, using conventional feedstuff as energy or protein source is quite expensive to develop and increase animal production.

Another factor is land for forage production, which is always limited in developing tropical countries. Whether land can be spared for such use depends largely on population pressures and the need to produce staple food for humans. High human population densities are almost always associated with soil of high potential productivity, high rainfall and adequate water for irrigation systems where up to three crops a year can be cultivated. Livestock have been integrated into these systems and are usually multi-purposed in terms of providing draught power in addition to milk, meat, hides and dung for fuels and fertilisers. Such animals depend on crop residues and by-products of agro-industries for feed stuff.

The other factors, agricultural by-products from crop production, have numerous uses in both developing and developed countries. It is often stressed that these by-products should be degraded or burnt in the field in order to recycle organic matter and minerals, but the importance of this has not been defined. It
materials in excreta is more important in the small scale farming system, such as those in South East Asia, where land available for grazing is limited, and animals often compete with humans for feed resources. However, these by-products are generally characterised by low digestibility and hence low intake, low nitrogen content and unbalanced mineral content. The low digestibility of by-products is due to their high cell wall content of lignin, silica, and low supply of other essential minerals. As such, they do not meet the maintenance requirements of ruminants.

Malaysia still imports most of the concentrates used in animal rations except for small amounts available locally. The amount of animal feed required is expected to increase with the increasing rate of population growth and consequently increasing demands in livestock production. There is no natural pasture land in Malaysia other than small and scattered areas of mixed grasses and weeds on wasteland, road shoulders, fringes of rubber, coconut and oil palm estates and abandoned paddy lands (Mustaffa, 1987). Furthermore, the prospect for increasing areas sown to improve pastures is rather limited because of the high investment cost and slow return. Therefore, inadequate supply of good quality feed is one of the main constraints to ruminant production in Malaysia.

One of the alternatives to overcome this constraint is to utilise by-products from agricultural crops and agro-industry as animal feeds. It has been estimated that more than 5.0 million tonnes of agro-industrial by-products are available in Peninsular Malaysia (Mustaffa, 1987). Some of these are already commercially well utilised e.g. palm kernel cake (pko) and rice bran. It is known that thousands
of tonnes of agro-industrial by-products are being burnt or dumped into rivers and ponds causing pollution to the environment. Effective utilisation of these by-products would serve two useful purposes, e.g. reducing the rate of pollution and providing new sources of feed stuffs for livestock.

Emphasis on food and diversification in agricultural results in a number of by-products being produced such as Roselle (Hibiscus sabdariffa, L.). Roselle pods and seeds are by-products obtained after the calyces are removed from the fruits. Roselle is one of the botanical species cultivated for its pleasant red colour calyxes which are used for making a common drink called karkade (Al-Wandawi et al., 1984). It was reported by Earle et al. (1960) and also Watt and Breyer (1962) that roselle seed has high content of oil and protein. The total protein content of roselle seed is 25.20% (Al-Wandawi et al., 1984) in comparison to 20.58% reported for mature okra seed (Karakoltsidid and Constantinides, 1975), which is considered to be potentially rich in protein with a high lysine level (Al-Wandawi, 1983). Currently the by-products of roselle are of no use and are sources of pollution. Malaysia produces more than 5400 tonnes of roselle pods yearly (Mardi, 1994).
Objectives of the Study

The objectives of the study are as the following:

1. To determine the chemical composition of roselle by-products

2. To determine the dry matter (DM) and organic matter (OM) in sacco degradability of roselle by-products

3. To determine the chemical composition of treated roselle by-products at different concentrations (2%, 4% and 6%) of sodium hydroxide and calcium hydroxide.

4. To determine the digestibility, feed intake (FI) and average daily gain (ADG) of sheep fed with untreated and treated roselle by-products with NaOH 2% and Ca(OH)\(_2\) 4%.
CHAPTER II

LITERATURE REVIEW

History and General Description of Roselle

The origin of the Roselle crop is not fully known but it is believed to be from West Africa. It is also thought that Roselle originated from India (Perry, 1980) and it is now widely cultivated throughout the tropics. It has been in Asia for at least three centuries and was taken early to the New World by the slave trade, reaching Brazil in the 17th century. It was recorded in Jamaica in 1700 (Purseglove, 1976).

In Malaysia, most of the Roselle is planted commercially by small holder farmers in Terengganu on Bris soil. The planting was later expanded to some parts of Johor in the Peninsular of Malaysia.

Botanical Description

The classification of Roselle is as follows:

Common Name: Roselle

Scientific Name: Hibiscus sabdariffa, L.

Family: Malvaceae

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<th>PERPUSTAKAAN USU</th>
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<tr>
<td>No. Pengsil</td>
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<tr>
<td>Sumber</td>
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<tr>
<td>Dinonikan</td>
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</tbody>
</table>
**Figure 2.1.** *Hibiscus sabdariffa*, L.: ROSELLE.  
A. fruiting shoot (X ½); B. leaf (X ½); C. flower from side (X ½); D. flower from above (X ½); E. flower in longitudinal section (X ½); F. fruit in longitudinal section (X ½).
Plant

Roselle is an annual plant. The plant is small in size like shrubs and can grow to a height of 205 cm. Its economic life is about 6 - 10 months. There are several branches from the main stem. The stem is dark red in colour. Usually the plant will lodge at maturity unless pruning is done.

Leaves

The leaf is green in colour. At juvenile stage, the leaf is ovate but later changes to three lobes with pointed edges measuring 7 - 15 cm.

Flowers

The plant starts flowering at 45 - 60 days after planting. The flower arises singly from every axil of leaves. The flower is light yellow and pink in colour with five calyces.

Fruits

Roselle fruits are dark reds in colour. The fruits mature about 35 days after flower expansion. Each fruit contains five calyces that are fused together at the lower part of the flower. This calyx is 4 - 5 cm long.
Figure 2.2: A. Roselle Fruits; B. Roselle Whole Pods; C. Roselle Seeds; D. Roselle Empty Pods
Ecology

Roselle is suitable for tropical climate with well distributed rainfall of 1500 - 2000 mm yearly, from sea level to about ± 600 m altitude. It can tolerate warmer and more humid climate than kenaf (Duke, 1979). The plant exhibits marked photoperiodism, not flowering at shortening days of 13.5 hours, but flowering at 11 hours daylight. In the United States, the plants do not flower until the short days of late fall or early winter. Since flowering is not necessary for fibre production, long light days for 3 - 4 months are the critical factor. Roselle requires a permeable soil, a friable sandy loam with humus being preferable. However, it will adapt to a variety of soils. It is not shade tolerant and must be kept weed free. It can tolerate floods, heavy winds or stagnant water. Ranging from warm temperate moist through tropical wet to very dry forest life zone, roselle is reported to tolerate annual precipitation of 6.4 to 42.9 dm (mean at 213 cases = 17.14), annual temperature of 12.5 to 23.5°C (mean of 213 cases = 23.11) and pH of 4.5 to 8 (mean of 119 cases = 61) (Duke, 1979).

Cultivation

Land Preparation: Twice ploughing are generally practised for land preparation

Seed Requirement: The recommended rate is 19 kg/ha for roselle/kenaf and 3 kg/ha for jute.

Plant Population: Optimum plant spacing is 30 x 10 cm, with 1 plant/hill or 40 x 40 cm with 4 - 5 plants/hill. This makes up the plant population of 333000 plants/ha.
Planting Time: Generally they are planed between early April and early May depending on the rainfall.

**Fertiliser Application:** The recommended rate is 313 kg/ha of 15 - 15 - 15 fertiliser applied once right after weeding approximately 30 days after planting.

Weed Control: Hand weeding once approximately 30 days after planting or spraying of metholachlor at the rate of 1.125 - 1.5 kg /ha immediately after planting when the soil is moist for pre emergence weed control is recommended (Department of Agriculture Thailand, 1996).

**Harvesting**

The roselle is harvested for the calyces of the fruits. About three weeks after the onset of flowering, the first fruits are ready for picking. The fruit consists of large reddish calyces surrounding the small seedpods. The capsules are easily separated, but need not be removed before cooking.

**Yield**

Calyx production per plant has ranged from 3 lbs (1.3 kg) in California to 4 lbs (1.8 kg) in Puerto Rico and 16 lbs (7.2 kg) in southern Florida. In Hawaii, roselle yielded 16000 - 19000 kg/ha. Dual-purpose plantings can yield 17000 kg/ha of herbage in 3 cutting and later 6300 kg/ha of calyces (Morton, 1987). Mardi (1994) reported that in Malaysia the yield averages 12000 kg/h.
Varieties

Two botanical varieties are recognised: var. *sabdariffa*, a bushy branched subshrub with red or green stems and red or pale yellow inflated edible calyces; var. *altissima* Wester, a tall, vigorous, practically un branched plant, 10 - 16 ft high, with fibrous, spiny, inedible calyces, grown for fibre (Purseglove, 1976).

Uses

The red acid succulent calyces of var. *sabdariffa* are boiled with sugar to produce sorrel drink. They are also made into jellies, sauces, chutneys and preserves. The tender leaves and stalks are eaten as salad and as a potherb and are used for seasoning curry. The seeds contain oil and are eaten in Africa. Var. *altissima* is grown for fibre in India, Java and the Philippines.

The seeds are somewhat bitter but have been ground to a meal for human food in Africa and have also been roasted as a substitute for coffee. The seeds are considered excellent feed for chickens. The residue after oil extraction is valued as cattle feed when available in quantity (Morton, 1987).

Characteristics of Roselle

Department of Agriculture Malaysia (1999) reported the characteristics of roselle as presented in Table 1.1
Table 1.1. Roselle Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Average Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height</td>
<td>205.3 cm</td>
</tr>
<tr>
<td>No. of 10 branches</td>
<td>17.6</td>
</tr>
<tr>
<td>No. of 20 branches</td>
<td>10.2</td>
</tr>
<tr>
<td>No. of fruits/tree</td>
<td>152</td>
</tr>
<tr>
<td>Wt. of fresh fruit/tree</td>
<td>1,793 gm (19,926 kg/ha)</td>
</tr>
<tr>
<td>Wt. of fresh calyx/tree</td>
<td>1,000 gram (11,115 kg/ha)</td>
</tr>
<tr>
<td>Wt. of dry calyx/tree</td>
<td>118 gm (1,307 kg/ha)</td>
</tr>
<tr>
<td>No. of fresh fruit/kg</td>
<td>84.8</td>
</tr>
<tr>
<td>No. of fresh calyx/kg</td>
<td>152</td>
</tr>
<tr>
<td>Wt. of calyx/wt. of fresh fruit</td>
<td>56.8%</td>
</tr>
<tr>
<td>Fresh to dry ratio</td>
<td>11.8%</td>
</tr>
</tbody>
</table>

Source: Department of Agriculture Malaysia (1999).

Availability of Roselle in Malaysia

In Terengganu, 337 ha of land have been cultivated with roselle since it was introduced in 1993, while Sarawak had begun to commercialise roselle product. In Malaysia, roselle yields 12000 kg/ha (Mardi, 1994). It can be estimated that Terengganu can produce 4044 tonnes of roselle per year.
Nutritive Value of Roselle

The fruits and stems of roselle (*Hibiscus sabdariffa*, L.) which is grown for its calyces, fibres and seeds have 22% and 2.10% of protein, 3.70% and 1.90% of ether extract, 25.60% and 62.40% of fibre, 33.30% and 22% of N-free extract and 90.20% and 90.90% of DM, respectively (Abdel, 1977).

From studies with cockerels, it was reported that ground roselle fruits or mixed fruits and stems could replace 25% of concentrate (Abdel, 1977).

Samy (1980) reported that roselle seeds contained 7.60% moisture, 24% crude protein, 22.30% fat, 15.30% fibre, 23.80% N-free extract, 7% ash, 0.30% calcium, 0.60% phosphorus and 0.40% sulphur. Seed extracted with ether had 0.70% fat, 29% protein and 32.90% N-free extract.

In studies with cockerels given extracted and non-extracted seeds, digested nutrients contained 15.4% and 27.5% of protein, 22.5% and 32.5% of N-free extract with 75.8% and 68.8% of total digestible nutrients, 84.1% and 64.2% of starch value and 3184 and 2891 kcal/kg metabolisable energy, respectively.

The nutritional value of roselle seeds meal (RSM) in broiler diets had been studied by Mohammed and Idris (1991) who concluded that it can replace groundnut meal with no adverse effects on chick growth. Backeit *et al.*, (1994) showed that RSM can support satisfactory egg production when included at concentrations up to 200g kg⁻¹.
In 100 g, the fruit contains 49 calories of gross energy, 84.5% H₂O, 1.9 g protein, 0.1 g fat, 12.3 g total carbohydrate, 2.3 g fibre, 1.2 g ash, 1.72 mg Ca, 57 mg P, 2.9 mg Fe, 300 µg β-carotene equivalent and 14 mg ascorbic acid (Duke, 1984).

In 100 g, the leaf is reported containing 43 calories, 85.60% H₂O, 3.3 g protein, 0.3 g fat, 9.2 g total carbohydrate, 1.6 g fibre, 1.6 g ash, 213 mg Ca, 93 mg P, 4.8 mg Fe, 4135 µg β-carotene equivalent, 0.17 mg thiamine, 0.45 mg riboflavin, 1.2 mg niacin and 54 mg ascorbic acid (Duke, 1984). According to Morton (1987), roselle seeds contain 12% moisture, 3.29% protein, 16.80% fatty oil, and 16.80% cellulose, 15.80% pentosans and 11.10% starch.

FAO (1978) found that roselle tops and roselle leaves contain CP (30.10; 25.70), CF (10.90; 11.40), Ash (9.10; 10.60), EE (1.90; 3.30) and NFE (48; 49).

Characteristics of Fibrous Agricultural By-Products

Agricultural fibrous by-products are generally low in nitrogen and low in digestibility. Substances masking cell wall structures, which prevent action, cause their low digestibility by micro-organisms and enzymes upon the substrates within the cell. According to Mc. Manus (1982), these masking substances are lignin, hemicellulose and certain proteins. This effect is further enhanced by mineralisation of silica in the external layer of the cell wall.
To degrade substrates within the cell, the cell wall fraction needs rupturing and under normal circumstances this is achieved by chewing and grinding in the mouth of the animal. In the case of very fibrous materials, such actions have limited effect on the cell wall itself. It is thus necessary to treat the fibre so that masking substances found in cell wall are solubilised enabling micro-organisms to penetrate and act upon the carbohydrates of the cell wall (Jalaludin, 1982).

**Improvement of Nutritive Value of Agricultural By-Products**

Cellulose makes up more than 50% of the total organic carbon in the biosphere (Lehninger, 1970). It is the major component of mature grass, straw, stover, bagasse and many other plants and agro-industrial by-products. In poor quality roughages, cellulose is associated with lignin and other compounds, which make it more or less unavailable for gastro-intestinal tract microbes. Increasing dry matter intake, digestibility and higher energy intake that result in higher nutrient intake can improve animal performance. In using agricultural by-products and non-conventional roughage, high intake is very seldom achieved (Minson, 1990; Alimon, 1993). Processing of fibrous products has been considered to be an effective way to improve feed stuffs, where increased intakes can also be achieved (Devendra, 1984), besides improving nutritive value. Generally, processing becomes important when maximum productivity is desired.

An appropriate system of processing and conservation is an important parameter that should be considered when utilising fibrous feed. It has been
established that the digestibility of highly lignified materials may be improved by chemical, physical and microbiological treatment (Sundstøl, 1988).

Although there are major improvement in procedures for processing low quality fibrous feed, some of the methods used are not practical for the Malaysian condition. The combination of two of more techniques or treatments may be necessary. The most significant effect of these processing methods is to increase the rate and extent of cellulose and hemicellulose digestion and consequently VFA production and live weight gain by ruminants.

Chemical Treatment

It has been known for many years that treatment of fibrous materials with alkali improves their digestibility. Sodium hydroxide \( \text{NaOH} \) is one of the most commonly used alkaline substances for this purpose. Alkali dissolves lignin and renders the cell wall constituents susceptible to microbial digestion. Alkali treatment of straw is used extensively in Europe (Cheeke, 1991).

Usually chemical treatment uses chemicals such as:

Ammonia: Anhydrous ammonia, aqueous ammonia, urea, and urine.

Sodium Hydroxide: wet treatment, semi-wet treatment and dry treatment.

Others chemicals: Calcium hydroxide, potassium hydroxide and calcium dioxide.

Nolte et al., (1987) found wood ashes to be as effective as sodium hydroxide in improving fibre utilisation in ruminants.
Physical Treatment

Chopping and grinding: The digestibility of low quality roughage can be improved by grinding or chopping the material to reduce particle size. This exposes more terminal ends of cellulose fibres to cellulase. However, the benefits gained in improved digestibility are usually offset by a more rapid passage of small particles from the rumen, which thus escapes microbial digestion. The major benefits of chopping or grinding roughage are reduced feed wastage and that they can be handled easily by mechanical equipment and tends to provide uniform product for consumption (Cheeke, 1991). Chopping is particularly useful in small-scale farms using fibrous by-products as feed (Kiflewahid, 1982). Ibrahim (1981) reported that grinding result in increasing dry matter intake (DMI) and the digestibility of low quality roughage was either not affected or may be actually reduced. He also stated that chopping and grinding of a material could influence the effectiveness of chemical treatment by increasing the surface area. Obviously, it has been established that grinding of low quality roughage increases the voluntary feed intake.

Pelleting: Pelleting is a physical process following grinding and mixing. To prepare a complete diet containing different ingredients like grain, ground low quality fibrous feeds, pellet processing is desirable as it is a process to mix all. Although pelleting may not result in significant advantage in terms of increasing nutritive value of roughage, it is preferable over loosely ground form as it makes feed easier to handle and reduces feed losses during feeding process (Kiflewahid, 1982; Thomas and Vander, 1996). Pelleting has a greater practical value for
feedlot operators or feed processing plant. Moreover, the major advantage over the chopped, fresh, silage and the ground material is that pelleted feed is easy to store. Pelleting is employed to prepare a homogenous mixture of ingredients (Thomas and Vander, 1996).

Steam: In 1970, Bender and co-workers published a method by which wood products were steamed to make them suitable as feed for ruminants. Boiling under high pressure or steaming has also been used to improve rice straw and other cereal straw, sugar cane bagasse, maize cobs, stover etc. The technology is based on the hydrolytic action of high temperature steam that breaks chemical bonds and causes degradation that increase digestibility of the final product (Walker, 1984). At high pressure, the optimal treatment time seems to be shorter than at low pressure (Hart et al., 1981). At pressures between 20 and 30 kg cm$^2$ treatment time of 1 - 1.5 min. seems adequate. Donefer (1977) reported that physical treatment with steam presently appears unfeasible at farm level and questionable at commercial level, because it is relatively expensive for the equipment and investment require.

Microbiological Treatment

Various aerobic fungi and bacteria can degrade lignin. A typical study is that of Ward and Perry (1982), who observed that culture of the fungus Trichoderm viride on corn cobs increased their nutritional value for sheep. Microbiological treatment method for improving poor quality forages and roughages has not been used in practice to date. But it may prove to be one of the
most promising in the future. The main problem in biological upgrading of lignocellulosic materials is to find suitable micro-organisms which decompose lignin without using too much of hemicellulose and cellulose. One class of micro-organisms known to possess such properties is the white rot fungi. Cellulase deficient mutants of these have been studied by Eriksson et al. (1980) in Sweden. According to Zadrazil (1984), white coloured, decomposed wood was used as animal feed in Southern Chile at the end of the nine-tenth century. This product which was called “palo podrido” had a high cellulose content and an in vitro digestibility of 30 - 60% (up 77% for individual samples) as compared with 0 - 3% for wood not decomposed. The study also revealed that the white rot fungi (Ganoderma aplanatum and Armillariella sp.) decomposed the wood. Zadrazil (1984), reviewing microbial conversion of ligno-cellulose into feed, draws the following conclusion from a great number of work on white rot fungi to improve the digestibility of cereal straw etc.

**Ruminant as a Converter of Fibrous Feed**

Ruminants have various advantages to other converters of feed especially when using low quality fibrous roughage. Ruminant can cope with cellulose rich products by means of ruminant micro-organisms and are able to utilise feed stuffs that are not directly competing with human (Steg et al., 1985). Ruminants are able to utilise more fibrous residues by means of their fore stomachs, which are well equipped with a wide range of microorganisms and considered as the best utilised agricultural by-products which are usually wastes. Microbial populations and fermentation patterns vary with the changing rumen environment. A
continuous supply of substrate, salivary buffering salts and the removal of end products and residues will result in a relatively stable rumen environment, thus promoting high microbial populations and increases of biomass. Moreover, non protein nitrogenous substances (NPN) are utilised by the ruminant for microorganisms growth in the rumen. About 70 - 90% of the supplied NPN are utilised by rumen micro-organisms (Boda, 1990). Furthermore, ruminants can use dietary lipids efficiently for production purpose (Preston and Leng, 1987). Akin (1986) and Buchop (1979) reported that the rumen micro-organisms are able to degrade plant cell wall carbohydrate effectively through extra-cellular or cell bound carbohydrates. Akin (1986) also reported that the anaerobic fungi are better able to degrade the more resistant ligno-cellulose tissue that are primarily not degraded by bacteria.

Determination of Digestibility

An essential feature of feedstuff evaluation is nutrient digestibility, which measures the amount of nutrient in a feed that is digested, absorbed, and thus available for metabolism. There are several procedures used to assess digestibility.

Direct measurement involves keeping an animal in a metabolism cage or crate and measuring the feed intake and the faeces excreted. The feed and faeces are analysed for nutrients of interest. The difference between the amount of nutrient consumed and excreted in the faeces is the amount digested and absorbed.
Another method of faecal collection is to equip the animal with a harness and collection bags so that faeces and urine (if necessary) can be collected. Digestibility can also be estimated by procedures that do not require a total faecal collection. An indigestible substance (revered to as a marker) can be added to the feed. By measuring the concentration of the marker in the feed and faeces, one can calculate the extent of loss from the gut of digestible compounds to the feed. This procedure can use “grab samples” of faecal material rather than a total collection. Chromium oxide, an indigestible bright green dye, is often used as a marker (Cheeke, 1991).

In simplest terms, in vitro digestibility determination involves a test tube containing a buffer solution, the test forage and rumen microbes, incubated at body temperature under anaerobic conditions. The buffer solution represents artificial saliva and buffers the acid produce during fermentation. The rumen microbes are obtained by collecting rumen fluid from a fistulated animal and straining the fluid to remove particles. The test forages are added individually in weighed amounts to tubes, which are gassed with carbon dioxide to create anaerobic condition. The tube are placed in a water bath and incubated at 37°C, usually for 24 to 48 hours. At the end of incubation, the tubes are filtered and indigestible residues are measured. The difference between the starting and ending weight represents the amount digested. By measuring the amount of dry matter, fibre and other nutrient fractions in the forage before and after incubation, the digestibility of each fraction can be determined. The values correlated well with data obtained in in vivo determinations (Judkins et al., 1990). The advantage of the in vitro technique is that hundreds of samples can be run easily, using only
a few grams of each feedstuff, whereas metabolism trials are lengthy, expensive and involves large amount of feed.

The nylon bag technique for determination of digestibility involves inserting the test forage sample in a nylon bag, which is then suspended in the rumen via a rumen fistula. After 24 or 48 hours, the bag is withdrawn and by-analysing the sample residue, the digestibility can be determined. This is also known as the in sacco or in situ procedure.

Supplementation of By-Pass Protein

Protein supplements that escape fermentation in the rumen (by-pass protein) have been very effective in enhancing growth when a low quality fibrous feed is used as basal feed (Leng, 1987). By-pass protein is essential in the diet to achieve a higher growth of ruminant (NRC, 1974 ; ARC, 1980). To get a maximum growth for ruminants, by-pass protein is necessary especially when crops or plant residues are used as basal feed. Small inputs of by-pass protein increased dramatically the growth rate and feed efficiency of cattle (Preston and Willis, 1974 ; Mc. Allan and Smith, 1983). Addition of fishmeal as a by-pass protein source to a basal diet of molasses ad libitum and restricted urea improve growth rate and feed conversion. Yahya et al. (1996) reported that high nutrient and digestibility could be achieved with sago pith meal when it is supplemented with 4% urea fishmeal. It has been reported that brewers dried grain, fishmeal coconut cake, cotton seed meal, meat meal and heat or formaldehyde treated feed materials are good sources of by-pass protein (Sampath, 1987). By-pass protein is
the most important supplement to increase animal productivity when it is fed with fibrous plant residues (Kempton et al., 1977).

Fibre Digestion in the Rumen

The universal end products of fermentation of all diets in the rumen are volatile fatty acids (VFA, such as acetate, propionate and butyrate), carbon dioxide and methane. Energy is lost as both heat and methane. The ATP produced by conversion of feed to VFA and intermediary compounds used in cell growth is the main source of energy for the growth of micro-organisms (Leng, 1982). The dietary composition has a marked influence on rumen pH and rumen fermentation (Antoniou and Hadjipanayiotou, 1985). With a change in diet composition from a low to a high fibre content, rumen pH and molar proportion of acetate should be expected to increase. The CHO fractions such as the cell contents are rapidly and completely digested in the rumen while the cell wall is variable and often incomplete. The OM digestibility of compound feeds appears to be strongly correlated to the fibre (CF, NDF, ADF) and lignin content of the feed (Linberg and Gonda, 1996).

Factors Affecting Digestibility of Roughage

The population of rumen microbes reflects the nature of the diet consumed. Roughage diets are high in cellulose, low in starch, and intermediate in soluble sugar, thus supporting a microbial flora of mainly cellulolytic and saccharolytic (sugar-digesting) bacteria. These organisms produce acetate as their
main fermentation end product. With high starch diet, amylolytic bacteria predominate; they ferment starch, sugars, and hemicellulose to propionate. The rate of digestion of cellulose is much slower than for soluble carbohydrates.

Various types of rumen fungi are intimately involved in the digestion of highly lignified cell wall material (Grenet, 1988; Akin, 1987). The action of fungi may be to degrade lignin, thus increasing accessibility of cell wall material to bacterial digestion. Rumen fungi have a good amino acid balance and high digestibility (Gulati et al., 1989), so they should be a valuable source of digestible amino acids in rumen fed with low quality roughages. A major animal factor influencing efficiency of roughage utilisation is the rumination rate. Rumination is important in helping to degrade fibrous material into smaller particle sizes, facilitating their digestion by microbes. The maximum time spent in rumination is 8 to 9 hours per day (Welch, 1982), so the more roughage that can be ruminated during the 8 to 9 hours the greater the efficiency of digestion, roughage intake and productivity. Intake of a forage by ruminants is closely related to digestibility. The voluntary intake also depends on palatability, which in turn is influenced by taste and physical properties.

**Evaluation of Fibrous Feed**

Preston (1986) described the evaluation techniques of a new fibrous feed ruminants. It includes determination of rate degradation (Orskov et al., 1980) in a nutritionally adequate diet of fistulated animals followed by chemical analyses (DM, CP, EE and OM). Other analyses like cell wall and cell content (Van Soest,
1982; Gohde and Becker, 1982) are also suggested. Preston (1986) also reported that the rate of degradation of the feed after 48 hours is more than 40%. The product is worth more consideration. The third step is to determine the rate of degradation in the fibrous feed based diet. The final step is suggested to determine the rumen fermentation parameters that feed the test in adequate quantities.

**External Factors that Affect the Rate of Outflow of Rumen Constituents**

Retention time of digesta particles and fluid in the reticulo-rumen are generally related to feed intake, the chemical and physical nature of the diet, the physiological state of the animal and the climatic conditions under which the animal lives. Their effects have been extensively reviewed (Faichney, 1986; Owens, 1986; Kennedy, 1988; Lechner et al., 1991) and are summarised below. Most investigators found that as DM intake increases, ruminal fluid volume, DM percentage, fluid and particle passage rate (FPR and PPR respectively) are increased. FPR increases more than PPR. Therefore, the amount of digesta leaving the rumen increases more than those present in the rumen, and the ruminal DM pool increases more than the fluid pool.

**Fluid Passage Rate (FPR)**

FPR appears to be a function of dietary factors that tend to increase rumen osmolarity (Harrison et al., 1975; Faichney and White, 1988) and hence, increasing the amounts of solutes from feed, saliva and fermentation products.
should enhance FPR. FPR is better for diets with a high roughage as opposed to concentrate content (Warner, 1981 and Owens, 1986), which reflects the increases of mastication, salivation, motility and stratification in the rumen when the percentage of fibre in the diet increases. Salts or buffer solubilized in the rumen could alter fluid kinetics directly through the effect of osmosis.

**Particle Passage Rate (PPR)**

DM in the rumen can be roughly considered as being distributed into 3 pools according to their size (or density) and the probability of their exit: solutes and small particles (arbitrarily defined as these particles which are not retained on 0.15 or 0.2 mm sieve, pool A) with the highest passage rate (but lower than FPR), intermediate particles (> 0.15 mm but < 1 - 1.8 mm sieve, pool B) with decreasing passage rate when the size increases and large particles with very low probability of exit (pool C). The size of this pool depends on the composition of the diet (% roughage); particles are comminuted by chewing and digestion so that after a meal, pool C decreases whereas pools A and B are more stables (Ichinohe et al., 1989).

**Feed Intake Level**

PPR increases as feed intake increases, although the strength of this relationship varies with the level of feed intake. At low levels, there is sometimes no response to the increase in the feed intake.
Diet Type

PPR and roughage level are positively related (Evans, 1981). The addition of roughage to a diet or the substitution of roughage for concentrate increases ruminal outflow, by the improving effect of fibre on motility, rumination, salivation and probably on sedimentation velocities of concentrate particles.

Feed Processing

Grinding and pelleting of forage decrease the bulk volume and result in an increase in voluntary feed consumption. There is conflicting result on the effect of this treatment on PPR. Some works reported an increase in PPR (Thomson, 1980; Warner, 1981; Fadlalla, 1987) and others a decrease (Weston, 1967; Faichney, 1983). Grinding negates the effect of roughage level on passage.

Animal and Climatic Factors

Rumen passage rate has been shown to increase throughout pregnancy and during lactation (Faichney, 1988). Inconsistent results have been obtained on the effect of age of the animal, although a lower passage rate in mature ruminants than in young animals has been observed (Warner, 1981). Ruminants adapt to the dry season by increasing rumen retention time primarily through an increase in their RR volume (Lechner et al., 1991).
Anti Nutritional Factors in Roselle By-Products

Roselle seed has properties similar to those of cotton seed oil and is used as a substitute for crude castor oil. Dried fruits contain two anthocyanins, gossipetin (hydroxyflavone) and hibiscin and also vitamin C and Ca-oxalate. Dry petals contain flavonol glucoside hibiscitrin (Salama, 1979). Ross (1989) and Ibrahim et al. (1971) reported that roselle plants contain oxalates. Oxalates act to precipitate serum calcium and cause hypo-calcemia. Crystals also form in the kidney and block action of the organ. Inclusion of di-calcium phosphate in the mineral mixture will usually prevent difficulties with the compounds.

El-Adawy and Khalil (1994) found that roselle seed consists of tannin. Analysis of tannins in roselle seed indicated that tannin in roselle seed was low (1.13% - 1.37%). Phytic acid analysis revealed the level to be 0.92% - 1.18%, which is considerably lower than that reported for beans (Khalil and El-Adawy, 1994). Al-Wandawi et al. (1984) reported that gossypol was found in the roselle as trace.
CHAPTER III

GENERAL MATERIALS AND METHODS

Animals

Thirteen one-and-a-half-year old local male sheep were used in the experiment. Four sheep were fistulated and kept in individual pens for about 3 months, which were used in the determination of degradability using the method described by Ørskov et al. (1980). The others were placed in metabolism pens for 6 months and were used in a feeding trial to determine the digestibility of untreated and treated roselle by-products, using the method described by Schneider and Flatt (1975). Before the commencement of the experiment, all the sheep were given antibiotic and dewormed.

Ambient Temperature

The house temperature and relative humidity were recorded in the morning and afternoon. In the morning, the temperature ranged from 25 - 26 °C and in the afternoon the temperature averaged from 28 - 37 °C. The relative humidity was 80 - 87% in the morning and 50 - 65% in the afternoon.
Digestibility Trial

Nine-one-and-a-half-year old local sheep were used in the experiment. These have an average body weight of 14.5 kg and were kept in the metabolism pens for 6 months, to determine the digestibility of roselle according to the procedures described by Schneider (1975). After two weeks of the adjustment period, total collection procedures for feed and feces were used to measure the daily feed intake and feces output for seven days. Dry matter of the faecal output and feed refusal were measured every day.

Diet

Roselle pods were obtained after the calyces are removed from the fruit, and were collected from Terengganu plantation. They were chopped and dried under the sun for three days. Dried chopped pods of 1 - 1.50 kg were soaked with 15 litter 2% NaOH for one hour and then kept in a basket for three days and given to the sheep ad libitum. Dried chopped pods were also treated with 4% Ca (OH)₂. For untreated roselle, they were only soaked with water. The procedures were the same as the above.

Sample Collection

Daily feed intake was determined by recording the feed offered and refused. Representative sample of feed, refused feed, faeces were collected, and
the dry matter determined daily. Ten percent of well-mixed faeces samples were retained for analysis. All the samples were ground to 1 - 2 mm and kept in the pillboxes before being analysed.

**In Sacco Degradability of Roselle By-Products**

Four one-and-half-year old fistulated male local sheep were used in the experiment. The sheep were kept in individual pens to determine the degradability of roselle, using the method describe by Ørskov *et al.* (1980). Dried chopped roselle pods and seed were ground through 3 - 4 mm screen. Five grams of each of the above samples were placed in nylon bags (9.0 x 6.0 cm, pore size 42 µm). The nylon bag with each sample were soaked in water for 2 minutes, then placed immediately into the rumen before the morning feed. The bags were incubated for 2, 6, 12, 24, 48 and 72 hours. At the end of each incubation period, the bags were withdrawn and washed thoroughly under running tap water with gentle squeezing until the rinse water became clear. The bags were then dried at 60° C for 72 hours in an air oven, and then were weighed and the samples were kept in pillboxes. For each incubation period, 3 bags were used. They were analysed for DM and OM to determine the percentage of losses and degradation parameters.

The design of experiment was a 2 x 2 Latin Square with two animals replicated in each experiment and the experiment has two periods of time.
The DM and OM losses at various incubation times were fitted according to the equation given by Ørskov (1979).

\[ P = a + b \left( 1 - e^{-ct} \right) \]

Where:

\( P \) = The cumulative amount degraded at time \( t \)

\( a \) = The rapidly soluble fraction

\( b \) = The potentially degradable fraction in the rumen

\( c \) = The fraction rate constant of \( b \)

\( a + b \) = The potential degradability

\( a \), \( b \) and \( c \) are degradation constants obtained by an iterative least square procedure (non-linear) in the SAS programme (1988, Appendix D).

These experiments were conducted at Ladang II and Nutrition Lab, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor.
CHAPTER IV

CHEMICAL COMPOSITION AND DEGRADABILITY OF ROSELLE PODS AND SEEDS

Introduction

The efficiency of animal production depends on the optimal utilisation of the feed for growth, development and reproduction. Thus as a prerequisite for the determination of biological efficiency it is necessary to have an indication of the suitability of the feed and nutritive value to meet the specific requirements of the animal.

Chemical analysis is the starting point for determining the nutritive value of feeds, but the value of a feeding stuff does not depend entirely on the amount of several nutrients it contains. The value of a feed depends on the amount of these nutrients that the animal can digest and use. The chemical composition alone of any feeding stuff is a very imperfect standard by which to judge its nutritive value. The first consideration is digestibility, since undigested nutrients do not enter the body proper at all.

It is, of course, only the digestible portion of the feed that can serve to maintain the vital functions and is of value for maintenance and the formation of animal product. It is now readily understood that the composition alone does not determine
the value of feed, but rather that the value depends on the composition, the digestibility and other factor (Schneider, 1975).

So far information on the utilisation of roselle pods and seeds as a ruminant feed is still lacking. Previous studies indicated that chemical composition of whole and defeated roselle seeds respectively was determined to be (in %): moisture 7.58, 8.18; Crude Protein (CP) 23.95, 29.04; digestible CP 15.36, 27.50; ether extract (EE) 22.34, 0.69; digestible EE 14.40, 0.68; N free extract (NFE) 23.81, 32.86; digestible NFE 22.53, 32.51; crude fibre (CF) 15.30, 20.04; digestible CF 5.53, 7.29. Digestibility was estimated using Rhode Island Red cocks (Samy, 1980). Backeit et al. (1994), studied the effects of feeding mechanically extracted roselle (Hibiscus sabdariffa) seed meal (RSM) at a level up to 200 kg⁻¹ on performance and egg quality of laying hens over a 14-week period. RSM contains 491.10 g crude protein, 59 g ether extract, 169.60 g crude fibre, 113.50 g ash, 6.80 g calcium, 6.60 g total phosphorus and 0.60 g magnesium kg⁻¹. As the inclusion rate of RSM increased, rate of lay, feed intake, and egg weight were increased (P < 0.01). Dietary treatments had no significant effect on feed conversion ratio, mortality, shell thickness, albumen height and yolk colour. It is concluded that RSM supported satisfactory egg production, when included in the diet at concentration up to 200 g kg⁻¹.

The objectives of this experiment were to determine the chemical composition, dry matter and organic matter degradability of roselle pods and seeds in sheep.
Materials and Methods

Collection of Roselle By-Products Sample

Roselle whole pods were collected from Terengganu, after the calyces were removed from the fruits. Then, the whole pods were chopped and dried under the sun for about 3 days. A representative sample, which was separated into whole pods, seeds and empty pods was taken and dried in a forced draught oven at 60°C until the weight was constant. After that the sample was ground to 1 - 2 mm and placed in the plastic bag. The bag was sealed and preserved in the freezer until further analysed.

Chemical Analyses

Dried roselle empty pods, seeds and whole pods were analysed for dry matter, ash, crude protein, ether extract, crude fibre, neutral detergent fibre, acid detergent fibre and Lignin. The details of the procedures are described in Appendix A.

In Sacco Degradability

Four one-and-a-half-year old fistulated male local sheep were used in the experiment. The design of the experiment was a 2 x 2 Latin Square with two animals replicated in each experiment. The sheep were kept in the individual pens to determine the degradability of roselle pods and seed using the method described by
Ørskov et al. (1980). Samples of roselle pods and seeds were ground through 3 - 4 mm screens. Five grams of each of the above samples were placed in nylon bags (9.0 x 6.0 cm, pore size 42 μm). The nylon bag with each sample were soaked in water for 2 minutes, then placed immediately into the rumen before the morning feed. The bags were incubated for 2, 6, 12, 24, 48 and 72 hours. The details of the procedures are described in Chapter III.

Results

Chemical Composition

The chemical compositions of roselle whole pods are presented in Table 4.1. The dry matter of whole pods were higher than empty pods and seeds (96.60%, 94.70% and 95.40%, respectively) while crude protein (CP) and ether extract (EE) of seeds were higher than the whole pods and empty pods (20.10%, 12.24%; 16.50%, 2.58%; 7.30%, 0.08%, respectively). Crude fibre (CF), neutral detergent fibre (NDF) and acid detergent fibre (ADF) in the pods were higher than whole pods and seeds (45.30%, 58.90%, 42.20%; 31.27%, 57.54%, 41.27% and 27.70%, 54.30%, 38.70%, respectively). Nitrogen free extract (NFE) content of whole pods were similar to those of the empty pods, but that of the seeds were lower (37.73%, 37.52% and 26.66%, respectively). Total carbohydrate empty pods and whole pods were higher than seeds (87.42%, 72.40% and 59.66%, respectively).
In Sacco Degradability of Roselle Pods and Seeds

The rumen degradability of dry matter and organic matter was low for both empty pods and seeds in Tables 4.2 and 4.3. Roselle seeds and empty pods that were incubated in the rumen sheep for 2, 6, 12 and 24 hours showed that dry matter and organic matter degradability were not significantly different (P > 0.05).

However the dry matter degradability of roselle seeds was more significantly different (P < 0.01) higher than pods for incubation at 48 and 72 hours (36.80%; 23.01% and 39.87%; 26.30%, respectively). Potential degradability empty pods and seeds were significantly different (P < 0.01), on DM, but not on OM (P > 0.05).

Organic matter degradability in seeds was significantly higher (P < 0.01) than empty pods for incubation time 48 and 72 hours (28.36%; 17.66% and 31%; 20.85%, respectively).
Table 4.1. Chemical Composition (%) of Air Dried Roselle

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Seeds</th>
<th>Empty Pods</th>
<th>Whole Pods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 5)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Moisture**</td>
<td>25.27 ± 2.03</td>
<td>25.23 ± 1.73</td>
<td>25.25 ± 0.54</td>
</tr>
<tr>
<td>Dry Matter</td>
<td>94.70 ± 1.14</td>
<td>95.40 ± 0.47</td>
<td>96.60 ± 0.47</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>20.10 ± 0.60</td>
<td>7.30 ± 0.42</td>
<td>16.50 ± 0.47</td>
</tr>
<tr>
<td>Ether Extract</td>
<td>12.24 ± 0.93</td>
<td>0.08 ± 0.02</td>
<td>2.58 ± 0.40</td>
</tr>
<tr>
<td>Crude Fibre</td>
<td>27.70 ± 0.52</td>
<td>45.30 ± 0.27</td>
<td>31.27 ± 0.18</td>
</tr>
<tr>
<td>Neutral Detergent Fibre</td>
<td>54.30 ± 0.46</td>
<td>58.90 ± 0.68</td>
<td>57.54 ± 0.44</td>
</tr>
<tr>
<td>Acid Detergent Fibre</td>
<td>38.70 ± 0.33</td>
<td>42.20 ± 0.19</td>
<td>41.27 ± 0.18</td>
</tr>
<tr>
<td>Acid Detergent Lignin</td>
<td>12.80 ± 0.61</td>
<td>12.50 ± 0.32</td>
<td>14.91 ± 0.31</td>
</tr>
<tr>
<td>Ash</td>
<td>8.00 ± 0.31</td>
<td>5.20 ± 0.16</td>
<td>8.52 ± 0.36</td>
</tr>
<tr>
<td>Nitrogen Free Extract*</td>
<td>26.66</td>
<td>37.52</td>
<td>37.73</td>
</tr>
<tr>
<td>Cellulose*</td>
<td>25.90</td>
<td>29.70</td>
<td>26.36</td>
</tr>
<tr>
<td>Hemicellulose*</td>
<td>15.60</td>
<td>16.70</td>
<td>16.27</td>
</tr>
<tr>
<td>Total Carbohydrate*</td>
<td>59.66</td>
<td>87.42</td>
<td>72.40</td>
</tr>
<tr>
<td>Non Fibrous Carbohydrate*</td>
<td>5.33</td>
<td>28.52</td>
<td>14.86</td>
</tr>
<tr>
<td>Ratio Parts of Roselle By-</td>
<td>44.27</td>
<td>55.73</td>
<td>100</td>
</tr>
<tr>
<td>Products</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Calculated

**Moisture content from fresh sample
Calculation:

Nitrogen Free Extract (%) = 100 - % (Moist. + CP + EE + Ash + CF)

Cellulose (%) = ADF % - ADL %

Hemicellulose (%) = NDF % - ADF %

Total Carbohydrate (%) = 100 - % (CP + EE + Ash)

Non Fibrous Carbohydrate (%) = Total Carbohydrate % - NDF %
<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Empty Pods</th>
<th>Seeds</th>
<th>S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.11</td>
</tr>
<tr>
<td>2</td>
<td>14.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.21</td>
</tr>
<tr>
<td>6</td>
<td>16.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.24</td>
</tr>
<tr>
<td>12</td>
<td>18.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.69</td>
</tr>
<tr>
<td>24</td>
<td>20.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.19</td>
</tr>
<tr>
<td>48</td>
<td>23.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.21</td>
</tr>
<tr>
<td>72</td>
<td>26.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.69</td>
</tr>
</tbody>
</table>

Degradation parameters

<table>
<thead>
<tr>
<th></th>
<th>Empty Pods</th>
<th>Seeds</th>
<th>S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>13.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.99</td>
</tr>
<tr>
<td>b</td>
<td>16.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.01</td>
</tr>
<tr>
<td>a + b</td>
<td>29.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.97</td>
</tr>
<tr>
<td>c</td>
<td>0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<sup>abc</sup>Different superscripts in the same row differ significantly (P < 0.05).

S.E.M: Standard error of means

a: The rapidly soluble fraction
b: The amount which would be degraded in time
a + b: Potential degradability
c: Rate of loss
Table 4.3. Percentage Organic Matter (OM) Loss and Degradation Parameters (a, b, a + b, and c) of Roselle Empty Pods and Seeds.

<table>
<thead>
<tr>
<th>Incubation Time</th>
<th>Empty Pods</th>
<th>Seeds</th>
<th>S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.43</td>
</tr>
<tr>
<td>2</td>
<td>9.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.87</td>
</tr>
<tr>
<td>6</td>
<td>11.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.02</td>
</tr>
<tr>
<td>12</td>
<td>13.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.49</td>
</tr>
<tr>
<td>24</td>
<td>15.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.61</td>
</tr>
<tr>
<td>48</td>
<td>17.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.81</td>
</tr>
<tr>
<td>72</td>
<td>20.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.27</td>
</tr>
</tbody>
</table>

Degradation parameters

<table>
<thead>
<tr>
<th></th>
<th>Empty Pods</th>
<th>Seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>9.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>b</td>
<td>18.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>a + b</td>
<td>27.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>c</td>
<td>0.03</td>
<td>0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Different superscripts in the same row differ significantly (P < 0.05)

S.E.M: Standard error of means

a: The rapidly soluble fraction
b: The amount which would be degraded in time
a + b: Potential degradability
c: Rate of loss
Discussion

Moisture

Morton (1987) reported that the moisture of roselle seed is 12.9% (analyses made in Philippines). El-Adawy and Khalil (1994) found that the moisture ranged from 9.25 - 11.66%. The present study indicated that roselle seeds, empty pods and whole pods have higher moisture content (25.27 ± 2.03, 25.23 ± 1.73 and 25.25 ± 0.54, respectively). These results might be due to the samples taken from Terengganu about three days after harvest. The harvest time could influence the condition of roselle by-products.

Dry Matter (DM)

The present study indicated that DM of roselle seed was higher than the previous study by Morton (1987) who found that roselle seed DM was 87.10%. However, Backeit et al. (1994) indicated that roselle seed DM was (96.93%). In Jamaica (FAO 1968) it was found that DM of roselle seed was 91.50%. El-Adawy (1994) found that roselle seed DM was around 88.34% - 90.50%.

From Table 4.1, DM of seed, empty pod and whole pod was 94.70%, 95.40% and 96.60%, respectively.
The variation of roselle seed DM could be attributed to the environmental factor or maturity of roselle seeds itself. Up to now, no data in the literature is related to the studying about roselle empty pods and whole pods are available for comparative purposes.

**Crude Protein (CP)**

Rao (1996) analysed two varieties of mesta (*Hibiscus sabdariffa* L.) seeds for their proximate composition. Their protein values ranged from 18.80% - 22.30%, while fat ranged from 19.10% - 22.80% and dietary fibre ranged from 39.50% - 42.60%, respectively. In the present study, it was indicated that Rao (1996) had shown roselle seed CP to be 20.10% and this result is similar to what, Morton (1987) found that roselle seed CP was lower (3.29%). In Jamaica (FAO, 1968), it was found that CP was 21.40 % of roselle seeds. These results were similar with the present study.

Earle *et al.* (1960) and Watt (1962) reported that roselle seed has high content of oil and protein. The total protein of roselle seeds was found to be 25.20 % (AL-Wandawi *et al*., 1984) in comparison to 20.58% reported for mature roselle seeds (Karokoltsidid, 1975). However, AL-Wandawi (1983) was considered to be a potential rich protein with high lysine level. According to El-Adawy (1994), roselle seeds contained 30.11% - 31.02% protein, 2.06% - 2.60% non-protein nitrogen. Moreover, Samy (1980) found that crude protein (CP) of whole and defeated roselle seeds was higher (23.95% and 29.04%) than the value found in the present study of
whole seeds (20.10%). From the previous study, it was indicated that CP ranged from 3.29% - 31.02%. The different compositions may be due to genetic and environmental factor or maturity of the roselle seed itself.

*Ether Extract (EE)*

Al Wandawi *et al.* (1984) found that the EE of roselle seed was 21.10% and Morton (1987) reported that EE of roselle seeds was 16%. The present study indicated that roselle seed EE was 12.24% and it was lower than the value analysed by Rao (1996), which was between 19.10 % - 22.80 %. In Jamaica, it was reported that roselle seed EE was 17.50% FAO (1968). However, Backert (1994) indicated that roselle seed EE was 16.96%. According to Samy (1980), EE of whole and defeated roselle seeds was found to be 22.34% and 0.69%, respectively.

Ahmed and Hudson (1982) found that the fat content of *Hibiscus sabdariffa* from different seed collections, representing different growing areas, showing differences in the fatty acid pattern. The fatty acids for myristic ranged from 0.20% - 0.50%, palmitic was 17.40% - 22.60%, stearic was 3.90% - 5.20% and oleic acid was 34.60% - 39.80%.

*Crude Fibre (CF)*

In this experiment, the CF of roselle seeds, empty pods and whole pods (in %) were 27.70 ± 0.52, 45.30 ± 0.27 and 31.27 ± 0.18, respectively. The results presented
here are in agreement with previous studies by Rao (1996) who showed that the CF of roselle seeds for two varieties (AMV-2 and Bhimil-1) were 39.50% and 42.60%, respectively.

Backeit et al. (1994) found that mechanically extracted roselle seeds had CF of 16.96%. Reports published by FAO (1978) indicated that CF of roselle seeds was 12%, which was lower than the present study with a value of 27.7%. According to Samy (1980) the CF content of whole and defeated roselle seeds were 15.30% and 20.04%, respectively. From the previous studies, roselle seed CF ranged from 12% - 42.60%. These data varied depending on locality. It was indicated that roselle seed CF was 27.7%.

Ash

In the present study the content of ash of the seeds, empty pods and whole pods is shown in Table 4.1. The results were higher than that analysed by FAO (1978). El Adawy and Khalil (1994) showed values of ash in roselle seeds to be 5.40%, ranging from 5.80% - 6.89%. Backeit et al. (1994) indicated that the ash content of roselle seeds was 11.35% which was higher than the values obtained in the present study which was 8%.
Nitrogen Free Extract (NFE)

Backeit et al. (1994) found that the NFE of mechanically extracted roselle seeds was 11.68%. In the present study, it was indicated that the NFE content of roselle seeds, empty pods and whole pods were 26.66%, 37.52% and 37.73%, respectively. Samy (1980) who supported these results reported that whole and defeated roselle seed NFE were 23.81% and 32.86%, respectively. However, reports by FAO (1978) showed that NFE of roselle seed was higher with a value of 43.70%.

Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL)

NDF consists mainly of cellulose, hemicellulose and lignin. ADF represents crude lignin and cellulose, and ADL represents lignin. Previous studies mentioned about chemical composition of roselle only for the seeds. They did not study about empty pods and whole pods and they also did not analyse NDF, ADF and ADL. While the present study mentioned about empty pods and whole pods and also NDF, ADF and ADL, it would be difficult to find out from available literature for comparison purposes.

Degradability of Roselle

DM and OM degradability of roselle empty pods and seeds were significantly higher (P< 0.01) at 48 h and 72 h (refer Table 4.2 and 4.3). Seeds were more
degradable than empty pods. The degradation values and degradation parameters of roselle seeds were higher than empty pods. Preston (1995) suggested that if the DM degradability on nutritionally adequate diet in the fistulated animals after 48h is more than 40 - 50%, then the product is worth more consideration. He also reported that if the degradation is low (10% to 30%) after 48 h of incubation, then the product is unsuitable for feeding directly to animals. In the present study it was indicated that roselle seeds and empty pods DM and OM after 48 h were 39.87%, 31% and 26.30%, 20.85%, respectively. However for maintenance, ruminant requires more than 55% DM and OM degradability (Djajanegeara, 1984). According to Preston (1986), satisfactory degradation was between 40% to 50% and poor degradation was between 10% to 20%.

Conclusion

It was found that the chemical composition of roselle empty pods and seeds were (see Table 4.1) relatively higher than other agricultural by-product, such as straws, stalks, bagasse, legume hulls and leaves. These by-products are not utilise and efficiently due to low protein component, digestibility and voluntary intake by ruminant.

DM and OM in saeco degradability of roselle seeds was higher than roselle pods after 48 h incubation time and was significantly different (P < 0.01). This result showed that DM and OM degradability of roselle pods and seeds was lower than 50%.
The present study indicated that roselle pods and seeds were not suitable as feed for animals directly. They need to be treated to improve their degradability.
CHAPTER V

CHEMICAL COMPOSITION OF ROSELLE WHOLE PODS TREATED WITH SOAKED SODIUM HYDROXIDE AND CALCIUM HYDROXIDE

Introduction

Fibrous crop by-products, in general are characterised by extensive lignification of cellulose, hemicellulose, low level of protein, soluble carbohydrates and minerals. As a consequence, the intake and digestibility of these by-products are not sufficient to sustain high levels of production (Khajaree, 1984). Roselle, like other fibrous by-products, is high in lignified fibre and low in degradability (see Chapter II).

The conventional method to improve the utilisation of roselle pods is by chemical treatment with sodium hydroxide and calcium hydroxide. Treatment of crop by-products with sodium hydroxide has been studied extensively and considerable amount of literature has accumulated concerning its use (see Jackson, 1977, 1978). This treatment has been tested experimentally in many Asian countries including Indonesia (Wignjosoesastro, 1980 and Sutadi et al., 1980), Malaysia (Devendra, 1979), Thailand (Holm, 1972) and in the sub continent in India (Singh, 1975) and Sri Lanka (Jayasuriya, 1979). It is generally concluded that sodium hydroxide is more effective than other chemical treatments in improving the potential digestibility of fibrous feeds.
The objective of this experiment was to determine the chemical composition of treated roselle whole pods at different concentrations (2%, 4% and 6%) of sodium hydroxide and calcium hydroxide.

Materials and Methods

Roselle whole pods were soaked with different concentration of sodium hydroxide 2%, 4% and 6% for one hour and were kept in the basket for three days. For 2% NaOH roselle pods used were 100 g DM/ 20 g NaOH/ l water, 4% NaOH: 100 g DM/ 40 g NaOH/ l water and 6% NaOH: 100 g DM/ 60 g NaOH/ l water. Each treatment had four replicate. For Ca(OH)$_2$, the procedure was the same with NaOH. After three days, the entire samples were put in 60$^\circ$ C oven until the weight was constant. The samples were ground through a 1 - 2 mm screen and prepared for chemical analyses (DM, Ash, CP, EE, NDF, ADF and Lignin). All the data was analysed by analysis of variance (ANOVA) according to the General Linear Model (GLM) procedure of the Statistical Analysis Institute (SAS, 1997.). Treatment means were compared by the least significant difference method (Duncan, 1997).

Results

The chemical composition of soaked roselle with NaOH and Ca(OH)$_2$ for various concentration levels (2%, 4% and 6%) are presented in Table 5.1. For
DM, the control was significantly different (P < 0.01) with all the treated roselle. The DM of control was more higher than that of others.

Chemical Composition

Ash for treated roselle was significantly (P < 0.01) higher than the control, where the ash value of a control, NaOH 2%, NaOH 4%, NaOH 6%, Ca(OH)_2 2%, Ca(OH)_2 4%, and Ca(OH)_2 6% were 3.54, 19.92, 25.94, 32.26, 17.87, 16.61, and 27.18, respectively.

Crude protein for treated roselle in general was lower than control. However, the control was not significantly different (P > 0.05) with Ca(OH)_2 2%, Ca(OH)_2 4% (11.03%, 11.15%, and 10.36%, respectively).

EE for treated roselle in general was significantly (P < 0.01) lower than the control. The EE value of the control for NaOH 2%, NaOH 4%, NaOH 6%, Ca(OH)_2 2%, Ca(OH)_2 4%, and Ca(OH)_2 6% were 7.98%, 4.57%, 2.76%, 1.53%, 5.60%, 4.58% and 3.57%, respectively. For NaOH 2% and Ca(OH)_2 4% there was no significant difference (P>0.05) in EE (4.57% and 4.58%).

NDF and ADL of the control were higher than treated roselle of NaOH 2%, NaOH 4%, NaOH 6%, Ca(OH)_2 2%, Ca(OH)_2 4% and Ca(OH)_2 6% (74.96%, 18%; 53.36%, 12.80%; 43.54%, 8.56%; 33.93%, 5.79%; 55.91%, 14.07%; 51.56%, 12.57% and 44.99%, 10.57%, respectively).
ADF of the control was not significantly different ($P > 0.05$) with NaOH 2% and Ca(OH)$_2$ 2% (46.43%, 47.14% and 39.37%, respectively), but significantly different with NaOH 4%, NaOH 6%, Ca(OH)$_2$ 4% and Ca(OH)$_2$ 6% (46.43%, 38.80%, 32.25%, 36.26% and 33.95%, respectively).

**Discussion**

The amount of dry matter of roselle treated with NaOH and Ca(OH)$_2$ was significantly lower ($P < 0.01$) than the DM control treatment (Table 5.1). Similar results were obtained by Wanapat *et al.* (1984) using barley straw. It appears that treated roselle has high ash than the control ($P < 0.01$).

The crude protein of treated roselle was lower than that of the control. These results presented here are in agreement with the previous studies by Tait and Beames (1988). According to these authors, that the effect of alkali on protein is to destroy some amino acids and reduce the availability of others due to such changes as racemization and the formation of cross linked compounds such as lysinoalanine. Kiflewahid (1982) said that Na OH treatment caused a decrease in CP, EE and CF but increased ash content in all by-products.

The present study indicated that EE of treated roselle with NaOH and Ca(OH)$_2$ decreased significantly ($P < 0.01$) by more than 40%. This result is supported by Wanapat *et al.* (1985) who mentioned that the EE content of treated barley straw decreased by 38.50%.
Table 5.1. Chemical Composition of Roselle Untreated and Treated with NaOH and Ca(OH)$_2$ at 2%, 4% and 6%.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Control</th>
<th>NaOH 2%</th>
<th>NaOH 4%</th>
<th>NaOH 6%</th>
<th>Ca(OH)$_2$ 2%</th>
<th>Ca(OH)$_2$ 4%</th>
<th>Ca(OH)$_2$ 6%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 4)</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
</tr>
<tr>
<td>DM</td>
<td>96.33 $^a\pm$ 0.28</td>
<td>92.77 $^c\pm$ 0.15</td>
<td>93.20 $^d\pm$ 0.16</td>
<td>92.83 $^e\pm$ 0.13</td>
<td>95.47 $^f\pm$ 0.18</td>
<td>95.82 $^b\pm$ 0.19</td>
<td>95.91 $^b\pm$ 0.42</td>
</tr>
<tr>
<td>Ash</td>
<td>3.54 $^a\pm$ 0.31</td>
<td>19.92 $^c\pm$ 0.27</td>
<td>25.94 $^b\pm$ 0.53</td>
<td>32.26 $^a\pm$ 0.44</td>
<td>17.87 $^d\pm$ 2.23</td>
<td>16.61 $^d\pm$ 1.24</td>
<td>27.18 $^b\pm$ 1.56</td>
</tr>
<tr>
<td>CP</td>
<td>11.03 $^a\pm$ 0.30</td>
<td>7.90 $^c\pm$ 0.89</td>
<td>6.54 $^d\pm$ 0.33</td>
<td>5.85 $^d\pm$ 0.13</td>
<td>11.15 $^a\pm$ 0.46</td>
<td>10.36 $^b\pm$ 0.32</td>
<td>9.98 $^b\pm$ 0.84</td>
</tr>
<tr>
<td>EE</td>
<td>7.89 $^a\pm$ 1.11</td>
<td>4.57 $^c\pm$ 0.17</td>
<td>2.76 $^c\pm$ 0.65</td>
<td>1.53 $^f\pm$ 0.12</td>
<td>5.60 $^b\pm$ 0.20</td>
<td>4.58 $^b\pm$ 0.32</td>
<td>3.57 $^d\pm$ 0.21</td>
</tr>
<tr>
<td>NDF</td>
<td>79.96 $^a\pm$ 0.47</td>
<td>53.36 $^c\pm$ 0.73</td>
<td>43.54 $^d\pm$ 1.2</td>
<td>33.93 $^e\pm$ 1.19</td>
<td>55.91 $^b\pm$ 2.99</td>
<td>51.56 $^c\pm$ 2.40</td>
<td>44.99 $^d\pm$ 1.20</td>
</tr>
<tr>
<td>ADF</td>
<td>46.43 $^a\pm$ 0.99</td>
<td>47.14 $^a\pm$ 1.28</td>
<td>38.80 $^b\pm$ 0.76</td>
<td>32.25 $^c\pm$ 1.05</td>
<td>39.37 $^b\pm$ 2.23</td>
<td>36.26 $^b\pm$ 1.08</td>
<td>33.95 $^b\pm$ 1.24</td>
</tr>
<tr>
<td>ADL</td>
<td>18 $^a\pm$ 0.20</td>
<td>12.80 $^b\pm$ 0.80</td>
<td>8.56 $^b\pm$ 0.52</td>
<td>5.79 $^c\pm$ 0.40</td>
<td>14.07 $^b\pm$ 0.57</td>
<td>12.57 $^b\pm$ 0.45</td>
<td>10.57 $^a\pm$ 0.35</td>
</tr>
</tbody>
</table>

$^a^b^c$ Different superscripts in the same row differ significantly (P < 0.01).
NDF and ADL of treated roselle decreased significantly ($P < 0.01$) to the control. This result is similar with Wanapat et al., 1984 and Kiflewahid, 1982. According to them, fibrous treated with alkaline can decrease NDF, ADF and CP content. When roselle whole pods were treated with alkali, a saponification of ester linkages between acetic acid and phenolic acid and polysaccharides and/or lignin may take place. If the linkages between lignin and hemicellulose are broken, this will make the latter and also cellulose (which is embedded in the lignin-hemicellulose complex) more accessible for hydrolysing enzymes (Theander and Aman, 1984; Grenet and Besle, 1991). Alkali treatment also partly solubilizes the hemicellulose and lignin of straw (Sundstøl et al., 1977; Aman and Theander, 1977).

In general, nutrient content of treated roselle with NaOH 2%, is similar to the treated roselle with Ca(OH)$_2$, especially found in nutrient such as: Ash, EE, NDF and ADL (19.92%, 16.61%; 4.57%, 4.58%; 53.36%, 51.56%; 12.8%, 12.57%, respectively). It was thought that the following experiment would investigate the effect of 2% NaOH and 4% Ca(OH)$_2$ in the nutritive intake, the digestibility and average daily gain by sheep.

**Conclusion**

Based on details from the findings of the present study, it appears that treated roselle pods with alkali such as NaOH and Ca(OH)$_2$ at various levels (2%, 4% and 6%) decrease chemical composition of roselle by-product, except ash.
The treated roselle with NaOH 2% and Ca(OH)_2 4% is better than others, especially in Ash, EE, NDF and ADL contents.
CHAPTER VI

DIGESTIBILITY, NUTRIENT INTAKE AND AVERAGE DAILY GAIN OF SHEEP FED UNTREATED ROSELLE AND TREATED ROSELLE WITH NaOH 2% AND Ca(OH)$_2$ 4%

Introduction

Increasing dry matter intake, digestibility and higher energy intake that result in a higher nutrient intake can improve animal performance. In using agricultural by-products and non-conventional roughage, high intake is very seldom achieved (Minson, 1990 and Alimon, 1993).

Like other agricultural by-products, roselle whole pods are high in lignified fibre (Chapter IV). The conventional method to improve the utilisation of roselle whole pods is by chemical treatment with NaOH and Ca(OH)$_2$.

Chemical analysis is the starting point for determining the nutritive value of treated roselle whole pods, but the value of treated roselle whole pods does not depend entirely on the amount of several nutrients it contains. The value of treated roselle whole pods depends on the amount of these nutrients that the animal can digest and use. The chemical composition alone of any feeding stuff is a very imperfect standard by which to judge its nutritive value. The first consideration is digestibility, since undigested nutrient do not enter the body proper at all (Schneider and Flatt, 1975).
The digestibility of treated roselle whole pods is an important factor in determining their nutritive value for two reasons. First, the higher the digestibility, the more nutrients are liberated for use by animals. Second, as digestibility increases, feed intake can increase because the turnover rate in the rumen increases. Ingesta do not leave the rumen until it has been digested to a small particle size. If this process is rapid, the digested feed can be replaced by further feed intake (Cheeke, 1991).

This study was designed to determine the digestibility, nutrient intake of treated roselle whole pods with NaOH 2% and Ca(OH)\(_2\) 4% and also ADG in sheep.

**Materials and Methods**

Schneider and Flatt (1975) conducted the digestion trial of the diets for six weeks of the feeding trial using the method described. Before the experiment started, all the sheep were given antibiotic and de wormed.

In this experiment, nine local sheep (14.50 kg) of about 1 - 1.5 years old were used. The sheep were put in the metabolic pens. They were assigned in 3 x 3 Latin Square design experimental with three replicates in each treatment. The dietary feed treatment was untreated roselle, roselle treated with NaOH 2% and the other roselle treated with Ca(OH)\(_2\) 4%. The details of the procedure have been described in General Materials and Methods (Chapter III). All the data was
subjected to analysis of variance (ANOVA) according to the General Linear Model (GLM) procedures of the Statistical Analysis Institute (SAS, 1997.). Treatment means were compared by the least significant difference method (Duncan).

**Results**

**Nutrient Content in the Treatment**

The nutrient content of the different feeds used in the feeding trial are presented in Table 6.1. Dry matter, crude protein and organic matter after treatment were decreased (g.kg\(^{-1}\)) (422.20 ± 0.28, 375.80 ± 0.15 and 441.70 ± 0.19; 110.30 ± 0.30, 79 ± 0.98 and 103.60 ± 0.32; 964.60 ± 0.31, 800.80 ± 0.27 and 883.90 ± 1.24, respectively).

**Table 6.1. Nutrient Content (g.kg\(^{-1}\)) of Untreated and Treated Roselle Whole Pods Used in the Feeding Trial**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Untreated</th>
<th>Na(OH) 2%</th>
<th>Ca(OH)(_2) 4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>422.20 ± 0.28</td>
<td>375.80 ± 0.15</td>
<td>441.70 ± 0.19</td>
</tr>
<tr>
<td>Crude protein</td>
<td>110.30 ± 0.30</td>
<td>79 ± 0.98</td>
<td>103.60 ± 0.32</td>
</tr>
<tr>
<td>Organic matter</td>
<td>964.60 ± 0.31</td>
<td>800.80 ± 0.27</td>
<td>883.90 ± 1.24</td>
</tr>
<tr>
<td>Acid detergent fibre</td>
<td>464.30 ± 0.99</td>
<td>471.40 ± 1.28</td>
<td>362.60 ± 1.08</td>
</tr>
<tr>
<td>Acid detergent lignin</td>
<td>180 ± 0.20</td>
<td>128 ± 0.80</td>
<td>125.70 ± 0.45</td>
</tr>
</tbody>
</table>
Nutrient Intake

The value of roselle pods offered and roselle pods refused are shown in (Table 6.2). It was observed that sheep refused more than 50% of the offered roselle when roselle untreated and treated with NaOH and Ca(OH)₂ were given. These results showed that roselle by-products were not palatable to the sheep.

The dry matter intake, organic matter intake and crude protein per unit metabolic body weight (g kg⁻¹·W₀·75) of treated roselle were higher than untreated roselle 25.22 ± 5.62, 31.03 ± 4.67 and 36.11 ± 6.88; 22.64 ± 5.02, 26.32 ± 3.95 and 30.53 ± 5.93; 3.32 ± 0.79, 3.45 ± 0.53 and 4.57 ± 0.87, respectively.

The average daily gain (g/d) of the sheep, which were fed with treated roselle, were higher than the untreated: 10.95 ± 2.56, 18.57 ± 2.88 and 15.95 ± 3.24, respectively). It is shown in Table 6.2.

Nutrient Digestibility

The apparent nutrient digestibilities of the different diet are presented in Table 6.3. DMD and OMD of treated roselle with NaOH were significantly (P < 0.01) higher than untreated roselle (46.76 ± 1.57 and 46.70 ± 1.68; 30.42 ± 2.60 and 33.69 ± 2.50, respectively). While treated roselle with Ca(OH)₂ was not significantly different (P > 0.05) (34.81± 2.19 and 38.42 ± 1.89 ; 30.42 ± 2.60 and 33.69 ± 2.50, respectively) there were some increases.
### Table 6.2. Nutrient Intake (% DM) of Roselle Pods (RP) Fed to Sheep

<table>
<thead>
<tr>
<th>Item</th>
<th>Untreated</th>
<th>Na (OH)</th>
<th>Ca(OH)$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=9)</td>
<td>(n=9)</td>
<td>(n=9)</td>
</tr>
<tr>
<td>RP offered (g)</td>
<td>348.78 ± 40.60</td>
<td>356.79 ± 40.26</td>
<td>416.58 ± 51.67</td>
</tr>
<tr>
<td>RP refused (g)</td>
<td>153.02 ± 15.72</td>
<td>111.77 ± 13.89</td>
<td>140.18 ± 14.84</td>
</tr>
<tr>
<td>RP DMI (g)</td>
<td>195.76 ± 46.84</td>
<td>245.02 ± 41.71</td>
<td>276.40 ± 56.42</td>
</tr>
<tr>
<td>TDMI (g.kg$^{-1}.W^{0.75}$)</td>
<td>25.22 ± 5.62</td>
<td>31.03 ± 4.67</td>
<td>36.11 ± 6.88</td>
</tr>
<tr>
<td>TOMI (g.kg$^{-1}.W^{0.75}$)</td>
<td>22.64 ± 5.02</td>
<td>26.32 ± 3.95</td>
<td>30.53 ± 5.93</td>
</tr>
<tr>
<td>TCPI (g.kg$^{-1}.W^{0.75}$)</td>
<td>3.32 ± 0.79</td>
<td>3.45 ± 0.53</td>
<td>4.57 ± 0.87</td>
</tr>
<tr>
<td>TNDFI (g.kg$^{-1}.W^{0.75}$)</td>
<td>16.52 ± 3.49</td>
<td>18.88 ± 2.85</td>
<td>20.43 ± 3.82</td>
</tr>
<tr>
<td>TADFI (g.kg$^{-1}.W^{0.75}$)</td>
<td>10.98 ± 2.45</td>
<td>13.98 ± 2.13</td>
<td>14.84 ± 2.80</td>
</tr>
<tr>
<td>TADLI (g.kg$^{-1}.W^{0.75}$)</td>
<td>3.92 ± 0.87</td>
<td>3.91 ± 0.60</td>
<td>4.84 ± 0.92</td>
</tr>
<tr>
<td>TDEI (Kj/kg.W$^{0.75}$)</td>
<td>117.49 ± 33.83</td>
<td>145.55 ± 22.31</td>
<td>181.55 ± 41.24</td>
</tr>
<tr>
<td>Initial Wt.(kg)</td>
<td>14.61 ± 0.48</td>
<td>14.86 ± 0.57</td>
<td>14.43 ± 0.45</td>
</tr>
<tr>
<td>Final Wt.(kg)</td>
<td>15.07 ± 0.53</td>
<td>15.64 ± 0.56</td>
<td>15.10 ± 0.51</td>
</tr>
<tr>
<td>ADG (g/day)</td>
<td>10.95 ± 2.56</td>
<td>18.57 ± 2.88</td>
<td>15.95 ± 3.24</td>
</tr>
</tbody>
</table>

**Notes:**

DMI, Dry matter intake; TDMI, Total dry matter intake; TOMI, Total organic matter intake; TCPI, Total crude protein intake; TNDFI, Total NDF intake; TADFI, Total ADF intake; TADLI, Total ADL intake; TDEI, Total digestible energy intake; ADG, Average daily gain.
Table 6.3. Nutrient Digestibility (%) of Roselle Untreated and Roselle Treated with Na(OH) 2% and Ca(OH) 2 4%.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Untreated (n=9)</th>
<th>Na(OH) 2% (n=9)</th>
<th>Ca(OH) 2 4% (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMD</td>
<td>30.42 ± 2.60 b</td>
<td>46.76 ± 1.59 a</td>
<td>34.81 ± 2.19 b</td>
</tr>
<tr>
<td>OMD</td>
<td>33.69 ± 2.50 b</td>
<td>45.70 ± 1.68 a</td>
<td>38.42 ± 1.89 b</td>
</tr>
<tr>
<td>CPD</td>
<td>28.97 ± 4.50 a</td>
<td>28.29 ± 2.60 a</td>
<td>33.32 ± 3.43 a</td>
</tr>
<tr>
<td>NDFD</td>
<td>24.98 ± 3.50 b</td>
<td>42.76 ± 2.63 a</td>
<td>30.77 ± 2.15 b</td>
</tr>
<tr>
<td>ADFD</td>
<td>26.39 ± 2.70 b</td>
<td>49.64 ± 5.95 a</td>
<td>32.92 ± 1.62 b</td>
</tr>
<tr>
<td>ADLD</td>
<td>12.45 ± 2.60 a</td>
<td>14.04 ± 2.55 a</td>
<td>10.97 ± 2.15 a</td>
</tr>
<tr>
<td>EED</td>
<td>70.42 ± 4.90 a</td>
<td>68.95 ± 6.50 a</td>
<td>78.69 ± 5.70 a</td>
</tr>
<tr>
<td>CFD</td>
<td>37.50 ± 3.35 c</td>
<td>56.28 ± 1.94 a</td>
<td>45.71 ± 1.51 b</td>
</tr>
<tr>
<td>DE</td>
<td>25.04 ± 3.01 a</td>
<td>31.91 ± 2.82 a</td>
<td>31.58 ± 2.70 a</td>
</tr>
</tbody>
</table>

Notes:
Means with different superscripts in the same row differs significantly (P < 0.01) DMD, Dry matter digestibility; OMD, Organic matter digestibility; CPD, Crude protein digestibility; NDFD, Neutral detergent fibre; ADFD, Acid detergent fibre digestibility; ADLD, Acid detergent lignin digestibility; EED, Ether extract digestibility; CFD, Crude fibre digestibility and DE, Digestible energy.
Discussion

Digestibility

Table 6.3 indicates that the contents of DMD and OMD of treated roselle with NaOH were significantly ($P < 0.01$) higher than those of untreated roselle. Roselle treated with Ca(OH)$_2$ had increased DMD and OMD, but the difference was not significant compared to untreated roselle. These results were similar with those by Djajanegara (1985) who reported that Ca(OH)$_2$ treatment on wheat straw increased, intake of organic matter, organic matter digestibility and NDF digestibility.

The effects of NaOH on digestibility are therefore well known. At its best, alkali treatment will improve OMD of straw by about 20% (Greenhalgh, 1980). In this experiment, roselle treated with NaOH had shown an increased DMD and OMD by 20% with values of (53.70% and 35.65%, respectively). According to Wanapat et al. (1985), straw treated with NaOH can increase DMD by 15%, OMD by 20 - 22% and CFD by 10 - 20%. In this study, CFD was increased by 50%. These effects may be due to solubilisation of hemicellulose, increasing the extent of cellulose and hemicellulose digestion, and increasing the rate of cellulose and hemicellulose digestion (Klopfenstein, 1978). The digestibility nutrients of roselle treated with NaOH had increased significantly ($P < 0.01$), except DE, CPD and ADLĐ increase but not significantly different($P > 0.05$) (see Table 6.3). It is thought that roselle treated with NaOH increased the DMD,
OMD and ADFD through effects of NaOH on cell wall structure which allowed greater accessibility of the structural carbohydrates to the rumen micro-organisms and their enzymes.

Digestibility nutrients of roselle treated with Ca(OH)$_2$ in general had increased but not significantly different with untreated roselle. These results were similar with those of Djajanegara et al. (1985). According to them, there was no difference in DMD between treatments.

**Nutrient Intake**

The intake of nutrient measured per unit of metabolic body weight ($W^{0.75}$) is shown in Table 6.2. The treated roselle showed a higher total dry matter intake (TDMI) compared with untreated roselle. This is supported by Kiflewahid (1982) and Djajanegara (1985) who reported that rice straw, corn stover and bagasse treated with NaOH or Ca(OH)$_2$ increased the dry matter intake, digestibility, ash and decrease crude protein. The higher total dry matter intake (TDMI) of treated roselle resulted a higher total organic matter intake (TOMI), higher total crude protein intake (TCPI), higher total acid detergent fibre intake (TADFI) and higher total neutral detergent fibre (TNDFI). Sundstøl (1988) who reported that both physical and chemical treatment improved the voluntary intake of low quality roughages supports this. The intake was increased by 15 - 20%. (Treated NaOH straw). Previous studies indicated that the intake of treated roselle increased by 25 - 41% (see Table 6.3).
Average Daily Gain

Average daily gain (g/day) of sheep fed with untreated and treated roselle with NaOH 2% and treated with Ca(OH)$_2$ are shown in Table 6.2. ADG of sheep fed with treated roselle was higher than that of untreated roselle 18.57 ± 2.88, 15.95 ± 3.24 and 10.95 ± 2.56, respectively. This might be due to the higher digestible nutrient intake (see Table 6.3). Table 6.2 presents that Ca(OH)$_2$ treatment found that nutrient intake was higher than others, but ADG was lower than NaOH treatment. It might due to oxalate of roselle that interfered with the calcium absorption and utilisation that can bond in to oxalate to form Ca-oxalate. This Ca-oxalate can influence digestibility (Table 6.3). According to Djajanegara et al. (1985), Ca(OH)$_2$ treatment improved the intake and digestibility of wheat straw by sheep, it only upgraded the diet to approach maintenance level. In this experiment (Table 6.2) treated roselle with Ca(OH)$_2$ also improved intake and digestibility of roselle in sheep.

Conclusion

Based on these data, DMD, OMD, NDFD and CF digestibility of treated roselle with NaOH 2% were significantly (P < 0.01) increased, while treated roselle with Ca(OH)$_2$ 4% was shown not significantly different (P > 0.05) except for CFD that was increased significantly (P < 0.01) than untreated roselle.
Nutrient intake of treated roselle with NaOH 2% and Ca(OH)₂ 4% were increased but not significantly different (P > 0.05), even though treated roselle with Ca(OH)₂ 4% was higher.

Average daily gain of sheep fed with treated roselle increased. Based on details from the findings of the present study, it appears that treated roselle with NaOH 2% and Ca(OH)₂ 4% can improve digestibility, nutrient intake and ADG.
CHAPTER VII

GENERAL DISCUSSION AND CONCLUSION

Roselle pods are by-products obtained after the calyces are removed from the fruits, which are used for juice, jam, etc. They were separated into seeds, empty pods and whole pods, which were analysed for its chemical composition.

The moisture of roselle seeds, empty pods and whole pods showed nearly the same 25.27 ± 2.03, 25.23 ± 1.73 and 25.25 ± 0.54, respectively. These results were higher than the results analysed by El-Adawy and Khalil (1994) who ranged 9.25 - 11.66% for roselle seeds. The difference was due to the maturity of roselle seeds, harvesting handling and sample processing. Up to now, no data in the literature studying about roselle empty pods and roselle whole pods is available for comparison purposes.

The present study indicated that CP of roselle seeds, empty pods and whole pods was 20.10 ± 0.60%, 7.30% ± 0.42 and 16.50% ± 0.47, respectively. This result was in agreement with Rao (1996), Al-Wandawi et al. (1984) and FAO (1968) 18.80% - 22.30%, 20.58% and 21.24%, respectively. However, it was lower than that reported by El-Adawy and Khalil (1994) by 30.11% - 31.02%. The differences in CP might be due to genetic and environmental factor or maturity of the roselle seeds.
According to Rao (1996), the EE content of roselle seeds was 19.10% - 22.80%, while Backeit et al. (1994) indicated the value to be about 16.96%. However, the present study indicated that EE of roselle seed was 12.24% ± 0.93, lower than reported in the previous study.

The CF of roselle seeds in this study indicated that it was 27.70%. However, it was higher than that of Backeit et al. (1994), FAO (1978), and Samy (1980) who reported that CF values were 16.96%, 12% and 15.30% - 20.04%, respectively. However, Rao (1996) found that CF of roselle seeds was higher. Their data were variable, which could be attributed to genetic and maturity of seeds.

The present study indicated that ash of roselle seeds was 8% ± 0.31, however, it was lower than that reported by Backeit et al. (1994), 11.35%. However, according to FAO, 1978 and El-Adawy and Khalil (1994) who found that ash seeds were lower (5.40% and 5.80% - 6.89%, respectively) than the present study. The differences might due to the sample processing or the harvesting handling.

The result of this study indicated that roselle seeds NFE were 26.66%. It was in agreement with Samy (1980), who found NFE was 23.81% - 32.86%. However, it was lower than that of FAO (1978) who reported that roselle seed NFE was 43.7%. The differences could be attributed to maturity, genetic and environment.
DM and OM degradability of seeds and empty pods after 48 h were 39.87%, 31% and 26.30%, 28.85%, respectively. It showed that seeds were more degradable than empty pods. This is due to roselle seeds lower in CF than empty pods (27.70% and 45.30%) and roselle seeds were higher in CP and EE (see Table 4.1). This can influence microbe in the rumen (Jouany, 1991). However, it was still low. It might be due to the characteristics of fibrous by-products, where their low digestibility is caused by substances making cell wall structure, which prevent micro-organism action and enzymes on the substrates within the cell. According to Djajanegara (1984), for maintenance, ruminant requires more than 55% DM and OM degradability.

In this study, roselle whole pods were soaked with different concentrations: 2%, 4% and 6% of NaOH and Ca(OH)₂. The results are shown in Table 5.1 which indicates that roselle whole pods after treated with different level of alkalis, had lower nutrient content with the exception of ash. Alkali causes the saponification of ester linkages between acetic acid, phenolic acid, polysaccharides and lignin. If the linkages between lignin and hemicellulose are broken, this will make the latter and also cellulose (which is embedded in the lignin-hemicellulose complex) more accessible for hydrolysing enzymes.

The effects of alkali on the digestibility are therefore well known. At its best, alkali treatment will improve digestibility. The present study, indicated that roselle treated with NaOH 2% and Ca(OH)₂ 4% were higher in DMD, OMD,
CFD, TDMI, TOMI, TNDFI and TADFII. This result is related to those of Wanapat et al. (1985) and Djajaneagra (1985).

The ADG of sheep fed with the treated roselle whole pods with NaOH 2% and Ca(OH)$_2$ 4%, were higher than untreated roselle whole pods $18.57 \pm 2.88$, $15.95 \pm 3.24$ and $10.95 \pm 2.56$, respectively. This result could be due to the higher digestible nutrient (see Table 6.3). Table 6.2 showed that roselle pods treated with Ca(OH)$_2$ 4%, had higher nutrient intake than other diets. However, the ADG of sheep was lower than the treatment with NaOH 2%. It could be due to the presence of oxalate in roselle pods, which may interfere with the calcium absorption and utilisation, whereby calcium could be bonded to oxalate to form Ca-oxalate. This Ca-oxalate can be influence digestibility (Table 6.3).

However, the use of alkali depends on many factors, including national policy on chemical treatment, the cost of chemical, applicability and practicability of the method and importantly acceptance by the farmers.

**Conclusion**

From the result of this study, it can be concluded that the nutrient content of roselle seeds, empty pods and whole pods were relatively higher than other agricultural by-products, such as sago waste, straws, cocoa pods, stalks, and sugar cane bagasse and can be considered a potential energy and protein source for small ruminants.
The potential degradability of seeds was higher than the empty pods after 48 h incubation, while the degradability of the seeds was acceptance; the degradability of the untreated empty pods was low and may not be suitable for ruminants.

Digestibility for DM, OM, NDF and CF of roselle whole pods treated with NaOH 2% were significantly (P < 0.01) increased.

Nutrient intake and Average daily gain of sheep treated with whole pods roselle with NaOH 2% and also with Ca(OH)₂ 4% were increased but not significantly different (P > 0.05). Based on these studies, it can be concluding that a treated roselle by-product is potential alternative roughage, but it cannot be used as a single diet.

Future Research Program

Further research could include: 1) Determining the other anti nutritive compounds in the roselle particularly, tannin and oxalate, 2) Improving the nutritive value of treated roselle supplement with urea, molasses to improve the animal performance, 3) Improving and utilisation through physiochemical and micro biological processing and supplementation.
BIBLIOGRAPHY


feedsRef.No.537.
TFEED8/refs/537/roselle.html

Agriculture Hand Book, No.379.

Gohde, G and K. Becker. 1982. Solubility of cell wall constituents as predictors
of organic matter digestibility in some tropical and subtropical by-

Greenhalgh, J.F.D., 1980. Use of straw and cellulosic wastes and methods of
improving their value. In By-products and Wastes in Animal Feeding
(Edited by Dr. E .R. Yrskov) Occasional Publication No. 3. British


Microbial Metabolism and Ruminant Digestion (Edited J. P. Jouany)

Nutritional availability of amino acids from the rumen an aerobic fungus
Neocallimastix sp. L.M1, in sheep. Journal Agriculture Science 13: 383 -
387.

Steam treatment of crop residues for increased ruminant digestibility.

Manipulation of rumen fermentation in sheep by increasing the rate of
flow of water from the rumen. Journal Agriculture Science(Cambridge),
85; 93 -101. In Rumen Microbial Metabolism and Ruminant digestion
(Edited J. P. Jouany, 1991) pp. 311 - 316.

Holm, J. 1972. The treatment of rice straw with Sodium Hydroxide and its
economic limitation in Northern Thailand. That. Journal Agriculture
Science 5: 89 - 100.

Roselle (Hibiscus sabdariffa). Sudan Journal Food Science Technology 3:
37.


Workers. *FAO Animal Production and Health* paper, No. 50/2. FAO, Rome, Italy.


Smith, O.B. 1992. Small ruminant feeding system for small scale farmers in humid West Africa. In the complementary of feed resources for Animal


APPENDIX A

PROXIMATE ANALYSIS

Determination of Moisture and Dry Matter

Dry matter was determined following the method of AOAC (1984). Porcelain crucible were soaked and cleaned with decon 98 and rinsed with distilled water, dried in the oven at 105 °C, the dried crucibles were cooled in the dessicator and weighed \( W_1 \). About 1 g of sample was placed in the previously weighed empty crucible and the weighed of sample and crucible was recorded \( W_2 \). The crucibles were covered with the lids and kept in the oven set at 105 °C for 24 hour until there is no further loss in weigh. After 24 hours of drying, the crucibles were removed from the oven, cooled in a dessicator, and weighed \( W_3 \). The crucibles were handled with metal tong. Each sample was replicated three times and the mean values were taken. The moisture and the dry matter were calculated by using the following formulae.

\[
\text{Moisture} \, (\%) = \frac{W_3 - W_2}{W_2 - W_1} \times 100
\]

Where:

\( W_2 = \text{Dry weigh of crucible} \)

\( W_2 = \text{Weight of fresh sample + crucible} \)

\( W_3 = \text{Weight of crucible + dry sample} \)

\( DM \, (\%) = 100 - \% \text{Moisture} \)
Determination of Ash

Porcelain crucibles were cleaned, dried and cooled in dessicator. The empty crucible was weight and about 1 g of samples was taken to determine the ash content. The crucibles, with sample were covered and placed in the muffle furnace. The temperature was increased gradually to 550° C or 600° C and the samples were ignited for 5 - 6 hours or until white, light grey or reddish ash is obtained. The crucibles were then transferred from the furnace into the dessicator, allowed to cool and weighed.

\[
Ash(\%) = \frac{\text{Weight of ash}}{S.D.W} \times 100
\]

Where:

\[ S.D.W = \text{Sample Dry Weight} \]

Crude Protein (CP)

The sample contained in the in the plastic containers was mixed up by thorough shaking by hand. The digestion tubes were placed in the tube holding rack and identified with marker. About 0.3 - 0.5 g of sample was placed in the digestion tube. After weighing all the sample one selenium tablet (Kjeldahl catalyst tablets, Ajaxchemical Pvt. Limited); and 5 ml of concentrated sulphuric acid (98% H₂SO₄, N-free) was added to the tubes. The tubes containing samples were vortex to mix and placed on the digestion block under the fume hood (Kjeldahl digestion System, KT Gerhardt, Germany). The temperature of the
heating block was set at 100° C and heated for 30 minutes; the temperature was increased to 150° C and heated for another 30 minutes. Following the methods of Thomas et al. (1967) the temperature was switched to 225° C, after appearing a ring like appearance on the surface of the tube a few drops of hydrogen peroxide (H₂O₂) was added. The H₂O₂ treatment was repeated for 4 - 5 times. The temperature of the heating block was then gradually increased to 400° C and allowed to heat until digestion complete (colourless). Two blank tubes were placed in each batch of forty tubes and each sample was replicated twice.

The digestion tubes containing the sample were allowed to cool and the digesta were transferred in 100 ml volumetric flask. The tubes were forcibly rinsed with distilled water, which was transferred to the volumetric flask. The volumetric flasks were then allowed to cool and pouring distilled water up to the mark made the final volume. The crude protein was determined by using the Kjeltec Auto Analyser. The auto analyser was calibrated with blank sample and the reading was recorded. Ten ml of the diluted sample was placed in the tube of auto analyser and the reading was recorded to get the CP. Following formula was used for calculation.

\[
Crude\text{ Protein} (\%) = \frac{1401 \times N \times ML (Tit - Blank) \times Dil \times Factor}{S.D.W.}
\]

Where:

\( N = \text{Normality of the Acid} \)

\( Dil = \text{Dilution Factor} \)

\( Tit. = \text{Titration Reading} \)

\( Factor = 6.25 \)

\( Blank = \text{Blank Reading} \)
Ether Extract (EE)

The ether extract or the lipid content was determined either by means of Soxtec System (Soxtec System HT 1043 Tecator). About 1g of sample was weighted out into an extraction thimble having porosity permitting rapid passage of petroleum benzene or petroleum ether. The extraction cups were cleaned, dried and cooled in the dessicator and weighed ($W_1$). The thimbles were placed into the extraction unit along with previously weighed extraction cups. About 50 ml of solvent (petroleum benzene) was used for extraction. The samples were allowed to extract for the first 30 minutes in the boiling point by keeping the indicator at boiling position and rinsed for another 30 minutes in the rinsing position and another 30 minutes for evaporation. After evaporating the solvent, the cups were released and dried in the oven for 2 - 3 hours at 105° C. The cups were then cooled in the dessicator. After being cooled in the dessicator the weight was taken with the extract ($W_2$). The percentage of lipid was calculated using the following equation.

$$EE(\%) = \frac{W_2 - W_1}{S.D.W} \times 100$$

Where:

$W_1 = Weight$ of empty dry cup

$W_2 = Weight$ of empty dry cup + Ether Extract

$S.D.W = Sample$ Dry Weight
APPENDIX B

FIBRE COMPONENT

Determination of Crude Fibre (CF)

About 1.5 g of sample was placed in 600 ml beaker and 150 ml of 1.25 % H$_2$SO$_4$ was added. After reaching the temperature to the boiling point the solution containing the sample was heated for 30 minutes. The contents of the beaker were filtered through a California Buchner funnel with Whatman 541 filter paper. The beaker was rinsed with hot water to take the entire sample particle. The residue was washed with hot water to drain out all the acids and the filtrate was transferred into the beaker with the filter paper.

One hundred and fifty ml of 1.25% NaOH was added and allowed to boil for another 30 minutes following the same procedure discussed above. After completion of boiling, the samples were filtered through a pre cleaned sintered glass crucible. The filter paper was washed and removed. The filtrate in the crucible was washed with warm water to drain out the NaOH for at least three times. It was then washed with ethanol followed by rinsing with diethyl ether.

The sintered glass crucible containing the residue was placed in the oven and dried at 105°C over night. The crucibles were taken out from the oven, cooled in the dessicator, and weighed ($W_1$). The glass crucibles were then placed in the muffle furnace and ignited for 4 hours at 550°C. The crucibles were
cooled to below 200° C and transferred from the furnace to the dessicator, allowed to cool and weight \( (W_2) \). The percentage of crude fibre was calculated by the following formula.

\[
Fibre(\%) = \frac{W_1 - W_2}{S.D.W.} \times 100
\]

Where:

\( W_1 = \text{Weight of sintered glass crucible} + \text{dry fibre} \)

\( W_2 = \text{Weight of sintered glass crucible} + \text{Ash} \)

\( S.D.W = \text{Sample Dry Weight} \)

**Determination of Acid Detergent Fibre (ADF)**

About 1 g sample was placed into a 600 ml beaker and 100 ml of cold solution of Cetyl Trimethyl Ammonium Bromide (CTAB) (2% w/v) in H\(_2\)SO\(_4\) (1 N) and a few drops of decalin was added to prevent foaming. The mixture was rapidly brought to the boiling point and allowed to boil gently and evenly reflux for one hour. The contents of the flasks were filtered through a previously tarred sintered glass crucible of pore size of 100 - 200 µ. The crucible was repeatedly washed with warmed distilled water (at least three times) to drain out the detergent, then with acetone and finally sucked to dry. The crucibles were removed from the filtering manifold, placed in an oven and allowed to dry at 105° C for overnight. The crucibles were then placed in a dessicator, allowed to cool and weighted. Following formula was used to determine the ADF content.
\[ A.D.F. (\%) = \frac{\text{Fibre Weight}}{\text{Original S.D.W.}} \times 100 \]

*Where:*

\[ \text{S.D.W.} = \text{Sample Dry Weight} \]

**Determination of Acid Detergent Lignin (ADL)**

The acid detergent lignin procedure includes the acid detergent fibre as a preparatory step. The detergent removes the protein and other acid soluble materials, which would interfere with the lignin determination. ADF residue is primarily ligno-cellulose and acid insoluble ash (mainly silica) of which the cellulose is dissolved in 72% H₂SO₄ solution. By ashing the residue, the crude lignin fraction, including the cutin could be determine.

The crucible was placed in the glass tray. One end of the tray was 2 cm higher to drain away the acid from the crucible. The contents of the crucible was covered with cooled 72% H₂SO₄ and stirred with a glass rod to obtain a smooth paste, breaking all lumps. The crucible were filled in about half way (20 ml) and the glass rod was remained in the crucibles were refilled with H₂SO₄ after one hour and stirred to drain the acid. It was repeated for three times. After 3 hours, the residue was filtered off as much as possible with vacuum. The residue was rinsed once again with concentrated H₂SO₄. The filtered was then washed with hot distilled water until free of acid (at least 5 times) and filtered it off. The outer side of the crucible was washed to remove the residual acid. The crucibles were then placed in the oven, dried at 105° C for over night, and weight (Wₐ). The
crucibles were ignited in a muffle furnace 500° C for four hours. They cooled to below 200° C, transferred to a dessicator and weighed ($W_2$).

\[
\text{Lignin} \% = \frac{W_1 - W_2}{D.S.W.} \times 100
\]

Where:

\[W_1 = \text{Weight of crucible} + \text{Lignin}\]
\[W_2 = \text{Weight of crucible} + \text{Ash}\]
\[D.S.W. = \text{Dry sample weight from ADF procedure.}\]

Neutral Detergent Fibre (NDF)

The method for determining NDF was the same as the one describe by Goering and Van Soes (1970). About 1 g of sample was taken in a 600 ml beaker and 100 ml detergent solution was added in which 5 drops of decalin was added to prevent foaming. Temperature was reduced to avoid foaming. The temperature was readjusted and refluxed for 60 minutes after the onset of boiling. The neutral detergent solution was prepared by dissolving sodium laural sulphate (30 g), di sodium hydrogen ethylene diamine tetra acetate dihydrate (18.61 g), di sodium hydrogen phosphate reagent (4.56 g), sodium borate decahydrate (6.81 g) and purified grade, 2-ethoxy ethanol (10 ml) in distilled water (1 L). The pH of neutral detergent solution should be 6.9 - 7.0.

The beaker was gently swirled to suspend solids before pouring the contents into a sintered glass crucible (Duran No.2) that was previously placed on
the filtration apparatus. The beaker was rinsed twice with hot water (80°C) and
the contents poured into the sintered glass crucible. The sample in the sintered
glass crucible was washed with hot water and twice with acetone. The sample
was dried at 105°C in an oven overnight and weighed. Recovery of NDF was
calculated as:

\[ N.D.F(\%) = \frac{W_2 - W_1}{D.S.W.} \times 100 \]

Where:

- \( W_1 \) = Weight crucible
- \( W_2 \) = Crucible + fibre weight
- \( D.S.W. \) = Dry sample weight
APPENDIX C

DETERMINATION OF GROSS ENERGY

The amount of heat that is released when a substance is completely oxidised in a bomb calorimeter is called Gross Energy (GE). The heat of combustion is usually measured in calories.

Gross energy value of the feed and faeces were determined using a bomb calorimeter (Parr Adiabatic Calorimeter, Parr Instrument Co., III, USA). The sample holder assembly in the holding rings on the front of the master cabinet so that the terminal on the sample holder mates with the terminal of the master cabinet. Approximately 0.5 - 1 g of the sample was made into a pellet by using a pellet press, and placed into crucible. The crucible was tarred before placing the sample. The sample weight was recorded and the crucible containing sample was placed into the sample cup holder. Ten centimetres of fuse wire for oxygen bomb (NO. 45C10 Parr instrument Co., Moline, ILL U.S.A.) was tied to electrodes on the bomb head and bent into a loop to touch the sample. The sample holder was then assembled onto the combustion chamber by placing the bomb cap in position and turning it until tight. The bomb was fitted with the oxygen cylinder and oxygen was passed to 25 Atmospheric pressure.

The oval bucket was filled in with two litre of water and placed into the Parr Adiabatic Calorimeter. The bomb was then placed in the placed in the oval
bucket. The temperature probes were lowered and power switched on. The bomb ignited and the temperature was recorded after it becomes stable.

The calorimeter lid was opened, the bomb removed and the pressure was discharged slowly. The bomb cap was unscrewed from the combustion chamber and it was removed. The entire inner bomb surface was rinsed with distilled water and the solution was collected in conical flask to which a few drops of methyl red indicator was added and titrated with Sodium Carbonate (Na₂CO₃) solution and the volume. The volume of Na₂CO₃ used was recorded. All unburned pieces of fuse wire were removed from the electrode; values were subtracted from the initial length and recorded. Before bomb calorimeter is used, that bomb calorimeter should be calibrated with benzoic acid. The procedure the same with run for the sample above. The energy value was determined by using the following formula.

\[
W_{\text{std}} = \frac{(HW_{\text{std}} + C_1 + C_2)}{\Delta t_{\text{std}}}
\]

Where:

\(H = 6318 \text{ cal} / g\)

\(W_{\text{std}} = \text{Weight of benzoic acid}\)

\(C_1 = \text{ml of Na}_2\text{CO}_3\)

\(C_2 = \text{Fuse used}\)

\(W t\ \text{std.} = \text{Change of temperature for benzoic acid}\)

\(W t\ \text{sample} = \text{Change of temperature for sample}\)
\[ H = \frac{Wst \times \Delta t \text{ (sample)} - (C_1 - C_2)}{\text{Weight of sample}} \]

\[ H = \text{Energy value for sample} \]
Figure 4.1. Percentage of Loss in DM of Roselle Pods and Seeds in the Rumen of Sheep

Figure 4.2. Percentage of Loss in OM of Roselle Pods and Seeds in the Rumen of Sheep
VITA

Tri Hesti Wahyuni was born on April 29, 1958 in Bina, North Sumatra, Indonesia. She graduated from Gajah Mada University with B.Sc. degree in Animal Science in 1980 and Engineering of Animal Science in 1982. After completing her degree, she joined Mabar Feed Meal Co., Ltd., in quality control sector. Then she worked in the Sibolangit Breeding Farm until 1985. She became a lecturer in the Department of Animal Science, Faculty of Agriculture Universitas Sumatra Utara, Medan since 1986. In November 1997, she joined the Department of Animal Science, Faculty of Agriculture to study her Master Degree at Universiti Putra Malaysia.