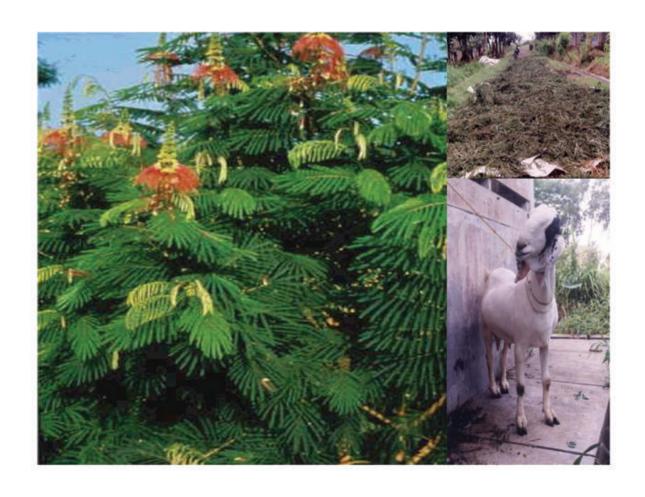
YUSUF SUBAGYO

EVALUATION OF Calliandra calothyrsus LEAVES AS SUPPLEMENT TO NAPIER GRASS ON INDONESIAN ETTAWAH CROSSBRED GOATS' PERFORMANCE





Cuvillier Verlag Göttingen

EVALUATION OF Calliandra calothyrsus LEAVES AS SUPPLEMENT TO NAPIER GRASS ON INDONESIAN ETTAWAH CROSSBRED GOATS' PERFORMANCE

DOCTORAL DISSERTATION SUBMITTED FOR THE DEGREE OF DOCTOR OF AGRICULTURAL SCIENCES OF THE FACULTY OF AGRICULTURAL SCIENCES

BY: YUSUF SUBAGYO BORN IN KEBUMEN, CENTRAL JAVA, INDONESIA

GEORG-AUGUST-UNIVERSITY, GÖTTINGEN, GERMANY MAY 2004

Bibliografische Information Der Deutschen Bibliothek

Die Deutsche Bibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über http://dnb.ddb.de abrufbar.

1. Aufl. - Göttingen : Cuvillier, 2004 Zugl.: Göttingen, Univ., Diss., 2004 ISBN 3-86537-194-9

D7	
Referee	Prof. Dr. Dr. h.c. Udo ter Meulen
Co Referee	Prof. Dr. Helge Böhnel
Date of examination	27 Mai 2004

Printed with the financial support of Quality Undergraduate Education (QUE) Programme within the framework of World Bank IBRD LOAN NO. 4043-IND

© CUVILLIER VERLAG, Göttingen 2004

Nonnenstieg 8, 37075 Göttingen

Telefon: 0551-54724-0 Telefax: 0551-54724-21

www.cuvillier.de

Alle Rechte vorbehalten. Ohne ausdrückliche Genehmigung des Verlages ist es nicht gestattet, das Buch oder Teile daraus auf fotomechanischem Weg (Fotokopie, Mikrokopie) zu vervielfältigen.

1. Auflage, 2004

Gedruckt auf säurefreiem Papier

LIST OF CONTENTS

CHAPTE	R	PAGE
	LIST OF CONTENTS	i
	LIST OF TABLES	vii
	LIST OF FIGURES	ix
	ABREVIATIONS	X
1.	INTRODUCTION	1
1.1. 1.2.	Background of the study Objectives of the study	1 3
2.	LITERATURE REVIEW	4
2.1. 2.1.1. 2.1.2 2.1.3. 2.1.4.	Calliandra calothyrsus Taxonomy Botanical description Habitat Variation within Calliandra calothyrsus	4 5 5 6 7
2.2. 2.2.1. 2.2.2. 2.2.2.1. 2.2.2.2. 2.2.3. 2.2.4. 2.2.4.1. 2.2.4.2. 2.2.4.3. 2.2.4.4. 2.2.5.	Use and management of <i>C. calothyrsus</i> Fodder Chemical composition Different between <i>C. calothyrsus</i> provenance Effect of drying on chemical composition Nutritive value Animal production Use of <i>C. calothyrsus</i> as a supplement to low quality diets Use of <i>C. calothyrsus</i> as a replacement for concentrates Use of <i>C. calothyrsus</i> in-non ruminant diets Use of <i>C. calothyrsus</i> in grazing systems Management for fodder production	7 8 8 9 10 11 12 12 13 14 14 15
2.3. 2.3.1. 2.3.2. 2.3.3. 2.3.4. 2.3.5. 2.3.5.1. 2.3.5.2. 2.3.5.3. 2.3.5.4. 2.3.6. 2.3.7.	Secondary plant compounds and tannins Occurrence of hydrolysable and condensed tannin in plants Occurrence of tannins in <i>Calliandra calothyrsus</i> Factors affecting concentration of condensed tannins in plants The beneficial effects of tannins The detrimental effects of tannins Tannins and toxicity Tannins and palatability Tannins effect on digestibility Tannins and digestive enzymes Physiological mechanism of adaptation to tannin Effects of tannins on nutritive value of ruminant feeds	17 18 19 21 21 23 23 24 24 25 25
2 3 7 1	Nitrogen metaholism	26

2.3.7.1.1. 2.3.7.1.2. 2.3.7.1.3. 2.3.7.2. 2.3.7.3. 2.3.7.4. 2.3.7.5.	Protein degradation Nitrogen absorption and amino acid supply to the small intestine Nitrogen retention Effect on pH Carbohydrate metabolism Palatability and voluntary feed intake Effects of condensed tannins on animal production	26 27 28 28 29 30 31		
2.4.	Detannification	31		
2.4.1.	Polyethylene glycol	32		
2.4.2.	Pre-treatments with chemicals	33		
2.4.3.	Drying	34		
2.4.4.	White rot fungi	34		
2.4.5.	Nutrient supplements	35		
3.	EXPERIMENT 1: PHYTOCHEMICAL ANALYSIS AND QUALITATIVE DETERMINATION OF CHEMICAL COMPOUNDS OF CALLIANDRA CALOTHYRSUS LEAVES USING GAS CHROMATOGRAPHY-MASS SPECTROMETRIC ANALYSIS	37		
3.1.	Objectives	37		
3.2.	Materials and methods			
3.2.1.	C. calothyrsus harvesting	37		
3.2.2.	Sample extraction	38		
3.2.3.	Toxicological test			
3.2.3.1.	Sample preparation			
3.2.3.2.	Hatchery of cyst of Artemia salina (Leach)			
3.2.3.3.	Determination of larvae mortility			
3.2.3.4.	Statistical analysis			
3.2.4.	Phytochemical analysis of C. calothyrsus	39		
3.2.4.1.	Crude extract			
3.2.4.2.	Identification of volatile oils, fat and high fatty acids, sterols and monoterpenoids	40		
3.2.4.3.	Identification of alkaloids and triterpenoids	40		
3.2.4.4.	Identification of flavonoids	41		
3.2.4.5.	Identification of flavonoids Identification of glycosids di/triterpenoids, saponins, and carbohydrates	41		
3.2.4.3.	Gas chromatography-mass spectrometric analysis	43		
3.2.5.1.	Extraction of <i>C. calothyrsus</i> leaves	43		
3.2.5.2.	Gas chromatography-mass spectrometric analysis	43		
3.3.	Results	45		
3.3.1.	Toxicological test	45		
3.3.2.	Phytochemical analysis	46		
3.3.3.	GC-MS analysis of C. calothyrsus leaves	47		
3.4.	Discussion 4			

3.5.	Conclusions	53
4	EXPERIMENT 2: EVALUATION OF NUTRITIVE VALUE, TANNIN CONTENT, TANNIN, METABOLISABLE ENERGY AND ACCEPTIBILITY OF <i>C. calothyrsus</i> LEAVES TO GOATS	54
4.1.	Objectives	54
4.2.	Materials and methods	54
4.2.1.	Determination of nutrient content	54
4.2.2.	Measurement of tannin content	55
4.2.2.1.	Sample preparation	55
4.2.2.2.	Reagents preparation	55
4.2.2.3.	Measurement of tannin content using butanol-HCl (Bu-HCl) method	56
4.2.2.3.1.	Extraction	57
4.2.2.3.2.	Determination	57
4.2.2.3.2.1.	Free tannin	57
4.2.2.3.2.2.	Protein-tannin complex	57
4.2.2.3.2.3.	Complex of fibre - tannin	58
4.2.2.4.	Measurement of tannin content using protein precipitation (P-P)	
	method	58
4.2.2.4.1.	Extraction	60
4.2.2.4.2.	Determination	60
4.2.2.4.2.1.	Free tannin	60
4.2.2.4.2.2.	Protein – tannin complex	60
4.2.2.4.2.3.	Complex of fibre - tannin	61
4.2.3	Determination of ME content in C. calothyrsus and the other feedstuffs	61
4.2.3.1.	Keeping and maintenance of fistulated animals	61
4.2.3.2.	Preparation of sample	62
4.2.3.3.	Gas Test Procedures	62
4.2.3.3.1.	Preparation of solution	62
4.2.3.3.2.	Formulation of buffer medium	63
4.2.3.3.3.	Collection of rumen liquor	63
4.2.3.3.4.	Sample incubation	63
4.2.4.	Acceptability of C. calothyrsus leaves to goats	64
4.3.	Results	65
4.3.1.	Chemical composition	65
4.3.2.	Tannin Content	65
4.3.3.	ME content	66
4.3.4.	Acceptability	67
4.4.	Discussion	67
4.4.1.	Chemical composition of C. calothyrsus leaves	67
4.4.2.	Tannin content of C. calothyrsus leaves	69
4.4.3.	ME content of C. calothyrsus leaves	71

4.4.4.	Acceptability of C. calothyrsus leaves to goats	71
4.5.	Conclusions	72
5.	EXPERIMENT 3: EFFECT OF SUPPLEMENTING NAPIER GRASS WITH C. calothyrsus LEAVES, COCONUT OIL MEAL AND MAIZE MEAL ON DIET UTILISATION BY GOATS	74
5.1.	Objectives	74
5.2. 5.2.1. 5.2.2. 5.2.3. 5.2.4. 5.2.4.1. 5.2.4.2. 5.2.4.3. 5.2.5.	Materials and methods Animals Housing Diets Experimental procedure Experimental design Feeding and management Sample collection and analysis Data analysis	74 74 75 75 75 76 76
5.3.	Results	77
5.3.1. 5.3.2.	Digestion coefficient and feed intake Rumen parameters	77 78
5.4.	Discussion	78
5.5.	Conclusions	81
6.	EXPERIMENT 4: EFFECT OF SUBSTITUTION OF DIFFERENT LEVELS OF <i>C. calothyrsus</i> LEAVES FOR COCONUT OIL MEAL AND SOYBEAN WASTE CAKE ON RUMINAL FERMENTATION AND BLOOD PARAMETERS OF LACTATING GOATS	82
6.1.	Objectives	82
6.2.	Materials and methods	82
6.2.1. 6.2.2. 6.2.3. 6.2.4. 6.2.4.1. 6.2.4.2. 6.2.4.3. 6.2.5.	Animals Housing Diets Experimental procedure Experimental design Feeding and management Sample collection and analysis Statistical analysis	82 82 83 83 83 84 84
6.3.	Results	85
6.3.1.	Ruminal fluid parameters	85

6.3.2.	Blood parameters	85
6.4.	Discussion	86
6.4.1. 6.4.2.	Ruminal parameters Blood parameters	86 88
6.5.	Conclusion	90
7.	EXPERIMENT 5: EFFECT OF SUBSTITUTION OF DIFFERENT LEVELS OF <i>C. calothyrsus</i> LEAVES FOR COCONUT OIL MEAL AND SOYBEAN WASTE CAKE ON GOATS PERFORMANCE	91
7.1.	Objectives	91
7.2.	Materials and methods	91
7.2.1. 7.2.2. 7.2.3. 7.2.4. 7.2.4.1. 7.2.4.2. 7.2.5.1. 7.2.5.2. 7.2.5.2.1 7.2.5.2.2. 7.2.5.2.2. 7.2.5.2.2.1 7.2.5.2.2. 7.2.5.2.3. 7.2.5.3. 7.2.6.	Animals Housing Diets Experimental procedure Experimental design Feeding and management Measurement of research parameter Reproductive performance Productive performance Measurement of milk yield Measurement of milk quality Sample collection of milk goats Procedures of milk quality test Economic calculation of lactating goat Statistical analysis	91 91 92 92 92 92 93 93 93 94 94 94 95 95
7.3.	Results	96
7.3.1. 7.3.2. 7.3.3.	Reproductive performance Productive performance Economic calculation	96 97 98
7.4.	Discussion	98
7.4.1. 7.4.2. 7.4.3.	Reproductive performance Productive performance Economic calculation of lactating goats	98 101 104
7.5.	Conclusions	106
8.	GENERAL DISCUSSION	108

9.	CONCLUSIONS	114
	SUMMARY	116
	REFERENCES	120

LIST OF TABLES

Table 2.1.	Range of values for the chemical composition (g/kg dry matter) and in vivo digestibilities (%) of oven dried <i>C. calothyrsus</i> and <i>Leucaena leucocephala</i>	9
Table 2.2.	Total phenoic, hydrolysable and condensed tannin in <i>C. calothyrsus</i> measured in different studies	20
Table 3.1.	Mortality rate of <i>Artemia salina</i> using Brine Shrimp Lethality Test (BSLT) method	45
Table 3.2.	Statistical analysis of the LC_{50} value of the different fractions of C . calothyrsus	46
Table 3.3.	Phytochemical analysis of the hexane, CHCl ₃ , Ethyl alcohol, and 50 % Alcohol extract fractions of <i>C. calothyrsus</i> leaves	47
Table 3.4.	Gas chromatography-mass spectrometric analysis of the different extract fractions of alcohol, hexane and chloroform of <i>C. calothyrsus</i> leaves	47
Table 4.1.	Solutions for use in the in vitro gas test	62
Table 4.2.	Chemical composition of C. calothyrsus leaves	65
Table 4.3.	Recovery test of the protein-precipitation method of <i>C. calothyrsus</i>	
Table 4.4.	Free and bound tannin content (% dry matter) in <i>C. calothyrsus</i> measured by P-P and Bu-HCl methods	66
Table 4.5.	Metabolisable Energy (ME) of C. calothyrsus and the other feedstuff	67
Table 4.6.	Average of feed intake and health condition of Indonesian Ettawah crossbred goats fed fresh <i>C. calothyrsus</i> leaves as a sole diet during 21 days	67
Table 5.1.	The diet composition on dry matter basis	75
Table 5.2.	Mean ans standard deviation of digestion coefficient (%) and and feed intake in goats fed Napier grass diets with various supplements	77
Table 5.3.	Mean and standard deviation of NH ₃ -N concentration and total VFA in rumen of goats fed Napier grass diets with various supplements	78
Table 6.1.	Feed ingredients and nutrient composition of the diets (100 %DM)	83
Table 6.2.	Ruminal volatile fatty acid (VFA) and ammonia nitrogen (NH3-N) of Indonesian Ettawah crossbred lactating goats fed diets containing different levels of dried C . calothyrsus leaves $(0-30\%)$	85
	vii	

Table 6.3.	The concentration of blood glucose and plasma urea of Indonesian Ettawah crossbred lactating goats fed diets containing different levels of dried C . calothyrsus $(0-30 \%)$	86
Table 7.1.	The reproductive performance of Indonesian Ettawah crossbred lactating goats fed diets containing different levels of dried C . calothyrsus leaves $(0-30\%)$ during 10 weeks of experiment	96
Table 7.2.	Average milk yields (ml), pH and specific gravity of Indonesian Ettawah crossbred lactating goats fed diets containing different levels of dried C . calothyrsus $(0-30 \%)$ during 10 weeks of experiment	97
Table 7.3.	Average milk composition of Indonesian Ettawah crossbreed lactating goats fed diets containing different levels of dried <i>C. calothyrsus</i> (0 – 30 %) during 10 weeks of experiment	98
Table 7.4.	The economic calculation of Indonesian Ettawah crossbred lactating goats fed different diets containing different levels of C . calothyrsus (0 – 30 %) during 10 weeks of experiment	98

LIST OF FIGURES			
Figure 2.1.	Calliandra calothyrsus plant	6	
Figure 3.1.	Flowchart of phytochemical analysis of C. calothyrsus leaves	42	
Figure 4.1.	Flowchart of the Bu-HCl method for the determination of tannins	56	
Figure 4.2.	Flowchart of the protein precipitationmethod for the determination of tannins	59	

LIST OF ABBREVIATIONS

ADF Acid detergent fibre

BSA Bovine serum albumin

Bu-HCl Butanol-HCl BW Body weight

C. calothyrsus Calliandra calothyrsus

CF Crude fibre
CL Crude lipid

CT Condensed tannin

D1-D4 Diet 1 – Diet 4

DM Dry matter

DMD Dry matter digestibility

DMI Dry matter intake

EE Ether extract
FT Free tannin

g gram

GC-MS Gas chromatography-mass spectrometry

HT Hydrolysable tannin

IVDMD In vitro dry matter digestibility

Kg Kilogram

LC₅₀ Lethal concentration of 50 ppm

ME Metabolisable energy
Mg/dl Milligram per decilitre

MJ Mega joule

MW Molecular weight

N Nitrogen

NDF Neutral detergent fibre
NFE Nitrogen free extract

NIST National Institute of Standard Technology

OM Organic matter

OMD Organic matter digestibility

P Probability

PAC Proanthocyanidins

PEG Polyethylene glycol

P-P method Protein-precipitation method

ppm Part per million

R1 – R4 Ration 1 – Ration 4

R² Correlation coefficient

SDS Sodium dodecyl sulphate

SPC Secondary plant compounds

TDN Total digestible nutrient

TI Tannin isolate

TF Tannin bound to fibre

TP Tannin bound to protein

VFA Volatile fatty acid

1. INTRODUCTION

1.1. Background of the study

In Indonesia inadequate year-round feed supply is probably the most important factor contributing to low animal output. This constraint is not peculiar to Southeast Asia but is common in most tropical and subtropical countries. The supply of animal protein is commonly restricted by an insufficient supply of high-quality forage. Tropical grasses are generally low in quality and they do not sustain high levels of animal productivity. Their deficiencies lead to an increase in the time the animals take to reach marketable weight, resulting in the production of lower quality products (Palmer and Ibrahim, 1996).

Inadequate nutrition in ruminant animals has often been associated with heavy economic losses to the farmers because of animal weight and condition losses, reduction in reproductive capacity and increased mortality rates (Simbaya, 2001). The gap between the availability and requirement of energy (TDN) is not wide (21 %), but the deficiency of protein sources is critical (54 %) (Pradhan 1995). Further, the traditional protein sources available are used mainly for the feeding of dairy animals (cattle and buffalo). This has resulted in low productivity of small ruminants.

To obtain optimum production of sheep and goats, attention has been given to exploiting alternative protein sources (Singhi *et al.*, 2000). Commercial concentrates have been used as supplements to basal diets of goats. However, the cost of traditional concentrates are escalating due to low availability and high demand from non-ruminant livestock industries, which are also growing rapidly in Indonesia. Therefore, development of non-traditional feed resources to replace the commercial concentrate in the country is important.

In order to improve the productive and reproductive capacity of smallholder ruminant animals, there is a need to look at ways of producing these feeds on the farms. One potential way of increasing the feed supply under smallholder conditions may be through the use of fodder trees and shrub legumes (Simbaya, 2001). Tree legume forages supply relatively cheap sources of protein for livestock. Due to their high nitrogen content, they could be satisfactory substitutes for the more expensive protein supplements in ruminant feeds. Feeding tree legumes to cattle has resulted in increased intake and live weight gains