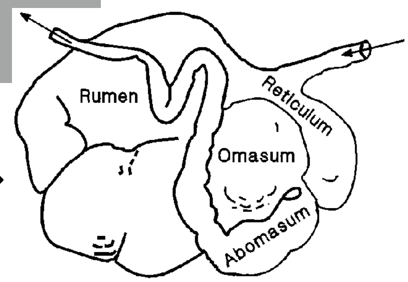
LEUCAENA LEUCOCEPHALA IN RUMINANT FEEDING MANAGEMENT:

Challenge and Opportunity on Production and Methane Emission Reduction

RUMINANTS



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1. Part of Thesis dissertation PhD , University of the Philippines Los Banos, major adviser Dr. Cesar C. Sevilla. Presented in ADSS at 16 March 2010, SEARCA
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FINAL EXAM-UPLB

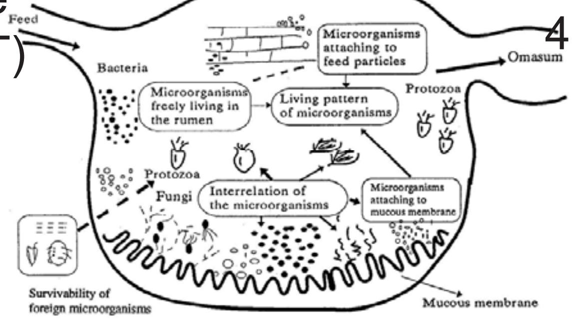
I. Background & Objectives
II. Materials & Method
III. Result & Discussion
IV. Summary, Conclusion & Recommendation
V. Thankyou

Bambang Suwignyo
March 16, 2010

Major : Animal Science
Minor : 1.Community Development
2.Environmental Sciences

BACKGROUND

1. Fibrous & poor (cellulose- hemicelluloses)
2. Concentrate
3. Others (LCT)



1. Maintenance
2. Meat, Milk
3. Faeces
4. Gas: CO_2, N_2O, CH_4 etc

Rumen- **Ruminant**

By pass

Carbohydrate, Protein

Protection
Tannin

Digestibility
Microorganism

OBJECTIVES

Determine the effect of tannin from *Leucaena leucocephala* on the rumen ecology of carabao and cattle fed with rice straw.

Specifically, it aimed to determine its effect on the rumen pH, temperature and ammonia-N levels, *in sacco* dry matter and protein digestibility, and the microbials population.

MATERIALS & METHODS

The study was conducted at Animal and Dairy Sciences Cluster from October 2008 to August 2009.

Rice Straw

Napier

Leucaena sp

- Diet 2.5% body weight, DM based
- Const : roughage 40 : 60
Napier, *Leucaena* sp
- 17 days feeding

- 3 fistulated Carabao and Cattle (BW 309 ± 67.4 kg ; 384 ± 36.14 kg)
- *Leucaena sp*. (5.1% tannin): 0%, 6%, 12% levels

PARAMETERS MEASURED, Study 1 & 2

- In Sacco rumen digestibility: DM, CP, NDF
- Feed Intake: DM, CP, NDF
- Rumen Fermentation Parameter : pH, temperature, Ammonia-N.
- Cellulolytic bacteria population
- Methanogenic bacteria population

Statistical Analysis

- Data collected were analyzed ANOVA using Predictive Analytics SoftWare (PASW; formerly SPSS) and Microsoft Excel.
- 2x 3 RCBD was used at this Study

RESULTS AND DISCUSSION

Study LCT

rumen *In sacco* digestibility

Feed Intake & Rumen Parameter

Extraction and Amplification

Celulolytic

Methanogenic

RESULTS AND DISCUSSION

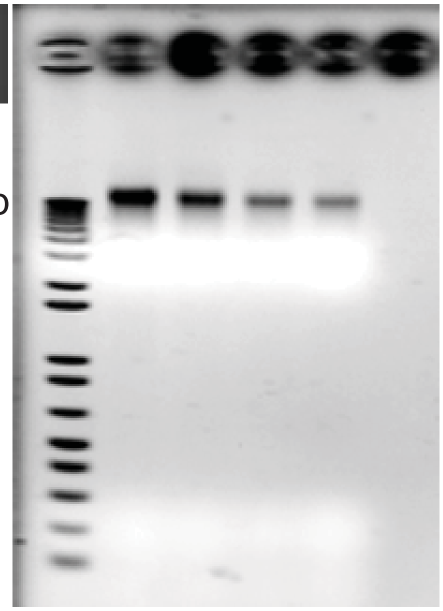
DNA EXTRACTION

Sharma et al., 2003

Nucleospin kit

Nano Drop

Gel



RESULTS AND DISCUSSION

- Pattern on the intensity of agarose gel band 0%, 6% and 12% (LCT).
- All bands in the *agarose* gel product conform with the expected bands for:
- *Fibrobacter succinogenes* (445 bp),
- *Ruminococcus albus* (176 bp) and
- *Ruminococcus flavefaciens* (295 bp).
- *Methanogens* (1.4 kbp)

Table 9. Rumen dry matter (%), crude protein (%) and neutral detergent fiber degradability (%) of rice straw with treatment LCT in carabao and cattle

Parameter measured	Animals	Level of LCT (%)			Average
		0	6	12	
Dry matter					
D	Carabao	15.0 ^{p}	15.0 ^{p}	13.0 ^{q}	14.33
	Cattle	15.0 ^{p}	15.2 ^{p}	13.5 ^{q}	14.57
a	Carabao	6.00	7.00	6.00	6.33
	Cattle	6.00	6.00	5.80	5.93
b	Carabao	20.5	20.5	12.0	17.67
	Cattle	24.2	24.0	17.7	21.97
c	Carabao	-1.83	-1.41	-1.79	-1.68
	Cattle	-1.38	-1.18	-1.67	-1.41

$^{p, q, r}$ superscript show significant differences among CVT levels
$^{y, z}$ superscript show significant differences between carabao and cattle

Crude protein					
D	Carabao	1.10	1.10	0.90	1.03
	Cattle	1.25	1.25	1.15	1.22
a	Carabao	0.50	0.50	0.50	0.50
	Cattle	0.50	0.52	0.45	0.49
b	Carabao	1.65	1.30	1.40	1.45
	Cattle	1.65	1.78	1.55	1.66
c	Carabao	-1.37	-1.31	-1.03	-1.24
	Cattle	-1.30	-0.93	-1.85	-1.36
Neutral detergent fiber D	Carabao	13.2 ^{p}	12.0 ^{p}	11.0 ^{q}	12.07
	Cattle	14.5 ^{p}	12.5 ^{p}	11.5 ^{q}	12.83
a	Carabao	6.0 ^{y}	5.5 ^{y}	5.5 ^{y}	5.67 ^{y}
	Cattle	5.0 ^{z}	5.0 ^{z}	5.0 ^{z}	5.0 ^{z}
b	Carabao	14.1	13.0	13.5	13.53
	Cattle	21.0	17.0	14.8	17.60
c	Carabao	-1.41	-1.39	-1.22	-1.34
	Cattle	-1.99	-1.68	-1.49	-1.72

$^{p, q, r}$ superscript show significant differences among CVT levels
$^{y, z}$ superscript show significant differences between carabao and cattle

Table 10. Feed Intake DM, CP, NDF carabao and cattle, CVT

Variable measured	Animals	Level of LCT (%)			Average
		0	6	12	
Dry matter intake (DMI)					
% body weight	Carabao	2.44	2.45	2.40	2.43
Total (kg)	Cattle	2.43	2.45	2.36	2.41
	Carabao	10.47 py	9.49 qy	8.15 ry	9.37
	Cattle	10.22 pz	9.31 qz	8.27 ry	9.26
Crude protein intake (CPI)					
% body weight	Carabao	0.26 p	0.28 q	0.30 r	0.28
Total (kg)	Cattle	0.26 p	0.28 q	0.30 r	0.28
	Carabao	1.12 py	1.10 qy	1.02 ry	1.08
	Cattle	1.09 pz	1.07 qz	1.06 qz	1.07
(NDFI)					
% body weight	Carabao	0.25 p	0.27 q	0.29 q	0.27
Total (kg)	Cattle	0.25 p	0.27 q	0.29 q	0.27
	Carabao	1.07 py	1.06 py	0.99 qy	1.04
	Cattle	1.05 pz	1.03 qz	1.03 qz	1.04

p, q, r superscript show significant differences among CVT levels
y, z superscript show significant differences between carabao and cattle

Table 11. Rumen pH, temperature, and ammonia-N of carabao and cattle in 0%, 6% and 12% LCT in different parameter

Variable measured	Animals	Level of LCT (%)			Average
		0	6	12	
pH pre-post feeding					
0 hour	Carabao	6.6	5.9	6.3	6.3
	Cattle	6.5	6.4	6.6	6.5
4 hours	Carabao	6.9	6.9	6.6	6.8
	Cattle	7.2	6.8	6.9	7.0
8 hours	Carabao	7.0	6.9	6.8	6.9
	Cattle	7.1	7.0	7.0	7.0

Rumen temperature					
0 hour	Carabao	39.6	38.2	38.9	38.9
	Cattle	38.9	38.5	38.6	38.7
4 hours	Carabao	40.1 ^{p}	39.4 ^{p}	39.1 ^{q}	39.5
	Cattle	38.9 ^{p}	38.9 ^{p}	38.1 ^{q}	38.6
8 hours	Carabao	39.2 ^{p}	39.2 ^{p}	39.0 ^{q}	39.1
	Cattle	39.7 ^{p}	39.3 ^{p}	38.4 ^{q}	39.1
Ammonia-N (NH_3)					
0 hour	Carabao	92.4 ^{p}	178.5 ^{q}	141.6 ^{q}	137.5
	Cattle	70.4 ^{p}	134.9 ^{q}	97.4 ^{q}	100.9
4 hours	Carabao	100.4 ^{p}	132.6 ^{q}	158.5 ^{q}	130.5
	Cattle	75.7 ^{p}	134.3 ^{q}	121.3 ^{q}	110.4
8 hours	Carabao	25.9 ^{p}	61.5 ^{q}	62.8 ^{q}	50.0
	Cattle	9.9 ^{p}	60.5 ^{q}	50.8 ^{q}	40.4

$^{p, q, r}$ superscript show significant differences among CVT levels

$^{y, z}$ superscript show significant differences between carabao and cattle

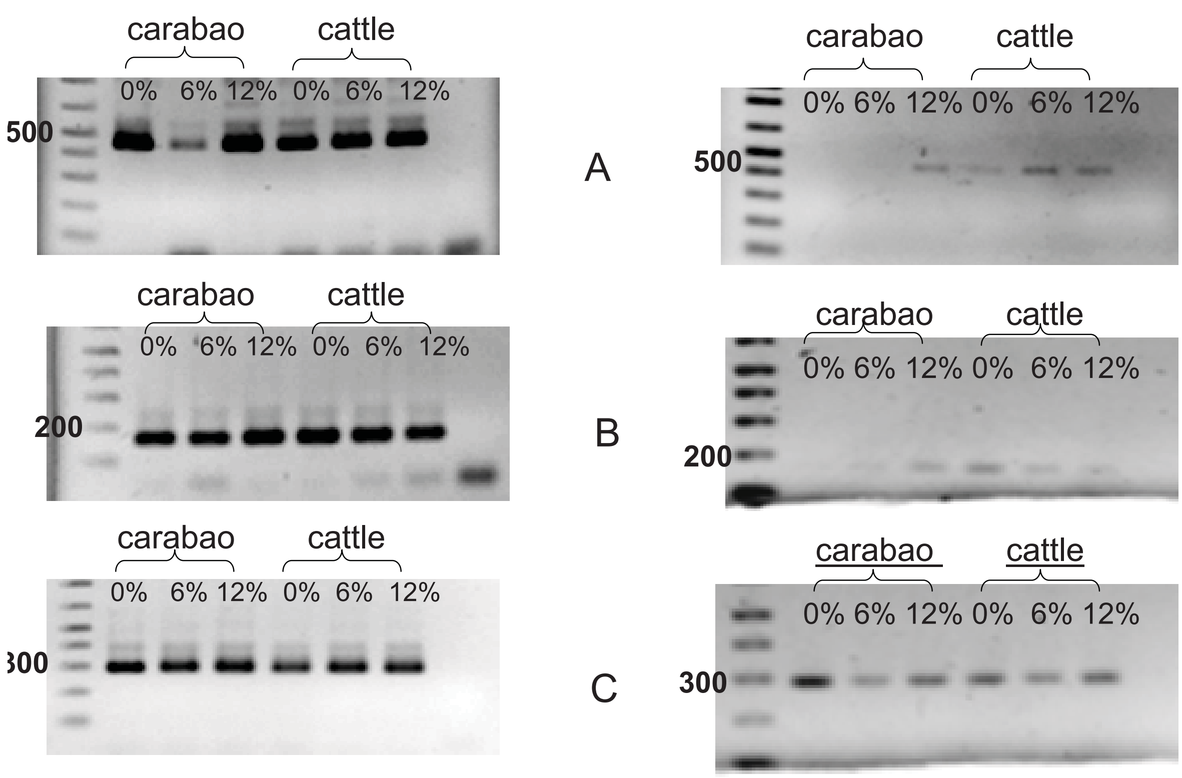


Fig.11. PCR amplification of *F. succinogenes* (A), *R. albus* (B), & *R. flavefaciens* (C). PCR 30 cycles in the left and 20 cycles in the right side; with LCT.

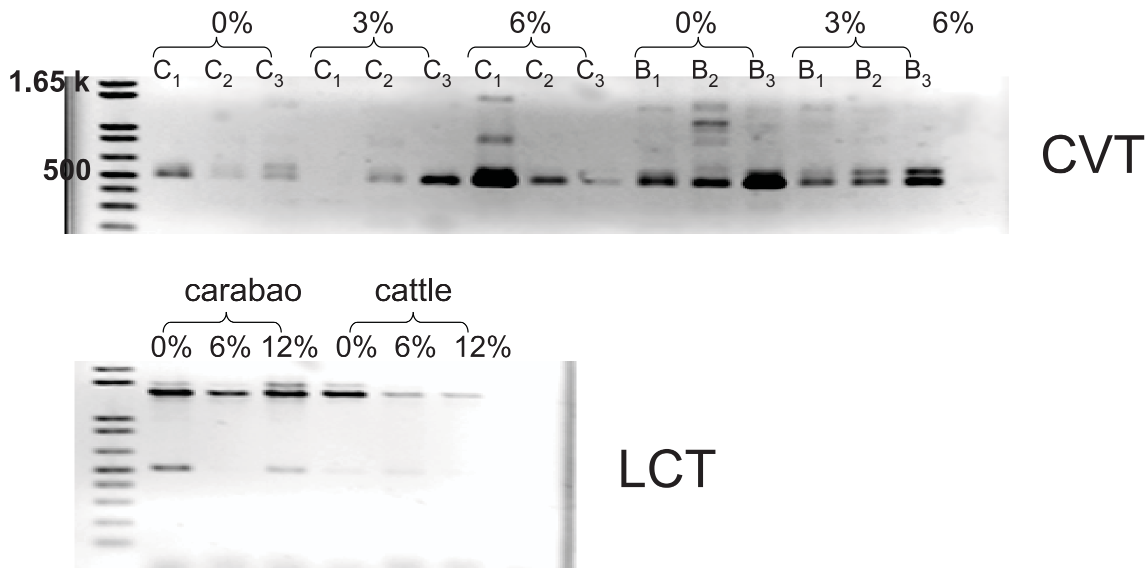


Figure 7. PCR amplification of Universal primers *Methanogens* (PCR 30 cycles)

SUMMARY

CVT

Intake (DM, CP, NDF)
In sacco #
intake

Carabao >>> Cattle
- c >>
- b <<
- DMI, CPI. NDFI >>
- NH3 >>

Leucaena sp

23.9 % CP

1 Intake >> CVT (DM, CP)
2. *In sacco* >> CVT (DM, CP, NDF)
3. NH3 >> CVT

- VFA up
- Branch VFA up
- Stimulate MO growth

PCR product

Methanogens F.succinogenes up

Propionate up Gluconeogenesis

Productivity up → milk yield, ADG

DMI
acetate
Propionate

10^7- 10^9 cell/ml
37% CH_4 emission
30 -40% gas → Environment

CONCLUSION

- Additives in the diet of ruminants affect the performance of microorganisms in the rumen or rumen ecology as a system and ultimately can affect performance of the ruminant itself. Tannin, condensed type is an important feed additive that inhibits the digestibility of carbohydrates and protein in the rumen of ruminants.
- *Leucaena leucocephala* enhances the population of *F. succinogenes* in rumen carabao and cattle, enhances *R. albus* in carabao, inhibits *R. albus, R. flavefaciens* and *methanogens* in cattle.

CONCLUSION

- Tannin from LCT able to decrease *in sacco* rumen digestibility. It maybe affect to increasing number of by pass protein that will be digested in the small intestinum.
- In this study *Leucane leucocephala* not only better as source of tannin but also as protein supplier that may stimulate the growth of cellulolytic bacteria to without any effect significant in the pH, temperature and NH_3-N.

CONCLUSION

- Intake of DM, CP, and NDF carabao significantly decreases by LCT, while cattle decreased by LCT only. Carabao has higher intake of DM, CP and NDF and rumen ammonia concentration compared with cattle.
- The pH, temperature and NH_3 rumen carabao and cattle doest not affected by tannin. However, base on data intake, *in sacco* and rumen parameter, carabao shows better performance compared with cattle.

RECOMMENDATION

Additional studies should be conducted to compare the effects of commercial vegetable tannin and *Leucaena leucocephala* with other indigenous sources of tannins on rumen ecology.

A study likewise, to determine more about the role of protozoa on methanogens and the possibility of increasing the efficiency of animal production as well as environmental benefit due to reduction of methane emission.

RECOMMENDATION

- Feed intake and digestibility was higher than cattle. In farming communites where there is abundant source of agricultural by-products but with low quality feedstuff like cereal straws, stovers, sugarcane bagasse, and fruit pulps, citrus pulp, and rice straw, and owing to the results of the study, it is better to raise ruminants like carabao instead of cattle. And this information could be integrated in the extension messages of community change agents as well as get them incorporated in the subject matter content of various science curriculum in both elementary and high school.

RECOMMENDATION

- Planting of *Leucaena leucocephala* trees as fence or strip cropping will become good investment for farmer who raises ruminants. *Leucaena leucocephala* give many benefit e.g increase soil quality, source of leaf protein for ruminant and source of tannin that will have a good impact in the term of ruminat nutrition and and global environmental contribution through reducing methanogens in the rumen.

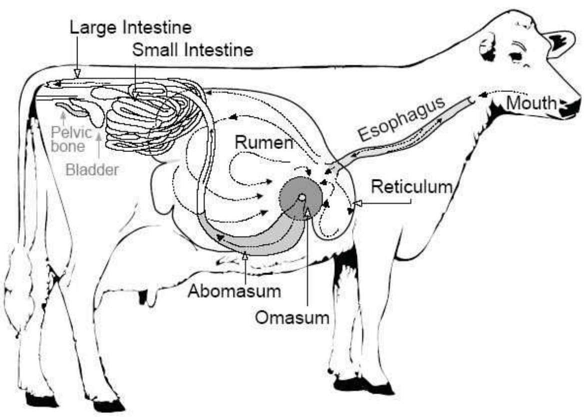
TERIMAKASIH

Thank you !

Salamat Po !

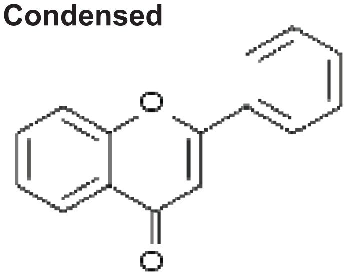
A ruminant

- multiple stomach where
- microorganism (bacteria, fungi and protozoa) has
- ability to digest the fibrous material and poor quality of feed that contains cellulose and hemicelluloses (Dehority, 1998; Kamra, 2005).



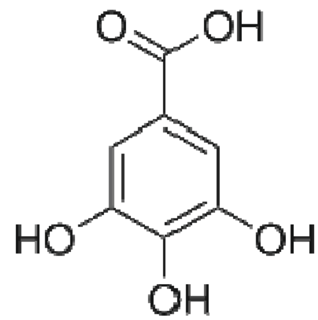
TANNIN

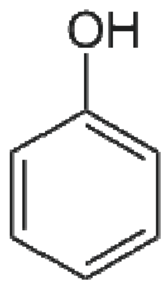
Condensed



Polyphenol

Hydrolysable





Phenol

Tannins used:

- would improve the efficiency of nutrient
- ensure safety for animals and human

Tannins

- present in several native shrubs
- may inhibit activity ruminal microorganisms (Nunez-Hernandez et al., 1991).
- unstable at the acid pH of the abomasum

Rumen microorganism population consists of:
•bacteria (10^{10}–10^{11} cells/ml, represent-ing more than 50 genera),
•ciliate protozoa (10^4–10^6/ml, from 25 genera),
•anaerobic fungi (10^3–10^5 zoospores/ml, repre-senting five genera)
•and bacteriophages (10^8–10^9/ml)

Three major *cellulolytic* bacteria:

- considered to be representative of the rumen (Forsberg et al., 1997).
- *Fibrobacter succinogenes* (19.2%),
- *Ruminococcus albus,* and *Ruminococcus flavefaciens* (59.8%).

Rumen Gases

- Between 30 and 40 percent of the total gas present in the rumen of cattle is methane,
- carbon dioxide can vary from 20 to 65 percent in cattle fed once in 24 hours. Usually, carbon dioxide forms about 60 percent of the gas present in animals that are fed *ad libitum* (Kamra, 2005).

Dairy cows typically produce 118 kg methane/ year, which is over twice that produced by other non-lactating cattle.

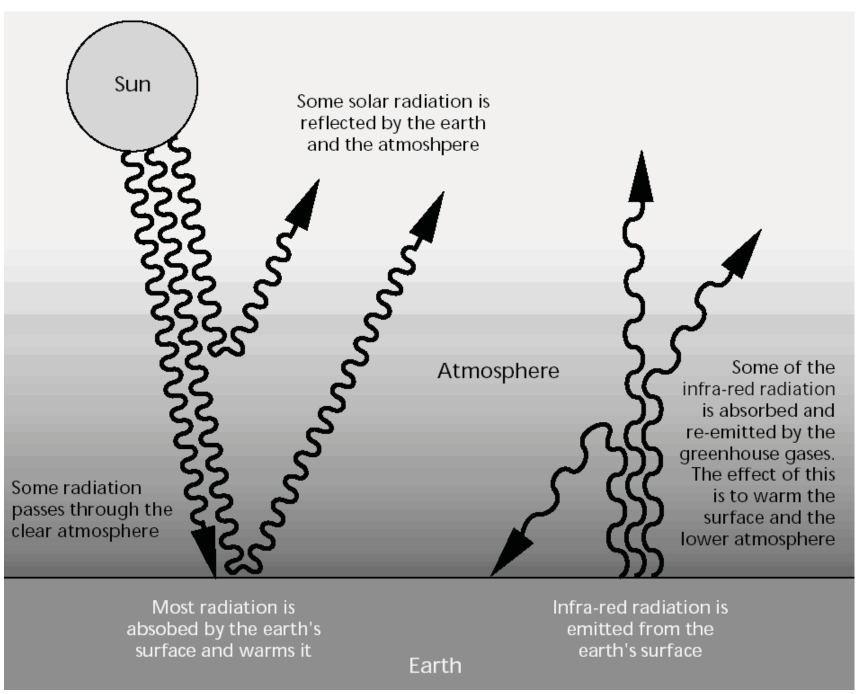
118 kg of methane is equivalent to 2.478 ton of CO_2 in inventories of GHG production (Frank O'Mara, 2004).

METHANE

$$CO_2 + 4H_2 \xrightarrow{\text{ATP generation}} CH_4 + 2H_2O$$

- (CH_4) is one of the largest sources of greenhouse gas from feedlot and dairy farm, aside from nitrous oxide (N_2O) and carbon dioxide (CO_2).
- A poor quality diet leads to inefficient digestion, which leads to increased methane production and lowered animal productivity
- CH_4 emissions represent an economic loss to the farmer where feed is converted to CH_4 rather than to product output. In fact, 8 to 12% of the digestible energy ingested by ruminants is lost in the rumen as methane
- *Methanogens* are present in the rumen in large numbers which vary from 10^7 to 10^9 cells/ml of rumen liquor depending upon the type of diet given to the animals, especially the fiber content in the ration.

GREEN HOUSE EFFECT by GHG



Protection

Efficiency can be increased by protecting nutrient in the diet especially protein from rumen microorganism degradation

Formaldehyde

- can be used to protect feed from rumen microbial digestion.
- Chemical material , costly, dangerous
- does not fit the local ruminant feeding system (Sevilla et al., 2003).

Tannins in legume

Leucaena leucocephala :

- Common legume,
- Cheap and easy to find.
- Source of protein
- Source of tannins

Atega et al., 2003; Orden et al, 2002, Sevilla et al., 2003).

Table 2. Chemical composition of feedstuff offered to animals (*)

Feed stuff	DM	CP	NDF	Tannin
Study				
Commercial concentrate	88.01	13.54	21.03	-
Napier grass	24.80	8.70	70.72	-
Leucaena leucocephala	23.60	23.90	36.58	5.108 [2)]

* Analysis result at Nutrition and DTRI laboratory, ADSC, UPLB
1. LQCC, 2008
2. Hess et al., 2007

Study

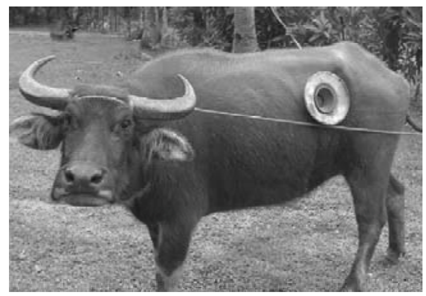
I. 40% concentrate : 60% Napier
II. 40% concentrate : 54% Napier : 6% LCT, DM basis
III. 40% concentrate : 48% Napier : 12% LCT, DM basis

I N SACCO

- Nylon bag 5 cm x 15 cm; pore size 50 u
- Rice straw, dried oven at 70°C
- ground using Willey mill at 1 mm sieve
- 5 g of rice straw into bag
- Incubated 2, 4, 8, 16, 24, and 48 hours
- Washed until water clear
- rinsed and then squeezed using the hand to remove the excess water
- dried at oven at 70°C and
- weighed to get the final weight
- Analyzed: moisture, crude protein (AOAC, 1984) and NDF (Van Soest et al., 1991).
- Computation **$D = a + b\,(1 - e^{-ct})$**

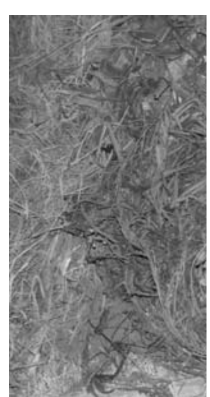
D = % degradation), a = intercept (soluble fractions),

b = fractions which potentially degraded, c = degradation rate of fraction b, and e = natural logarithm



FEED INTAKE

- Feed refusal was collected from each animal at 800 h,
- weighed, mixed, sub-sampled and bulked in bags
- dried oven at 70 ^{o}C
- ground using Willey mill at 1 mm sieve
- Analyzed: moisture, crude protein (AOAC, 1984) and NDF (Van Soest et al., 1991).



RUMEN pH, AMMONIA-N & TEMPERATURE

- Sample pH, temperature and ammonia taken from rumen *canula* at pre-feeding, 4 and 8 hours post-feeding
- pH and temperature measured directly after it collected
- Portable pH meter
- Portable thermometer
- Ammonia-N sample: portion of 20 ml sub-samples acidified with 2 ml 6 N HCl to inhibit microbial activity and frozen at -20 ^{o}C until analyzed

Ammonia-N

pH

Three specific primers cellulolytic bacteria

1. Fibrobacter succinogenes
a.Fs219f (5P-GGT ATG GGA TGA GCT TGC-3P) and
b.Fs654r (5P-GCC TGC CCC TGA ACT ATC-3P), (446- bp)

2. Ruminococcus albus primers,
a.Ra1281f (5P-CCC TAA AAG CAG TCT TAG TTC G-3P) and
b.Ra1439r (5P-CCT CCT TGC GGT TAG AAC A-3P), (175-bp).

3. Ruminococcus flavefaciens primers,
a.Rf154f (5P-TCT GGA AAC GGA TGG TA-3P) and
b.Rf425r (5P-CCT TTA AGA CAG GAG TTT ACA A-3P), (295 bp)

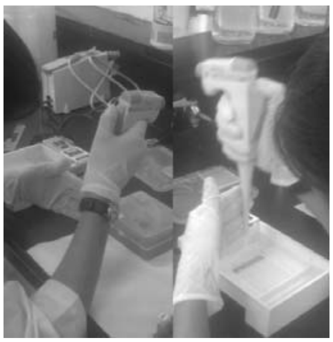
Universal primers methanogenic bacteria

a. 0025e F CTG GTT GAT CCT GCC AG
b. 1492 R GGT TAC CTT GTT ACG ACTT (1.4 kbp)

deposited in Gen Bank.

PCR

Extraction
- Rumen fluid , pump
- Conical tube, freezer
- DNA extraction (Sharma et al., 2003)



Amplification
- Adjust dilution, **quantity**
- **3 specific primers**, *cellulolytic*
- **PCR** machine

Electrophoresis
- Gel agarose, photodoc
- Picture

PCR

- The PCR reaction mixture contained 2.0 μl of 10 ×; 0.6 Taq polymerase buffer (50 m*M* MgCl); 0.5 μl of forward primer (20 μM); 0.5 μl of reverse primer (10 μM); 0.16 μl of dNTP (25 m*M*), 0.04 μl of Taq polymerase (0.2 u/ μl); 1 μl of DNA (6.84 ng/μl); add ddH2O (sterile) to total volume 20 μl.
- The reaction was carried out in a PCR G-Storm, as follows: 30 s at 94 ^{o}C for denaturing, 30 s at 60 ^{o}C for annealing and 30 s at 72 ^{o}C for extension (30 cycles), except for 9 min denaturation in the first cycle and 10 min extension in the last cycle.
- Number of cycle in the PCR set was reduced into 20 cycles for optimization purpose.
- Products of PCR were separated on agarose gel, stained with ethidium bromide, and photographed.
- Adobe Photoshop program was used to edit the gel image.

Figure 13. Outline of the pathways of carbohydrate and protein degradation in the rumen (Kempton et al., 1978).

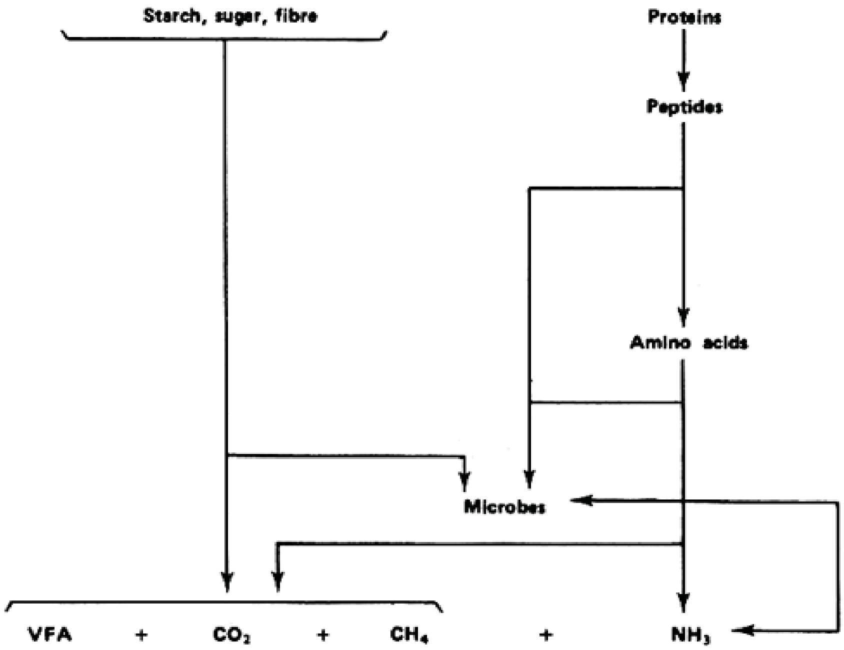
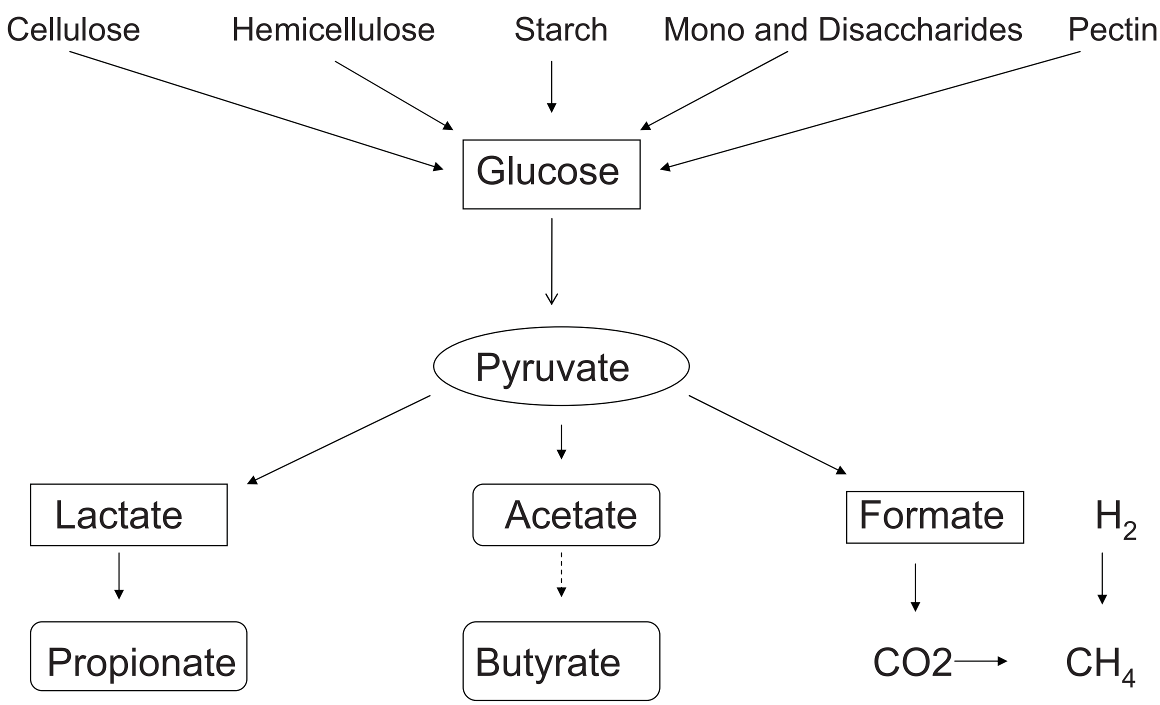


Figure 14. Carbohydrate utilization by rumen microbes (Allison and Leek, 1993)



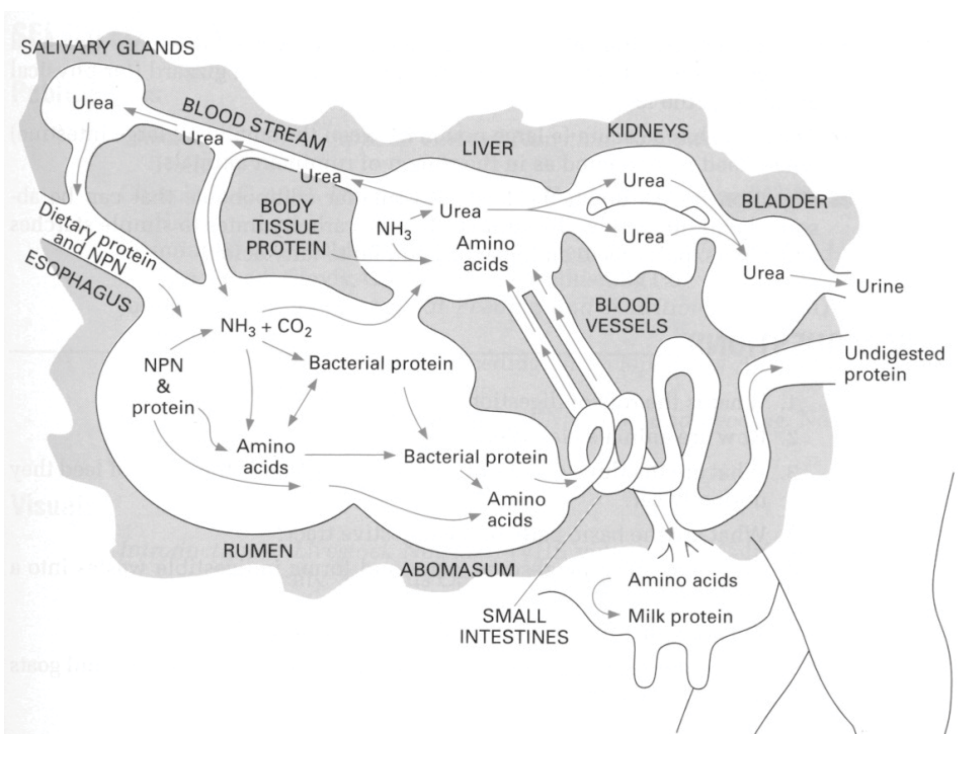
VFA

In the rumen:

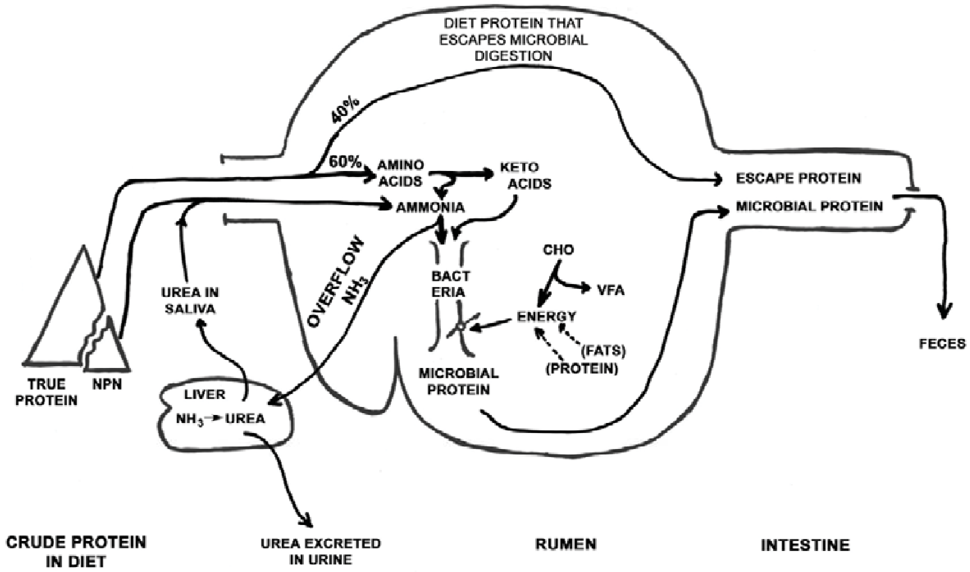
- Acetic acid, 60-70 %,
- propionic acid, 15-20 %,
- butyric acid it usually represents and 10-15 %

of the fatty acid mixture in animals fed hay or other roughages

Figure 16. Protein pathways in the ruminant (Bryant and Moss, 2009)



PROTEIN (NITROGEN) UTILIZATION BY THE RUMINANT



ENERGY PATHWAY IN THE RUMINANT

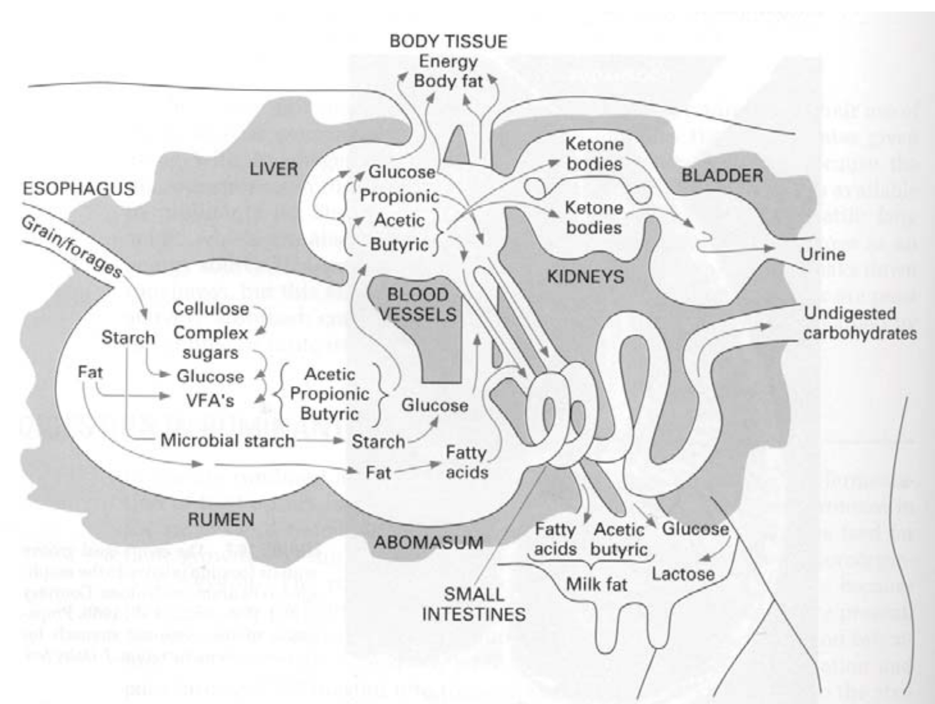


FIGURE 16.8 Energy pathways in the ruminant. Courtesy of J. Bryant and B. R. Moss, Montana State University.

Figure 15. Degradation and digestion of dietary protein in the ruminant (Kempton et al., 1978).