

Contents

	Page
Table of contents	i
Abbreviation	vi
List of tables	viii
List of figures	x
1 Introduction	1
2 Literature Review	3
2.1 Mimosine	3
2.1.1 Mimosine in Leucaena	3
2.2 <i>Leucaena leucocephala</i> (leucaena)	4
2.2.1 Description	5
2.2.2 Some common names of <i>Leucaena leucocephala</i>	6
2.2.3 Environmental adaptation	6
2.3 Nutritive value of Leucaena	7
2.3.1 Leaves	7
2.3.2 Seeds	11
2.4 Use of Leucaena in livestock production	12
2.4.1 Leucaena as cattle feed	12
2.4.2 Leucaena as sheep feed	13
2.4.3 Leucaena as goat feed	14
2.4.4 Leucaena in poultry ration	15
2.4.5 Leucaena as feed for other animals	16
2.5 Adverse effects of Leucaena Mimosine on livestock production	16
2.5.1 Cattle	16
2.5.2 Sheep	18
2.5.3 Goat	18
2.5.4 Poultry	19
2.5.5 Rats and mice	20
2.6 Acceptable daily intake of Mimosine for the animals	21

2.7 Some metabolic pathways of Mimosine toxicity	21
2.8 Possible solutions to alleviate Mimosine toxicity	25
2.8.1 Development of Mimosine low cultivars	25
2.8.2 Heat treatment to the Leucaena leaves	26
2.8.3 Water washing, boiling or soaking	27
2.8.4 Detoxifying Mimosine with mineral supplementation	27
2.8.5 Control of Mimosine toxicity in ruminants by using ruminal microbes	28
2.8.5.1 Natural distribution of the Mimosine detoxifying ruminal bacteria	29
2.8.5.2 Persistence of ruminal 3,4-DHP degrading bacteria	30
2.8.5.3 Isolation and characterization of Mimosine detoxifying bacteria	31
2.8.5.4 Detection of Mimosine detoxifying ruminal bacteria	31
2.8.5.5 Ruminal inoculation methods	34
2.8.5.6 Production of bacterial culture containing <i>S.</i> <i>jonesii</i>	34
3 Selection, isolation, characterization and detection of properties of Mimosine degrading ruminal bacteria from German steers	36
3.1 Materials	36
3.2 Methods	36
3.2.1 Fermenter System	36
3.2.1.1 Fermenter vessel	37
3.2.1.2 Medium supply	38
3.2.1.3 Treatment with mimosine	38
3.2.1.4 Control of incubation temperature and pH	38
3.2.1.5 Out let of the overflow of fermenter fluid	38
3.2.1.6 Fermentation process	39
3.3 Detection of Mimosine degradability	39
3.3.1 Colourimetric assay	40

3.3.2 Preparation of momosine standard curve	40
3.4 Isolation and characterisation of Mimosine degrading ruminal bacteria	40
3.4.1 Bacteriological staining	40
3.4.2 DNA isolation	41
3.4.3 PCR amplification process (16S PCR)	41
3.4.4 Purification of PCR fragments for sequencing	42
3.4.5 Gene sequencing	42
3.5 Comparison of some physical properties, morphology of colonies and biochemical properties of isolate 3948 with reference strains	43
3.5.1 Morphology of colonies	43
3.5.2 Bacterial mobility	43
3.5.3 Capsule staining	44
3.5.4 Tests for biochemical properties	44
3.5.4.1 Microbial community analysis test (Biolog)	44
3.5.4.2 API test	45
3.6 Results	45
3.6.1 Standard curve for Mimosine content in rumen juice	45
3.6.2 Mimosine degradation	46
3.6.3 Microscopic appearance of isolate 3948	48
3.6.4 Comparison of some properties of isolate 3948 with reference strains	49
3.6.4.1 Physical properties	49
3.6.4.2 Biochemical properties	49
3.7 Discussion	54
3.7.1 Mimosine degradation	54
3.7.2 Identification of isolate 3948	56
3.7.3 Dominant phenotype of Mimosine degrading ruminal bacteria	57
3.8 Conclusion	58

4 Feeding trials	59
4.1 Multiplication of Mimosine degrading bacteria using IBT-Göttinger Bioreactor	59
4.1.1 Materials	59
4.1.2 Fermenter vessel	61
4.1.3 Medium supply	62
4.1.4 Inoculum preparation	62
4.1.5 Control of incubation temperature and pH	62
4.1.6 Gaseous exchange system	62
4.1.7 Air filters	63
4.1.8 Sampling and harvesting system	63
4.1.9 Counting bacteria by using most probable number (MPN)	63
4.2 Preservation of fermenter suspension for field experiment	64
4.2.1 Entrapment of bacteria in alginate beads	64
4.2.1.1 Counting bacteria after entrapment	65
4.2.2 Freeze-drying and counting the cells after freeze-drying	65
4.3 Digestion trial in Myanmar sheep	66
4.3.1 Materials and methods	66
4.3.1.1 Source of rice straw	66
4.3.1.2 Manipulation of feedstuffs	66
4.3.1.3 Preliminary experiment to estimate toxic level of leucaena	67
4.3.1.4 Experimental animals, management of animals and experimental period	67
4.3.1.5 Experimental design, period and sample collection	67
4.3.2 Observations	69
4.3.3 Chemical analysis	69
4.3.4 Statistical analysis	70
4.4 Results	70
4.4.1 Bacterial cell counts	70

4.4.1.1 After production	70
4.4.1.2 In alginate beads	70
4.4.1.3 After freeze-drying	70
4.4.2 Dry matter intake and toxic level of Leucaena in Myanmar sheep	70
4.4.3 Clinical symptoms of Leucaena toxicosis	71
4.4.4 Chemical composition of feedstuffs and diets	71
4.4.5 Voluntary feed intake	72
4.4.6 Digestibilities of nutrients and nutritive values of diets	73
4.4.7 Daily nutrient intakes and digestible nutrient intakes	75
4.4.8 Nitrogen utilization	78
4.5 Discussion	80
4.5.1 Toxic symptoms of Mimosine	80
4.5.2 Chemical composition of Leucaena and diets	81
4.5.3 Nutrient intakes and nitrogen retention	82
4.5.4 Correspondence of Mimosine on digestibility of nutrients	85
4.6 Conclusion	87
5 General discussion	88
5.1 Some probiotic effects of <i>K. pneumoniae</i>	89
5.2 Effect of infusion of preserved Mimosine degrading ruminal bacteria on Mimosine detoxification	90
6 Conclusion	93
7 Summary	94
7 Zusammenfassung	96
8 References	98
Appendices	123
Acknowledgement	136