EFFECT OF ANHYDROUS AMMONIA GAS TREATMENT OF LOW-QUALITY CEREAL STRAWS ON CHEMICAL COMPOSITION AND *IN VITRO* RUMINAL FERMENTATION

T.F. Buthane[1], L.E. Motsei[2], C.M. Mnisi[2] & K.E. Ravhuhali[2]

[1]Grootfontein Agricultural Development Institute, PBag X529, Middelburg (EC), 5900, South Africa

[2]Department of Animal Science, Faculty of Natural and Agricultural Science, North-West University, Mafikeng, 2790, South Africa

[*]E-mail: ThaboBU@dalrrd.gov.za

INTRODUCTION

Under free-range production systems, ruminants are highly dependent on the properties of forage to maximise voluntary feed intake, supply essential nutrients, and promote ease of utilisation (Ravhuhali et al., 2018). In the dry seasons, available grasses have poor nutritive value with protein being the major limiting nutrient. Additionally, the use of commercial supplements is prohibitive for small-holder farmers due to lack of capital. For many years, cereal straw has been used as an alternative source of feed for ruminants owing to the reduction of grazing lands for cultivation of commercial crops (Misra et al., 2013; Shrivastava et al., 2014). However, the utility of cereal straws in ruminant diets may be limited by their low crude protein content and high levels of cellulose, hemicellulose and lignin (Mahesh & Madhu, 2014; Singh et al., 2016). Simmons et al. (2010) reported that the presence of lignin in cereal straw inhibits the hydrolysis process and consequently reduces digestibility.

In view of that, various strategies have been explored to improve the utilisation of cereal straws by ruminants (Zhang et al., 2007; Nguyen et al., 2012). The treatment of low-quality feeds with ammonia is an effective and affordable strategy to improve their feed value (Sarnklong et al., 2010; Beigh et al., 2017). Sarnklong et al. (2010) reported that ammonia treatment has the potential to hydrolyse the cell wall of roughages thereby reducing the fibre content. Brand et al. (1991) reported an increase in crude protein and a decrease in crude fibre when maize straw was treated with urea. Several studies have found that ammonia treatment of cereal straw increased intake and digestibility (Habib et al., 1998; Cömert et al., 2015).

Despite reports on the efficiency of ammonia treatment in low-quality feeds, there have been conflicting reports on the optimum application rate of anhydrous ammonia gas (AAG) in ruminant diets, which could be attributed to different ammonia products, various application methods, as well as the nutrient composition and harvesting stage of the straws. In addition, there is limited information on the use of AAG on the nutritive value of maize (*Zea mays*), wheat (*Triticum sativum*) and oats (*Avena sativa*) straws in South Africa. This study was, therefore, designed to investigate the effect of treating maize, wheat and oats straw with graded levels of AAG on chemical composition and *in vitro* ruminal fermentation.

MATERIALS AND METHODS

The study was conducted at Molelwane Research farm of the North-West University, Mafikeng. The maize wheat and oats cereal straws were collected at maturity stage post-harvest from Bakgat Voere Farm (25°27′ S, 27°56 E) located in Brits (North West Province). The crops were grown under ambient temperatures ranging from 2.1 °C to 29.3 °C, with an average annual rainfall of 540 mm. Anhydrous ammonia gas (density of 0.77 142 kg/m^3 and purity of 97%) was supplied by Puregas (PTY) Ltd (Gauteng, South Africa).

Five samples from each of the three cereal straw (5 kg/bag) was independently placed in a closed polythene plastic bag (60 x 100 cm), and thereafter treated with AAG at a rate of 0, 2.5, 3.5, 4.5 and 5.5% per bag. The treated straws were replicated three times with each replicate sample being treated independently of each other. Treatment with AAG was conducted by connecting a pipe to the polythene bags 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, South Africa). The ammoniated samples were subsequently stored in a temperature-controlled (30°C) room for seven days. Thereafter, the ammoniated samples were air-dried for 24 h and then ground (2 mm; Polymix PX MFC-90 D) and stored in respective sample bottles pending laboratory analyses.

For laboratory dry matter (DM) determination, approximately 1 g of each treatment sample was placed into pre-weighed crucibles and oven-dried at 105 °C for 12 h (AOAC, 2012; method no. 973.18). Organic matter content was determined after incinerating the dried samples in a muffle furnace set at 600 °C for 6 h (AOAC, 2012; method no. 973.18). Crude protein was determined using the standard macro-Kjeldahl method (AOAC, 2012; method no. 976.06). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by refluxing samples (0.45 - 0.5 g) in ANKOM F57 filter bags with neutral detergent and acid detergent solutions for 60 min and 75 min, respectively, using the ANKOM^{2000} Fibre Analyser (ANKOM Technology, New York), according to Van Soest et al. (1991). Alpha-amylase was used for the NDF analysis. Acid detergent lignin (ADL) was determined by treating the ADF residue in ANKOM F57 filter bags with 72% sulphuric acid. Mineral content (calcium, phosphorus magnesium, manganese, copper and zinc) was analysed following the guidelines of the Agri-Laboratory Association of Southern Africa (AgriLASA, 1998).

The Reading Pressure Technique (RPT) developed by Mauricio et al. (1999) was used to measure *in vitro* ruminal gas production parameters. Each sample was weighed (1 ± 0.001 g) into individual RPT bottles (125 mL, Thermo Scientific, USA). Thereafter, an ANKOM buffer solution (pH 6.8) was prepared as described in the ANKOM Technology Method 3 for *in vitro* true digestibility. Ninety millilitres (90 mL) of the buffer was then added to each of the RPT bottles, including two blanks bottles and subsequently transferred to an incubator set at 39 °C overnight. The following morning, rumen inoculum was collected prior to feeding from a ruminally cannulated Bonsmara cow (~600 kg), which was fed with a total mixed rations but also allowed to graze, and had free access to fresh, clean water. The rumen fluid was collected into two pre-warmed thermos flasks and immediately taken to the laboratory where it was blended and strained using a two-layer muslin cloth. Upon straining, the inoculum was held at 39 °C under a stream of carbon dioxide gas and then added (25 mL) to the RPT bottles and immediately incubated. Headspace gas pressure was measured using a pressure transducer (22IC, Gems Sensors and Controls, United Kingdom) and measurements were taken at 12, 24, 36 and 48 h after inoculation. From these measurements, gas volume (mL), cumulative gas production, effective gas (Egas) and potential gas (Pgas) production were calculated (Ørskov & McDonald, 1979).

As the experiment was a completely randomized design , data were analysed using a two-way ANOVA on chemical composition and *in vitro* ruminal fermentation data using the GLM procedure of SAS (2010).

RESULTS AND DISCUSSION

The effect of cereal straw type and ammonia treatment on the chemical components is presented in Table 1. OM, CP, NDF, ADF and ADL differ significantly among cereal straw types, while CP and NDF differ significantly among ammonia treatments. Significant two-way interactions between cereal type and ammonia treatment were observed for CP, NDF and ADF.

Table 1. Effect of cereal straw type and ammonia treatment on chemical components

Component	Straw type (ST)	Ammonia treatment (AT)	ST × AT
Dry matter	NS	NS	NS
Organic matter	***	NS	NS
Crude protein	***	***	*
NDF	***	***	***
ADF	***	NS	*
ADL	***	NS	NS

NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL= acid detergent lignin. ST × AT = interaction between cereal straws and ammonia treatment. NS = P>0.05; * = P<0.05; *** = P<0.001

The effect of anhydrous ammonia gas treatment rate of maize, oats and wheat straws on chemical composition is presented in Table 2. Untreated straws had a lower CP content (P<0.05) than the treated straws. This indicates that AAG treatment improves the CP content of cereal straws, which corroborate the findings of Adesogan et al. (2019) who stated that ammonia is made up of nitrogen molecules which increases the nitrogen fraction of feedstuffs. Wheat straw had a lower CP content (P<0.05) than oats and maize straws. Ammonia treated maize straw had the highest CP content, followed by oats straw and then wheat straw. The highest CP content was observed in the 4.5% ammonia treated maize straw. An increase in CP content means that there would be enough dietary protein for efficient microbial fermentation of substrates (Aderinboye et al., 2016).

Table 2. The effect of anhydrous ammonia gas (AAG) treatment rate of maize, oats and wheat straws on chemical composition (g/kg DM)

AAG Rate[1]	Maize			Oats			Wheat		
	DM	CP	NDF	DM	CP	NDF	DM	CP	NDF
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}
[2] ±SE	5.3	7.7	25.0	5.3	7.7	25.0	5.3	7.7	25.0

[a,b,c] Means in a column with different lowercase superscripts denote significant differences (P<0.05) between anhydrous ammonia treatments.
[A,B,C] means in a row with different uppercase superscripts denote significant differences (P<0.05) between cereals.
[1]Rate: 0 = untreated cereal straws; 2.5 = cereal straws treated with 2.5% of anhydrous ammonia gas; 3.5 = cereal straws treated with 3.5% of anhydrous ammonia gas; 4.5 = cereal straws treated with 4.5% of anhydrous ammonia gas; 5.5 = cereal straws treated with 5.5% of anhydrous ammonia gas. [2]SE = standard error of the mean.

For the untreated straws, wheat had a higher NDF content than oats and maize straw, which did not differ (P>0.05). The untreated maize and wheat straw had a higher NDF content (P<0.05) than the treated maize and wheat straws. For the treated straws, maize had a lower NDF content (P<0.05) than oats and wheat straws, which were similar (P>0.05). Ammonia treatment reduced the NDF content of maize and wheat straws, indicating that the utilisation of these treated straws would be higher than that of untreated straws. This is because low NDF values are associated with high DM intake in ruminants. The increase in CP and decrease in NDF content was consistent with the reports by Figueiras et al. (2010) and Das et al. (2017), who reported that when fibre molecules are broken down, the nitrogen that is bound to the fibre is released, thus increasing the nitrogen content of a feed. According to Brar et al. (2019), an increase in CP and a decrease in NDF and ADL contents translate to an improvement in the nutritive value of the straws, which ultimately improves dry matter intake and animal performance. Almeida et al. (2019) reported similar results that ammonia treatment improved the nutritional value of grass hay by increasing the nitrogen content while reducing the fibre content.

Wheat straw had the highest ADF content (P<0.05) followed by oats straw and then maize straw. Ammonia treatment had no effect (P>0.05) on the ADF content of wheat and oats straws. The untreated maize straw had a higher ADF content (P<0.05) than those treated with 2.5% and 4.5% of AAG. Untreated wheat straw had the highest ADL content (P<0.05), followed by oats straw and then maize straw. The decrease in ADF and

ADL concentrations in the treated maize and oats straws indicated the potential to increase ruminal digestibility due to lower lignification (Du et al., 2016). According to Oji et al. (2007), a reduction in cellulose content of a feed increase fermentation efficiency, hence maize and oats straws can be better utilized compared to wheat straw. According to NRC (2018), the lignin content of forage can be used as an indicator of NDF degradation, but vary amongst forages (Tesfayohannes et al., 2013; Santana-Neto et al., 2019).

In vitro ruminal gas production is important in determining fermentation patterns of rumen microbes, and allows the collection of digestion kinetics data that is applicable in feeding programs (Davies et al., 2000). This technique has gained a lot of attention because it is cost effective, is not labour intensive, and is suitable for large-scale evaluation of feed in controlled environments (Sallam et al., 2007; Mnisi & Mlambo, 2017). The rate of gas produced over time of maize, oats and wheat straws treated with anhydrous ammonia gas is depicted in Figure 1. Significant differences among cereal straws were observed in the rate of gas produced at 12, 24, 36 and 48 h post-inoculation. At 12 h post-inoculation, maize produced the highest ($P<0.05$) rate of gas (40.6 mL/h OM), followed by wheat (28.95 mL/h OM), while oats straw produced the least gas (23.73 mL/h OM). This could be attributed to the lower fibre and ADL content of the treated maize straw. Nonetheless, no differences ($P>0.05$) were observed among the cereal straws in terms of the rate of gas produced at 24, 36 and 48 h after incubation. There was no significant ammonia treatment rate effect nor an interaction between cereal type and ammonia treatment rate on gas production rate.

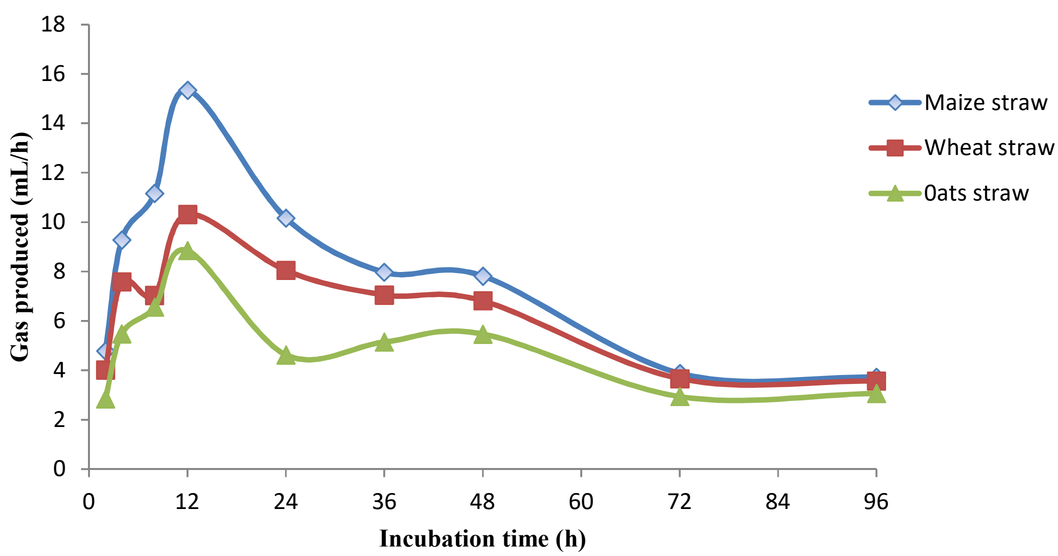

Figure 1. Rate of gas produced over time (mL/h OM) of maize, oats and wheat straws treated with anhydrous ammonia gas

Cumulative gas production (mL/g OM) of maize, oats and wheat straws treated with anhydrous ammonia gas is given in Table 3. Maize straw had higher ($P<0.05$) cumulative gas production at 12, 24, 36 and 48 h post-inoculation than oats straw (Table 3). Wheat straws had similar ($P>0.05$) cumulative gas production until 48 h post-inoculation as maize and oats straws. The higher cumulative gas production of maize straw could be due to maize having lower fibre and ADL values than oats and wheat straws, making it more accessible to microbial enzymes that catalyse their hydrolysis (Zhang et al., 2019; Diether & Willing, 2019). There was no significant ammonia treatment rate effect nor an interaction between cereal type and ammonia treatment rate on gas production rate.

Table 3. Cumulative gas production (mL/g OM) of maize, oats and wheat straws treated with anhydrous ammonia gas

Parameters	**Maize**	**Oats**	**Wheat**	**SE**	**ST**	**AT**	**ST × AT**
Cumgas12	40.56[b]	23.73[a]	28.95[ab]	4.64	*	NS	NS
Cumgas24	50.73[b]	28.36[a]	36.99[ab]	5.89	*	NS	NS
Cumgas36	58.70[b]	33.50[a]	44.04[ab]	6.78	*	NS	NS
Cumgas48	66.52[b]	38.97[a]	50.85[ab]	7.67	*	NS	NS

[a,b,c] Means in a row with different superscripts denote significant differences (P<0.05).
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.
SE = standard error of the mean. ST = Straw type; AT = Ammonia treatment; ST × AT = interaction between straw type and ammonia treatment. * = P<0.05; NS = not significant (P>0.05)

In vitro ruminal gas production parameters of wheat, oats and maize straws treated with anhydrous ammonia gas are summarised in Table 4. There were significant differences among the straw types on fraction *c*, lag time and effective gas production (Table 4). Maize straw had the highest (P<0.05) gas production rate constant for the slowly fermentable fraction (*c*) and a longer lag time than oats and wheat straws, which could be as a result of the ammonia solubilizing the fibre content of maize and the rumen microbes having a better attachment on oats and wheat straws (Tesfayohannes et al., 2013). Maize straw had higher (P<0.05) effective gas production than oats straw, but no differences were observed on fraction *a* and *b*. Contrary to the findings of this study, Kakengi et al. (2003) reported that highly fibrous diets would be poorly degraded in the rumen and, as a result, have low gas production from the rapidly fermentable fraction. The lag phase for wheat and oats straws was shorter than that of maize straw, suggesting that the rumen microbes firstly attached to these straw.

Table 4. *In vitro* ruminal gas production parameters (mL/g OM, unless stated otherwise) of wheat, oats and maize straws treated with anhydrous ammonia gas

Parameters	**Maize**	**Oats**	**Wheat**	**±SE**	**ST**	**AT**	**ST × AT**
a	4.53	2.89	4.32	0.520	NS	NS	NS
b	67.38	40.69	53.09	7.879	NS	NS	NS
c (%/h)	0.058[b]	0.043[a]	0.048[a]	0.002	***	NS	NS

Lag time (h)	1.358[b]	0.989[a]	1.034[a]	0.101	*	NS	NS
Pgas	71.91	43.59	57.40	8.362	NS	NS	NS
Egas	54.22[b]	31.56[a]	40.99[ab]	6.219	*	NS	NS

[a,b] Means in a row with different superscripts denote significant differences (P<0.05)
a = the immediate fermentable fraction; b = the slowly fermentable fraction; c = the gas production rate constant for the insoluble fraction b; Pgas = potential gas production; Egas = effective gas production. SE = standard error of the mean. ST = Straw type; AT = Ammonia treatment; ST × AT = interaction between Straw type and ammonia treatment.
* = P<0.05; *** = P<0.001; NS = not significant (P>0.05)

CONCLUSION

It can be concluded that treatment with anhydrous ammonia gas in general improved the proximate constituents of the cereal straws, but was most effective on maize straw. In addition, ammonia treatment influenced the *in vitro* ruminal fermentation parameters of the straws. Treated maize straw had a higher fermentability than oats and wheat straws.

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