

Ammonia pre-treatment of low quality cereal residues for ruminant nutrition

TF Buthane

 **orcid.org/0000-0003-0764-0222**

Dissertation accepted in fulfilment of the requirements for the
degree **Master of Science in Animal Science** at the
North West University

Supervisor: Dr LE Motsei

Co-supervisor: Dr CM Mnisi

Graduation ceremony: April 2020

Student number: 28511700

DECLARATION

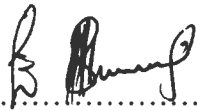
I, Thabo Frans Buthane, affirm that:

- i. This is my original research.
- ii. The use of information and resources from other sources has been fully acknowledged.
- iii. This thesis is not submitted for any degree or examination at any other university other than the North-West University.
- iv. Reported results were generated by me and not by any other scholar or organisation.

Student: T.F. Buthane

Signed: T.F. Buthane Date: 28/07/2020

Supervisor: Dr L.E. Motsei

Signed:  Date: 28/07/2020

Co-Supervisor: Dr C.M. Mnisi

Signed:  Date: 28/07/2020

GENERAL ABSTRACT

The use of crop residues is commonplace in semi-arid regions of the developing world, where farmers cannot afford bought-in supplements during the dry seasons. Cereal crop residues form the bulk of alternative forage that can be used under extensive farming systems. However, the poor nutritive value of cereal straws, characterized by high fibre and low protein content, means that strategies must be developed to enhance its utilisation by ruminants. This study was, therefore, designed to improve the nutritive value of maize (*Zea mays*), wheat (*Triticum aestivum*) and oats (*Avena sativa*) straws using incremental levels of anhydrous ammonia gas. A total of 15 replicate samples from each of the three cereal straws were pre-treated with anhydrous ammonia gas at a rate of 0, 2.5, 3.5, 4.5 and 5.5% for a period of seven days. The samples were subsequently air-dried for 24 h, ground (2 mm), and analysed for chemical composition (Chapter 3), nitrogen buffer solubility and *in vitro* ruminal dry matter degradability (Chapter 4), and *in vitro* ruminal gas production (Chapter 5). Results from Chapter 3 showed that for wheat straw, there were significant quadratic effects for DM and OM, but not ($P > 0.05$) for CP and NDF in response to ammonia treatment levels. Crude protein linearly increased whereas NDF linearly decreased with ammonia treatment. For maize straw, there were significant quadratic effects on CP, NDF and ADF with ammonia treatment levels. Organic matter linearly increased whereas ADL linearly decreased with ammonia treatment. For oats straw, there were neither linear nor quadratic effects ($P > 0.05$) for OM, ADF and ADL, however, there were quadratic trends for DM and CP in response to ammonia treatment. Neutral detergent fibre linearly decreased with ammonia treatment. There were no significant effects observed for cereal type, ammonia, and their interaction on DM content. Cereal type had significant effects on OM and ADL content. There were significant effects for cereal type, ammonia and their interaction on CP and NDF contents. For ADF content, there was a significant effect for cereal type, and cereal type \times ammonia

interaction but not for ammonia. Untreated straws had the least CP content than treated straws, but had the highest ($P < 0.05$) NDF, ADF and ADL content. Results from Chapter 4 revealed that there were significant quadratic effects for N, BISN, BSN and SI, for which an optimum ammoniation rate for oats straws was calculated to be 3.3%. However, for maize straw, linear effects ($P < 0.05$) were observed for BSN, BISN, and SI. A quadratic trend was observed for N, for which an optimum ammoniation rate for maize straws was calculated to be 4.29%. Linear effects were observed for DMD12 and DMD24 in ammoniated wheat straws. For maize and oats straws, there were significant linear and quadratic trends for DMD12, DMD24, DMD36 and DMD48 in response to ammonia treatment. Maize straws had the highest ($P < 0.05$) DMD12, DMD24, DMD36 and DMD48 when compared to oats and wheat straws. Untreated straws had the least DMD12, DMD24, DMD36 and DMD48 ($P < 0.05$) than the ammoniated straws, which did not differ ($P > 0.05$). Results from Chapter 5 indicated that for wheat straws, there were neither linear nor quadratic ($P > 0.05$) effects observed for rate of gas produced and cumulative gas production after 12, 24, 36 and 48 h of incubation. No linear and quadratic ($P > 0.05$) trends were observed for gas produced from the immediately soluble fraction (a), gas produced from slowly soluble fraction (b), lag time, potential gas production (P_{gas}) and effective gas production (E_{gas}) in response to ammonia treatment. The gas production rate constant (c) for the insoluble fraction b linearly increased with anhydrous ammonia levels. For oats straw, the rate of gas produced at 12 h linearly decreased with anhydrous ammonia levels. There were neither linear nor quadratic ($P > 0.05$) effects observed for fraction c and lag time, but there were quadratic trends for cumgas12, cumgas24, cumgas36, cumgas48, fractions a and b , P_{gas} and E_{gas} for which an optimum ammoniation rate for oats straws was calculated to be 3.5%. For maize straw, there were neither linear nor quadratic ($P > 0.05$) trends for cumgas24, cumgas36, fraction b and c , lag time, P_{gas} as well as the rate of gas produced at 8, 12, 24 and 48 h of incubation. Cumgas12, cumgas48, a , and E_{gas} linearly declined with ammonia levels. Maize had the highest rate of

gas produced at 12 h (40.562 mL/h), followed by wheat (28.951 mL/h) and oats (23.734 mL/h). There was no differences ($P > 0.05$) observed between the three cereal crop residues in terms of gas produced at 24, 36 and 48 h of incubation. Maize straw had higher ($P < 0.05$) fraction c , lag time and E_{gas} after inoculation than oats straw. Wheat straw had the same ($P > 0.05$) fraction c , lag time and E_{gas} after inoculation as oats straw. It was concluded that anhydrous ammonia gas treatment does improve the nutritive value of poor quality cereal straws but the optimum ammoniation rate varies with type of straw.

Keywords: Buffer nitrogen solubility; Cereal straws; Chemical composition; *In vitro* ruminal dry matter degradability; *In vitro* ruminal gas production

ACKNOWLEDGEMENTS

I would like to acknowledge PUREGAS (PTY) Ltd, for their financial support towards this research study. My gratitude extends further to the programme manager, Mr Wihan Fourie, for his support and guidance throughout this research.

My unfathomable gratitude goes to Dr L.E. Motsei and Dr C.M. Mnisi for their unwitting academic support, guidance and patience throughout this research project.

Further gratitude goes to Prof. V. Mlambo for his support. The assistance of Mr M.W. Mankga, Mr K.P. Meso and the entire academic staff members in the Department of Animal Science, North-West University, Mafikeng campus, is duly acknowledged.

The support and encouragement of my entire family and friends is highly appreciated.

DEDICATION

I dedicate this thesis to both my beloved late aunt, Ms N.O. Mnambathi, and sister, Ms T.J. Buthane.

TABLE OF CONTENTS

DECLARATION	i
GENERAL ABSTRACT	ii
ACKNOWLEDGEMENTS	v
DEDICATION	vi
LIST OF TABLES	x
LIST OF FIGURES	xii
LIST OF APPENDICES	xiii
LIST OF ABBREVIATIONS	xiv
1 CHAPTER ONE - GENERAL INTRODUCTION	1
1.1 Background	1
1.2 Problem statement	2
1.3 Justification	3
1.4 Overall Objectives	4
1.5 Hypothesis	4
1.6 Summary	5
1.7 References	5
2 CHAPTER TWO - LITERATURE REVIEW	10
2.1 Introduction	10
2.2 Potential feed sources for ruminants	11
2.3 Maize (<i>Zea mays</i>)	12
2.4 Wheat (<i>Triticum astivum</i>)	12
2.5 Oats (<i>Avena sativa</i>)	13
2.6 Possible strategies to improve the utilization of cereal straws	14
2.6.1 Grinding and pelleting	15
2.6.2 Treatment with sodium hydroxide	16
2.6.3 Urea treatment	17
2.6.4 Ammonia treatment	17
2.7 Use of ammoniation to improve nutritive value	18
2.8 Recommended ammonia dosages	18
2.9 Ammonia treatment methods	19
2.9.1 Stack method	19
2.9.2 Silo or bunker method	20
2.10 Factors influencing effectiveness on ammoniation	20

NWU
LIBRARY

2.10.1	Temperature and length of treatment	20
2.10.2	Moisture content	21
2.11	Effects of ammoniated feedstuffs on ruminant productivity	22
2.11.1	Associative effects	23
2.12	Environmental implications of feeding low quality feeds	23
2.13	Summary	24
2.14	References.....	26
3	CHAPTER THREE - CHEMICAL COMPOSITION OF WHEAT, OATS AND MAIZE STRAWS AMMONIATED WITH INCREMENTAL LEVELS OF ANHYDROUS AMMONIA GAS.....	34
	Abstract.....	34
3.1	Introduction.....	35
3.2	Materials and methods	37
3.2.1	Study site and ingredient sources.....	37
3.2.2	Ammoniation and processing procedure	38
3.2.3	Chemical analyses.....	38
3.2.4	Statistical analysis.....	40
3.3	Results.....	41
3.4	Discussion.....	56
3.5	Conclusion	57
3.6	References.....	59
4	CHAPTER FOUR - IN VITRO RUMINAL DRY MATTER DEGRADABILITY AND NITROGEN BUFFER SOLUBILITY OF WHEAT, OATS AND MAIZE STRAWS AMMONIATED WITH INCREMENTAL LEVELS OF ANHYDROUS AMMONIA GAS	63
	Abstract.....	63
4.1	Introduction.....	64
4.2	Materials and methods	65
4.2.1	Study site and ammoniation process.....	65
4.2.2	Buffer Nitrogen Solubility	65
4.2.3	In vitro ruminal dry matter degradability.....	66
4.2.4	Statistical analysis.....	67
4.3	Results.....	68
4.3.1	Buffer Nitrogen Solubility	68
4.3.2	In vitro ruminal dry matter degradability.....	69
4.4	Discussion.....	75
4.5	Conclusion	77

4.6	References.....	78
5	CHAPTER FIVE - IN VITRO RUMINAL GAS PRODUCTION OF WHEAT, OATS AND MAIZE STRAWS AMMONIATED WITH INCREMENTAL LEVELS OF ANHYDROUS AMMONIA GAS	82
5.1	Abstract.....	82
5.2	Introduction.....	83
5.3	Materials and methods	84
5.3.1	Study site and ammoniation process.....	84
5.3.2	In vitro ruminal fermentation.....	84
5.3.3	Statistical analysis.....	85
5.4	Results.....	86
5.5	Discussion	94
5.6	Conclusion	96
5.7	References.....	98
6	GENERAL DISCUSSION, CONCLUSIONS AND FUTURE RESEARCH	102
6.1	General discussion and conclusions.....	102
6.2	Future research.....	103
6.3	References.....	104
7	LIST OF APPENDICES.....	105
	Appendix 1:.....	105
	Appendix 2:.....	106
	Appendix 3:.....	107
	Appendix 4:.....	108

LIST OF TABLES

Table 2.1. Chemical composition of maize, wheat and oats cereal straws (% DM)	14
Table 2.2. Recommended temperature and length of ammonia treatment	21
Table 2.3. Effect of ammonia treatment on moisture and mould contamination	22
Table 3.1. Chemical composition (g/kg DM, unless stated otherwise) of anhydrous ammonia gas treated wheat straws.....	42
Table 3.2. Chemical composition (g/kg DM, unless stated otherwise) of anhydrous ammonia gas treated maize straws.....	43
Table 3.3. Chemical composition (g/kg DM, unless stated otherwise) of anhydrous ammonia gas treated oats straw	44
Table 3.4. Level of significance of effects of the main factors on chemical composition of anhydrous ammonia gas treated maize, oats and wheat straws	45
Table 3.5. The effect of anhydrous ammonia on chemical composition of maize, oats and wheat straws and interaction.....	46
Table 4.1. <i>In vitro</i> ruminal dry matter degradability (g/kg DM) of anhydrous ammonia gas treated wheat straws.....	69
Table 4.2. Responses of <i>in vitro</i> ruminal dry matter degradability (g/kg DM) of oats straws with incremental levels of anhydrous ammonia gas.....	71
Table 4.3. Responses of <i>in vitro</i> ruminal dry matter degradability (g/kg DM) of maize straws with incremental levels of anhydrous ammonia gas.....	72
Table 4.4. Level of significance of effects of the main factors on <i>in vitro</i> ruminal dry matter degradability of anhydrous ammonia gas treated wheat, oats and maize straws.....	73
Table 4.5. The effect of anhydrous ammonia on <i>in vitro</i> ruminal dry matter degradability (g/kg DM) of maize, oats and wheat straws.	74
Table 5.1. Rate of gas produced (mL/g OM) by anhydrous ammonia gas treated oats straws	87

Table 5.2. Cumulative gas production (mL/g OM) of anhydrous ammonia gas treated oats straws	88
Table 5.3. Level of significance of effects of the main factors on the rate of gas produced per period (mL/h) of ammoniated cereal straws	89
Table 5.4. Level of significance of effects of the main factors on cumulative gas production (h).....	91
Table 5.5. Cumulative gas production (mL/g OM) of ammoniated cereal straws	92
Table 5.6. Level of significance of effects of the main factors on the <i>in vitro</i> ruminal gas production parameters (mL/g OM, unless stated otherwise)	93
Table 5.7. The effects of main factors on <i>in vitro</i> gas production (mL/g OM, unless stated otherwise) parameters on wheat, oats and maize straws.....	94

LIST OF FIGURES

Figure 3.1. Effect of ammonia treatment on organic matter content of cereal straws	48
Figure 3.2. Effect of ammonia treatment on acid detergent fibre of cereal straw	48
Figure 3.3. Effect of ammonia and cereal straw on acid detergent fibre (g/kg DM).....	49
Figure 3.4. Effect of ammonia treatment on acid detergent lignin of cereal straws	50
Figure 5.1. Rate of gas produced per period (h) by anhydrous ammonia treated wheat, oats and maize straws	90

LIST OF APPENDICES

Appendix 1: Recipe for phosphate-borate buffer (Licitra <i>et al.</i> , 1996)	105
Appendix 2: Recipe for the <i>in vitro</i> ruminal gas production buffer	106
Appendix 3: Ethics Certificate of the Study	107
Appendix 4: Language Editing Certificate	108



LIST OF ABBREVIATIONS

ADF	:	Acid detergent fibre
ADL	:	Acid detergent lignin
ANOVA	:	Analysis of variation
AOAC	:	Association of Official Analytical Chemists
BISN	:	Buffer insoluble nitrogen
BSN	:	Buffer soluble nitrogen
CP	:	Crude protein
DM	:	Dry matter
Egas	:	Effective gas produced
GLM	:	General linear model
LSMEANS	:	Least square means
N	:	Nitrogen
NDF	:	Neutral detergent fibre
GLM	:	General linear model
NRC	:	National Research Council
OM	:	Organic matter
PDIFF	:	Probability of difference
Pgas	:	Potential gas produced
SAS	:	Statistical analysis system
SI	:	Solubility Index

1 CHAPTER ONE - GENERAL INTRODUCTION

1.1 Background

Due to the rapidly growing human population and increased market demands of ruminant meat and milk, producers are expected to double production by selecting animals with superior genetic material, high residual feed intake and efficient feed conversion ratio in order to ensure food and nutrition security (Kelley *et al.*, 1991). As such, high-quality rations are required to meet the animal's daily nutrient requirements but their use comes at a higher cost because they are expensive and sometimes inaccessible for smallholder farmers. This has prompted the need to explore least cost alternative sources of nutrients that can be offered to ruminants without compromising their performance and health status. Several exploratory studies have been designed to investigate ways of improving available forages for ruminants (Mahesh & Madhu, 2014), which included feed ammoniation. Natural pastures (grasses), crop residues and agro-industrial by-products are an important staple feed for ruminants in most parts of the country (Beyene & Mlambo, 2012). Numerous grass species in arid and semi-arid regions of Southern Africa serve as a major source of feed for both domestic and wildlife herbivores (Beyene & Mlambo, 2012). However, the productivity of these animals depends on the availability of quality pasture (Berhane *et al.*, 2006). Due to seasonal fluctuations, especially in the dry seasons, the quantity and quality of grasses become low and poor, respectively (Beyene & Mlambo, 2012). Furthermore, the protein content decreases whilst the indigestible fibre content increases as a result of lignification (Evitayani *et al.*, 2005).

For many decades, resource-poor farmers have resorted to the use of crop straws from various cereal plants (wheat, maize, oats and etc.) as an alternative source of feed for ruminants, especially during the prolonged periods of the dry seasons where the available natural pastures are of poor quality and low in quantity (Ravhuhali *et al.*, 2019). These crop

residues are readily available after harvesting and are a source of cheap roughage for ruminants. However, according to Leng (1990), these cereal straws have high fibre and lignin content, and low crude protein. Indeed, Bath *et al.* (1997) reported that the utility of these cereal straws may be limited by their low protein (3 – 6%) and energy (5.8 - 6.5 MJ/kg) content and low digestibility (40 - 50%) due to high fibre and lignin levels. Thus a need arises to explore ways of improving the nutritive value of these available cereal crop straws. Various strategies have been explored to improve the nutritive value of cereal straws which include physical and chemical ways (Nguyen *et al.*, 2012). Physical methods used are grinding and pelleting, which improves feed utilisation and digestibility. The use of sodium hydroxide, urea and ammonia are some of the chemical ways, which are used to improve the nutritive value of cereal crop straws. Several exploratory studies were designed to investigate ways of improving available forages for ruminants (Mahesh & Madhu, 2014), which included feed ammoniation.

1.2 Problem statement

The increasing demand for agricultural livestock produce adds immense pressure to farmers to increase the supply of food (Mahesh & Madhu, 2014). However, in times where grasses as major sources of feeds are suboptimal and cannot be relied upon, there is a need to evaluate alternative sources such as crop residues (Bhuvnesh *et al.*, 2014). Cereal straws have become an important feed source due to the reduction of grazing lands owing to the cultivation of cash crops, development of infrastructure and human settlement, as well as urbanisation (Jodha, 1986). The major challenge with crop residues is their low nutritive value and poor digestibility due to the presence of cellulose, hemicellulose and lignin as well as the deficiency in vitamins and minerals (Bath *et al.*, 1997; Mahesh & Madhu, 2013). Lignin forms a crystalline structure with cellulose, which protects highly digestible compounds from enzymatic hydrolysis (Henics, 1987; Karunanandaa *et al.*, 1995). Thus the presence of lignin

in cereal straws inhibits the hydrolysis process and thus reduce their digestibility (Simmons *et al.*, 2010).

There is, therefore, a need to modify the non-starch polysaccharides while improving the protein content of these straws by enhancing the hydrolysis process (Hendriks & Zeeman, 2009). The nutritive value of crop residues can be improved by treatment with anhydrous ammonia (Horton *et al.*, 1982). The use of anhydrous ammonia to improve the nutritive value of crop residues has not been widely investigated in South Africa. There are also discrepancies on the optimal level required to treat these cereal straws due to different application methods and the chemical composition of the straws, which is influenced by the growth environment.

1.3 Justification

Various strategies have been evaluated to improve the nutritive value of crop residues by increasing nitrogen content whilst reducing fibre levels (Mahesh & Madhu, 2014). Some of the strategies employed to improve the nutritive value of these residues include physical and chemical methods (Nguyen *et al.*, 2012). The physical method includes milling, grinding, pelleting, soaking, β -irradiation and pressure steaming (Yang *et al.*, 2002; Jafari *et al.*, 2007; Viola *et al.*, 2008; Zhao *et al.*, 2009). These methods are known to increase voluntary intake of low quality straws by reducing their physical forms. Indeed, Alawa and Owen (1984) reported that milling improves the dry matter intake by 29% but depresses digestibility. Uden (1988) stated that grinding and pelleting of grass hay reduces dry matter degradability in cows from 73% to 67%. Furthermore, β -irradiation of lignocellulosic feeds has the potential to increase digestibility but there are limitations to the use of this technique as it requires sophisticated and expensive equipment (Walker, 1984). Horton *et al.* (1991) reported that pressure steaming increases energy availability by solubilisation of cellulose and hemicellulose, however, this method results in dry matter loss (Rangnekar *et al.*, 1982).

Chemical treatment methods involve the use of sodium hydroxide, urea and ammonia. These methods allow the breakdown of cell wall by a hydroxide group that increases microbial attachment (Fahey *et al.*, 1993). Ammoniation of straws with gaseous or liquid ammonia has received considerable attention in many countries (Sundstel & Coxworth, 1984). Ammoniation of low quality feed is reported to reduce neutral detergent fibre and acid detergent fibre contents and increases dry matter digestibility (Paterson *et al.*, 1980; Yalchi, 2010).

1.4 Overall Objectives

The broad objective of the study was to enhance the nutritive value of low-quality cereal straws (maize, wheat and oats) using graded levels of anhydrous ammonia gas. The following specific objectives guided the study:

- a) To determine the chemical composition and buffer nitrogen solubility of low-quality cereal straws treated with graded levels of anhydrous ammonia gas.
- b) To determine the *in vitro* ruminal nitrogen degradation and *in vitro* ruminal gas production of maize, wheat and oats straws treated with graded levels of anhydrous ammonia gas.
- c) To determine the optimum anhydrous ammonia gas treatment level of maize, oats and wheat straws measured by chemical composition, buffer nitrogen solubility, *in vitro* ruminal nitrogen degradation and *in vitro* ruminal gas production parameters.

1.5 Hypothesis

- a) The study tested the alternative hypothesis that anhydrous ammonia gas treatment enhances the chemical composition, and nitrogen buffer solubility, *in vitro* ruminal nitrogen degradability and *in vitro* ruminal gas production of maize, wheat and oats straws.

- b) The study tested the alternative hypothesis that anhydrous ammonia gas treatment improves *in vitro* ruminal nitrogen degradability and *in vitro* ruminal gas production of maize, wheat and oats straws.
- c) Chemical composition, buffer nitrogen solubility, *in vitro* ruminal fermentation of maize, wheat and oats respond to anhydrous ammonia gas treatment in an asymptotic fashion.

1.6 Summary


During the dry seasons, natural pastures are limiting and do not meet the animal's nutrient requirements and, as such, there is a need to improve the nutritive value of readily available alternative source of feed. Resource-poor farmers have resorted to the use of cereal straws, however, their utilisation is limited by its poor digestibility, low protein content and deficiency in both vitamins and minerals. The treatment of these cereal crop residues with anhydrous ammonia could promote better utilisation of these residues in ruminants, resulting in improved voluntary feed intake and performance. Anhydrous ammonia treatment of cereal crop residues will enable resource-poor farmers to implement less-expensive feeding strategies, especially during the dry season.

1.7 References

- Alawa, J.P. & Owen, E., 1984. The effect of milling and sodium hydroxide treatment on intake and digestibility of wheat straw by sheep. *Anim. Feed Sci. Technol.* 11, 149-157.
- Boudra, H., Rouillé, B., Lyan, B. & Morgavi, D.P., 2015. Presence of mycotoxins in sugar beet pulp silage collected in France. *Anim. Feed. Sci. Technol.* 205, 131-135.

- Bath, D., Dunbar, J., King, J., Bery, S. & Olbrich, S., 1997. By-products and unusual feedstuffs. *Feedstuffs*, 69, 32-38.
- Berhane, G., Eik, L.O. & Tolera, A., 2006. Chemical composition and *in vitro* gas production of vetch (*Vicia sativa*) and some browse and grass species in northern Ethiopia. *Afr. J Range. For. Sci.* 23, 60-75.
- Beyene, S.T. & Mlambo, V., 2012. Botanical and chemical composition of common grass species around dip-tank areas in semi-arid communal rangelands of Swaziland. *Trop. Subtrop. Agroecosyst.* 15, 142-143.
- Bhuvnesh, S., Kavish, K.J., Anup, K. & Ramesh, C.K., 2014. Bioprocessing of wheat straw into nutritionally rich and digested cattle feed. *Sci. Rep.* 4, 6360.
- Evitayani, L., Warly, A., Fariani, T., Ichinohe, T. & Fujihara, T., 2005. Seasonal changes in nutritive value of some grass species in West Sumatra, Indonesia. *Anim. Sci. J.* 76(5), 461-468.
- Fahey, G.C., Bourquin, L.D., Titgemeyer, E.C. & Atwell, D.G., 1993. Post-harvest treatment of fibrous feedstuffs to improve the nutritive value. Forage cell wall structure and digestibility. *Amer. Soc. Agron. Madison, Wisconsin.* pp. 715-766.
- Flachowsky, G., Ochrimenko, W.L., Schneider, M. & Richter, G.H., 1996. Evaluation of straw treatment with ammonia sources on growing bulls. *Anim. Feed Sci. Technol.* 60, 117-130.
- Hendriks, A.T.W.M. & Zeeman, G., 2009. Pre-treatments to enhance the digestibility of lignocellulosic biomass. *Biores. Technol.* 100, 10-18.

- Henics, Z., 1987. Wheat straw upgraded by *Pleurotus ostreatus*. World. Rev. Anim. Prod. 3, 189-205.
- Horton, G.M.J., Nicholson, N.H. & Christensen, D.A., 1982. Ammonia and sodium hydroxide treatment of wheat straw in diets for fattening steers. Anim. Feed Sci. Technol. 7(1), 1-10.
- Horton, G.M.J., Pate, F.M. & Pitman, W.D., 1991. The effect of steam pressure treatment, pelleting and ammoniation on the feeding value of sugarcane bagasse for cattle. Can. J. Anim. Sci. 71, 79-86.
- Jafari, M.A., Nikkhah, A, Sadeghi, A.A. & Chamani, M., 2007. The effect of *Pleurotus* spp. Fungi on chemical composition and *in vitro* digestibility of rice straw. Pak. J. Biol. Sci. 10, 2460-2464.
- Jodha, N.S., 1986. Common property resources and dynamics of rural poverty: Field Evidence from Dry Regions of India. EPW, India.
- Karunanandaa, K., Varga, G.A., Akin, D.E, Rigsby, L.L. & Royse, D.J., 1995. Botanical fractions of rice straw colonized by white-rot fungi: change in the chemical composition and structure. Anim. Feed Sci. Technol. 55, 179-199.
- Kelley, T.G., Rao, P.P. & Walker, T.S., 1991. The relative value of cereal straw fodder in semi-arid tropics of India: Implications for cereal breeding programs in ICRISAT. Resource Management Program, Economics group progress report 105, Hyderabad, India.
- Leng, R.A., 1990. Factors affecting the utilization of poor quality forages by ruminants particularly under tropical conditions. Nutr. Res. Rev. 3, 277-303.
- Mahesh, M.S. & Madhu, M., 2014. Crop residues for sustainable livestock production. J. Adv. Dairy Res. 2, 2.

- Nguyen, V.N., Wanapat, M., Khejornsart, P. & Kongmun, P., 2012. Nutrient digestibility and ruminal fermentation characteristics in swamp buffaloes fed chemically treated rice straw and urea. *Trop. Anim. Health Prod.* 44, 629-636.
- Paterson, J.P., Klopfenstein, T.J. & Peterson, L., 1980. Corn plant residue, ammonia treatment. *Nebraska Beef Cattle Report.* 80, 24-25.
- Rangnekar, D.V., Badve, V.C., Kharat, S.T., Sobale, B.N. & Joshi, A.L., 1982. Effect of high pressure steam treatment on chemical composition and digestibility *in-vitro* of roughages. *Anim. Feed Sci. Technol.* 7, 61-70.
- Ravhuhali, K., Mlambo, V., Beyene, S. & Palamuleni, L.G., 2019. A comparative analysis of the morphology and nutritive value of five South African native grass species grown under controlled conditions. *Afr. J. Range For. Sci.* 36(1), 67-70.
- Simmons, B.A., Loque, D. & Ralph, J., 2010. Advances in modifying lignin for the enhanced biofuels production. *Curr. Opin. Plant. Biol.* 13(3), 313-320.
- 
- Sundstel, F. & Coxworth, E.M., 1984. Straw and other fibrous by products as feed. Amsterdam, Netherlands. pp. 196-239.
- Uden, P., 1988. The effect of grinding and pelleting hay on digestibility, fermentation rate, digesta passage and rumen and faecal particle size in cows. *Anim. Feed Sci. Technol.* 19,145-157.
- Viola, E., Zimbardi, F., Cardinale, M., Cardinale, G., Braccio, G. & Gambacorta, E., 2008. Processing cereal straws by steam explosion in a pilot plant to enhance digestibility in ruminants. *Biores. Technol.* 99(4), 681-689.

- Walker, H.G., 1984. Physical treatment in straws and other fibrous by-products as feed. Amsterdam, Netherlands. pp. 79-105.
- Yalchi, T., 2010. Effects of urea and aqueous ammonia treatment on the nutritive value of triticale straw. *J. Food Agri. Environ.* 8(1), 69-72.
- Yang, W.Z, Beauchemin, K.A. & Rode, L.M., 2002. Effects of particle size of alfalfa-based dairy cow diets on site and extent of digestion. *J. Dairy Sci.* 85, 1958-1968.
- Zhao, X.G., Wang, M., Tan, Z.L., Tang, S.X., Sun, Z.H., Zhou, C.S. & Han, X.F., 2009. Effects of rice straw particle size on chewing activity, feed intake, rumen fermentation and digestion in goats. *Asian-Australas. J. Anim. Sci.* 22, 1256-1266.
- Zorrilla-Rois, J., Owens, F.N., Horn, G.W. & McNew, R.W., 1985. Effect of ammoniation of wheat straw on performance and digestion kinetics in cattle. *J. Anim. Sci.* 60, 841-821.

2 CHAPTER TWO - LITERATURE REVIEW

2.1 Introduction

According to Red Meat Research and Development South Africa (RMRD SA, 2012), ruminant production in South Africa is mostly based on extensive grazing systems but more than 75% of the cattle slaughtered in the formal sector are finished in feedlots on maize grain and its by-products (RMRD SA, 2012). However, in recent years because of low rainfall and an increase in ambient temperatures, there has been a decline in quality natural pastures for ruminants (Rojas-Downing *et al.*, 2017). Consequently, farmers are often forced to use conventional feed supplements, which are rather expensive and uneconomical. However, the competitive demand for conventional feed resources for both livestock and human has over the years ignited an unprecedented increase in the cost of conventional supplements making them inaccessible to many livestock farmers (Henning, 1999). The increasing costs of conventional feeds, coupled with a decline in quantities and quality of natural pastures make it imperative for farmers to explore new approaches that optimize among others, the utilization of low quality forages including hays and crop straws. South Africa is one of the major producers of cereals and maize grain in the world (FAO, 2016). From cereal grain production, high amounts of crop straws are produced and often left unutilized or underutilized after harvesting. For example, Gertenbach and Dugmore (2004) estimated that in the production of maize, the grain yield is similar to the yield of straws and may have the same mass. Furthermore, there is an abundance of mature, highly fibrous grass species that are found in high rainfall areas of South Africa with potential to be used as alternative sources of feed for ruminants. Although these low quality roughages and crop residues (wheat, oats, maize, barley, rice and etc.) have great potential to be used in animal diets, they are characterized by low crude protein levels (2 – 6%), high lignin (10 – 25%) and low digestible (34 – 65%) and metabolisable (10.3 MJ/kg) energy that may negatively affect

productivity (Mupangwa *et al.*, 2002; Kraidees, 2005; Jabbar *et al.*, 2009). Poor quality forages have low digestibility and may reduce voluntary intake, impair ruminal function and subsequently affect animal performance (Van Soest, 2006; Sarklong *et al.*, 2010). To change the negative effects of poor quality roughages, chemical and physical treatments has been suggested as a strategy to improve quality of these feedstuffs (Nguyen *et al.*, 2012).

Treatment of stovers before feeding may be essential to improve utilization (Woyengo *et al.*, 2004). Literature reveals that chemical treatment of poor quality roughages improves their nutritive value (Owen & Jayasuriya, 1989) because of their ability to disrupt plant cell wall by partial breakdown of lignin-carbohydrate complex and by so doing improves their utilization in the rumen by increasing the availability of fermentable energy to ruminal microbes (Mahesh & Madhu, 2013). The use of chemical treatment including sodium hydroxide (Dass & Kundu, 1994; Pires *et al.*, 2010), urea (Wang *et al.*, 2004; Jabbar *et al.*, 2009) and ammonia (Wang *et al.*, 2004) has been extensively explored. Anhydrous ammonia has the potential to increase the *in vitro* digestibility of wheat straws (Tejada *et al.*, 1979). However, in South Africa, the use of chemicals to treat poor quality cereal straws and its effect on ruminant performance is limited.

2.2 Potential feed sources for ruminants

Grass and legume hays, cereal grain and oil seed by-products are often considered as poor quality roughages López *et al.* (2005). This includes maize stover and cobs, sorghum stalks, wheat, rice and barley straws, cotton by-products, soybean hay and hulls and peanut hulls. Wheat, maize, barley, oats and rice straws are major agro-residues in terms of volumes generated (14 million tons) in South Africa (Batidzirai *et al.*, 2016). Although they have vast energy potential, their energy is bound in lignocellulolytic matter. This causes a major setback on the utility of this energy source because the animals cannot utilize them to their fullest potential due to poor digestibility.

Cereal straws are particularly high in fibre, structural carbohydrates, lignin and other compounds (xenobiotics, trycin flavones), which makes the important nutrients less available to microbes in the rumen (Liu *et al.*, 2008). In addition, the lignin cell wall material often constitutes up to 80% of the dry matter (Jabbar *et al.*, 2009), leading to slow rate of ruminal degradation, high rumen load, low rumen fractional outflow rates, poor growth and poor ultimate productivity (Arisoy, 1998; Mupangwa *et al.*, 2002).

2.3 Maize (*Zea mays*)

Maize is grown throughout the world. Different types of maize are grown with the distinction made from the kernel colour, white, yellow, red and black. Countries from Africa, prefer white maize. Uses of maize range from being used for human consumption, animal consumption to production of fuel. This has made the production of maize to be increased significantly in turn the straw production increased as well. According to Carvalho *et al.* (2008), maize straws have shown great potential to be used for fuel production. In Poland, the maize straw production is estimated to be around 4 million tons annually (Wojcieszak *et al.*, 2018). During the dry period, farmers use maize straw as roughage for their livestock (Lukuyu *et al.*, 2011).

2.4 Wheat (*Triticum aestivum*)

It is estimated that 765.6 metric tons of wheat are produced monthly in the world with the European Union (152 million tons) being the highest producer and followed by China (132 million tons) (FAO, 2019). There are six varieties of wheat namely hard red winter, soft red winter, hard red spring, hard white, soft white and durum. These varieties are identified by the hardness of the grain, colour of the kernel and the time of planting. Wheat has number of uses which includes as feed for livestock (cattle and poultry), as a base for alcoholic beverage production and it is milled to produce flour which is used to make a variety of products

(bread) (Cappelli *et al.*, 2019). In some countries the wheat straws are used for roofing (thatch) their houses whilst commonly, the straws are used as roughage by ruminant farmers (Aswini, 1990).

2.5 Oats (*Avena sativa*)



In 2017, the world production of oats was estimated to be 26 million tons, with Russia (5.4 million tons) being the highest producer, followed by Canada (3.7 million tons) (FAO, 2019). Oats is used both for human and animal consumption. Commonly, oats is used in the equine industry as feed. The straw is appreciated as bedding in the equine sectors and as feed during the dry season by ruminant farmers.

Table 2.1. Chemical composition of maize, wheat and oats cereal straws (% DM)

¹ Parameters	Straws			References
	Maize	Wheat	Oats	
Dry matter	76.14 - 89.9	92.7 – 94.3	92.9 – 93.9	Sousal <i>et al.</i> (2019)
Crude protein	3-5	4.6	4.8	Olson <i>et al.</i> (1974)
ME	35.10	1.0 – 2.1	36.3 – 53.7	Galila <i>et al.</i> (2012)
NDF	66 - 92	75 – 82	70	Olson <i>et al.</i> (1974)
ADF	45.94	51 -64	47	Sousa <i>et al.</i> (2019)
Lignin	5.5 – 8.8	8.5 -15	14	Olson <i>et al.</i> , (1974)
Calcium	0.61	0.13 – 0.33	0.18 – 0.45	Anderson & Hoffman (2006)
Phosphorus	0.07 - 0.14	0.09 – 0.30	0.13 – 0.36	Li <i>et al.</i> (2014)
Potassium	0.68 – 1.88	0.84 – 2.0	0.18 – 0.45	Li <i>et al.</i> (2014)
Sodium	0.04	0.05	0.0027	McCartney <i>et al.</i> (2006)
Iron	0.05178	0.0139	0.0099	Galila <i>et al.</i> (2012)
Zinc	0.00418	0.0016	0.0015	Galila <i>et al.</i> (2012)

¹Parameters: ME = Metabolisable energy; NDF = Neutral detergent fibre; ADF = Acid detergent fibre.

2.6 Possible strategies to improve the utilization of cereal straws

The utility of poor nutritive value cereal straws in ruminant diets can be enhanced by physical, chemical and biochemical treatments that break down lignin and high fibrous material (Flachowsky *et al.*, 1999. These treatments are also known to increase nitrogen content and promote the bioavailability and accessibility of nutrients by rumen microbes (Flachowsky *et al.*, 1999. However, field application of these treatment approaches depends on economic and practical considerations. In many instances, the feasibility and economic

advantage of feeding pre-treated poor quality forages depends on the local prices of feed and produce as well as the type and level of animal production (Schiere & De Wit, 1993). Generally, two approaches are used to assess the economics of straw treatment: (1) comparison of the cost of a unit of total digestible energy (TDN) and crude protein; (2) the use of least cost ration formulation (Schiere & De Wit, 1993). These approaches indicate that cereal straw treatment is economically attractive when treated straw is cheaper than other supplements for cows of medium production, and when animal products are sold at a remunerative price. Ultimately, the objective of employing any of the treatment methods is to improve the quality of roughages to enable them to be incorporated in least cost rations that can substitute conventional diets. Physical treatment such as grinding and pelleting can be applied specifically to improve handling of the roughages and to increase surface area for chemical treatment (McDonald *et al.*, 2002). The major chemicals used in these treatment methods are alkali in nature and include urea, ammonia, hydrogen peroxide and sodium hydroxide (Wang *et al.*, 2004). Alkaline agents can be absorbed into the cell wall where they chemically break down the ester bond between lignin and hemicellulose and cellulose, and as a consequence, improve the structure lignin of the roughage (Lam *et al.*, 2001; Sarnklong *et al.*, 2010). The breakdown of the bonds between lignin and hemicellulose, and cellulose components may enable the attachment or reattachment of rumen microbes to structural carbohydrates, therefore, enhancing degradability and palatability of the roughages.

2.6.1 Grinding and pelleting

Grinding increases particle density allowing for rapid outflow rate from the rumen to the small intestine shortly after ingestion (Hooper & Welch, 1985). This in turn reduces the retention time of feed in the rumen but correlatively increases feed intake by the host animal. Due to the less retention time of feed in rumen, degradability is to some extent impeded. Whereas, pelleting changes the physiological and chemical characteristics of the ingredients,

due to the heat that is applied during the process (Zijlstra *et al.*, 2009). The process is believed to increase dry matter intake by at least 8% (McCroskey *et al.*, 1961). However, Greenhalgh and Reid (1973) reported a decrease in dry matter digestibility of pelleted forages as compared to their normal physical form. This could be due to the increase in rumen passage flow time to the intestines. When pelleted feed was given to pigs in place of mesh diets, feed intake improved (Steidinger *et al.*, 2000). According to Richert and DeRouchey (2010), pelleted feeds increase feed efficiency by 6 to 7%. Pelleting and grinding application with cereal straws (wheat, oats and maize) causes a problem with degradability as the rumen retaining time is less due to the particle size, which is not favourable.

2.6.2 Treatment with sodium hydroxide

Fahey *et al.* (1993), reported that treatment with sodium hydroxide (NaOH) improves the DM intake of the crop residues by 22% and results in an increase in DM digestibility by 30%. This was also in agreement with the findings of Klopfenstein *et al.* (1972). Sodium hydroxide is an effective chemical for improving the digestibility (Arisoy, 1998) and palatability (Chaudhry & Miller, 1996) of various low quality roughages. Although sodium hydroxide appears to be an effective chemical treatment at solubilizing the cell wall fractions, it also has negative effect on digestibility as sodium hydroxide treated feed shift the site of fibre digestion to the lower gut (Berger & Klopfenstein, 1980). Cattle fed roughages treated with sodium hydroxide have been reported to have a negative minerals balance (Peterson, 2014). According to Arndt (1980) sodium hydroxide alters rumen fermentation or function, which prevents the full benefit of using sodium hydroxide. Sundstol and Coxwoth (1984) also reported that the application of sodium hydroxide cause environmental pollution, due to high content of sodium in leaching into ground water .

2.6.3 Urea treatment

Treatment with urea can provide a nearly equal improvement of the degradability and nitrogen content of forages as other chemical methods (Sarnklong *et al.*, 2010), because it is very effective. The ability of urea to enhance the nitrogen content of ruminant's diet either as supplement or as chemical reagent for improving low quality roughages has been demonstrated for many years. In most studies urea is used as an alternative to anhydrous ammonia, based on the fact that urea would be hydrolysed into ammonia by urease. Urea in the rumen is converted to ammonia by bacterial urease, which weakens the lignified outer walls (Chenost, 1995). The weakening of lignin by urea is accomplished by ammonia which is produced when urea undergoes ureolysis (Wanapat *et al.*, 1996). Rumen bacteria require ammonia for the synthesis of microbial protein, fermentation and the production of acetate (Russell *et al.*, 1992). Abate and Melaku (2009) reported that urea treatment increase the nutritive value of barley straws and improve overall intake, nutrient digestibility, volatile fatty acid production and increased passage rate of particles in the rumen at dosage of 5% urea.

2.6.4 Ammonia treatment

While the treatment of urea needs to be converted into ammonia to improve nutritive value of low quality roughages, ammonia can be used directly. The use of ammonia has shown desirable results in the enhancement of the nutritive value of low quality roughages. This treatment does not only increase the degradability and nitrogen content of the roughages, but also preserves the roughages from inhibiting fungal growth (Calzado & Rolz, 1990; Hadjipanayiotou *et al.*, 1993). Mir *et al.* (1993) reported an increase in crude protein content in alfalfa hay containing 20 - 30% moisture, where the ammoniated hay did not have mould

even after 14 weeks of storage. Orden *et al.* (2000) also reported that ammoniation of low quality roughages increased nitrogen content and cellulose degradability.

2.7 Use of ammoniation to improve nutritive value

Sundstol and Coworth (1984) reported that the efficacy of the process is determined by the amount of ammonia used, length of treatment, the moisture content and the treated substrate. It is therefore imperative that the duration, type of roughage, treatment dosage and temperature interactions be taken into consideration. Jabbar *et al.* (2009) reported a positive correlation between protein content of straws and urea levels. Furthermore, the duration of incubation had an influence on protein content of the straws, with the protein content increasing from week one to week eight. Hadjipanayiotou and Enomides (1997) reported that temperature (40-60°C) improves the effect of urea on protein content of treated straws. Whereas, Schneider and Flachowsky (1990) recommended an ammonia dosage of 3 – 4% per DM with a moisture content of 30% in cereal straws. Ammoniation of about 1.5 - 2.0% of hay per DM was reported to be fungicide and can effectively preserve roughages containing up to 30% moisture, thus preventing mould formation during storage (Mir *et al.*, 1993).

2.8 Recommended ammonia dosages

Literature reveals that the most effective ammonia treatment dosage level is 3% ammonia per DM of low quality roughage (Sundstol & Owen, 1984; Cardoso *et al.*, 2004). Sundstol *et al.* (1978) also reported significant improvement in *in vitro* digestibility when dosage of ammonia was increased from 1 - 2.5% to 2.5- 4% of the straw. It was also observed that high doses of ammonia result in high nitrogen retention, which is unsuitable for animals (Dryden & Leng, 1986). Normally lower doses of 1.0 to 1.5% of NH₃ and 1.9 to 2.8% of urea are used to preserve forage with moisture content that is between 20% and 50%. However, the effectiveness of the ammonia dosage may be influenced by the type of straws treated. For



example, Arndt (1980) reported an increase in *in vitro* digestibility when a dosage of 5.2% of ammonia was used in the treatment of alfalfa, barley, bean, fescue, oats, ryegrass, rice and wheat straw. Furthermore, Oji *et al.* (1977) reported an increase in cellulose digestibility when corn-stover was treated with ammonia at a rate of 3 to 5%. However, all these observations show that there is an opportunity to determine the optimum ammonia inclusion level.

Ammonia dosage probably lies between 2.5 and 3.5 percent of DM (Sundstol *et al.*, 1978). Depending on the form of ammonia, the possible optimum dosages (per 100 kg of straw) are: 2.5 - 3.5 kg for anhydrous ammonia; 4.5 - 6.2 kg for urea; 13.8-19.3 kg (8 - 12 kg in practice) for ammonium bicarbonate; and 10.3 - 14.5 kg for aqueous ammonia (20% N) (FAO, 2002). The following equation adapted from Sundstol *et al.* (1978) can be used to calculate the optimum dosage:

$$\text{Dosage (kg)} = \frac{\text{economic optimum for dosage}}{\text{nitrogen content of ammonia source} \times 1.21}$$

2.9 Ammonia treatment methods

2.9.1 Stack method

In this method, the loose straw is chopped and stacked preferably on an elevated dry land (FAO, 2002). Chopping before treatment facilitates feeding, saves plastic and reduces the danger of puncturing the plastic. The moisture content of the straw to be treated should be adjusted to 20% or more during stacking, this is because anhydrous ammonia requires a low-moisture straw compared with urea and ammonium bicarbonate. However, after treatment, it is difficult to ventilate the straw, and as a consequence it can easily become mouldy. A wooden bar, which will be pulled out when ammonia injection starts, is placed before

stacking so that the ammonia can be injected easily and conveniently. The stack is sealed with a non-toxic polyethylene sheet and injected with 3% dry matter anhydrous ammonia (FAO, 2002). At the end, the opening left after removing the pipe is sealed with a good quality tape. In this method, measurement of the stack density is important to inject the correct amount of ammonia. The appropriate ammonia inclusion is of importance as over treatment has no effect and under treatment is ineffective.

2.9.2 Silo or bunker method

The silo or bunker method has the advantage that silos or bunkers may be used for either ammoniating or ensiling all-year-round (FAO, 2002). Moreover, silos/bunkers are easy to manage and avoid rodent damage to plastic films. Silos or bunkers constructed with cement are the best, since they save on plastic (only one sheet is needed, to cover) and minimize repairs. Once a silo or bunker is constructed, it can be used for several years. In addition, the bunker facilitates the estimation of straw weight because if the dimensions of the bunker are known, the fill capacity can be estimated (FAO, 2002). There are many types of bunkers, but rectangular bunkers are normally recommended. They can be built on the surface, underground, or half-and-half. By adding internal walls, the bunkers can be divided into double or twin bunkers for sequential treatment.

2.10 Factors influencing effectiveness on ammoniation

2.10.1 Temperature and length of treatment

There is a significant interaction effect observed on temperature and the length of treatment. At lower temperatures and lower moisture levels, there is a slow reaction and that can partly be compensated by a longer treatment period. Ballet *et al.* (1997) stated that ammoniation period is affected by temperature, which determines the speed at which the reaction between ammonia and the feedstuff occurs, as well as the extent of improved digestibility (Cloete &

Kritzinge, 1984). When the temperature decreases, the length of time required to complete the reaction is increased (Sundstol *et al.*, 1978). Table 2.2 shows the recommended temperatures with their respective treatment times.

Table 2.2. Recommended temperature and length of ammonia treatment

Ambient temperature	Treatment time
< 5°C	>8 weeks
5 – 15°C	4 - 8 weeks
15 – 30°C	1 - 4 weeks
>30°C	<1 week

Source: Sundstol *et al.* (1978)

2.10.2 Moisture content

Sundstol *et al.* (1978) reported ammoniation to be faster and more effective at high moisture levels. According to Wanapat *et al.* (1996), the moisture content of the roughages should not be less than 30% and not great than 60% when treated. Below 30%, the reaction may not take place. This may be due to difficulties faced when compressing the mass of roughages and expel the air when the roughage is in a loose form. This will result in NH_3 and excess of oxygen in the medium leading to poor ammonia treatment and mould development. Moisture content above 60% would result in a discrepancy of forage mass, insufficient diffusion of ammonia and the ammonia bound to water instead of the plant cell. This would in turn result in the development of moulds because of the moisture and inadequate ammonia to treat roughages. Muñoz *et al.* (1991) reported an effective response when the moisture content was

raised from 20% to 30%, however, increasing the moisture level to 40% negatively affected the forage quality. Ibrahim *et al.* (1986) also estimated optimum moisture content to be 30%.

Table 2.3 Effect of ammonia treatment on moisture and mould contamination

Treatment	Moisture (%)	Mould contamination (%)
1% NH ₃ plastic removed at 4 days	33.7	73
1% NH ₃ plastic removed at 21 days	36.6	36
2% NH ₃ plastic removed at 4days	37.0	0

2.11 Effects of ammoniated feedstuffs on ruminant productivity

The effects of ammonia treatment on digestibility and nutritive value of low quality roughages can be noticeable on the ultimate responses of the animals. Klett *et al.* (1972) reported no improvement in gain, feed consumption, feed efficiency or carcass traits in steer fed ammoniated rice hulls, sorghum stubble and wheat straw. However, Mir *et al.* (1993) reported an increase in crude protein in alfalfa hay, as well as improvement in animal performance. Horton and Steacy (1979) reported that 3.5% ammoniated wheat straw fed to steers improved intake, gain or energy value of the roughages, due to improved nitrogen content of the roughages. This was also in agreement with a report from FAO (2016), which reported differences in digestibility of sheep fed treated and untreated straw. Nevertheless, according to Fike *et al.* (1995) ammoniation alone does not meet the nutrient requirements of ruminant animals beyond maintenance level even though there is improved intake and ruminal degradability. Hence there is a need to include the treated roughage in complete rations that meet the entire nutrient requirements of ruminants for optimum production.

2.11.1 Associative effects

Information on the use of ammonia or ammoniation is inconsistent particularly with regards to toxicity in animals. According to Russell *et al.* (1992) as well as Gillespie and Flanders (2009) excess ammonia results in an increased nitrogen excretion and energy loss, it may even cause urea toxicity. When excess amount of ammonia is produced in the rumen or used in treatment of forages, it can be absorbed into the blood stream and negatively affect the animals. Normally, the liver can detoxify ammonia into urea efficiently (Antonelli *et al.*, 2007). However, at a higher concentration in blood, the ammonia overpowers hepatocytes capacity of detoxification, and increases its levels in blood, cerebrospinal fluid and other tissues, resulting in ammonia poisoning (Antonelli *et al.*, 2007). Furthermore, Perdok and Leng (1987) reported that cattle fed on roughages treated with hydrous ammonia develop hyperexcitability syndrome. Some problems result when the treated roughage was of high quality (>5% CP) with high soluble sugar content and had more than 20% moisture content when treated (Gillespie & Flanders, 2009).

2.12 Environmental implications of feeding low quality feeds

Feeding low quality roughages in ruminants is often associated with a significant loss of energy as a result of prolonged ruminal fermentation and methane (CH₄) gas production, with the latter also contributing significantly to global warming through greenhouse gas emissions (Johnson & Johnson, 1995). It is well documented that ruminants on low quality feeds possibly produce over 75% of the methane, from the world's ruminant contribution to atmospheric methane (Benchaar *et al.*, 2001). According to Johnson and Johnson (1995) cattle can produce 250 – 500 litres of methane per day per animal, representing a loss of 2 to 12% of dietary energy in ruminants (Johnson and Johnson, 1995). Higher levels of methane emissions are observed on pastures with lower feed digestibility (Iqbal & Hashim, 2014). Therefore, a greater amount of methane production can be reduced by modifying the

composition of animal feeds. Benchaar *et al.* (2001) reported a 15% reduction in methane production, when forage intake and digestibility were increased. Therefore, possible strategies to improve the quality of roughage have been suggested. Ammoniation is known to improve the nutritive value and digestibility of low quality roughage. Ammonia treatment acts mainly on the linkages among cell wall components, thus increasing accessibility for rumen microorganisms.

2.13 Summary

Cereal straws are widely used by resource poor farmers as animal feed during the dry period. They are used as alternative roughage due to their abundance during the dry period as it correlates with the cereal harvest. The major challenge with the use of cereal crop straws is their low nutritive value. The straws are characterised by low crude protein and high fibre content. These factors inhibit the efficient use of cereal straws by ruminants. A number of techniques to improve the nutritive value of the cereal straws have been recommended. The techniques include amongst others physical and chemical treatment. The main objective is to modify the structural make-up of the cereal straws. According to literature, the physical treatment focuses on cutting, grinding and pelleting of cereal straws. The physical treatment method has shown improvements in terms of intake but the negative effect was the high rumen-gut rate of passage. When focusing on chemical treatment, literature has indicated that alkaline solutions like urea, sodium hydroxide and ammonia have a potential to enhance the nutritive value of cereal straws. For effective treatment when using these alkali chemicals, conditions like the environmental temperature, treatment length and moisture content of the cereal straw have to be taken into consideration. Literature has indicated that the advantage of using urea and ammonia is that they modify the fibre of the cereal straw and also give off nitrogen which is used by rumen microbes. To achieve optimum ruminant production, the rumen microbes must be able to efficiently ferment the substrate. Alkali treatment of cereal

straws has shown to improve the low nutritive value of cereal straws. This study thus seeks to investigate the effect of anhydrous ammonia gas treatment on improving the nutritive value of maize, oats and wheat straws.

2.14 References

- Abate, D. & Melaku, S., 2009. Effect of supplementing urea-treated barley straw with lucerne or vetch hays on feed intake, digestibility and growth of Arsi bale sheep. *Trop. Anim. Health Prod.* 41, 579-86.
- Antonelli, A.C., Torres, G.A.S., Soares, P. & Mori, C.S., 2007. Ammonia poisoning causes muscular but not liver damage in cattle. *Arq. Bras. Med. Vet. Zoo.* 58, 8-13.
- Arisoy, M., 1998. The effect of sodium hydroxide treatment on chemical composition and digestibility of straw. *J. Vet. Anim. Sci.* 22, 165-170.
- Arndt, D.L., 1980. Chemically treated low quality roughages for ruminants. PhD Thesis, Texas Tech University. Texas, USA.
- Aswini, P., 1990. Wheat Production in India, Constraints and prospects, Mittal Publications, New Delhi, India, 1(100), 59.
- Ballet, N., Besle, J.M. & Demarquilly, C., 1997. Effect of ammonia and urea treatments on digestibility and nitrogen content of dehydrated lucerne. *Anim. Feed Sci.* 67(1), 69-82.
- Batidzirai, B., Valk, M., Wicke, B., Junginger, M., Daioglou, V., Euler, W. & Faaij, A.P.C., 2016. Current and future technical ,economic and environmental feasibility of maize and wheat residues supply biomass energy application: Illustrated for South Africa. *Biomass Bioenerg.* 92, 106-129.
- Benchaar, C., Pomar, C. & Chiquette, J., 2001. Evaluation of dietary strategies to reduce methane production in ruminants: A modelling approach. *Can. J. Anim. Sci.*, 81, 563-574.
- Berger, R. & Klopfenstein, T., 1980. Effect of sodium hydroxide on efficiency of rumen digestion. *J. Anim. Sci.* 50(4), 745-749.



- Calzado, J. F. & Rolz, C., 1990. Estimation of the growth rate of *Pleurotus* on stocked straw. J. Ferment. Bioeng. 69, 70-71.
- Cardoso, G. C., Garcia, R., Souza, A. L., Pereira, O. G., Andrade, C. M. S., Pires, A. J. V. & Bernardino, F. S., 2004. Performance of simental steers fed sorghum silage, 20 sugar cane and straw rice treated or not with anhydrous ammonia. Rev. Braz. Zootec. 33, 2132-2139.
- Carvalho, L., Lundgren, J. & Wopienka, E., 2008. Challenges in small-scale combustion of agricultural biomass fuels. Int. J. Environ. Eng. 9(1-3), 127-142.
- Cappelle, E.R., Filho, S.V., Da Silva, J.F.C, & Cecon, P.R., 2001. Estimates of energy value from chemical characteristics of feedstuff. Rev. Braz. Zootec. 30, 1837-1856.
- Cappelli, A., Guerrini, L., Parenti, A., Palladino, G. & Cini, E., 2019. Effects of wheat tempering and stone rotational speed on particle size, dough rheology and break characteristics for a stone-milled weak flour. J. Cereal Sci. 91, 102879.
- Chaudhry, A.S. & Miller, E.L., 1996. The effect of sodium hydroxide and alkaline hydrogen peroxide on chemical composition of wheat straw and voluntary intake, growth and digesta kinetics in store lambs. Anim. Feed Sci. Technol. 60, 69-86.
- Chenost M., 1995. Optimizing the use of poor quality roughages through treatments and supplementation in warm climate countries with particular emphasis on urea treatment. <http://www.fao.org/ag/AGA/FRG/ECONF95/PDF/CHENOST.PDF>
- Cloete, S.W.P. & Kritzinger, N.M., 1984. A laboratory assessment of various treatment conditions affecting the ammoniation of wheat straw by urea. 2. The effect of temperature, moisture and incubation period. S. Afri. J. Anim. Sci. 14, 55-58.
- Coxworth, E, Kernan, J., Nicholson, H. & Champlin, R., 1977. Improving the feeding value of straw for ruminant animals. In: On Farm Waste Utilization for Feed Opportunities and Profits for Livestock Producers, Agricultural Economics Research Council of Canada. Canada.

- Dass, M.M. & Kundu, S.S., 1994. Effects of calcium hydroxide, urea and calcium hypochloride treatment on composition and digestibility of wheat straw. *Indian. J. Dairy Sci.* 47, 59-61.
- Dryden, G. & Leng, R.A., 1986. Treatment of barley straw with ammonia and Sulphur dioxide gases under laboratory conditions. *Anim. Feed Sci. Technol.* 14, 41-54.
- Fahey, G.C., Bourquin, L.D., Titgemeyer, E.C. & Atwell, D.G., 1993. Postharvest treatment of fibrous feedstuffs to improve their nutritive value. *Madison.* pp. 715-766.
- FAO, 2002. *Animal Production Based on Crop Residues - Chinese experiences.* Food & agriculture organization of the United Nations, Rome, Italy.
- FAO, 2016. *Treating straw for animal feeding Agriculture and consumer protection.* Food and Agriculture Organization of the United Nations, Rome, Italy.
- FAO, 2019a. *Food outlook, biannual report on global food markets.* Food and agricultural organization of the United Nations, Rome, Italy.
- FAO. 2019. *Oats production in 2017.* Food and Agriculture Organization of the United Nations, Rome, Italy.
- Fike, G.D., Simms, D.D., Cochran, R.C., Vanzant, E.S., Kuhl, G.L. & Brandt, R.T., 1995. Protein supplementation of ammoniated wheat straw: effect on performance and forage utilization of beef cattle. *J. Anim. Sci.* 73, 1595-1601.
- Flachowsky, G., Kamra, D.N. & Zadrazil, F., 1999. Cereal straws as animal feed-possibilities and limitations. *J.Appl. Anim.* 16(2), 105-118.
- Gertenbach, W.D. & Dugmore, T.J., 2004. Crop residues for animal feeding. *S. Afr. J. Anim. Sci.* 5.
- Gillespie, J.R. & Flanders, F., 2009. Feeding and management of the cow-calf herd. *Modern Livestock and poultry production.* 8th Ed. pp. 288-289.

- Greenhalgh, J.F.D. & Reid, G.W., 1973. The effects of pelleting various diets on intake and digestibility in sheep and cattle. *Anim. Prod.* 16, 223-233.
- Hadjipanayiotou, M.L., Verheaghe, T.G. & Shaker, B., 1993. Ammoniation of straw using urea, ammonia gas or ammonium hydroxide. *Livestock Res. Rural Dev.* 5, 1- 4.
- Hadjipanayiotou, M. & Economides, S., 1997. Assessment of various treatment conditions affecting the ammoniation of long straw by urea. *Livest. Res. Rural Dev.* 9, 5.
- Henning, P.H., 1999. Feeding program. *Feedlot management hand book*, ANPI, ARC.
- Horton, G.M. J. & Steacy, G. M., 1979. Effect of anhydrous ammonia treatment on the intake and digestibility of cereal straws by steers. *J. Anim. Sci.* 48, 1239-1249.
- Hooper, A.P. & Welch, J.G., 1985. Effects of particle size and forage composition on functional specific gravity. *J. Dairy Sci.* 68, 1181-1188.
- Ibrahim, M.N.M., Schiere, J.B. & Perea, H.G.D., 1986. Effect of method of urea solution application on the nutritive value of treated rice straw. *Agric. Wastes.* 18, 225-232.
- Iqbal, M.F. & Hashim, M.M., 2014. Dietary manipulation to combat methane production. *J. Anim. Plant. Sci.* 24, 91- 93.
- Jabbar, M.A., Muzafar, H., Khattak, F.M., Pasha, T.N. & Khalique, A., 2009. Simplification of urea treatment method of wheat straw for its better adoption by the farmers. *S. Afri. J. Anim. Sci.* 39, 58-61.
- Johnson, K.A. & Johnson, D.E., 1995. Methane emissions from cattle. *J. Anim. Sci.* 73, 2483-2492.
- Klett, R. H., Maben, B. G. & Sherrod L. B., 1972. Ammoniated roughages in steer finishing rations. *ICASALS*. pp. 104, 55.
- Klopfenstein, T., Krause, V.E., Jones, M.J. & Woods, W., 1972. Chemical treatment of low quality roughages. *J. Anim. Sci.* 35, 418-422.

- Kraidees, M.S., 2005. Influence of urea treatment and Soybean Meal (Urease) Addition on the utilization of Wheat Straw by Sheep. *Asian-Australas. J. Anim.* 18, 957-965.
- Lam, T. B. T., Kadoya, K. & Iiyama, K., 2001. Bonding of hydroxycinnamic acids to lignin: ferulic and p-coumaric acids are predominantly linked at the benzyl position of lignin, not the *b*-position, in grass cell walls. *Phytochemistry.* 57, 987-992.
- Liu, O., Luo, L. & Zheng, L., 2008. Lignins: Biosynthesis and Biological Functions in Plants. *Int. J. Mol. Sci.* 19(2), 335.
- López, S., Davies, D.R., Giráldez, F.J., Dhanoa, M.S. & James, J.D., 2005. Assessment of nutritive value of cereal and legume straws based on chemical composition and *in vitro* digestibility. *J. Sci. Food. Agric.* 85(9), 1550-1557.
- Lukuyu, B., Franzel, S., Ongadi, P.M. & Duncan, A.J., 2011. Livestock feed resources: Current production and management practices in central and northern rift valley provinces of Kenya. *Livestock Res. Rural Dev.* 23, 5.
- Mahesh, M.S. & Madhu, M., 2013. Biological treatment of crop residues for ruminant feeding: A review. *Afr. J. Biotechnol.* 12, 4221- 4231.
- McCroskey, J.E., Pope, L.S., Stephens, D.F & Waller, G., 1961. Effect of pelleting steer-fattening rations of different concentrate to roughage ratios. *J. Anim. Sci.* 20, 42.
- McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. Morgan CA., 2002. *Animal Nutrition.* 6th ed. Harlow. England.
- Mir, Z., Jan, E.Z. Robertson, J. A., McCartney, D. H. & Mir, P.S., 1993. Effects of ammoniation of brome-alfalfa and alfalfa hay, stored as large round bales on preservation and feed quality. *Can. J. Anim. Sci.* 71, 755 -765.

- Muñoz, F., Joy, M., Faci, R. & Alibés, X., 1991. Treatment of ligno-cellulosic residues with urea. Influence of dosage, moisture, temperature and addition of ureases. *Ann. Zootech.* 40, 215-225.
- Mupangwa, J.F., Ngongoni, N.T., Daka, D.E. & Hamudikuwanda, H., 2002. The effect of supplementing a basal diet of veld grass hay with increasing levels of velvet bean hay 23 (*Mucuna pruriens*) on nutrient parameters in sheep. *Livest. Res. Rural Dev.* 14, 4.
- Nguyen, V.N., Wanapat, M., Khejornsart, P. & Kongmun, P., 2012. Nutrient digestibility and ruminal fermentation characteristics in swamp buffaloes fed chemically treated rice straw and urea. *Trop. Anim. Health Pro.* 44, 629-636.
- Oji, U. I., Mowat, D.N. & Winch, J.E., 1977. Alkali treatments of corn stover to increase nutritive value. *J. Anim. Sci.* 44, 798.
- Orden, E. A., Yamaki, K., Ichinohe, T. & Fujihara, T., 2000. Feeding value of ammoniated rice straw supplemented with rice bran in sheep: II. *In situ* rumen degradation of untreated and treated rice straw. *Asian. Austral. J. Anim. Sci.* 13, 906-912.
- Owen, E. & Jayasuriya, M.C.N., 1989. Use of crop residues as animal feeds in developing countries. *Research and Development in Agriculture.* 6(3), 129-138.
- Perdok, H.B. & Leng, R.A., 1987. Hyperexcitability in cattle fed ammoniated roughages. *Anim. Feed. Sci. Technol.* 17, 121-143.
- Peterson, S. 2014. Feeding alkaline treated and processed crop residue to feedlot cattle. University of Nebraska. USA.
- Pires, A.J.V., Carvalho, G.G.P. & Ribeiro, L.S.O., 2010. Chemical treatment of roughage. *R. Braz. Zootec.* 39,192-203.
- Richert, B .T. & DeRouchey, J.M., 2010. Swine feed processing and manufacturing. In: Meisinger, D.J. (Ed.), *National Swine Nutrition Guide*. Pork Center of Excellence. Ames, IA. USA. pp. 245-250.

- RMRD SA, 2012. Research and development plan for the large and small stock meat industries in South Africa, 2012-2013. www.rmrdsa.co.za
- Rojas-Downing, M.M., Nejadhashemi, A.P., Harrigan, T. & Woznicki, S.A., 2017. Climate change and livestock: Impacts, adaptation, and mitigation. *Clim. Risk Manag.* 16, 145-163.
- Russell, J.B., O'Connor, J.D., Fox, D.G., Soest, P.J.V. & Sniffen, C.J., 1992. A net carbohydrate and protein system for evaluating cattle diets. *J. Anim. Sci.* 70, 3 551–3561.
- Sarklong, C., Cone, J.W., Pellikaan, W. & Hendriks, W.H., 2010. Utilization of rice straw and different treatments to improve its feed value for ruminants. *Asian-Australas. J. Anim. Sci.* 23, 680 - 692.
- Schiere, J.B. & De Wit, J., 1993. Feeding standards and feeding systems. *Anim. Feed. Sci. Technol.* 43, 121-134.
- Schneider, M. & Flachowsky, G., 1990. Studies on ammonia treatment of wheat straw: Effect of level of ammonia, moisture content, treatment time and temperature on straw composition and degradation in the rumen of sheep. *Anim. Feed Sci. Technol.* 29, 251-264.
- Steidinger, M.U., Goodband, R.D., Tokach, M.D, & Dritz, S., 2000. Effects of pelleting and pellet conditioning temperatures on weanling pig performance. *J. Anim. Sci.* 78, 3014-3018.
- Sundstol, F., Coxworth, E. & Mowat, D.N., 1978. Improving the nutritive value of straw and other low quality roughages by treatment with ammonia. *World animal review.* 26, 13-21.

- Sundstol, F. & Coxworth, E. M., 1984. Ammonia treatment. In: Sundstol, F. and Owen, E. (eds.) Straw and other fibrous by products as feed. Amsterdam, Netherlands. pp, 196-239.
- Tejada, R., Murillo, B. & Cabezas, M.T., 1979. Ammonia treated wheat straw as a substitute for maize silage for growing lambs. *Trop. Anim. Prod.* 4, 2.
- Van Soest, P.J., 2006. Rice straw, the role of silica and treatments to improve quality. *Anim. Feed Sci.* 130. 137-171.
- Wanapat, M., Munoz, F., Kayouli, C. & Chenost, M., 1996. Method for improving the nutritive values of fibrous feed. *Ann. Zootech.* 45, 89-103.
- Wang, Y., Spratling, B.M., ZoBell, D.R., Wiedmeier, R.D. & McAllister, T.A., 2004. Effect of alkali pre-treatment of wheat straw on the efficacy of exogenous fibrolytic enzymes. *J. Anim. Sci.* 82,198-208.
- Woyengo, T.A., Gachuiiri, C.K., Wahome, R.G. & Mbugua, P.N., 2004. Effect of protein supplementation and urea treatment on utilization of maize stover by Red Maasai sheep. *S. Afr. J. Anim. Sci.* 34, 1.
- Wojcieszak, D., Przybył, J., Dach, J., Zaborowicz, M. & Staszak, Z., 2018. Economic Assessment of the Technology Harvesting Maize Straw for Biogas Production. *BIO. Web. Conf.* 10.
- Zijlstra, R.T., Tibbie, S., Van Kempen, T.A.T.G., 2009. Feed manufacturing technology and feed intake in young pigs. In: Torrallardona, D. & Roura, E. (Eds.), Voluntary feed intake in pigs. Wageningen. Netherlands. pp, 277-292.
- Zorrilla-Rios, J., Horn, G.W., Phillips, W.A. & McNew, R.W., 1991. Energy and protein supplementation of ammoniated wheat straws diets for growing steers. *J. Anim. Sci.* 69, 1809-1819.



3 CHAPTER THREE - CHEMICAL COMPOSITION OF WHEAT, OATS AND MAIZE STRAWS AMMONIATED WITH INCREMENTAL LEVELS OF ANHYDROUS AMMONIA GAS

Abstract

Cereal crop residues are characterised by low digestibility, low nitrogen content and deficiency in both vitamins and minerals. Pre-treatment of these cereal crop residues with anhydrous ammonia could promote better utilisation by ruminants, resulting in improved animal performance. This study was, therefore, designed to investigate the effect of treating wheat, oats and maize cereal straws with incremental levels of anhydrous ammonia gas on proximate and mineral components. In an environmentally controlled room, the three cereal straws were pre-treated with anhydrous ammonia gas at a rate of 0, 2.5, 3.5, 4.5 and 5.5% for a period of seven days. The samples were subsequently air-dried for 24 hours, ground (2 mm sieve) and stored in their respective bottles. For wheat straw, crude protein linearly increased [$y = 39.23 (\pm 9.15) x + 24.20 (\pm 7.13)$; $R^2 = 0.761$; $P = 0.0001$] whereas NDF linearly decreased [$y = 710.29 (\pm 34.14) x - 5.89 (\pm 26.60)$; $R^2 = 0.511$; $P = 0.0065$] in response to anhydrous ammonia gas treatment. There were quadratic trends for CP [$y = 93.45 (\pm 8.44) + 44.07 (\pm 6.58) x - 5.27 (\pm 1.17) x^2$; $R^2 = 0.873$; $P = 0.0007$] and NDF [$y = 508.15 (\pm 19.01) - 123.46 (\pm 14.81) x + 14.65 (\pm 2.64) x^2$; $R^2 = 0.915$; $P = 0.0001$] in response to anhydrous ammonia treatment of maize straws. There were neither linear nor quadratic effects ($P > 0.05$) for OM, ADF and ADL in response to anhydrous ammonia gas levels but quadratic effects were observed on CP for oats straws. Maize straws had the highest CP content when compared to oats and wheat straws, which were similar ($P > 0.05$). Maize straws had the least NDF ($P < 0.05$) when compared to oats and wheat straws, which were the same ($P > 0.05$). There was a quadratic trend for Ca [$y = 6.29 (\pm 0.7) + 1.68 (\pm 0.6) x - 0.26 (\pm 0.1) x^2$; $R^2 = 0.443$; $P = 0.0291$] in response to anhydrous ammonia gas treatment of wheat straws. A

quadratic effect was observed for Cu [$y = 7.18 (\pm 1.1) - 2.23 (\pm 0.8) x + 0.34 (\pm 0.1) x^2$; $R^2 = 0.470$; $P = 0.0467$ in response to anhydrous ammonia gas treatment of maize straws. There were neither linear nor quadratic effects ($P > 0.05$) on the mineral content of oats straws, in response to anhydrous ammonia treatment. It was concluded that anhydrous ammonia gas treatment enhance the nutritive value of wheat, oats and maize straws. This was indicated by the increase in the CP content and the reduction of the straws fibre content.

Keywords: Ammoniation; Chemical composition; Cereal straw; Feed sources; Ruminants

3.1 Introduction

The chemical composition of feedstuffs is of utmost importance to feed manufacturers as they strive to create balanced rations. Ruminant production is highly dependent on the ability of a forage to maximise voluntary feed intake, supply essential nutrients, and promote ease of utilisation. Forage quality depends on its physical and chemical characteristics and the determinants of the quality are the crude protein content and digestibility (Barnes *et al.*, 2003). Fibre is a fraction of the plant cell wall and it comprises of carbohydrates. Fibre is made up of three components, namely, cellulose, hemicellulose and lignin. Lignin gives the plant rigidity but it has been reported to have a negative effect on digestion. Lignification of plant cell wall increases with the maturity of the plant and increased lignification is associated with reduced digestion (Cherney *et al.*, 1993). The ruminal pH has an effect on ruminal digestion. Lack of fibre or insufficient fibre, reduces the chewing time of ruminants thus inversely reducing the production of saliva which consequently causes a decrease in ruminal pH. As rumen pH decreases, fibrolytic bacteria in the rumen become less active and fibre digestion is decreased. When ruminal pH falls below 5.8 - 5.9, the rumen is mildly acidic and fibre digestion in the rumen ceases completely (Allen, 1997). There is a negative correlation between lignin content and dry matter digestibility (Jung *et al.* 1997).

One objective of feeding livestock is to increase their energy intake in the form of fibre and subsequently improve their performance. Crop residues are deficient in essential nutritional components such as protein and energy, and are high in fibre, which consequentially reduce their intake and digestibility in ruminants (Smith, 1993). Wheat straws have been found to have an average protein content of 2-6%, NDF of 77-80%, lignin of 10-25% and dry matter digestibility of 34-50% , whilst maize straws have a protein content of 3-5%, NDF of 66-92%, lignin of 5.5-8.8% and dry matter digestibility of 38-65% (Olson *et al.*, 1974). Generally, straws from different cereals have an average of 3.1 - 4.1% crude protein , 67.2-86% of NDF, 46.3-59.1% of ADF and 4.6-10.7 of ADL (Sipos *et al.*, 2010; Xing, 1995). It is thus important to introduce strategies that can improve the nutritive value of these residues whilst reducing the content of structural carbohydrates and lignocellulosic molecules in order to increase voluntary dry matter intake and performance. These strategies include physical, chemical and biological methods. Physical methods include soaking, grinding, pelleting and steaming under pressure. Chemical treatment focuses on using alkali chemicals like urea, ammonia and sodium hydroxide. Biological methods include the use of enzymes or microorganisms. According to Castro and Machado (1990), steam treatment of sugarcane basses increased its digestibility by 68.96%. Tuyen *et al.* (2012), concluded that the use of white rot fungi on wheat straw, applied enzymatic mechanisms that broke down lignin and improved its nutritive value. White rot fungi also broke down the lignin and lignocellulose structures of maize straw (Tuyen *et al.*, 2012). Treatment of maize straws with urea caused an increase in crude protein whilst the crude fibre decreased when the level of urea increased (Brand *et al.*, 1991). Ammoniation of maize straws generally causes an increase in digestibility and intake (Flachowsky, *et al.*, 1996). Aitchison *et al.* 1988, found that feeding sheep with ammonia treated straws caused a 21% increase in dry matter intake. Similarly, Fahmy and Ørskov (1984) reported that the OM intake of ammonia treated barley straw was 73% higher than for the untreated straw and the intake of digestible organic matter was

improved by 98%. Even though fibre plays a major role in dry matter intake, the quality of the forage in terms of its crude protein content is of vast importance as well.

During the dry season, the nutritive value of forage drastically drops as the crude protein of forage decreases, whilst the NDF and ADF content increase (Evitayani *et al.*, 2005). Cereal crops are harvested during the winter period and the straws are used as feed by resource poor farmers (Schiere, 2010). In order to improve the nutritive value of forage, various strategies have been applied so as to assist rumen microorganisms to digest structural carbohydrates (Alseny *et al.*, 2015). Several research studies have been conducted on the use of biological treatment on crop residues to improve the accessibility of cellulosic fractions, thus improving their digestibility (Zhang *et al.*, 2007). Fungi (*P. chrysosporium*) degrades lignin to the extent of 65 to 70% while others like *Ganoderma applanatum* and *Coriolus versicolor* degrade over 45% of lignin in lignocellulosic material (Gupta *et al.*, 1993). This research study was, therefore, designed to evaluate the effect of treating maize, oats and wheat cereal straws with incremental level of anhydrous ammonia gas with the hypothesis that anhydrous ammonia gas enhances the chemical components of cereal straws.

3.2 Materials and methods

3.2.1 Study site and ingredient sources

The research study was conducted at the North-West University, Molelwane farm located in the semi-arid regions of the North West province of South Africa. The geographical coordinates are as follows: 25°79' South, 25° 62' East. Annual temperatures around the area range from 3°C to 37°C with annual rainfall ranging between 300 mm and 600 mm. The straws for oats (*Avena sativa*), maize (*Zea mays*) and wheat (*Triticum sativum*) were collected after the ripening, reproductive and ripening stages of growth, after harvest of the crops from Bakgat Voere Farm (Brits, North West province) which has a loamy clay soil and

geographical coordinates of 25°27' South 27°56 East. The temperatures range from a minimum of 2.1°C to a maximum of 29.3°C during the winter and autumn season, with an average rainfall of 540 mm that is mostly received between November and February. The anhydrous ammonia gas used to treat the cereal straws was sourced from a company named Puregas (Germiston, Gauteng province).

3.2.2 Ammoniation and processing procedure

Each of the three cereal straws (5 kg) were independently placed in closed polythene plastic bags (60 x 100 cm) before being treated with anhydrous ammonia gas. The anhydrous gas used had a density of 0.77 142 kg/m³ and purity of 97%. The cereal straws were thereafter treated with anhydrous ammonia gas at a rate of 0, 2.5, 3.5, 4.5 and 5.5%, with each treatment replicated five times, making a total of 75 samples. The ammoniation was done by connecting a pipe to the plastic bag and infusing the ammonia gas to each replicate treatment using a 2150 series glass tube flow meter calibrated in hours/litre flow (CF Technologies, Johannesburg, Gauteng province). The ammoniated treatments were then kept in a temperature-controlled room for a period of one week. After a week, the ammonia treated straw samples were air-dried for 24 hours and then ground (Polymix PX MFC-90 D) to pass through a 2 mm sieve and stored in respective sample bottles pending laboratory analyses.

3.2.3 Chemical analyses

The milled cereal straws were analysed for dry matter (DM), organic matter (OM), total nitrogen (N), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and minerals in the Animal Science laboratory of the North-West University. For laboratory DM determination, approximately 1 g of each treatment sample was placed into pre-weighed crucibles and oven-dried at 105°C for 12 hours. After 12 h, the

crucibles were taken from the oven and placed into the desiccator to cool and then weighed. The loss in weight was measured as moisture content and DM was calculated as the difference between the initial sample and moisture content. Organic matter content was determined by ashing the dried samples in a muffle furnace set at 600°C for 6 h. After 6 h, the crucibles were removed from the furnace and placed into a desiccator to cool and thereafter weighed. The loss in weight was measured as OM content and the residue was measured as ash. Total nitrogen content was determined using the standard macro-Kjeldahl method (AOAC, 2005; method no. 984.15) and converted to crude protein (CP) by multiplying the percentage N content by a factor of 6.25. Neutral detergent fibre and ADF were determined by refluxing 0.45 - 0.5 g of samples weighed in ANKOM F57 filter bags with neutral detergent and acid detergent solutions for 60 min and 75 min, respectively, using the ANKOM²⁰⁰⁰ Fibre Analyser (ANKOM Technology, New York), according to van Soest *et al.* (1991) and α -amylase was added during NDF determination.. Acid detergent lignin was determined by treating the ADF residue in ANKOM F57 filter bags with 72% sulphuric acid to dissolve the cellulose and estimated after drying in an oven set at 105°C for a period of 12 h. Mineral content was analysed by using the dry ashing macro and trace minerals methods, following the guidelines provided by the Agri-Laboratory Association of Southern Africa (Agri-LASA, 1998). Samples that were used to determine the DM were further incinerated in a muffle furnace for 12 h. The ash was weighed and digested with 1 mL of 55% nitric acid and 10 mL of 32% hydrochloric acid using a Microwave Reaction System Model 3000. Samples were digested for 45 minutes, cooled, and transferred into respective volumetric flasks (100 mL), which were eventually topped-up with distilled water and left standing for 24 h to allow the sediment to settle down. After 24 h, samples were slowly transferred to McCartney bottles without disturbing the sediment. The concentrations of Ca, P, Mn, Fe, Co, Cu and Zn were then determined using an ICP Mass Spectrometer (Perkin-Elmer, 1982, NexION 300Q).

3.2.4 Statistical analysis

The NORMAL option in the Proc Univariate statement was used to test for normality of the measured parameters. The chemical composition data were evaluated for linear and quadratic effects using polynomial contrasts. Response surface regression analysis (Proc RSREG; SAS, 2010) was applied to estimate the optimum ammoniation rate for each cereal straw, according to the following quadratic model:

$$y = c + bx + ax^2$$

Where, y = response variable; a and b are the coefficients of the quadratic equation; c is the intercept; x is spawning rate, and $-b/2a$ is the x value for optimal response.

In a completely randomised design, two-way analysis of variation (ANOVA) was carried out on the chemical composition data using the general linear models (GLM) procedure of SAS (2010) as follows:

$$Y_{ijk} = \mu + S_i + A_j + (S \times A)_{ij} + E_{ijk}$$

Where: Y_{ijk} = the observation of the dependent variable, μ = the fixed effect of the population mean for the variable, S_i = the effect of the cereal straws (maize, oats and wheat), A_j = the effect of ammoniation rate, $(S \times A)_{ij}$ = the interaction effect of cereal straws and ammoniation rate, and E_{ijk} = the random error effect associated with the observation ijk , in which will be deduced to be normally and independently distributed. For all the statistical tests, the significance was declared at $P \leq 0.05$. The least square means (LSMEANS) were compared using the probability of difference (PDIFF) option in the LSMEANS statement of SAS.

3.3 Results

For wheat straw, Table 3.1 shows that there were significant quadratic effects on DM [$y = 938.8 (\pm 7.10) - 15.058 (\pm 5.53) x + 2.265 (\pm 0.98) x^2$; $R^2 = 0.409$; $P = 0.0403$] and OM [$y = 870.194 (\pm 7.85) - 22.74 (\pm 6.11) x + 4.056 (\pm 1.09) x^2$; $R^2 = 0.542$; $P = 0.0029$] in response to ammonia treatment. However, no linear effects ($P > 0.05$) were observed. No significant quadratic effects were observed for CP and NDF with ammonia gas levels. Crude protein linearly increased [$y = 39.23 (\pm 9.15) x + 24.20 (\pm 7.13)$; $R^2 = 0.761$; $P = 0.0001$] whereas NDF linearly decreased [$y = 710.29 (\pm 34.14) x - 59.89 (\pm 26.60)$; $R^2 = 0.511$; $P = 0.0065$] with ammonia treatment.

Table 3.1. Chemical composition (g/kg DM, unless stated otherwise) of anhydrous ammonia gas treated wheat straws

² Parameters	¹ Ammoniation rate					³ SEM	R ²	Significance	
	0	2.5	3.5	4.5	5.5			Linear	Quadratic
DM (g/kg)	939.3	911.1	919.9	914.5	924.5	5.29	0.409	0.108	0.040
OM	870.1	838.0	844.0	845.4	869.5	17.71	0.542	0.588	0.003
CP	39.4	85.7	99.0	106.3	108.7	7.79	0.761	<.000	0.125
NDF	706.7	620.2	557.5	560.2	574.9	25.00	0.511	0.007	0.216
ADF	437.0	445.5	411.4	416.0	418.9	28.60	0.033	0.537	0.944
ADL	160.0	153.1	118.0	132.4	143.6	14.15	0.107	0.356	0.488

¹Ammoniation rate: 0 = untreated wheat straws; 2.5 = wheat straws treated with 2.5% of anhydrous ammonia gas; 3.5 = wheat straws treated with 3.5% of anhydrous ammonia gas; 4.5 = wheat straws treated with 4.5% of anhydrous ammonia gas; 5.5 = wheat straws treated with 5.5% of anhydrous ammonia gas.

²Parameters: DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin.

³SEM = Standard error of mean.

For maize straw, Table 3.2 shows that there were significant quadratic effects on CP [$y = 93.45 (\pm 8.44) + 44.07 (\pm 6.58) x - 5.27 (\pm 1.17) x^2$; $R^2 = 0.873$; $P = 0.0007$], NDF [$y = 508.15 (\pm 19.01) - 123.46 (\pm 14.81) x + 14.65 (\pm 2.63) x^2$; $R^2 = 0.915$; $P = 0.0001$] and ADF [$y = 273.77 (\pm 16.48) - 69.86 (\pm 12.84) x + 9.48 (\pm 2.29) x^2$; $R^2 = 0.773$; $P = 0.0014$] in response to ammonia treatment. However, no linear effects ($P > 0.05$) were observed. No significant quadratic effects were observed for OM and ADL with ammonia gas levels. Organic matter linearly increased [$y = 870.5 (\pm 2.85) x + 2.61 (\pm 2.22)$; $R^2 = 0.433$; $P = 0.0108$] whereas ADL

linearly decreased [$y = 89.58 (\pm 11.82) x - 15.79 (\pm 9.21)$; $R^2 = 0.385$; $P = 0.0249$] with ammonia treatment.

Table 3.2. Chemical composition (g/kg DM, unless stated otherwise) of anhydrous ammonia gas treated maize straws

² Parameters	¹ Ammoniation rate					³ SEM	R ²	Significance	
	0	2.5	3.5	4.5	5.5			Linear	Quadratic
DM (g/kg)	926.2	929.5	923.6	924.8	921.4	5.29	0.0927	0.4169	0.4849
OM	870.8	875.2	878.7	881.6	880.8	17.71	0.4325	0.0108	0.7968
CP	93.3	174.8	172.1	195.4	173.1	7.79	0.8726	<.0001	0.0007
NDF	513.9	259.6	278.2	266.4	258.2	25.00	0.9151	<.0001	0.0001
ADF	278.2	133.5	165.6	161.5	166.6	28.60	0.7727	0.0004	0.0014
ADL	89.4	58.6	60.7	42.9	54.0	14.15	0.3852	0.0249	0.3488

¹Ammoniation rate: 0 = untreated wheat straws; 2.5 = wheat straws treated with 2.5% of anhydrous ammonia gas; 3.5 = wheat straws treated with 3.5% of anhydrous ammonia gas; 4.5 = wheat straws treated with 4.5% of anhydrous ammonia gas; 5.5 = wheat straws treated with 5.5% of anhydrous ammonia gas.

²Parameters: DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin.

³SEM = standard error of mean.

For oats straw, Table 3.3 shows that there were no linear and quadratic effects ($P > 0.05$) for OM, ADF and ADL in response to ammonia gas levels. However, there were significant quadratic effects on DM [$y = 932.63 (\pm 3.18) - 6.19 (\pm 2.48) x + 0.90 (\pm 0.44) x^2$; $R^2 = 0.145$; $P = 0.0475$] and CP [$y = 79.35 (\pm 12.00) + 34.55 (\pm 9.36) x - 4.17 (\pm 1.66) x^2$; $R^2 = 0.367$; $P =$

0.016] in response with ammonia treatment. Neutral detergent fibre linearly decreased [$y = 569.99 (\pm 46.16) x - 52.13 (\pm 35.97)$; $R^2 = 0.106$; $P = 0.0460$] with ammonia treatment.

Table 3.3. Chemical composition (g/kg DM, unless stated otherwise) of anhydrous ammonia gas treated oats straw

¹ Parameters	Ammoniation rate					SEM	R ²	Significance	
	0	2.5	3.5	4.5	5.5			Linear	Quadratic
DM (g/kg)	932.3	927.0	924.9	927.3	932.5	5.29	0.150	0.079	0.048
OM	759.9	835.3	801.1	813.7	804.0	17.71	0.015	0.431	0.873
CP	104.0	168.2	162.1	154.3	147.2	7.79	0.367	0.0001	0.016
NDF	483.6	552.4	521.0	497.5	521.8	25.00	0.106	0.046	0.395
ADF	266.2	333.3	340.8	321.4	351.5	28.60	0.007	0.718	0.708
ADL	105.1	89.9	88.9	90.3	92.8	14.15	0.064	0.150	0.395

¹Ammoniation rate: 0 = untreated wheat straws; 2.5 = wheat straws treated with 2.5% of anhydrous ammonia gas; 3.5 = wheat straws treated with 3.5% of anhydrous ammonia gas; 4.5 = wheat straws treated with 4.5% of anhydrous ammonia gas; 5.5 = wheat straws treated with 5.5% of anhydrous ammonia gas.

²Parameters: DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin.

³SEM = standard error of mean.

Table 3.4. Level of significance of effects of the main factors on chemical composition of anhydrous ammonia gas treated maize, oats and wheat straws

Factors	¹ Parameters					
	DM	OM	CP	NDF	ADF	ADL
Cereal	NS	***	***	***	***	***
Ammoniation rate	NS	NS	***	***	NS	NS
Cereal x Ammoniation rate	NS	NS	*	***	*	NS



¹Parameters: DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL= acid detergent lignin; NS = $P > 0.05$; * = $P < 0.05$; *** = $P < 0.001$.

Table 3.4 shows that there were no ($P > 0.05$) cereal, ammoniation rate, and interaction of cereal x ammoniation rate effects on DM content. There were cereal effect ($P < 0.05$) on OM and ADL content, but not for ammoniation rate, and cereal and ammoniation rate interaction effect. There were significant differences for cereal, ammoniation rate and their interaction on CP and NDF contents. For ADF content, there was a significant difference ($P < 0.05$) for cereal, and cereal and ammonia interaction but not for ammonia.

Table 3.5. The effect of anhydrous ammonia on chemical composition of maize, oats and wheat straws and interaction

	Maize			Oats			Wheat		
	DM	CP	NDF	DM	CP	NDF	DM	CP	NDF
0.0	926.2	93.3 ^{ab}	513.9 ^{bA}	932.3	104.0 ^{aB}	483.6 ^{aA}	939.3	39.4 ^{aA}	706.7 ^{bB}
2.5	929.5	174.8 ^{bb}	259.6 ^{aA}	927.0	168.2 ^{bb}	552.4 ^{aB}	911.1	85.7 ^{ba}	620.2 ^{aB}
3.5	923.6	172.1 ^{bb}	278.2 ^{aA}	924.9	162.1 ^{bb}	521.0 ^{aB}	919.9	99.1 ^{ba}	557.5 ^{aB}
4.5	924.8	195.4 ^{bc}	266.4 ^{aA}	927.3	154.3 ^{bb}	497.5 ^{aB}	914.5	106.3 ^{ba}	560.2 ^{aB}
5.5	921.4	173.1 ^{bb}	258.2 ^{aA}	932.5	147.2 ^{bb}	521.8 ^{aB}	924.5	108.7 ^{ba}	574.9 ^{aB}
SEM	5.29	7.71	25.00	5.29	7.71	25.00	5.29	7.71	25.00

^{a,b,c} Means in a column with different lowercase superscripts denote significant differences ($P < 0.05$). ^{A,B,C} Means in a row with different uppercase superscripts denote significant differences ($P < 0.05$). SEM = standard error of mean.

Table 3.5 shows the chemical composition of maize, oats and wheat straws treated with incremental levels of anhydrous ammonia gas. Ammonia treatment had no significant effect on DM content. Untreated maize straws had the least CP content (93.34 g/kg DM) when compared to the treated maize straws, which did not differ ($P > 0.05$). Untreated maize straws had the highest NDF content (513.94 g/kg DM) when compared to treated maize straws, which were the same ($P > 0.05$). Untreated oats straws had the least CP content (104.02 g/kg DM) when compared to the treated oats straws, which were similar ($P > 0.05$). There were no differences ($P > 0.05$) observed between treated and untreated oats straws on NDF content.

Untreated wheat straw had the least CP content (39.41 g/kg DM) when compared to the treated wheat straws, which were the same ($P > 0.05$). Untreated wheat straw had the highest NDF content (706.73 g/kg DM) than the treated wheat straws, which were the same (P

>0.05). Untreated wheat straws had the least CP content (39.41 g/kg DM) when compared to untreated oats and maize straws, which did not differ ($P > 0.05$). Untreated wheat straws had the highest NDF content (706.73 g/kg DM) when compared to untreated oats and maize straws, which were similar ($P > 0.05$).

At 2.5% anhydrous ammonia treatment, wheat straws had the least CP content (85.70 g/kg DM) when compared to oats and maize straws, which did not differ ($P > 0.05$). Whereas, maize straws had the least NDF content (259.64 g/kg DM) when compared to oats and wheat straws, which did not differ ($P > 0.05$) when treated with 2.5% anhydrous ammonia. At 3.5% anhydrous ammonia treatment, wheat straws had the least CP content (99.05 g/kg DM) when compared to oats and maize straws which were the same ($P > 0.05$). Maize straws had the least NDF content (278.21 g/kg DM) when compared to oats and wheat straws, which did not differ ($P > 0.05$) when treated with 3.5% anhydrous ammonia. When treated with 4.5% anhydrous ammonia, maize straws had the highest CP content (195.37 g/kg DM), followed by oats straws (154.29 g/kg DM) and the least CP was from wheat straws (106.33 g/kg DM). Maize straws had the least NDF content (266.44 g/kg DM) when compared to oats and maize straws, which did not differ ($P > 0.05$) when treated with 4.5% anhydrous ammonia. At 5.5% anhydrous ammonia treatment, wheat straws had the least CP content (108.67 g/kg DM) when compared to oats and maize straws, which did not differ ($P > 0.05$). Maize straws had the least NDF content (258.19 g/kg DM) when compared to oats and wheat straws, which did not differ ($P > 0.05$).

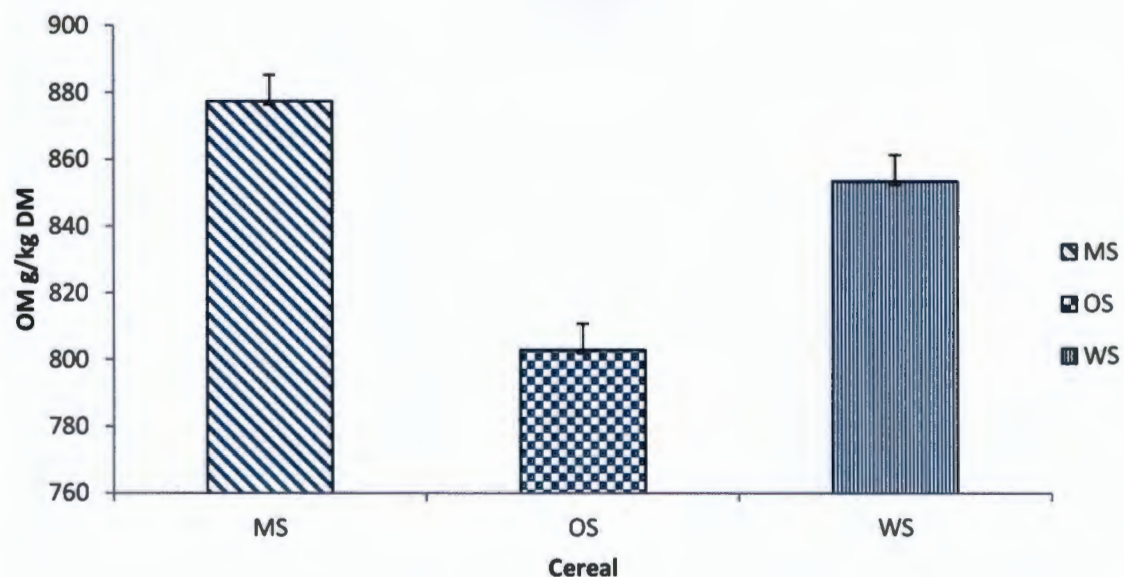


Figure 3.1. Effect of ammonia treatment on organic matter content of cereal straws

Figure 3.1 shows that ammonia-treated maize straw (877.42 g/kg DM) had the highest OM content ($P < 0.05$) followed by wheat straw (853.41 g/kg DM) and oats straw (802.80 g/kg DM), which had the least OM content.

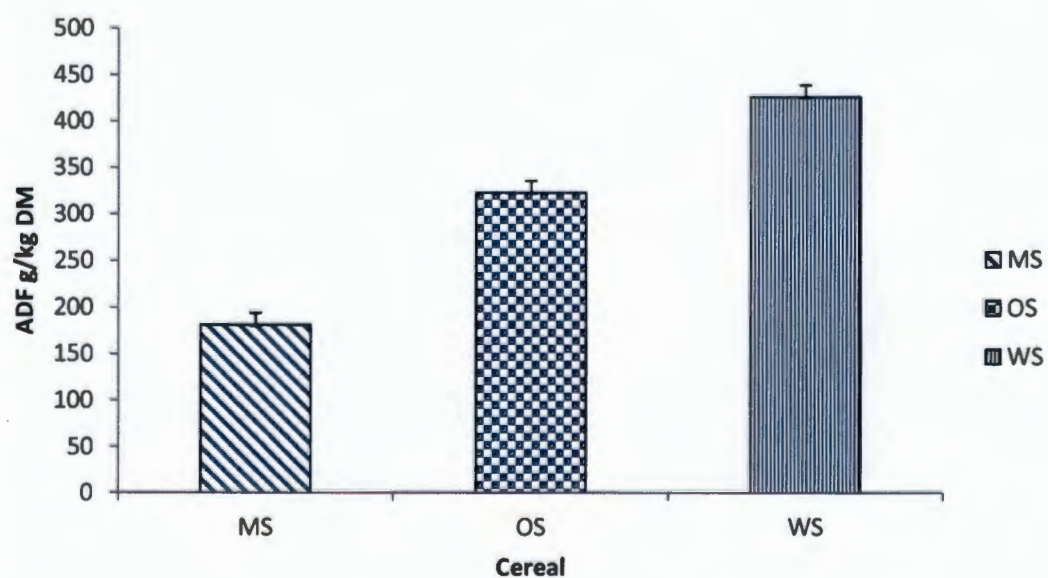


Figure 3.2. Effect of ammonia treatment on acid detergent fibre of cereal straw

Figure 3.2 shows that ammonia treated wheat straw (425.76 g/kg DM) had the highest ADF content ($P < 0.05$) followed by oats straw (322.65 g/kg DM) and maize straw (181.08 g/kg DM) which had the least ADF content.

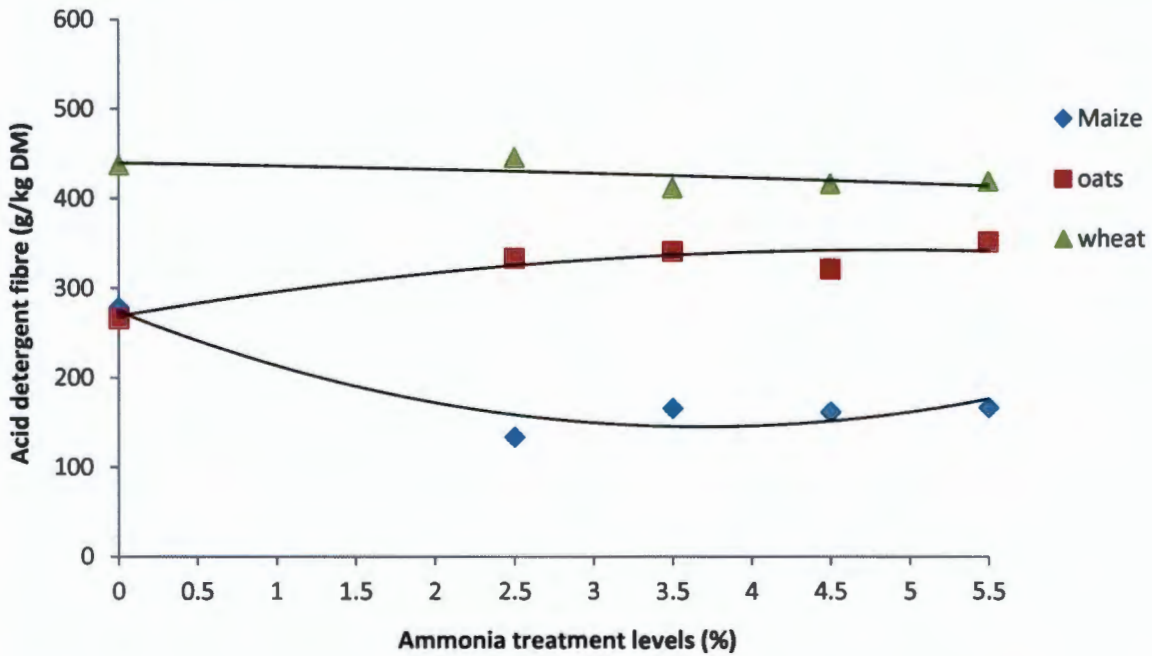


Figure 3.3. Effect of ammonia and cereal straw on acid detergent fibre (g/kg DM)

Figure 3.3 shows that untreated wheat straw (437.03 g/kg DM) had the highest ADF content followed by maize straws (278.17 g/kg DM) and oats straws (266.19 g/kg DM), which had the least ADF content. At 2.5% anhydrous ammonia treatment, wheat straws had the highest ADF content (445.46 g/kg DM) when compared to oats and maize straws. Maize straws had the least ADF content (133.51 g/kg DM), followed by oats straws (333.32 g/kg DM) when treated with 2.5% anhydrous ammonia. At 3.5% anhydrous ammonia treatment, wheat straws had the highest ADF content (411.42 g/kg DM) when compared to oats and maize straws. Maize straws had the least ADF content (165.59 g/kg DM), followed by oats straws (340.82 g/kg DM) when treated with 3.5% anhydrous ammonia. At 4.5% and 5.5% anhydrous ammonia treatment, wheat straws had the highest ADF content (416.00 g/kg DM and 418.90

g/kg DM respectively) when compared to oats and maize straws. Maize straws had the least ADF content (161.53 g/kg DM and 166.59 g/kg DM respectively), followed by oats straws (321.41 g/kg DM and 351.49 g/kg DM) respectively.

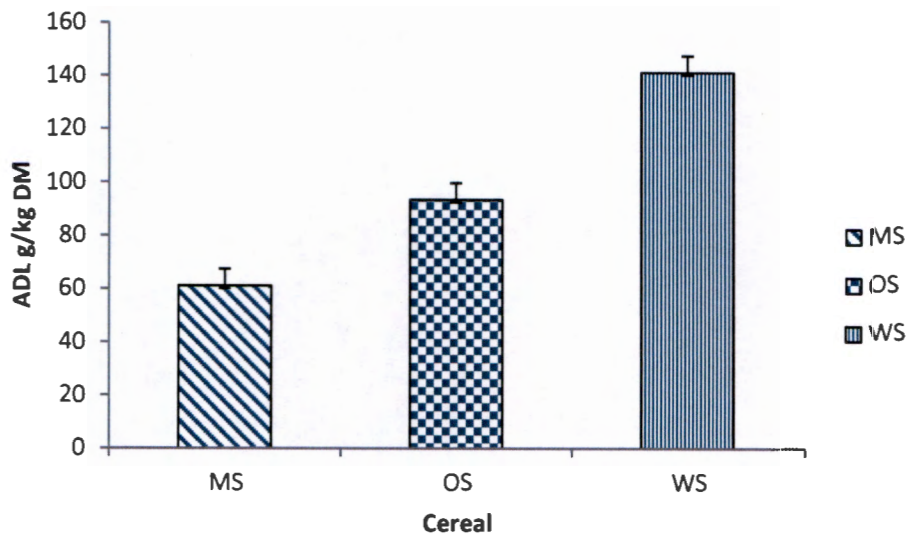


Figure 3.4 Effect of ammonia treatment on acid detergent lignin of cereal straws

Figure 3.4 shows that ammonia treated wheat straw (141.43 g/kg DM) had the highest ADL content ($P < 0.05$) followed by oats straw (93.40 g/kg DM) and maize straw (61.11 g/kg DM) which had the least ADL content.

Table 3.6. Mineral composition of anhydrous ammonia gas treated wheat straws

² Parameters	¹ Ammoniation rate					³ SEM	R ²	Significance	
	0	2.5	3.5	4.5	5.5			Linear	Quadratic
Mg (g/kg DM)	1.29	1.76	1.66	2.02	1.50	0.25	0.290	0.175	0.150
P (g/kg DM)	0.46	0.61	0.65	0.74	0.40	0.13	0.269	0.685	0.074
Ca (g/kg DM)	6.29	8.80	9.15	8.41	7.71	0.72	0.443	0.144	0.029
Mn (mg/kg DM)	9.76	14.05	13.13	18.84	10.11	7.72	0.201	0.401	0.185
Cu (mg/kg DM)	2.98	7.95	3.48	7.91	2.55	1.46	0.477	0.476	0.010
Zn (mg/kg DM)	19.41	18.77	12.49	42.33	20.26	5.86	0.060	0.434	0.843
Fe (mg/kg DM)	134.1	141.1	187.0	304.5	140.0	176.6	0.087	0.394	0.622
Co (mg/kg DM)	0.11	0.11	0.11	0.16	0.11	0.14	0.043	0.509	0.879

¹Ammoniation rate: 0 = untreated wheat straws; 2.5 = wheat straws treated with 2.5% of anhydrous ammonia gas; 3.5 = wheat straws treated with 3.5% of anhydrous ammonia gas; 4.5 = wheat straws treated with 4.5% of anhydrous ammonia gas; 5.5 = wheat straws treated with 5.5% of anhydrous ammonia gas.

²Parameters: Mg = magnesium; P = Phosphorus; Ca= calcium; Mn = manganese; Cu = copper; Zn = zinc; Fe = iron; Co = cobalt.

³SEM = Standard error of mean.

**NWU
LIBRARY**

For wheat straw, Table 3.6 shows that there were significant quadratic effects on Ca [$y = 6.29 (\pm 0.7) + 1.68 (\pm 0.6) x - 0.26 (\pm 0.1) x^2$; $R^2 = 0.443$; $P = 0.0291$] and Cu [$y = 3.31 (\pm 1.0) + 2.12 (\pm 0.8) x - 0.43 (\pm 0.1) x^2$; $R^2 = 0.477$; $P = 0.0105$] in response to anhydrous ammonia gas treatment.

Table 3.7. Mineral composition of anhydrous ammonia gas treated maize straws

² Parameters	¹ Ammoniation rate					³ SEM	R ²	Significance	
	0	2.5	3.5	4.5	5.5			Linear	Quadratic
Mg (g/kg DM)	3.12	2.38	3.45	2.71	2.58	0.25	0.166	0.342	0.398
P (g/kg DM)	1.29	0.95	1.65	1.26	1.14	0.13	0.179	0.700	0.211
Ca (g/kg DM)	8.48	7.81	9.95	6.33	6.48	0.72	0.280	0.194	0.247
Mn (mg/kg DM)	41.83	29.77	46.55	32.18	31.40	7.72	0.219	0.213	0.413
Cu (mg/kg DM)	7.29	5.22	3.76	4.15	4.90	1.46	0.470	0.136	0.047
Zn (mg/kg DM)	35.87	26.89	45.98	38.28	47.62	5.86	0.166	0.211	0.837
Fe (mg/kg DM)	387.56	197.50	471.58	489.21	309.50	176.61	0.058	0.948	0.476
Co (mg/kg DM)	0.21	0.43	0.21	0.14	0.14	0.14	0.183	0.195	0.823

¹Ammoniation rate: 0 = untreated maize straws; 2.5 = maize straws treated with 2.5% of anhydrous ammonia gas; 3.5 = maize straws treated with 3.5% of anhydrous ammonia gas; 4.5 = maize straws treated with 4.5% of anhydrous ammonia gas; 5.5 = maize straws treated with 5.5% of anhydrous ammonia gas.

²Parameters: Mg = magnesium; P = Phosphorus; Ca = calcium; Mn = manganese; Cu = copper; Zn = zinc; Fe = iron; Co = cobalt.

³SEM = Standard error of mean.

For maize straw, Table 3.7 shows that there were significant quadratic effects on Cu [$y = 7.18 (\pm 1.1) - 2.23 (\pm 0.8) x + 0.34 (\pm 0.1) x^2$; $R^2 = 0.470$; $P = 0.0467$] in response to anhydrous ammonia gas treatment. There were no linear or quadratic effects ($P > 0.05$) for Mg, Ca, Mn, Zn, Fe and Co content in response to ammonia treatment. For oats straw, Table 3.8 shows that there were no linear and quadratic effects ($P > 0.05$) in response to anhydrous ammonia treatment on Mg, Ca, P, Mn, Cu, Zn, Fe and Co content.

Table 3.8. Mineral composition of anhydrous ammonia gas treated oats straws

² Parameters	¹ Ammoniation rate					³ SEM	R ²	Significance	
	0	2.5	3.5	4.5	5.5			Linear	Quadratic
Mg (g/kg DM)	1.92	1.49	1.90	1.89	1.82	0.25	0.187	0.793	0.255
P (g/kg DM)	0.90	0.82	0.80	1.07	0.92	0.13	0.195	0.369	0.408
Ca (g/kg DM)	7.30	5.04	7.21	7.51	7.55	0.72	0.276	0.891	0.148
Mn (mg/kg DM)	64.36	45.70	72.17	60.46	81.82	7.72	0.414	0.560	0.069
Cu (mg/kg DM)	4.04	1.60	2.38	3.35	3.87	1.46	0.146	0.405	0.539
Zn (mg/kg DM)	9.90	8.87	11.03	5.85	12.17	5.86	0.149	0.306	0.930
Fe (mg/kg DM)	713.80	563.08	395.06	658.93	806.30	176.61	0.295	0.742	0.137
Co (mg/kg DM)	1.92	1.49	1.90	1.89	1.82	0.14	0.161	0.695	0.314

¹Ammoniation rate: 0 = untreated oats straws; 2.5 = oats straws treated with 2.5% of anhydrous ammonia; 3.5 = oats straws treated with 3.5% of anhydrous ammonia; 4.5 = oats straws treated with 4.5% of anhydrous ammonia; 5.5 = oats straws treated with 5.5% of anhydrous ammonia.

²Parameters: Mg = magnesium; P = Phosphorus; Ca = calcium; Mn = manganese; Cu = copper; Zn = zinc; Fe = iron; Co = cobalt.

³SEM = Standard error of mean.

Table 3.9 shows that there was a significant cereal effect observed on Mg, P, Ca, Mn, Zn, Fe and Co content but no differences for Cu ($P > 0.05$). Anhydrous ammonia gas treatment had no effect ($P > 0.05$) on mineral content. There was a significant cereal and ammonia interaction effect on Ca and Co concentrations.

Table 3.9. Level of significance of effects of the main factors on mineral content of anhydrous ammonia gas treated maize, oats and wheat straws

¹ Parameters								
Factors	Mg	P	Ca	Mn	Cu	Zn	Fe	Co
Cereal	***	***	NS	***	NS	***	***	***
Ammonia	NS	NS	NS	NS	NS	NS	NS	NS
Cereal x Ammonia	NS	NS	*	NS	NS	NS	NS	*

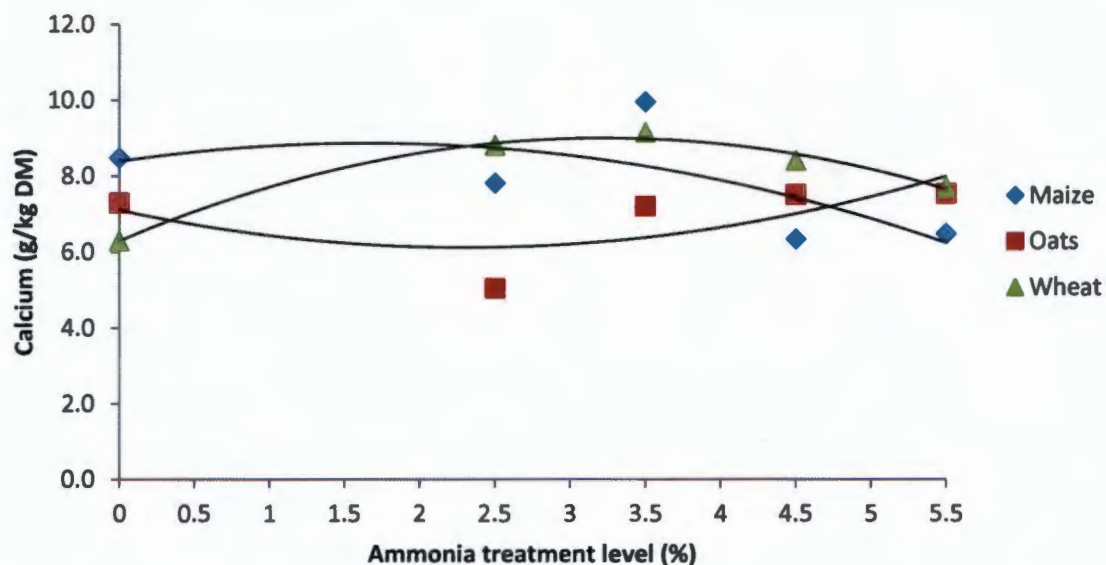
¹Parameters: Mg = magnesium; P = Phosphorus; Ca = calcium; Mn = manganese; Cu = copper; Zn = zinc; Fe = iron; Co = cobalt. NS = $P > 0.05$; * = $P < 0.05$; *** = $P < 0.001$.

Table 3.10 shows that maize straws had the highest Mg content (2.85 g/kg DM) when compared to oats and wheat straws, which did not differ ($P > 0.05$). Maize straws had the highest P content (1.26 g/kg DM), followed by oats straws (0.90 g/kg DM) and the least P was from wheat straws (0.57 g/kg DM). Oats straws had the least Ca content (6.92 g/kg DM) when compared to maize and wheat straws, which were the same ($P > 0.05$). Oats straws had the highest Mn content (64.90 mg/kg DM), followed by maize straws (36.35 mg/kg DM) and the least Mn content was from wheat straws (13.18 mg/kg DM). Maize, wheat and oats straws had no significant differences ($P > 0.05$) on Cu content. Maize straws had the highest Zn content (38.92 mg/kg DM) followed by wheat straws (22.65 mg/kg DM) and the least Zn content was from oats straws (9.57 mg/kg DM).

Table 3.10. The effect of anhydrous ammonia on mineral content of maize, oats and wheat straws

Cereal	¹ Parameters					
	Mg	P	Ca	Mn	Cu	Zn
Maize	2.85 ^a	1.26 ^a	7.81 ^a	36.35 ^a	5.06	38.93 ^a
Oats	1.80 ^b	0.90 ^b	6.92 ^b	64.90 ^b	3.05	9.57 ^b
Wheat	1.64 ^b	0.57 ^c	8.07 ^a	13.18 ^c	4.97	22.65 ^c
² SEM	0.12	0.06	0.34	3.62	0.69	2.75

¹Parameters: Mg = magnesium; P = Phosphorus; Ca = calcium; Mn = manganese; Cu = copper; Zn = zinc; Fe = iron. ^{a,b,c} Means in a column with different lowercase superscripts denote significant differences ($P < 0.05$). ²SEM = standard error of the mean.



3.5. Effect of ammonia and cereal straw on calcium concentration (g/kg DM)

Figure 3.5 shows that at 3.5% anhydrous ammonia treatment, maize straws had highest Ca content (9.95 g/kg DM) when compared to oats and wheat straws. Oats straws had the least Ca content (7.21 g/kg DM), followed by wheat straws (9.15 g/kg DM) when treated with 3.5% anhydrous ammonia.

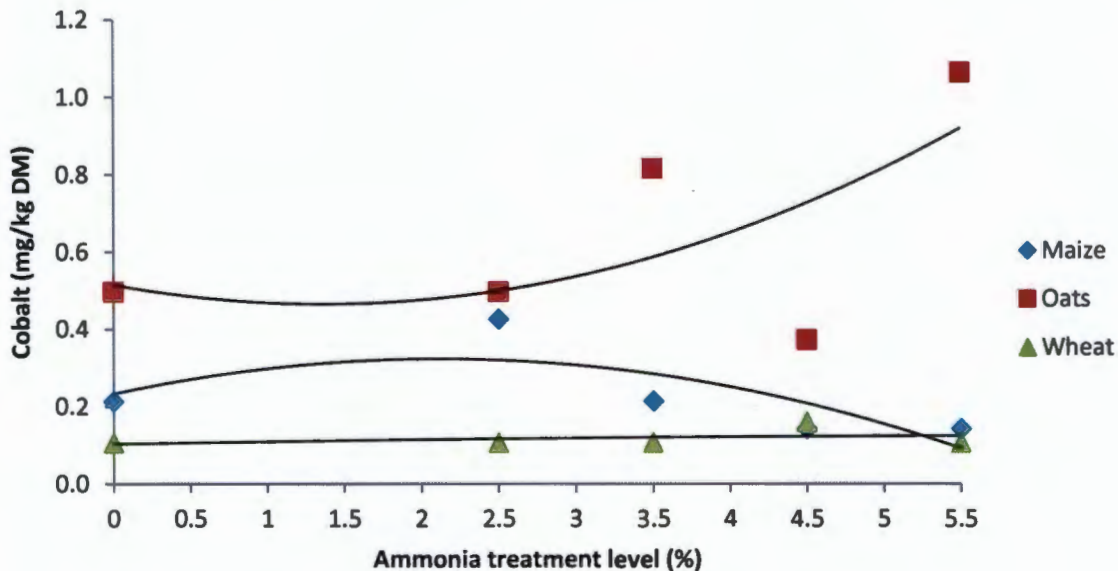


Figure 3.6. Effect of ammonia and cereal straw on cobalt concentration (mg/kg DM)

Figure 3.6 shows that at 5.5% anhydrous ammonia treatment, oats straws had highest Co content (1.06 mg/kg DM) when compared to maize and wheat straws. Wheat straws had the least Co content (0.11 mg/kg DM), followed by maize straws (0.14 mg/kg DM) when treated with 5.5% anhydrous ammonia.

3.4 Discussion

Chemical composition of feed is an important indicator of potentially available nutrients in a feed (Norton & Poppie, 1995). Good forage should provide highly digestible nutrients that satisfy the requirements of livestock (Kisitu, 2010). As expected, maize straws treated with anhydrous ammonia had lower NDF content. Oats cereal straws treated with anhydrous

ammonia had the second lowest NDF whilst untreated oats cereal straws had the lowest NDF but no differences were observed on NDF content of the untreated and treated straws. These results were also in agreement to those found by Brand *et al.* (1985). The lack of difference on the NDF content of the untreated and treated straws could be because ammonia treatment affects the chemical composition of straws when the NDF content of untreated straws is 86% (Joy *et al.*, 1992; Fahmy & Klopfenstein, 1994). Wheat cereal straws treated with 3.5% ammonia had the least NDF, this was in agreement with the findings of Habib *et al.* (1998) and Woyengo *et al.* (2004). Maize and wheat straws treated with anhydrous ammonia had a decrease in ADF and ADL. Treated oats straw had higher ADF and these findings were similar to those of Herrera-Saldana *et al.* (1982), as they found that treatment of wheat straws with ammonia increased the ADF content. Maize straws treated with anhydrous ammonia had a CP content increase (195.37 g/kg DM). The above results are in agreement with those found by McDonald *et al.* (1995), that treatment of cereal straws with alkaline increases their crude protein. The increase in CP as compared to the decrease in NDF and ADL content could improve the degradability and preservation of these cereal straws (Hadjipanayiotou *et al.*, 1993). Anhydrous ammonia gas treated wheat straws showed an increase in Ca and P mineral content. Maize and oats straws treated with anhydrous ammonia treatment showed a decrease in Ca and P mineral content as compared to the untreated straws. These results are in agreement with those of Peterson (2014) that feeding alkali treated crop residues results in mineral deficiency. The decrease in mineral content of anhydrous ammonia gas treated straws will result in mineral deficiency in ruminants fed the treated straws.

3.5 Conclusion

The treatment with anhydrous ammonia gas levels improved the chemical composition of maize, oats and wheat cereal straws by increasing the crude protein content and reducing the

fibre levels. This in turn, improved the buffer nitrogen solubility, *in vitro* ruminal nitrogen degradability and *in vitro* gas production of the maize, oats and wheat cereal straws. It was concluded that anhydrous ammonia gas treatment of cereal straws can be a sustainable and alternative strategy for resource-poor farmers to improve the nutritive value of forages during the dry winter period.

3.6 References

- AgriLASA, 1998. Feed and Plant Analysis Methods. Agri Laboratory Association of Southern Africa, Pretoria, South Africa.
- Allen, M., 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* 80, 1447-1462.
- Alsersy, H., Salem, A.Z.M., Borhami, B.E., Olivares, J. Gado, H.M., Mariezcurrena M.D., Yacuot, M.H., Kholif, A.E., El-Adawy, M. & Hernandez, S.R., 2015. Effect of Mediterranean saltbush (*Atriplex halimus*) ensilaging with two developed enzyme cocktails on feed intake, nutrient digestibility and ruminal fermentation in sheep. *J. Anim. Sci.* 86, 51-5.
- AOAC, 2005. In Official Methods of Analysis of AOAC International, 18th ed. Association of Official Analytical Chemists, Arlington, VA, USA.
- Aitchison, E., Rix, G.S. & Rowe, J.R., 1988. The effect of urea treatment of straw and lupin supplementation on intake, live weight changes and wool growth in sheep. *Anim. Prod.* 17, 134-137.
- Barnes, R.F., Nelson, C.J., Collins M. & Moore, K.J., 2003. Forages: an introduction to grassland agriculture. Ames. Iowa. USA.
- Brand, A.A., Cloete, S.W.P. & Franck, F., 1991. The effect of supplementing untreated, urea supplemented and urea ammoniated wheat straw with maize meal and/or fish meal in sheep. *S. Afr. J. Anim. Sci.* 21, 48-54.
- Brand, A.A., Cloete, S.W.P. & Kritzinger, N.M., 1985. The effect of anhydrous ammonia on the nutritive value of whole oats grain. *S. Afr. J. Anim. Sci.* 15, 2-9.
- Castro, F.B. & Machado, P.F., 1990. Feeding value of steam sugar cane bagasse in ruminant ration. *Livest. Res. Rural Dev.* 2 (1), 12-16.

- Cherney, D.J.R., Cherney, J.H. & Lucey, R.F., 1993. *In vitro* digestion kinetics and quality of perennial grasses as influenced by forage maturity. *J. Dairy Sci.* 76, 790-797.
- Evitayani, L., Warly, A., Fariani, T., Ichinohe, T. & Fujihara, T., 2005. Seasonal changes in nutritive value of some grass species in West Sumatra, Indonesia. *Anim. Sci. J.* 76(5), 461-468.
- Flachowsky, G., Ochrimenko, W. J., Schneider, M. & Richter, G.H., 1996. Evaluation of straw treatment with ammonia sources on growing bulls. *Anim. Feed Sci. Technol.* 60, 117-130.
- Gupta, B.N., Singh, G.P. & Singh, K., 1993. Biological treatment of lignocellulosics as feed for animals-an overview. In: Singh, K. & Schiere, J.B. (Eds) *Feeding of ruminants on fibrous crop residues*. Indian Council of Agricultural Research, Krishi Bhavan, New Delhi. pp. 209- 221.
- Herrera-Saldana, R., Church, D.C. & Kellems, R.O., 1982. The effect of ammoniation treatment on intake and nutritive value of wheat straws. *J. Anim. Sci.* 54, 3.
- Fahmy, S.T.M. & Ørskov, E.R., 1984. Digestion and utilization of straw. 1. Effect of different chemical treatments on degradability and digestibility of barley straw by sheep. *Anim. Prod.* 38, 69-74.
- Fahmy, S.T.M. & Klopfenstein, T.J., 1994. Treatment with different chemicals, their effects on the digestibility of maize stalks. *Anim. Feed Sci. Technol.* 45, 309-316.
- Habib, G., Hassan, M.F. & Siddiqui, M.M., 1998. Degradation characteristics of straws from different wheat genotypes and their response to urea ammonia treatment. *Anim. Feed Sci. Technol.* 72, 373-386.
- Hadjipanayiotou, M.L., Verheaghe, T.G. & Shaker, B., 1993. Ammoniation of straw using urea, ammonia gas or ammonium hydroxide. *Livest. Res. Rural Dev.* 5, 1- 4.
- Joy, M., Alibes, X. & Muñoz, F., 1992. Chemical treatment of lignocellulosic residues with urea. *Anim. Feed Sci. Technol.* 38. 319-333.

- Jung, H.G., Mertens, D.R. & Payne, A.J., 1997. Correlation of acid detergent lignin and Klason lignin with digestibility of forage dry matter and neutral detergent fiber. *J. Dairy Sci.* 80, 1622-1628.
- Kisitu, B., 2010. Growing fodder for livestock. Calliandra and elephant grass. TECA-FAO.
- McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A., 1995. Animal nutrition. Fifth Edition. Longman Scientific & Technical. pp. 300-304.
- Norton, B.W. & Poppi, D.P., 1995. Composition and nutritional attributes of pasture legumes. Tropical legumes in Animal Nutrition. CAB international, FAO.
- Olson, H., Santo, K.A.S. & McCoy, G.C., 1974. Percentage roughage in corn and milo complete feeds. *J. Dairy Sci.* 57, 1260.
- Peterson, S. 2014. Feeding alkaline treated and processed crop residue to feedlot cattle. University of Nebraska. Nebraska, USA.
- Schiere, J.B., 2010. Cereal straws as ruminant feeds: problems and prospects revisited. *Anim. Nutr. Feed Technol.* 10, 127-153.
- Schneider, M. & Flachowsky, G., 1990. Studies of ammonia treatment on wheat straw: effects of level of ammoniation, moisture content, treatment time and temperature on straw composition and degradation in the rumen of sheep. *Anim. Feed Sci. Technol.* 29, 251-264.
- Sipos, P., Nabradi, A. & Gyori, Z., 2010. Examination of chemical composition calorific value of cereal straws. *Acta. Agron. Hung.* 58, 97-101.
- Smith, O.B., 1993. Feed resource for intensive smallholder systems in the tropics: the role of crop residues. In Proceedings of the XVII International Grassland Congress. Rockhampton, Australia. pp. 18-21.

- Tuyen, V.D., Phuong, H., Cone, J., Baars, J., Sonnenberg, A. & Hendriks, W., 2013. Effect of fungal treatments of fibrous agricultural by products on chemical composition *and in vitro* rumen fermentation and methane production. *Bioresource Technol.* 129, 256-263.
- Tuyen, V.D., Cone, J., Baars, J., Sonnenberg, A. & Hendriks, W., 2012. Fungal strain and incubation period affect chemical composition and nutrient availability of wheat straw for rumen fermentation. *Bioresource Technol.* 111, 336-342.
- Van Soest, P.J. & Wine, R.H., 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *AOAC.* 50, 50-55.
- Woyengo, T.A., Gachuri, C.K., Wahome, R.G. & Mbugua, P.N., 2004. Effect of protein supplementation and urea treatment on utilization of maize stover by Red Massai sheep. *S. Afr. J. Anim. Sci.* 34, 1.
- Xing, T., 1995. Nutrition value and utilization of crop residues. Changsha. China.
- Zhang, X., Xu, C. & Wang, H., 2007. Pre-treatment of bamboo residues with *Coriolus versicolor* for enzymatic hydrolysis. *J. Biosci. Bioeng.* 104, 149-151.

4 CHAPTER FOUR - IN VITRO RUMINAL DRY MATTER DEGRADABILITY AND NITROGEN BUFFER SOLUBILITY OF WHEAT, OATS AND MAIZE STRAWS AMMONIATED WITH INCREMENTAL LEVELS OF ANHYDROUS AMMONIA GAS

Abstract



This study investigated the effect of treating cereal straws (wheat, oats and maize) with incremental levels of anhydrous ammonia gas on *in vitro* ruminal dry matter degradability and nitrogen solubility in buffer. The cereal straws were treated with anhydrous ammonia gas in a rate of 0%, 2.5%, 3.5%, 4.5% and 5.5%. For wheat straw, no linear or quadratic ($P > 0.05$) effects were observed for total nitrogen (N), buffer insoluble nitrogen (BISN), buffer soluble nitrogen (BSN) and solubility index (SI) in response to anhydrous ammonia gas treatment. There were significant quadratic effects for N, BISN, BSN and SI, for which an optimum ammoniation rate for oats straw was calculated to be 3.3%. However, for maize straw, linear effects ($P < 0.05$) were observed for BSN, BISN, and SI. A quadratic trend was observed for N [$y = 14.95 (\pm 1.35) + 7.05 (\pm 1.05) x - 0.84 (\pm 0.19) x^2$; $R^2 = 0.873$; $P = 0.0007$], for which an optimum ammoniation rate for maize straw was calculated to be 4.29%. Linear effects were observed for DMD12 and DMD24 in ammoniated wheat straws. For maize and oats straws, there were significant linear and quadratic trends for DMD12, DMD24, DMD36 and DMD48 in response to ammonia treatment. Maize straw had the highest ($P < 0.05$) DMD12, DMD24, DMD36 and DMD48 when compared to oats and wheat straws. Untreated straws had the least DMD12, DMD24, DMD36 and DMD48 ($P < 0.05$) than the treated straws, which were the same ($P > 0.05$). It was concluded that ammoniation of cereal straws improves *in vitro* ruminal dry matter degradability and N solubility. Maize straw showed better response to ammonia treatment followed by oats and wheat straw. It was recommended

that higher ammoniation rates be considered for wheat straw because it had linear responses for *in vitro* ruminal dry matter degradation.

Keywords: Ammoniation, Buffer solubility, Cereal straws, *In vitro* ruminal degradability, Ruminants

4.1 Introduction

Cereal straws have lower digestibility because of their high cellulose and lignin content (Horton & Steacy, 1979). A major limiting factor with regards to the degradation of cereal straws is their poor nutritive value characterised by low crude protein and high cell wall content (Paiva, 1995). Several studies have reported a low protein content averaging from 2 – to 6%, with a neutral detergent fibre (NDF) content of 77 to 80% and lignin content of 10 to 25% for wheat straws (Jabbar *et al.*, 2009; Du *et al.*, 2016; Su *et al.*, 2017). For maize straws, a low protein content of 3 to 5%, NDF content of 66 to 84%, and a lignin content of 5.5 to 8.8% have been reported (Olson *et al.*, 1974; Li *et al.*, 2014; Du *et al.*, 2016). Whereas oats straws have been reported to have a low protein content of 4.8 to 6%, NDF content of 62.6 to 79.3% and a lignin content of 6.43 to 14% (Van Soest, 2006; Kafilzadeh *et al.*, 2012; Cui *et al.*, 2016). All these studies indicate that the nutritive value of these straws needs to be improved in order to optimise their utilisation by ruminant animals. Thus the use of least-cost strategies such as ammonia treatment is inevitable. Ammonia treatment has the potential to hydrolyse the cell wall of the cereal straws, causing a reduction in the NDF content (Gobbi, 2005). The rate of dry matter degradation in the rumen is important in determining the amount of nutrients that can be absorbed by ruminants. Indeed, Preston and Leng (1987) as well as Sniffen (1980) postulated that the rate and extent of dry matter degradation and nitrogen solubility are important for assessing the efficiency of the rumen and the feed value of fibrous feeds. According to Nugent and Mangan (1978), the solubility of cereal residues determines the ability of rumen microbes to absorb proteins. When a forage is highly soluble

it implies that there is less lignin monomers and fibrous molecules, which allows increased ruminal microbial activity (Chesson & Ørskov, 1984). Treatment with anhydrous ammonia gas has been used to break down lignocellulosic bonds present in cereal crop residues (Silva *et al.*, 1989). Graham and Aman (1984) reported that the ammoniation of cereal straws increases their crude protein content and *in vitro* digestibility. Various studies have found that ammonia treatment of cereal straws increase *in situ* ruminal degradability of dry matter (Habib *et al.*, 1998). But, none of these studies have attempted to determine the optimum ammoniation rate of these straws for cost-effective dietary formulation and increased production efficiency. This study was, therefore, designed to evaluate the *in vitro* ruminal dry matter degradability and N solubility in a buffer of maize, oats and wheat cereal straws treated with incremental levels of anhydrous ammonia gas. The study tested the hypothesis that *in vitro* ruminal dry matter degradability and N solubility of maize, oats and wheat cereal straws respond to incremental rates of ammoniation in an asymptotic fashion.

4.2 Materials and methods

4.2.1 Study site and ammoniation process

The study site and ammoniation process were similar to the ones described in Chapter 3.

4.2.2 Buffer Nitrogen Solubility

The buffer nitrogen solubility of cereal straw samples was determined according to the modified Licitra *et al.* (1996) method using the ANKOM Daisy^{II} incubator consisting of thermostatic chamber (39°C) with four rotating jars which were filled with 1500 mL of a borate-phosphate and 10% sodium azide buffer solution. Milled samples were weighed into the pre-weighed ANKOM F57 filter bags (0.45 - 0.5 g), heat sealed and inoculated for 3 h in the four jars. The ANKOM F57 filter bags were withdrawn 3 hours after inoculation and put in an air drying pan to remove the excess solution then oven dried for 12 hours at 105°C.

After drying, the samples were removed from the oven and placed in a desiccator to cool before weighing. Nitrogen (N) residue was determined using the standard macro-Kjeldahl method (AOAC, 2005; method no. 984.13) and estimated as insoluble N content of the samples. Soluble N content was calculated as the difference between the total and insoluble N contents. Buffer solubility index was calculated as a ratio between the soluble N and the total N contents.

4.2.3 In vitro ruminal dry matter degradability

The *in vitro* ruminal DM degradability of cereal straw samples was determined according to the ANKOM Technology Method 3 for *in vitro* true digestibility (ANKOM Technology Corp., Fairport, NY) using the ANKOM Daisy^{II} incubator consisting of a thermostatic chamber (39°C) with four rotating jars. ANKOM F57 filter bags were pre-soaked in acetone for 5 min and air-dried overnight, and then weighed the following day to acquire the bag weight. Milled samples were weighed into the pre-weighed ANKOM F57 filter bags (0.45 - 0.5 g) and heat sealed (Hand impulse sealer 12 inch 300 mm, Abuks inc, Worli Mumbai). The ANKOM Daisy^{II} jars were filled with 1600 mL of ANKOM buffer solution (Phosphate-borate buffer) by Licitra *et al.* (1996) and left to warm up at 39°C for 12 h. The following morning, rumen inoculum was collected prior feeding from a ruminally cannulated Bonsmara cow with a body weight of approximately 550 kg which was fed grass (*Eragrostis* spp.) hay as a basal diet and had access to clean water *ad libitum*.

Rumen fluid was collected into two pre-warmed thermos flasks and immediately taken to the Animal Science laboratory of the North-West University in Molelwane farm, where it was blended and strained through two layers of pre-warmed muslin cloths. The blending was carried out in order to dislodge microbes that are attached to fibrous substrates. After straining, the inoculum was held at 39°C under a stream of carbon dioxide gas. The four

Daisy^{II} jars, each containing 1600 mL of buffer and cereal straw samples sealed in the ANKOM F57 filter bags, were then inoculated with 400 mL of the rumen inoculum into each digestion jar. Each jar was purged with deoxygenated CO₂ before being placed into the incubator, and two blanks were placed in each jar. The ANKOM F57 filter bags were withdrawn at 12, 24, 36 and 48 hours after inoculation and thereafter washed with tap water for 20 min. After washing, the samples were oven-dried at a temperature of 105°C for 12 h and thereafter weighed to determine *in vitro* true digestibility (IVTD) using the following formula:

$$IVTD (\% DM \text{ basis}) = \frac{100 - (W3 - (W1 \times C1))}{W2 \times DM} \times 100$$

Where, W1 = Bag weight (g), W2 = Sample weight (g), W3 = Final bag weight after *in vitro* treatment (g), and C1 = Blank bag correction factor (final oven-dried weight ÷ original blank bag weight).

4.2.4 Statistical analysis

Normality of all measured parameters was tested using the NORMAL option in the Proc Univariate statement (SAS, 2010). Polynomial contrasts were used to evaluate the total nitrogen, buffer soluble nitrogen, buffer insoluble nitrogen, solubility index and *in vitro* ruminal dry matter degradability data for linear and quadratic effects. Response surface regression analysis (Proc RSREG; SAS, 2010) was employed to estimate the optimum ammoniation rate for each cereal straw as described in Chapter 3. In a completely randomised design, two-way ANOVA was used to analyse the *in vitro* ruminal DM degradability and N solubility data using the GLM procedure of SAS (2010). The main effects were the cereal straws, ammoniation rate and the interaction between the cereal straws and ammoniation rate. The linear statistical model employed in Chapter 3 was used for this study. For all statistical

tests, significance was declared at $P \leq 0.05$. The LSMEANS were compared using the PDIFF option in the LSMEANS statement of SAS.

4.3 Results

4.3.1 Buffer Nitrogen Solubility

For wheat straw, there were neither linear nor quadratic ($P > 0.05$) trends observed for total nitrogen (N), buffer insoluble nitrogen (BISN), buffer soluble nitrogen (BSN) and solubility index (SI) in response to incremental levels of anhydrous ammonia gas treatment. For oats straws, there were significant quadratic responses for total N [$y = 17.03 (\pm 0.99) + 5.57 (\pm 0.70) x - 0.82 (\pm 0.12) x^2$; $R^2 = 0.860$; $P < 0.0001$], BISN [$y = 717.7 (\pm 58.07) - 178.49 (\pm 40.99) x + 27.16 (\pm 6.94) x^2$; $R^2 = 0.639$; $P = 0.0024$], BSN [$y = 282.29 (\pm 58.07) + 178.49 (\pm 40.99) x - 27.16 (\pm 6.94) x^2$; $R^2 = 0.639$; $P = 0.002$] and SI [$y = 28.23 (\pm 5.81) + 17.85 (\pm 4.09) x - 2.72 (\pm 0.69) x^2$; $R^2 = 0.639$; $P = 0.002$] in response to anhydrous ammonia gas treatment levels. Using these quadratic equations, the optimum ammoniation rate for oats straws was calculated to be 3.3%. For maize straw, there were significant quadratic trends for total N [$y = 14.95 (\pm 1.35) + 7.05 (\pm 1.05) x - 0.84 (\pm 0.19) x^2$; $R^2 = 0.873$; $P = 0.0007$], whilst significant linear effects were observed for BISN [$y = 579.20 (\pm 81.92) - 178.09 (\pm 63.84) x$; $R^2 = 0.557$; $P = 0.005$], BSN [$y = 420.79 (\pm 81.92) + 178.09 (\pm 63.84) x$; $R^2 = 0.557$; $P = 0.005$] and solubility index [$y = 42.08 (\pm 8.19) + 17.81 (\pm 6.38) x$; $R^2 = 0.5567$; $P = 0.0050$]. The optimum ammoniation level for anhydrous ammonia gas in maize straws was calculated for N to be 4.29%.

4.3.2 In vitro ruminal dry matter degradability

For wheat straw, Table 4.1 shows that there were linear effects ($P < 0.05$) for DMD12 [$y = 64.63 (\pm 20.7) + 32.58 (\pm 20.7) x$; $R^2 = 0.349$; $P = 0.029$] and DMD24 [$y = 113.98 (\pm 16.9) + 1.35 (\pm 16.9) x$; $R^2 = 0.334$; $P = 0.037$], which linearly increased with ammonia treatment levels. There was no linear and quadratic ($P > 0.05$) effects observed after DMD36 and DMD48 for ammoniated wheat straws.

Table 4.1. *In vitro* ruminal dry matter degradability (g/kg DM) of anhydrous ammonia gas treated wheat straws

² DMD	¹ Ammoniation rate					³ SEM	Significance	
	0	2.5	3.5	4.5	5.5		Linear	Quadratic
DMD12	68.52	119.86	133.82	203.89	150.71	20.67	0.029	0.606
DMD24	123.38	94.47	155.86	236.52	170.17	16.87	0.037	0.501
DMD36	211.10	121.76	194.73	272.87	184.57	17.22	0.635	0.375
DMD48	253.7	172.33	224.47	303.65	221.14	17.74	0.778	0.308

¹Ammoniation rate: 0 = untreated wheat straws; 2.5 = wheat straws treated with 2.5% of anhydrous ammonia gas; 3.5 = wheat straws treated with 3.5% of anhydrous ammonia gas; 4.5 = wheat straws treated with 4.5% of anhydrous ammonia gas; 5.5 = wheat straws treated with 5.5% of anhydrous ammonia gas.

²DMD: DMD12 = dry matter degradability at 12 h after inoculation; DMD24 = dry matter degradability at 24 h after inoculation; DMD36 = dry matter degradability at 36 h after inoculation; DMD48 = dry matter degradability at 48 h after inoculation.

³SEM = standard error of mean.

For oats straw, Table 4.2 shows that there were significant linear and quadratic trends for DMD12, DMD24, DMD36 and DMD48 with ammonia treatment levels. For maize straw, Table 4.3 shows that there were significant linear and quadratic trends for DMD12, DMD24, DMD36 and DMD48 with ammonia treatment levels.

Table 4.2. Responses of *in vitro* ruminal dry matter degradability (g/kg DM) of oats straws to incremental levels of anhydrous ammonia gas

¹ DMD	Equations	R ²	Optimum	Linear	Quadratic
DMD12	$y = 20.23 (\pm 10.8) + 96.26 (\pm 8.5) x - 11.73 (\pm 1.5) x^2$	0.950	4.10	***	***
DMD24	$y = 46.08 (\pm 15.5) + 134.29 (\pm 12.1) x - 17.49 (\pm 16.9) x^2$	0.940	3.84	***	***
DMD36	$y = 131.21 (\pm 13.4) + 119.94 (\pm 10.4) x - 14.69 (\pm 1.8) x^2$	0.950	4.08	***	***
DMD48	$y = 154.2 (\pm 12.6) + 112.8 (\pm 16.8) x - 13.01 (\pm 2.9) x^2$	0.881	4.34	***	**

¹DMD: DMD12 = dry matter degradability at 12 h after inoculation; DMD24 = dry matter degradability at 24 h after inoculation; DMD36 = dry matter degradability at 36 h after inoculation; DMD48 = dry matter degradability at 48 h after inoculation.

Significance: NS = $P > 0.5$; * = $P < 0.05$; *** = $P < 0.001$.

Table 4.3. Responses of *in vitro* ruminal dry matter degradability (g/kg DM) of maize straws to incremental levels of anhydrous ammonia gas

¹ DMD	Equations	R ²	Optimum	Linear	Quadratic
DMD12	$y = 16.56 (\pm 10.7) + 118.2 (\pm 8.3) x - 14.09 (\pm 1.5) x^2$	0.969	4.19	***	***
DMD24	$y = 23.89 (\pm 17.5) + 208.95 (\pm 13.6) x - 27.98 (\pm 2.4) x^2$	0.965	3.74	***	***
DMD36	$y = 54.31 (\pm 14.1) + 227.99 (\pm 11.0) x - 29.26 (\pm 1.9) x^2$	0.982	3.89	***	***
DMD48	$y = 108.4 (\pm 16.7) + 201.43 (\pm 12.9) x - 25.59 (\pm 2.3) x^2$	0.970	3.94	***	***

¹DMD: DMD12 = dry matter degradability at 12 h after inoculation; DMD24 = dry matter degradability at 24 h after inoculation; DMD36 = dry matter degradability at 36 h after inoculation; DMD48 = dry matter degradability at 48 h after inoculation. Significance: *** = $P < 0.001$.

Table 4.4 shows that there were significant differences ($P < 0.05$) for DMD12, DMD24, DMD36 and DMD48 on cereal, ammonia and the interaction between cereal and ammonia.

Table 4.4. Level of significance of effects of the main factors on *in vitro* ruminal dry matter degradability of anhydrous ammonia gas treated wheat, oats and maize straws

¹ DMD	Factors		
	Cereal	Ammonia	Cereal x Ammonia
DMD12	***	***	**
DMD24	***	***	***
DMD36	***	***	***
DMD48	***	***	***

¹DMD: DMD12 = dry matter degradability at 12 h after inoculation; DMD24 = dry matter degradability at 24 h after inoculation; DMD36 = dry matter degradability at 36 h after inoculation; DMD48 = dry matter degradability at 48 h after inoculation.

Significance: ** = $P < 0.01$; *** = $P < 0.001$.

Table 4.5 shows that maize straw had the highest DMD12 (200.35 g/kg DM) when compared to oats and wheat straws, which did not differ ($P > 0.05$). Maize straws had the highest DMD24 (306.46 g/kg DM), followed by oats straw (234.44 g/kg DM) and the least was from wheat straw (156.08 g/kg DM). Maize straw had the highest DMD36 and DMD48 when compared to oats and wheat straws.

Table 4.5. The effect of anhydrous ammonia on *in vitro* ruminal dry matter degradability (g/kg DM) of maize, oats and wheat straws.

¹ Ammoniation rate	Maize				Oats				Wheat			
	DMD12	DMD24	DMD36	DMD48	DMD12	DMD24	DMD36	DMD48	DMD12	DMD24	DMD36	DMD48
0	14.6 ^{Aa}	19.1 ^{aA}	49.7 ^{aA}	103.9 ^{aA}	17.4 ^{aA}	46.7 ^{aA}	128.7 ^{aB}	149.4 ^{aA}	68.5 ^{aB}	123.4 ^{abB}	211.1 ^{bcC}	253.8 ^{bcB}
2.5	236.9 ^{bA}	397.09 ^{bA}	468.9 ^{bA}	479.7 ^{bA}	201.2 ^{bAB}	267.9 ^{bB}	356.0 ^{bB}	382.8 ^{bB}	119.9 ^{aB}	94.5 ^{aC}	121.8 ^{aC}	172.3 ^{aC}
3.5	242.8 ^{bA}	394.90 ^{bA}	467.9 ^{bA}	470.5 ^{bA}	207.2 ^{bAB}	307.5 ^{bB}	351.2 ^{bB}	366.4 ^{bB}	133.8 ^{aB}	155.9 ^{abC}	194.7 ^{bC}	224.5 ^{abC}
4.5	264.8 ^{bA}	382.24 ^{bA}	482.3 ^{bA}	495.7 ^{bA}	202.8 ^{bB}	295.1 ^{bB}	375.9 ^{bB}	388.3 ^{bB}	203.9 ^{bB}	236.5 ^{cb}	272.9 ^{cC}	303.7 ^{cC}
5.5	242.7 ^{bA}	338.97 ^{bA}	431.6 ^{bA}	448.4 ^{bA}	203.3 ^{bB}	254.9 ^{bB}	348.9 ^{bB}	391.6 ^{bA}	150.7 ^{aB}	170.2 ^{bcC}	184.6 ^{abC}	221.1 ^{abB}
SEM	20.67	16.87	17.22	17.74	20.67	16.87	17.22	17.74	20.67	16.87	17.22	17.74

¹Ammoniation rate: 0 = untreated wheat straws; 2.5 = wheat straws treated with 2.5% of anhydrous ammonia gas; 3.5 = wheat straws treated with 3.5% of anhydrous ammonia gas; 4.5 = wheat straws treated with 4.5% of anhydrous ammonia gas; 5.5 = wheat straws treated with 5.5% of anhydrous ammonia gas. ^{a,b,c} Means in a column with different lowercase superscripts denote significant differences ($P < 0.05$).

^{A,B,C} Means in a row with different uppercase superscripts denote significant differences ($P < 0.05$).

SEM = standard error of mean.

Table 4.5 shows that untreated maize straws had the least DMD12, DMD24, DMD36 and DMD48 ($P < 0.05$) when compared to the treated maize straws, which did not differ ($P > 0.05$). Untreated oats straws also had the least DMD12, DMD24, DMD36 and DMD48 ($P < 0.05$) than the treated oats straws, which were the same ($P > 0.05$). Untreated wheat straws had the least DMD12, DMD24, DMD36 and DMD48 ($P < 0.05$) than the treated wheat straws, which were similar ($P > 0.05$). Wheat straws treated with 2.5% had the least DMD at 24 hours (94.5 g/kg DM), 36 hours (121.8 g/kg DM), and 48 hours (172.3 g/kg DM) when comparing the treated wheat straws.

4.4 Discussion

Optimum production of ruminants is influenced by the quality of the forage, which is reflected by high voluntary intake and digestibility. The determination of forage digestibility using *in vivo* methods is labour intensive, time consuming and unsuitable for large-scale feed evaluation. Thus use of *in vitro* digestibility techniques have gained a lot of attention because they are conducted under controlled environment and large number of samples can be evaluated simultaneously. In this study, oats straws showed quadratic responses for nitrogen buffer solubility for which an optimal anhydrous ammonia treatment level was calculated to be 3.3%. This suggests that treatment beyond 3.3% decreases the solubility of oats straws. The decline in solubility for treatment beyond 3.3% could be due to the increase of NDF and ADF when treating oats straws as depicted in Chapter 3. For some agricultural by-products like canola hulls, ammoniation seems to increase the NDF content (McKinnon *et al.*, 1995). It has been proven that the response to ammoniation by agricultural by-products is dependent on the hemicellulose content, with the latter being that dicotyledonous by-products with high hemicellulose contents are more responsive to alkaline treatments than those with low hemicellulose content (Adebowale & Nakashima, 1992). Maize straws treated with

anhydrous ammonia gas showed major increases on *in vitro* ruminal dry matter degradability. Both linear and quadratic effects were observed on anhydrous ammonia gas treated maize straws. The linear effects indicate that there is potential of more incremental level treatment of anhydrous ammonia gas, to improve further the *in vitro* ruminal dry matter degradability of maize straws, but quadratic effects were observed on maize straws with an optimal anhydrous ammonia treatment of 4.3%. This means that anhydrous ammonia gas treatment of maize straws beyond 4.3%, does not offer any significant improvements on the *in vitro* ruminal dry matter degradability of maize straws.

In this study, wheat straw treated with anhydrous ammonia gas showed an increase in *in vitro* ruminal dry matter degradability from 12 to 48 h of incubation. This was in agreement with the finding of Sahoo *et al.* (2000) that wheat straw treatment with aqueous ammonia increases apparent digestibility. The highest degradability was observed after 12 h of inoculation with 4.5% ammonia treatment. Linear effects were observed on the *in vitro* dry matter degradability of wheat straws, meaning that there was a need for more anhydrous ammonia treatment. The increase in degradability could be associated with the reduction in the NDF values as reported in Chapter 3. According to NRC (2001), the lignin content of forage can be used as an indicator of NDF rumen degradability but this correlation differs between forages (Jung *et al.*, 1997). As expected, Chapter 3 indicated a decline in NDF, ADF and lignin content of the straws. This study thus indicated a negative correlation between *in vitro* ruminal degradability in association with NDF, ADF and lignin contents. Indeed, Arzani *et al.* (2006) as well as Damiran *et al.* (2008) showed that the *in vitro* ruminal dry matter degradability of *Medicago sativa* and *Calamagrostis rubescens* was negatively correlated with NDF and ADF content.

At all the incubation hours, wheat, oats and maize straws treated with anhydrous ammonia had higher *in vitro* ruminal DMD than untreated straw. This was in agreement with previous

research reporting that ammonia treatment increase *in vitro* ruminal DMD of wheat, oats and maize cereal straws (Vlemmix & Van Bruchem, 1993; Oliveros *et al.*, 1993; Madrid *et al.*, 1997).

4.5 Conclusion

This study shows that the *in vitro* ruminal dry matter degradability and nitrogen solubility of the straws were enhanced by anhydrous ammonia gas treatment. It was, therefore, concluded that ammoniation of cereal straws improves these parameters. Maize straw showed higher improvement due to ammonia treatment followed by oats and wheat straw. It was recommended that higher ammoniation rates be considered for wheat straw because it had linear responses for *in vitro* ruminal dry matter degradation and no responses for buffer solubility.

4.6 References

- Adebowale, E.A. & Nakashima, Y., 1992. Rumen degradation of some *Leguminosae* and *Graminae* roughages: effect of chemical pre-treatment with or without cellulase preparation on dry matter and cell wall disappearance. *Anim. Feed. Sci. Technol.* 38, 219-235.
- Arzani, H., Basiri, M., Khatibi, F. & Ghorbani, G., 2006. Nutritive value of some Zagros Mountain rangeland species. *Small Rumin. Res.* 65,128-135.
- AOAC, 2005. In *Official Methods of Analysis of AOAC International*, 18th ed. Association of Official Analytical Chemists, Arlington, VA, USA.
- Chesson, A. & Ørskov, E.R., 1984. Microbial degradation in the digestive tract. In: *Straw and other fibrous by-products as feed*. ed, Sundstel, F., Amsterdam, Netherlands.
- Cui, J.H., Yang, H.J., Yu, C.Q. & Bai, S., 2016. Effect of urea fertilization on biomass yield, chemical composition, *in vitro* rumen digestibility and fermentation characteristics of forage oat straw in Tibet of China. *J. Agric. Sci.* 154(5), 914-927.
- Damiran, D., DelCurto, T., Bohnert, D.W. & Findholt, S.L., 2008. Comparison of techniques and grinding size to estimate digestibility of forage based ruminant diets. *Anim. Feed Sci. Technol.* 141, 15-35.
- Du, S., Xu, M. & Yao, J., 2016. Relationship between fibre degradation kinetics and chemical composition of forages and by-products in ruminants. *J. Appl. Anim. Res.* 44(1), 189-193.
- Gobbi, K.F., 2008. Leaf tissues degradation of signal grass hay pre-treated with urea and submitted to *in vitro* digestion. *Braz. J. Vet. Res. Anim. Sci.* 37, 802-809.

- Graham, H. & Aman, P., 1984. A comparison between degradation *in vitro* and *in sacco* of constituents of untreated and ammonia-treated barley straw. *Anim. Feed Sci. Technol.* 10, 199-211.
- Horton, G.M.J. & Steacy, G.M., 1979. Effect of anhydrous ammonia treatment on the intake and digestibility of cereal straws by steers. *J. Anim. Sci.* 48, 1239-1249.
- Jabbar, M.A., Muzafar, H., Khattak, F.M., Pasha, T.N. & Khalique, A., 2009. Simplification of urea treatment method of wheat straw for its better adoption by the farmers. *S. Afr. J. Anim. Sci.* 39, 58-61.
- Jung, H.G., Mertens, D.R. & Payne, A.J., 1997. Correlation of acid detergent lignin and klason lignin with digestibility of forage dry matter and neutral detergent fiber. *J. Dairy Sci.* 80, 1622-1628.
- Kafilzadeh, I.F., Heidary, I.N. & Bahraminejad, S., 2012. Variety effect on composition, kinetics of fermentation and *in vitro* digestibility of oat (*Avena sativa L.*) straw and its neutral detergent fibre. *S. Afr. J. Anim. Sci.* 42, 4.
- Licitra, G., Hernandez, T.M. & Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim. Feed Sci. Technol.* 57, 347-358
- Li, H.Y., Xu, L., Liu, W.J., Fang, M.Q. & Wang, N., 2014. Assessment of the nutritive value of whole corn stover and its morphological fractions. *Asian-Australas. J. Anim. Sci.* 27(2), 194-200.
- Madrid, J., Hernandez, F., Pulgar, M.A. & Cid, J.M., 1997. *In-vivo* digestibility of treated and untreated barley straw: results of direct and by-difference digestibility trials. *Anim. Feed Sci. Technol.* 65, 129-138.
- McKinnon, J.J., Mustafa, A.F. & Cohen, R.D.H., 1995. Nutritional value and processing of canola hulls for ruminants. *Can. J. Anim. Sci.* 75, 231-237.

- Nugent, I.H.A. & Mangan, J.L., 1978. Rumen proteolysis of fraction I leaf protein, casein and bovine serum albumin. *Proc. Nutr. Soc.* 37(2), 48A.
- NRC, 2001. Nutrient requirements of dairy cattle. 7th ed. National Academy Press. Washington DC, USA.
- Oliveros, B.A., Britton, R.A. & Klopfenstein, T.J., 1993. Ammonia and/or calcium hydroxide treatment of maize stover: intake, digestibility and digestion kinetics. *Anim. Feed Sci. Technol.* 44(1-2), 59-72.
- Olson, H., Santo, K.A.S. & McCoy, G.C., 1974. Percentage roughage in corn and milo complete feeds. *J. Dairy Sci.* 57(10), 1260 - 1262.
- Paiva, J.A.J., 1995. Effects of anhydrous ammonia levels and ammonization periods on the degradability of dry matter and cell wall constituents of maize straw (*Zea mays* L.). *Braz. J. Zootec.* 24, 693-705.
- Preston, T.R. & Leng, R.A., 1987. Matching Ruminant Production Systems with Available Resources in the Tropics and Subtropics. Penambul Books. Armidale, Australia, pp. 245.
- Sahoo, B., Saraswat, M.L., Haque, N. & Khan, M.Y., 2000. Energy balance and methane production in sheep fed chemically treated wheat straw. *Small Rumin. Res.* 35, 13-19.
- Silva, A.T., Greenhalgh, J.F.D. & Orskov, E.R., 1989. Influence of ammonia treatment and supplementation on the intake, digestibility and weight gain of sheep and cattle on barley straw diets. *Anim. Prod.* 48, 99-108.
- Sniffen, C.J., 1980. Dynamics of protein solubility and degradability in ruminant rations. *Proc. Distillers Feed Conf.* pp. 69.
- Su, H., Akins, M.S., Esser, N.M., Ogden, R., Coblenz, W.K., Kalscheur, K.F. & Hatfield, R., 2017. Effects of feeding alfalfa stemlage or wheat straw for dietary energy dilution on

nutrient intake and digestibility, growth performance, and feeding behaviour of Holstein dairy heifers. *J. Dairy Sci.* 100(9), 7106-7115.

Tiller, J.M. & Terry, R.A., 1963. A two stage technique for the *in vitro* digestion of forage crops. *Grass For. Sci.* 18(2), 104-111.



Van Soest, P. J., 2006. Rice straw, the role of silica and treatments to improve quality. *Anim. Feed Sci. Technol.* 130, 137-171.

Vlemmix, P.J.M. & Van Bruchem, J., 1994. Effect of ammonia treatment of wheat straw with or without supplementation of potato protein on intake, digestion and kinetics of comminution, rumen degradation and passage in steers. *Br. J. Nutr.* 72, 147-165.

Zadrazil, F. & Puniya, A.K., 1995. Studies on effect of particle size during solid state fermentation of sugarcane bagasse into animal feed using white rot fungi. *Biores. Technol.* 54, 85-87.

5 CHAPTER FIVE - IN VITRO RUMINAL GAS PRODUCTION OF WHEAT, OATS AND MAIZE STRAWS AMMONIATED WITH INCREMENTAL LEVELS OF ANHYDROUS AMMONIA GAS

Abstract

This study investigated the effect of treating cereal straws (wheat, oats and maize) with incremental levels of anhydrous ammonia gas on *in vitro* ruminal fermentation, rate of gas produced per period and *in vitro* ruminal gas production parameters. The cereal straws were treated with anhydrous ammonia gas at a rate of 0, 2.5, 3.5, 4.5 and 5.5%. For wheat straws, there were neither linear nor quadratic effect ($P > 0.05$) observed for *in vitro* ruminal gas production parameters (fractions a and b , P_{gas} and E_{gas}) and cumulative gas production at 12, 24, 36 and 48 h. The only linear effects observed were on gas production rate constant for the insoluble fraction (c) [$y = 0.035 (\pm 0.0036) + 0.0031 (\pm 0.0028) x$; $R^2 = 0.388$; $P = 0.018$]. There were significant quadratic ($P < 0.05$) effects observed on cumulative gas after 12, 24, 36 and 48 h of incubation on oats straws in response to incremental anhydrous ammonia gas. Significant quadratic effects were also observed for the gas produced by immediate soluble fraction (a), gas produced by slowly soluble fraction (b), P_{gas} [$y = 51.93 (\pm 2.19) - 6.25 (\pm 1.17) x + 0.84 (\pm 0.31) x^2$; $R^2 = 0.607$; $P = 0.017$] and E_{gas} [$y = 38.11 (\pm 1.96) - 5.24 (\pm 1.53) x + 0.74 (\pm 0.27) x^2$; $R^2 = 0.552$; $P = 0.019$] on oats straw in response to incremental anhydrous ammonia gas treatment. The optimum ammoniation rate for oats straws was calculated to be 3.5%. For maize straws, significant linear effects were observed for fraction a and E_{gas} [$y = 60.61 (\pm 3.91) - 1.09 (\pm 3.05) x$; $R^2 = 0.324$; $P = 0.036$]. There were no significant differences ($P > 0.05$) observed for fractions a and b and P_{gas} between the wheat, oats and maize cereal straws in response to incremental anhydrous ammonia gas treatment. Wheat straws had the same ($P > 0.05$) fraction c , lag time and E_{gas} after inoculation as oats straws whilst the highest lag time was on maize straws and was different ($P < 0.05$). These

results indicate that anhydrous ammonia gas treatment has a potential to improve the ruminal fermentation of cereal straws (wheat, oats and maize). This is based on the fact that there was improved *in vitro* ruminal gas produced after 12 hours of incubation depending on the cereal straws. This indicates that rumen microbes were able to ferment the straws and produce gas at a shorter period as shown on the lag-time results.

Keywords: Cereal straws; Fermentation efficiency; *In vitro* ruminal gas production; Nutritive value

5.1 Introduction

Wheat, oats and maize straws form a major part of ruminant diets, and they are commonly used as an alternative energy source for livestock animals that are extensively reared. However, when fed to ruminant animals, their voluntary intake is also low due to their poor nutritive value. In addition, their digestibility or utilisation is very poor because of high fibre content (Wang *et al.*, 2014). This is because the cell walls of these straws are made up of crystalline cellulose, hemicellulose and pectin (Singh *et al.*, 2014). The degree of crystallinity of cellulose affects its degradability by rumen microbes whereby a higher degree of crystallinity results into slow degradation of the wheat straws (Fan *et al.*, 1980). Thus it is important to measure the ruminal fermentation kinetics of these straws in order to assess their nutritive value. These parameters give pivotal information of the immediately or slowly fermentable components of feed (Getachew *et al.*, 2001). The *in vitro* ruminal gas production technique is important as it allows the recording of gas produced at different times during the incubation period and allows the calculation of the rate of digestion (Liu *et al.*, 2002). When forage is incubated, carbohydrates are fermented by ruminal microbial cells and in the process they multiply, produce gas and volatile fatty acids (Getachew *et al.*, 1998; France *et al.*, 2000). The gas produced is a result of carbohydrates being fermented to acetate, propionate and butyrate (France *et al.*, 2000; Getachew *et al.*, 2000).

In vitro ruminal gas production is also important in determining fermentation patterns of micro-organisms and allow collection of digestion kinetics data that is applicable in feeding programs (Sunvold *et al.*, 1995; Pitt *et al.*, 1999). During fermentation, rumen microbes degrade substrates and produce gas and the amount of gas produced is dependent on the amount of the substrate (Davies *et al.*, 2000). Chapter 3 showed that ammonia treatments of the cereal straws have the potential to degrade fibre (NDF, ADF and ADL) and also improve their nitrogen content. Chapter 4 also showed that untreated straws had the least dry matter degradability than the treated straws. This Chapter was, therefore, designed to evaluate the *in vitro* ruminal gas production of wheat, oats and maize cereal straws treated with incremental level of anhydrous ammonia gas. The study tested the hypothesis that *in vitro* ruminal gas production of ammoniated wheat, oats and maize cereal straws respond in an asymptotic fashion.

5.2 Materials and methods

5.2.1 Study site and ammoniation process

The study site and ammoniation process were similar to the ones described in Chapter 3.

5.2.2 In vitro ruminal fermentation

The Reading Pressure Technique developed by Mauricio *et al.* (1999) was used to measure *in vitro* ruminal gas production for the ammoniated cereal straw samples. Each sample was weighed (1 g) into individual serum bottles (125 mL, thermo scientific, USA). The buffer solution (potassium phosphate) used in Chapter 3 was similarly prepared for this experiment, where 90 mL of the buffer was added to each of the bottles, including two blanks bottles. The bottles were then transferred to the incubator set at 39°C for a period of 12 h before inoculating with rumen fluid. After 12 h, rumen fluid was collected from the same Bonsmara donor cow and processed as described in Chapter 4. The inoculum was then added (25 mL) to

all the serum bottles and immediately incubated. Headspace gas pressure was measured using a pressure transducer (22IC, Gems sensors, Hampshire, United Kingdom), and measurements were taken at 12, 24, 36 and 48 h after inoculation. Headspace gas pressure measurements were then converted into gas volume (mL) using the following standard equation: $y = 0.034x^2 + 6.2325x + 1.8143$, where, y = gas volume per period (mL), x = gas pressure per period (psi).

Cumulative gas production parameters were estimated by fitting data into the Ørskov and McDonald (1979) non-linear model:

$$y = a + b (1 - e^{-c(t-l)})$$

Where, y = gas produced at time t ; a = the gas production from the immediately soluble fraction (mL); b = the gas production from the slowly soluble fraction (mL); c = the gas production rate constant for the insoluble fraction b ; t = incubation time (h); l = lag time (h).

Effective gas (E_{gas}) was calculated using the equation: $E_{gas} = \frac{a+bc}{k+c}$, where K = assumed solid flow rate at 2% per hour and a , b and c are constants from Ørskov and McDonald (1979) equation. Potential gas (P_{gas}) was calculated as the summation of fraction a and b .

5.2.3 Statistical analysis

The NORMAL option in the Proc Univariate statement was used to test for normality of the measured parameters. Rate of gas produced per period, *in vitro* ruminal cumulative gas production and *in vitro* ruminal gas production parameters data were evaluated for linear and quadratic effects using polynomial contrasts. To estimate the optimum ammoniation rate for each cereal straw, RSREG Proc of SAS (2010) was applied as stated in Chapter 3. In a completely randomised design, GLM procedure of SAS (2010) was used to analyse the rate of gas produced per period, *in vitro* ruminal cumulative gas production and *in vitro* ruminal

gas production parameters data using the statistical model employed in Chapter 3. For all the statistical tests, significance was declared at $P \leq 0.05$. The LSMEANS were compared using the PDIFF option in the LSMEANS statement of SAS.

5.3 Results

For wheat straw, there were neither linear nor quadratic ($P > 0.05$) effects observed for rate of gas produced after 12, 24, 36 and 48 h of incubation as well as cumulative gas production after 12, 24, 36 and 48 h of incubation. No significant linear and quadratic trends were observed for gas produced from the immediately soluble fraction (a), gas produced from slowly soluble fraction (b), lag time (h), potential gas production (P_{gas}) and effective gas production (E_{gas}) in response to incremental levels of anhydrous ammonia gas treatment. There were significant linear effects observed for the gas production rate constant for the insoluble fraction (c) [$y = 0.035 (\pm 0.0036) + 0.0031 (\pm 0.0028) x$; $R^2 = 0.388$; $P = 0.018$] in response to incremental anhydrous ammonia gas levels.

For oats straw, there were significant linear effects observed for rate of gas produced at 12 h [$y = 10.15 (\pm 0.4160) - 0.932 (\pm 0.3242) x$; $R^2 = 0.5082$; $P = 0.016$], which linearly decreased with anhydrous ammonia gas treatment levels. There were significant quadratic trends for rate of gas produced at 24, 36 and 48 h in response to incremental levels of anhydrous ammonia gas treatment (Table 5.1).

Table 5.1. Rate of gas produced (mL/g OM) by anhydrous ammonia gas treated oats straws

¹ Parameters	Oats		² Significance	
	Equations	R ²	Linear	Quadratic
R24	$y = 5.23 - 0.52x + 0.062x^2$	0.627	*	*
R36	$y = 6.10 - 0.61x + 0.072x^2$	0.739	**	*
R48	$y = 6.51 - 0.60x + 0.064x^2$	0.735	**	*

¹Parameters: R24 = rate of gas produced at 24 h after inoculation; R36 = rate of gas produced at 36 h after inoculation; R48 = rate of gas produced at 48 h after inoculation.

²Significance: NS = $P > 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

NWU
LIBRARY

For oats straws, there were neither linear nor quadratic ($P > 0.05$) effects observed for fraction c and lag time in response to incremental anhydrous ammonia treatment levels. Table 5.2 shows that there were quadratic trends for cumgas12, cumgas24, cumgas36, cumgas48, fractions a and b , Pgas and Egas for which an optimum ammoniation rate for oats straws was calculated to be 3.5%.

Table 5.2. Cumulative gas production (mL/g OM) of anhydrous ammonia gas treated oats straws

¹ Parameters	Oats		² Significance	
	Equations	R ²	Linear	Quadratic
Cumgas12	$y = 28.94 - 4.48 x + 0.66 x^2$	0.4576	NS	*
Cumgas24	$y = 34.37 - 4.99 x + 0.72 x^2$	0.4910	*	*
Cumgas36	$y = 40.48 - 5.61 x + 0.79 x^2$	0.5466	*	*
Cumgas48	$y = 46.98 - 6.21 x + 0.86x^2$	0.5954	*	*
<i>a</i>	$y = 4.36 - 1.33 x + 0.20 x^2$	0.4391	NS	*
<i>b</i>	$y = 47.56 - 4.91 x + 0.64 x^2$	0.6148	*	*
<i>P</i> gas	$y = 51.93 - 6.25 x + 0.84 x^2$	0.6068	*	*
<i>E</i> gas	$y = 38.11 - 5.24 x + 0.74 x^2$	0.5519	*	*

¹Parameters: Cumgas12 = cumulative gas produced at 12 h after inoculation; Cum24 = cumulative gas produced at 24 h after inoculation; Cumgas36 = cumulative gas produced at 36 h after inoculation; Cumgas48 = cumulative gas produced at 48 h after inoculation; *a* = gas produced from immediately soluble fraction; *b* = gas produced from slowly soluble fraction; *P*gas = potential gas production; *E*gas = effective gas production.

²Significance: NS = $P > 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

For maize straw, there were neither linear nor quadratic ($P > 0.05$) responses observed for rate of gas produced at 8, 12, 24 and 48 h of incubation in response to incremental levels of anhydrous ammonia gas treatment. There was a significant linear effect for rate of gas produced at 36 h of incubation [$y = 8.51 (\pm 0.42) + 0.11 (\pm 0.33) x$; $R^2 = 0.363$; $P = 0.036$], which linearly increased with ammonia levels. There were significant linear effects for cumgas12 [$y = 47.40 (\pm 2.31) - 4.62 (\pm 1.80) x$; $R^2 = 0.479$; $P = 0.016$], cumgas48 [$y = 73.67$

$(\pm 4.95) - 0.37 (\pm 3.85) x$; $R^2 = 0.302$; $P = 0.047$], fraction a [$y = 7.04 (\pm 0.61) - 1.39 (\pm 0.48) x$; $R^2 = 0.644$; $P = 0.0009$] and E_{gas} [$y = 60.61 (\pm 3.91) - 1.09 (\pm 3.05) x$; $R^2 = 0.324$; $P = 0.036$], which linearly declined in response to anhydrous ammonia gas treatment levels. There were neither linear nor quadratic ($P > 0.05$) effects observed for $cumgas_{24}$, $cumgas_{36}$, fraction b and c , lag time and P_{gas} .

Table 5.3 below shows the statistical significance of the main factors and their interaction on the rate of gas produced per period. There were no differences ($P > 0.05$) observed for rate of gas produced at 12, 24, 36 and 48 h for ammonia treatment, as well as for the interaction between cereal and ammonia treatment. However, there were significant cereal straw effect on the rate of gas produced at 12 h.

Table 5.3. Level of significance of effects of the main factors on the rate of gas produced per period (mL/h) of ammoniated cereal straws

Factors	Rate of gas produced per period			
	12	24	36	48
Cereal straw (CS)	*	NS	NS	NS
Ammonia treatment (AT)	NS	NS	NS	NS
CS \times AT	NS	NS	NS	NS

Significance: NS = $P > 0.05$; * = $P < 0.05$.

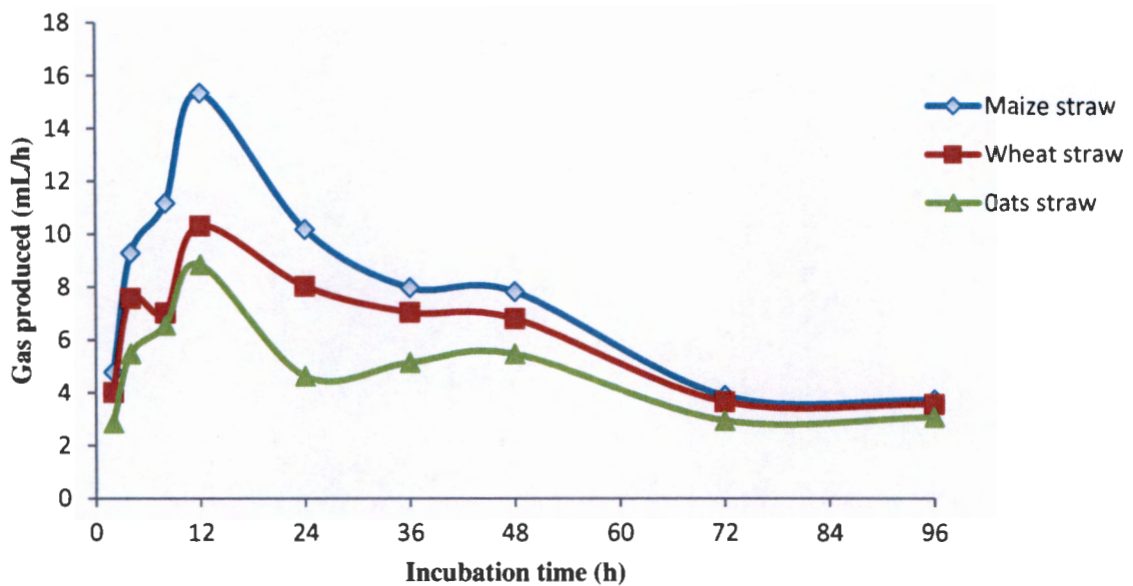


Figure 5.1. Rate of gas produced per period (mL/h) by anhydrous ammonia treated wheat, oats and maize straws

Figure 5.1 shows that the highest rate of gas produced at 12 hours was from the maize (40.56 mL/h), followed by wheat (28.95 mL/h) and the least gas produced was from oats (23.73 mL/h). There were no differences ($P > 0.05$) observed between the three cereal crop residues in terms of gas produced at 24, 36 and 48 h of incubation.

Table 5.4 shows the statistical significance of the main effects and their interactions on cumulative gas production at 12, 24, 36 and 48 h after inoculation. There were no significant differences ($P > 0.05$) observed for cumulative gas production for ammonia, and the interaction for all the incubation period. However, there were significant cereal effect on cumulative gas production at 12, 24 and 36 h, respectively.

Table 5.4. Level of significance of effects of the main factors on cumulative gas production

Factors	Cumulative gas production			
	Cumgas12	Cumgas24	Cumgas36	Cumgas48
Cereal straws (CS)	*	*	*	NS
Ammonia treatment (AT)	NS	NS	NS	NS
CS × AT	NS	NS	NS	NS

Significance: NS = $P > 0.05$; * = $P < 0.05$; *** = $P < 0.001$.

Table 5.5 shows that the cumulative gas production of wheat, oats and maize straws treated with anhydrous ammonia gas. Maize straws had higher ($P < 0.05$) cumulative gas production at 12, 24, 36 and 48 h after inoculation than oats straws. Wheat straws had the same ($P > 0.05$) cumulative gas production at 12, 24, 36 and 48 h after inoculation as maize straws. There were no significant differences observed for wheat and oats straws in terms of cumulative gas production at 12, 24, 36 and 48 h after inoculation.

Table 5.5. Cumulative gas production (mL/g OM) of ammoniated cereal straws

¹ Parameters	Cereals			² SEM
	Maize	Oats	Wheat	
Cumgas12	40.562 ^b	23.734 ^a	28.951 ^{ab}	4.639
Cumgas24	50.729 ^b	28.360 ^a	36.992 ^{ab}	5.889
Cumgas36	58.70 ^b	33.504 ^a	44.042 ^{ab}	6.784
Cumgas48	66.52 ^b	38.97 ^a	50.85 ^{ab}	7.673

¹Parameters: Cumgas12 = cumulative gas production at 12 h after inoculation; Cumgas24 = cumulative gas production at 24 h after inoculation; Cumgas36 = cumulative gas production at 36 h after inoculation; Cumgas48 = cumulative gas production at 48 h after inoculation.

²SEM = standard error of mean.

Table 5.6 shows the level of significance of the main factors on *in vitro* ruminal gas production parameters. There were significant differences for cereal on fraction *c*, lag time and Egas. There were no differences ($P > 0.05$) observed for ammonia treatment, and the interaction between cereal and ammonia treatment for all *in vitro* ruminal gas production parameters.

Table 5.6 Level of significance of effects of the main factors on the *in vitro* ruminal gas production parameters

¹ Parameters	Factors		
	Cereal straws (CS)	Ammonia treatment (AT)	CS × AT
<i>a</i>	NS	NS	NS
<i>b</i>	NS	NS	NS
<i>c</i> (%/h)	***	NS	NS
Lag time (h)	*	NS	NS
<i>P</i> _{gas}	NS	NS	NS
<i>E</i> _{gas}	*	NS	NS

¹Parameters: *a* = the immediately fermentable fraction; *b* = the slowly fermentable fraction; *c* = the gas production rate constant for the insoluble fraction *b*; *P*_{gas} = potential gas production; *E*_{gas} = effective gas production.

Significance: NS = $P > 0.05$; * = $P < 0.05$; *** = $P < 0.001$.

Table 5.7 shows that there were no difference ($P > 0.05$) observed on fraction *a*, fraction *b* and *P*_{gas} for all the cereal straws. Maize straws had higher ($P < 0.05$) fraction *c*, lag time and *E*_{gas} after inoculation than oats straws. Wheat straws had the same ($P > 0.05$) fraction *c*, lag time and *E*_{gas} after inoculation as oats straws. There were no significant differences observed for wheat and oats straws in terms of fraction *c*, lag time and *E*_{gas} after inoculation.

Table 5.7. The effects of main factors on *in vitro* ruminal gas production parameters on wheat, oats and maize straws

¹ Parameters	Cereal			² SEM
	Maize	Oats	Wheat	
A	4.526	2.893	4.317	0.522
B	67.38	40.69	53.09	7.879
c (%/h)	0.058 ^b	0.043 ^a	0.048 ^a	0.002
Lag time (h)	1.358 ^b	0.989 ^a	1.034 ^a	0.101
P _{gas}	71.91	43.59	57.40	8.362
E _{gas}	54.22 ^b	31.56 ^a	40.99 ^{ab}	6.219

¹Parameters: *a* = the immediate fermentable fraction; *b* = the slowly fermentable fraction; *c* = the gas production rate constant for the insoluble fraction *b*; *P_{gas}* = potential gas production; *E_{gas}* = effective gas production.

²SEM = standard error of mean.

5.4 Discussion

The *in vitro* ruminal gas production technique is widely used to evaluate the nutritive value of animal feeds. This technique is mostly utilised due to its ability to evaluate various feed material including agricultural by-products, plants and compound feed. Moreover, this technique has proved to be cheaper, adaptable, efficient and more precise than the *in vivo* technique (Sallam *et al.*, 2007). In this study, wheat straws did not show any quadratic nor linear effects on the rate of gas produced, cumulative gas production and fermentation parameters (fraction *a*, fraction *b*, lag time, *P_{gas}* and *E_{gas}*) except on fraction *c*, which showed linear effects. The intake of feed is associated with the rate of gas produced for the

insoluble fraction *b* (Khazaal *et al.*, 1995). There is thus an expectation that ammoniated wheat straws will have a linear increased intake correlating positively to anhydrous ammonia inclusion levels, due to the linear effects observed on fraction *c*. As there were no quadratic effects observed on anhydrous ammonia treated wheat straws, it is indicative that the various treatment levels were not sufficient to determine an optimum ammoniation rate.

Quadratic effects were observed on anhydrous ammonia treated oats straws for *in vitro* ruminal gas production, for which an optimal ammoniation level was calculated to be 3.5%. This indicates that treatment of oats straws with anhydrous ammonia gas should be at a maximum of 3.5% to achieve the best fermentation of the straws as the *in vitro* ruminal gas production indicates the fermentation of the substrate. Anhydrous treatment of oats straws with levels beyond 3.5%, does not make any significant difference on the *in vitro* ruminal gas production.

According to Theodorou *et al.* (1994), as the incubation time increases, the gas production also increase due to ruminal microbe fermenting the carbohydrates. The cumulative gas production of maize and wheat straws from 12 to 48 hours after incubation was higher when compared to that of oats straws. When evaluating the Chapter 3 results on the NDF content, it was evident that anhydrous ammonia gas treatment decreased the NDF and ADF content of maize and wheat straws thus as expected in this Chapter the straws were fermented and thus producing *in vitro* ruminal gas. This is in agreement with Jalilvand *et al.* (2008) and Musco *et al.* (2016) that the *in vitro* ruminal gas production of forage depends on the NDF content with the later that more *in vitro* ruminal gas is produced when the NDF content is low and vice versa. This means that ruminal microbes were able to ferment the straws and thus produce gas. Less *in vitro* ruminal gas was produced by anhydrous ammonia treated oats when compared to maize and wheat straws. As explained above, this could be due to the increase of NDF content when oats straws were treated with anhydrous ammonia gas when observed in

Chapter 3. This means that the ruminal microbes take longer to ferment the oats straws due to the NDF content and this is indicated by the low fraction *a* as opposed to maize and wheat straws. Maize straws did not show any quadratic response on *in vitro* ruminal gas production to incremental anhydrous ammonia treatment levels. The only linear responses observed on maize straws were on rate of gas produced after 36 h incubation, cumgas12, cumgas48, fraction *a* and Egas. Due to only linear effects being observed on maize straws to response of incremental anhydrous ammonia treatment, the optimum anhydrous ammonia gas inclusion could not be determined. This suggests that there is potential of more anhydrous ammonia gas level treatment to have a significant effect on the *in vitro* ruminal gas production of maize straws. According to Tesfayohannes *et al.* (2013), ammoniation of wheat straws, maize straws, oats straws and *Eragrostis curvula* hay improves their fermentation but the response is dependent on the type of feed. Low nutritive value feed, show more improvement in fermentation than good quality feed which is attributed to the decrease of NDF after treatment (Goto *et al.*, 1991). This is as result of the ammonia solubilizing the fibre and the rumen microbes having a better attachment on the feed (Fahey *et al.*, 1993; Tesfayohannes *et al.*, 2013). The lag time of wheat and oats straws was shorter than that of maize straws, suggesting that the rumen microbes firstly utilised these two straws over maize straws.

5.5 Conclusion

The results of this study shows that anhydrous ammonia gas treatment of wheat and maize straws improves the *in vitro* ruminal gas production. The high cumulative gas production of maize and wheat straws after 12 h of incubation indicates that ruminal microbes were able to ferment these straws much faster than oats straw. Oats straw had a lower cumulative gas production after 12 hours incubation, which could be due to the higher fibre content found in Chapter 3. For oats straw, the optimal treatment level is 3.5%. For maize and wheat straw the

optimal treatment level could not be deduced but it is evident that there is a need to increase the inclusion level from 5.5% as only linear effects were observed.



5.6 References

- Davies, Z.S., Mason, D., Brooks, A.E., Griffith, G.W., Merry, R.J. & Theodorou, M.K., 2000. An automated system for measuring gas production from forage inoculated with rumen fluid and its use for determining the effect of enzymes on grass silage. *J. Anim. Feed Sci. Technol.* 83, 205-221.
- Fahey, G.C., Bourquin, L.D., Titgemeyer, E.C. & Atwell, D.G., 1993. Postharvest treatment of fibrous feedstuffs to improve the nutritive value. In: H.G. Jung, D.R. Buxton, R.D. Hatfield, J. Ralph (eds). *Forage cell wall structure and digestibility*. ASA-CSSA-SSSA., Madison, WI, pp. 715-766.
- Fan, L.T., Lee, Y.H. & Beardmore, D.R., 1980. Mechanism of enzymatic hydrolysis of cellulose: Effect of major structural features of cellulose on enzymatic hydrolysis. *Biotechnol. Bioeng.* 22(1), 419.
- France, J., Dijkstra, J., Dhanoa, M.S., Lopez, S. & Bannink, A., 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations. *Br. J. Nutr.* 83, 43-150.
- Getachew, G., Makkar, H.P.S. & Becker, K., 1998. The *in vitro* gas coupled with ammonia nitrogen measurement for evaluation of nitrogen degradability in low quality roughages using incubation medium of different buffering capacity. *J. Sci. Food Agric.* 77, 87-95.
- Getachew, G., Makkar, H.P.S. & Becker, K., 2000. Stoichiometric relationship between short chain fatty acid and *in vitro* gas production in presence and absence of polyethylene glycol for tannin containing browses, EAAP Satellite Symposium, Gas production:

fermentation kinetics for feed evaluation and to assess microbial activity. Wageningen, Netherlands.

Getachew, G, DePeters, E.J., Robinson, P.H. & Taylor, S.J., 2001. *In vitro* rumen fermentation and gas production: Influence of yellow grease, tallow, corn oil and their potassium soaps. *Anim. Feed Sci. Technol.* 93, 1-15.

Goto, M., Gordon, A.H. & Chesson, A., 1991. Effect of gaseous ammonia on barley straws showing different rumen degradability. *J. Sci. Food Agric.* 56(2), 141-153.

Jalilvand, G., Odongo, N.E., López, S., Naserian, A., Valizadeh, R., Eftekhari Shahrodi, F., Kebreab, E. & France, J., 2008. Effects of different levels of an enzyme mixture on *in vitro* gas production parameters of contrasting forages. *Anim. Feed Sci. Technol.* 146, 289-301.

Khazaal, K.A., Dentino, M.T., Ribeiro, J.M & Ørskov, E.R., 1995. Prediction of apparent digestibility and voluntary intake of hays fed to sheep: comparison between using fibre components, *in vitro* digestibility or characteristics of gas production or nylon bag degradation. *Anim. Sci.* 61, 527-538.

Liu, J.X., Susenbeth, A. & Sudekum, K.H., 2002. *In vitro* gas production measurements to evaluate interactions between untreated and chemically treated rice straw, grass and mulberry leaves. *J. Anim. Sci.* 80, 517.

Mauricio, R.M., Mould, F.L., Dhanoa, M.S., Owen, E., Channa, K.S. & Theodorou, M.K., 1999. A semi-automated *in vitro* gas production technique for ruminant feedstuff evaluation. *Anim. Feed Sci. Technol.* 79, 321-330.

Musco, N., Koura, I.B., Tudisco, R., Awadjiè, G., Adjolohoun, S., Cutrignelli, M.I., Mollica, M.P., Houinato, M., Infascelli, F. & Calabrò, S., 2016. Nutritional

- characteristics of forage grown in south of Benin. *Asian Australas. J. Anim. Sci.* 29, 51-61.
- Ørskov, E.R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.* 92, 499-503.
- Pitt, R.E., Cross, T.L., Pell, A.N., Scholfield, P. & Doane, P.H., 1999. Use of *in vitro* gas production models in ruminal kinetics. *J. Dairy Sci.* 76, 1063-1073.
- Sallam, S.M.A., Nasser, M.E.A., El-Waziry, A.M., Bueno, I.C.S. & Abdalla, A.L., 2007. Use of an *In vitro* rumen gas production technique to evaluate some ruminant feedstuff. *J. Appl. Sci. Res.* 3(1), 34-41.
- Singh, D., Zeng, J., Gao, D. & Chen, S., 2014. Effects of lignin modification on wheat straw cell wall deconstruction by *Phanerochaete chrysosporium*. *Biotechnol. Biofuels.* 7,161.
- Sunvold, G.D., Hussein, H.S., Fahey, G.C.J., Merchen, N.R. & Reinhart, G.A., 1995. *In vitro* fermentation of cellulose ,beet pulp, citrus pulp, and citrus pectin using faecal inoculum from cats, dogs, horses, humans and pigs and ruminal fluid from cattle. *J. Anim. Sci.* 73, 3639-3648.
- Tesfayohannes, S.T., Nsahlai, I.V. & Bengaly, K., 2013. Effect of urea treatment and concentrate proportions on dry matter degradation of different roughages in the rumen of jersey cows. *Global J. Sci. Frontier Res.* 13, 8.
- Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B. & France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Anim. Feed Sci. Technol.* 48, 185-197.

Wang, B., Mao, S.Y., Yang, H.J., Wu, Y.M., Wang, J.K., Li, S.L., Shen, Z.M. & Liu, J.X.,
2014. Effects of alfalfa and cereal straw as a forage source on nutrient digestibility
and lactation performance in lactating dairy cows. *J. Dairy Sci.* 97, 7706-7715.

6 GENERAL DISCUSSION, CONCLUSIONS AND FUTURE RESEARCH

6.1 General discussion and conclusions

Forage plays a vital role in ruminant production. Good quality forage is one that brings out positive response in terms of ruminant production (Taysom, 2000). The information on forage quality is important to ruminant nutritionists in formulating balanced rations because balanced feeding is important for optimum ruminant production. Livestock offtake for resource-poor farmers is low due to scarcity of feed and unbalanced feeding practises (Tona 2018). This is more evident during the dry winter season when mostly low quality cereal crop straws are used as feed. These cereal crop straws do not meet the nutritional requirements of ruminants as they are characterised by low protein content and high fibre content (Rao & Hall, 2003; Mahesh & Madhu, 2013). A lot of research has thus been done to improve the nutritive value of these cereal crop straws and thus assisting the ruminants in efficient utilization of these straws (Dolberg, 1992; Hanafi *et al.*, 2012). One method of improving the low quality cereal straws is by anhydrous ammonia treatment, which was investigated in this study.

In this study, anhydrous ammonia gas treatment improved the chemical composition of maize, wheat and oats straws. This was seen by the improvement of CP content and the decreased NDF and ADF content of maize, wheat and oats straws significantly. Maize straws were the ones with the highest CP and fibre improvements. When evaluating the *in vitro* ruminal gas production of the straws, it was determined that the optimum inclusion level on oats straws is 3.5%. The optimum inclusion level for improved *in vitro* dry matter degradability and nitrogen buffer solubility for maize straws is 4.29% that of oats straws is 3.3%. The high content of NDF, ADF and ADL of the straws (Wheat, oats and maize) as

depicted in Chapter 3 played a major effect on the *in vitro* ruminal dry matter degradability and nitrogen buffer solubility. Anhydrous ammonia treated maize straw had the least NDF, ADF and ADL when compared to the other straws (wheat and oats), as a result they had better *in vitro* ruminal dry matter degradability and nitrogen buffer solubility. Treated oats straw had the second highest *in vitro* ruminal dry matter degradability and nitrogen buffer solubility followed by wheat straw, which had the least. Higher levels of anhydrous ammonia gas are needed to improve the *in vitro* ruminal dry matter degradability and nitrogen buffer solubility of wheat straw. Maize straw treated with anhydrous ammonia have shown the potential to be included in ruminant diets due to their higher CP, *in vitro* ruminal dry matter degradability, nitrogen buffer solubility and lower fibre content as compared to wheat and oats straws.

6.2 Future research

There is an opportunity to further investigate other methods (urea and sodium hydroxide treatment) apart from anhydrous ammonia treatment that could improve the nutritive value of cereal straws. This study only involved laboratory analyses, it is thus recommended that a feeding trial be conducted in order to determine the actual feed value of ammoniated cereal straws based on growth performance, health and meat quality parameters.

6.3 References

- Dolberg, F., 1992. Progress in the utilization of urea-ammonia treated crop residues: Biological and socio-economic aspects of animal production and application of the technology on small farms. *Bras. Soc. Anim. Proc.* 4(2), 1992.
- Hanafi, E.M., Khadrawy, E.I., Ahmed, W.M. & Zaabal, M.M., 2012. Some observations on Rice straw with emphasis on updates of its management. *World. Appl. Sci. J.* 16(3), 354–361.
- Mahesh, M.S. & Madhu, M. 2013. Biological treatment of crop residues for ruminants feeding. A review. *Afr. J. Biotechnol.* 12, 4221–4231.
- Rao, P.P. & Hall, A.J., 2003. Importance of crop residues in crop–livestock systems in India and farmers perceptions of fodder quality in coarse cereals. *Field Crops. Res.* 84, 189–198.
- Taysom, D., 2002. Managing forage resources. US dairy forage research centre. Madiso, W. US.
- Tona, G.O., 2018. Current and Future Improvements in Livestock Nutrition and Feed Resources, *Animal Husbandry and Nutrition*, Banu Yücel and Turgay Taşkin, IntechOpen, DOI: 10.5772/intechopen.73088. Available from: <https://www.intechopen.com/books/animal-husbandry-and-nutrition/current-and-future-improvements-in-livestock-nutrition-and-feed-resources>.

7 LIST OF APPENDICES

Appendix 1: Recipe for phosphate-borate buffer (Licitra *et al.*, 1996)

1. Borate-phosphate buffer, (pH 6.7 - 6.8) including

a. Monosodium phosphate ($\text{NaH}_2\text{OPO}_4 \cdot \text{H}_2\text{O}$)	:	12.20 g/L
b. Sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$)	:	8.91 g/L
c. Tertiary butyl alcohol	:	100 mL/L

2. Sodium azide (10%) solution freshly prepared.



The buffer and the solution was combined by adding 50 mL of borate-phosphate and 1 mL of 10% sodium azide

Appendix 2: Recipe for the *in vitro* ruminal gas production buffer

Reagents

Buffer Solution A (g/L)

a. Potassium dihydro phosphate (KH_2PO_4)	:	10.0
b. Magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	:	0.5
c. Sodium chloride (NaCl)	:	0.5
d. Calcium chloride dehydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)	:	0.1

Buffer Solution B (g/L)

a. Sodium carbonate (Na_2CO_3)	:	15.0
b. Sodium sulphide ($\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$)	:	1.0

Buffer A and B was combined at a ratio of 1:5, and according to the ANKOM Technology Method no. 3 (ANKOM, 2005).