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Rumen degradation characteristics of two tropical forages supplemented with monensin in the rumen of N'dama Steer

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Abstract

Rumen degradation characteristics of Panicum maximum and Gmelina arborea forages in response to monensin supplementation were studied in a 2 x 4 factorial experiment using an N'dama fistulated steer. Monensin had no significant effect (P>0.05) on the soluble fraction 'a' of nutrients but showed reductions (P<0.05) in the degradable fractions 'b' of dry matter, crude protein, neutral detergent fibre and acid detergent fibre at 30 and 45 mg/kg DM supplementation levels relative to the control. These reductions were within the range of 45.76 - 49.52, 37.88 - 43.57, 44.60 - 45.31 and 45.18 - 46.85% for DM, CP, NDF and ADF, respectively. The potential degradation 'p' of DM, NDF, and ADF reduced (P<0.05) at 30 and 45 mg/kg DM while 'p' for CP reduced at 45 mg/kg DM. Forage type had significant effect (P<0.05) on nutrient degradation with Panicum maximum having lower nutrient degradation characteristics values than those of Gmelina arborea. While monensin reduced nutrient degradation from Panicum maximum at 30 and 45 mg/kg DM, reduction in nutrients degradation from Gmelina arborea was observed only at 45 mg/kg DM. Comparatively, at similar levels of 15, 30 and 45 mg of monensin/kg DM, Panicum maximum had lower (P<0.05) degradation values to those observed for Gmelina arborea. The reduction effect of monensin on nutrient degradation from both forages suggests an inhibition of microbial digestion. This could increase rumen fill, reduce ruminal outflow rate and possibly increase the proportion of un-degradable proteins leaving the rumen for the lower tract. The particular level of monensin supplementation at which such reductions would occur depends on forage type.

Keyords: Gmelina arborea, Panicum maximum, Nutrient degradation, Fistulation, N'dama steer.

Introduction

Forage feeds consumed by ruminants are initially exposed to microbial degradation in the rumen prior to gastric and intestinal digestion. Ruminants derive substantial part of their nutrients from the microbes and their degradation activity. These microbes are able to make useful contribution to particle size reduction of forages during digestion (Mc Allister *et al.*, 1994; Bowman and Firkin, 1996). The extent to which forages are digested in the rumen is determined by the chemical and physical nature of the fibre, which in turn depends on the genetic make up of the plant and the environment where it grows (Osuji *et al.*, 1995). Tropical forages are often characterized by high fibre and low

nitrogen content which could limit efficient digestion. Since most of the ruminant livestock in developing countries feed mainly on cellulosic crop residues, pasture, trees and bushes, it is important therefore, that the rumen microbial fermentation be unimpeded so that the potential nutritive value of these roughages are extracted (Orskov, 1995). Monensin is an ionophore antibiotic which was first discovered in 1967 and it has been extensively used as a feed additive for young ruminants (Gomez *et al.*, 1990) and routinely as an additive for feedlot and grazing cattle (Russell and Strobel, 1989). It was approved by the U. S. FDA as a feed additive in the mid 1970's (Edrington *et al.*, 2003), and the initial work on monensin in beef animals was carried out in the feedlot system of the USA. The primary site of action of monensin in the digestive tract is the rumen and as such it is referred to as a 'rumen modifier' (Macgregor, 1988). Monensin exerts its effect primarily by selectively altering the balance of ruminal microbes (Bergen and Bates, 1984). It is reported to exhibit both anti-bacterial and anti-coccidial activities (EFSA, 2004). Since monensin acts strictly within the rumen and microbial degradation of forages initially occurs in the rumen, this study was carried out to evaluate the influence of monensin on ruminal degradation of nutrients from *Panicum maximum* and *Gmelina arborea* leaves which serve as forage resources for ruminants in many parts of the tropics.

Materials and Methods

Experimental site: The study was carried out on the Research farm of the Institute of Agricultural Research and Training (IAR & T), Ibadan, Nigeria (Longitude 7° 15'-7°30' N and Latitude 3°45'-4°0' E).

Experimental animal and its management: A fistulated N'dama steer weighing about 300 kg was used for this study. The bull was kept intensively and maintained on grass and concentrates for the period of the study. It was allowed access to clean drinking water *ad libitum*.

Experimental samples: The samples used for degradation study were *Panicum maximum* and *Gmelina arborea* leaves. Fresh samples of the two forages were harvested during the dry season from the University of Agriculture, Abeokuta. These samples were weighed and oven dried at 60°C for 48 hours to a constant weight for dry matter determination. Dried samples were then ground to pass through a 1 mm screen and 2.5mm mesh sieve for rumen degradation. Dried samples of the two forages were incorporated with monensin at four different levels of 0, 15, 30 and 45mg/kg dry matter. These levels of monensin formed the treatments T_1, T_2, T_3 and T_4, respectively for each of the forage feeds.

Experimental design: The experiment was laid out in a 2 x 4 factorial arrangement with the experimental model; $Yijk = \mu + Ti + Fj + (TF)ij + \Sigma ijk$ where, Yijk = the kth observation in the ith and jth treatments; μ = overall mean; T i = effect of monensin treatment; Fj = effect of forage type (Panicum and Gmelina); TF)ij = interaction effect between monensin treatment and forage type; Σijk = residual error

Rumen degradability measurement: The nylon bag technique as described by Orskov *et al.* (1980) was used to determine the dry matter disappearance of the two forage samples, *Panicum maximum* (a pasture grass) and *Gmelina arborea* (a browse species) in response to monensin supplementation at varying levels of 0, 15, 30 and 45 mg/kg DM. Nylon bags (5x16cm) of known weights with approximate pore size of 41 microns were used. 5g of each sample per treatment was

weighed in triplicates into nylon bags which were firmly fitted unto slit plastic tubes well labeled according to the incubation hour and inserted into the rumen of the steer. They were thereafter sequentially withdrawn at 3, 6, 12, 24, 48, 72 and 96h and immediately kept inside a flask containing ice cubes to terminate fermentation. Zero hour washing loss was determined by soaking 3 replicates of each sample in warm water at 39°C for 30 minutes. All the bags were then rinsed under tap water until the rinse water became clear, then placed on metal trays, oven dried at 60°C for 48h and weighed to determine the DM loss. The procedure was repeated 3 times to make 3 replicates per treatment. To determine degradation characteristic constants, the data of dry matter disappearance were fitted into the exponential equation $p=a + b (1- e^{-ct})$ as reported by Orskov and McDonald (1979) where p = potential degradability in time t; a = rapidly degradable fraction at zero hour; b = insoluble but degradable fraction at time t; c= fractional rate constant at which the fraction described by b will be degraded per hour and t= time of incubation. The cell wall disappearance was estimated by refluxing the nylon bag residue with neutral detergent solution and acid detergent solution for neutral detergent fibre (NDF) and acid detergent fibre (ADF) determination, respectively.

Chemical analysis: Dry matter and crude protein contents in samples incubated and residues evacuated from the rumen at specified incubation times were determined by the methods of AOAC (1996). The neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined by the methods of Van Soest and Robertson (1985).

Statistical analysis: Data generated from the degradation studies were subjected to the factorial analysis of variance procedure of statistical analysis system (SAS, 1999) in a two-way interaction. Significant differences between treatment means were compared using the Duncan's multiple range test according to the procedure of SAS (1999) at 5% probability level.

Table 1. Crude protein and fibre composition of forages used for degradation.

Composition	*Panicum maximum*				*Gmelina arborea*			
(%)	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Crude protein	8.12	8.76	7.79	7.68	12.28	11.76	11.24	11.08
NDF	64.36	63.98	63.16	62.76	59.12	58.54	58.76	59.02
ADF	43.26	41.82	40.95	40.97	40.31	39.68	39.75	39.27
Lignin	9.82	9.24	9.97	9.92	8.76	8.21	8.43	8.26

T_1, T_2, T_3 and T_4 represent monensin inclusion levels of 0, 15, 30 and 45mg/kg DM in each of the forages

Results and Discussion

The crude protein and cell wall constituents of *Panicum maximum* and of *Gmelina arborea* leaves supplemented with monensin at varying levels are presented in Table 1. Crude protein values of *P. maximum* were lower than those of *G. arborea* leaves. The fibre fraction values were also higher for *P. maximum* than for *G. arborea*. The low crude protein value of *P. maximum* confirms the report of Ash (1990) that tropical pasture grasses are frequently low in protein and cannot support high levels of ruminant animal production. A critical level of 70 g/kg DM has been reported by Minson (1990) below which

feed intake will be depressed. Similarly, a crude protein level below 10% has been reported to cause inhibition in microbial activity (Kilic and Saricrek, 2008). Crude protein values of *Gmelina arborea* were within the range of 10 37.9% reported for browse plants in Nigeria (Mecha and Adegbola, 1980). The higher crude protein content of *Gmelina arborea* leaves suggests it as a suitable protein supplement to poor quality grasses.

Effect of monensin inclusion on nutrient degradation characteristics of the two forages is shown in Table 2. The soluble fraction (a) of the different nutrients were not affected (P>0.05) by monensin inclusion. However, the degradable fraction (b) of dry matter, crude protein and fibre fraction was reduced (P<0.05) at 30 and 45 mg level of monensin inclusion. The reduction effect on dry matter degradation could be attributed to a possible reduction in the rate of microbial attachment and colonization of the forages within the rumen which probably reduced the rate of microbial reduction of forage particle size. Contrary to this result, Bogaert *et al.* (1989) reported that the addition of similar ionophores, lasalocid and catinomycin, to feed had no effect on dry matter digestibility, though their studies were conducted *in sacco*. The reduction

Table 2. Single effect of monensin supplementation on nutrient degradation characteristics of forages

Degradation characteristics (%)	T_1	T_2	T_3	T_4	SEM
Dry matter					
a	18.42	18.96	19.31	18.91	0.64
b	56.49^{a}	52.82^{ab}	49.52^{bc}	45.76^{c}	3.28
p	74.91^{a}	71.78^{ab}	68.83^{bc}	64.68^{c}	3.46
c	0.023	0.017	0.018	0.019	0.003
Crude protein					
a	23.20	24.42	23.82	24.21	0.51
b	47.66^{a}	45.47^{ab}	43.57^{b}	37.88^{c}	0.92
p	70.86^{a}	69.89^{a}	67.40^{a}	62.08^{b}	1.09
c	0.043^{a}	0.041^{ab}	0.034^{b}	0.028^{c}	0.003
Neutral detergent fibre					
a	20.25	19.88	18.94	19.16	0.21
b	51.87^{a}	48.17^{ab}	45.31^{b}	44.60^{b}	0.91
p	72.12^{a}	68.05^{ab}	64.25^{b}	63.76^{b}	0.10
c	0.031	0.029	0.031	0.024	0.002
Acid detergent fibre					
a	18.19	18.13	17.87	18.09	0.28
b	53.99^{a}	50.59^{a}	46.85^{b}	45.18^{b}	1.48
p	72.18^{a}	68.71^{ab}	64.72^{bc}	63.27^{c}	1.47
c	0.030^{a}	0.027^{a}	0.022^{b}	0.021^{b}	0.002

a, b, c Means along the same row with different superscripts are significantly different (P<0.05). T_1, T_2, T_3 and T_4 represent monensin inclusion levels of 0, 15, 30 and 45mg/kg DM. a = rapidly degradable fraction. b = insoluble but degradable fraction. p = potential degradation. c = fractional rate constant at which b will be degraded per hour

effect of monensin on crude protein degradation suggests possible inhibition of rumen microbes to utilize dietary crude protein. Several authors have reported a reduction in peptidolysis due to the inhibitory effects of ionophores on certain proteolytic bacteria. Gomez *et al.* (1991) found that the addition of certain ionophores decreased the breakdown of dietary protein in the rumen and lowered microbial synthesis. Similarly, Chalupa (1988) reported reduction effects on protein degradation by ionophores which are believed to be caused by changes in the bacterial population. The slowed release or breakdown of crude protein with monensin supplementation implies possible increases in the amount of rumen undegradable protein reaching the lower gut. This could be an indication for improved utilization of dietary protein provided there is an efficient digestion of the crude protein in the small intestine. Improvement in nitrogen flow from the rumen when ruminants were fed monensin had been reported (Bergen and Bates, 1984) and was associated with observed decrease in proteolysis, peptide breakdown and deamination. Henderson *et*

Table 3. Single effect of forage type on nutrient degradation characteristics of forages

Degradation characteristics (%)	*Panicum maximum*	*Gmelina arborea*	SEM
Dry matter			
a	16.82[a]	20.98[b]	0.46
b	46.58[a]	55.72[b]	2.32
p	63.40[a]	76.70[b]	2.45
c	0.018	0.021	0.002
Crude protein			
a	20.43[a]	27.40[b]	0.36
b	40.29[a]	47.01[b]	0.65
p	60.72[a]	74.40[b]	0.77
c	0.033	0.040	0.002
Neutral degradation			
a	17.81[a]	21.31[b]	0.15
b	43.53[a]	51.44[b]	0.64
p	61.34[a]	73.50[b]	0.71
c	0.031[a]	0.026[b]	0.001
Acid degradation			
a	15.90[a]	20.24[b]	0.20
b	44.95[a]	53.35[b]	1.05
p	60.85[a]	73.77[b]	1.04
c	0.030	0.030	0.001

[a, b, c] *Means along the same row with different superscripts are significantly different (P<0.05)*

a = rapidly degradable fraction. b = insoluble but degradable fraction. p = potential degradation . c= fractional rate constant at which b will be degraded per hour

Table IV. Interaction effect of monensin supplementation and forage type on nutrient degradation characteristics of forages

Degradation characteristics	T_1		T_2		T_3		T_4		SEM
(%)	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	
Dry matter									
a	16.41	20.44	17.02	20.90	16.77	21.85	17.09	20.74	0.91
b	54.27^{a}	58.71^{a}	49.22^{bd}	56.43^{ac}	45.02^{b}	54.01^{a}	37.81^{c}	$53.71d^{de}$	4.64
p	70.68^{ad}	79.15^{b}	66.24^{d}	77.33^{b}	61.79^{c}	75.86^{ab}	54.90^{e}	74.45^{a}	4.89
c	0.016	0.029	0.016	0.019	0.019	0.017	0.020	0.017	0.004
Crude protein									
a	18.68^{a}	27.72^{b}	21.63^{a}	27.21^{b}	21.36^{a}	26.28^{b}	20.04^{a}	28.37^{b}	0.72
b	45.72^{ab}	49.60^{a}	42.45^{bc}	48.49^{a}	40.27^{c}	46.88^{a}	32.71^{d}	43.05^{c}	1.30
p	64.40^{a}	77.32^{c}	64.08^{a}	75.70^{cd}	61.63^{a}	73.16^{cd}	52.75^{b}	71.42^{d}	1.54
c	0.043^{a}	0.043^{a}	0.040^{a}	0.041^{ac}	0.028^{b}	0.039^{c}	0.020^{b}	0.036^{c}	0.004
Neutral detergent fibre									
a	18.72	21.78	18.07	21.69	17.21	20.67	17.24	21.08	0.30
b	50.15^{a}	53.58^{a}	44.19^{c}	52.17^{ab}	40.16^{d}	50.45^{ab}	39.60^{d}	49.59^{b}	1.29
p	68.87^{a}	75.36^{bd}	62.26^{c}	73.84^{c}	57.37^{e}	71.12^{ad}	56.84^{e}	70.67^{ad}	1.41
c	0.025^{a}	0.036^{b}	0.029^{a}	0.029^{a}	0.036^{b}	0.025^{a}	0.033^{b}	0.014^{c}	0.002
Acid detergent fibre									
a	15.41^{a}	20.97^{b}	15.76^{a}	20.49^{b}	15.69^{a}	20.05^{b}	16.72^{a}	19.45^{b}	0.39
b	53.37^{a}	54.60^{a}	47.65^{b}	53.52^{a}	40.27^{c}	53.43^{a}	38.51^{c}	51.85^{a}	2.09
p	68.78^{a}	75.57^{b}	63.41^{c}	74.01^{be}	55.96^{d}	73.48^{be}	55.23^{d}	71.30^{e}	2.09
c	0.027^{a}	0.032^{b}	0.026^{a}	0.027^{a}	0.021^{ed}	0.023^{c}	0.023^{c}	0.019^{d}	0.002

a, b, c, d, e Means along the same row with different superscripts are significantly different ($P<0.05$). T_1, T_2, T_3 and T_4 represent monensin inclusion levels of 0, 15, 30 and 45mg/kg DM in each of the forages. F_1 and F_2 represent Panicum maximum and Gmelina arborea forages, respectively. a = rapidly degradable fraction. b = insoluble but degradable fraction. p = potential degradation. c= fractional rate constant at which b will be degraded per hour

al. (1981) reported toxic effect of monensin on Ruminococci. Studies of Chen and Wolin (1979) on the effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen saccharolytic bacteria in a complex medium containing rumen fluid showed that *Ruminococcus albus, Ruminococcus flavefaciens, and Butyrivibrio fibrisolvens* were inhibited by 2.5 µg of monensin per ml. *Ruminococcus* species are known to be the fibre degrading organisms in the rumen and therefore an inhibitory effect of monensin on these organisms in the rumen will consequently reduce ruminal fibre digestion Contrary to this report however, Osborne (2004) found that the rate of ruminal forage fibre degradability was similar between control and monensin-treated cows.

Forage type effect (Table 3) on ruminal degradation of nutrients, showed that *Gmelina arborea* leaves had a higher ($P<0.05$) nutrient degradation characteristics values than *Panicum maximum*. This might be due to differences

between the two forages in terms of particle size, crude protein and fibre composition, plant age or stage of re-growth. The differences in nutrient degradability between the two forages could also be a likely reflection of the rate at which microorganisms attached themselves to the forages within the rumen. The critically low protein content of *Panicum maximum* which was below 10% might have contributed to its significantly lower degradation. Since the utilization of forages is largely dependent on microbial degradation, the higher degradation value obtained for Gmelina suggests the presence of more degradable and fermentable carbohydrates than is contained in Panicum leaves.

Table 4 shows the combined effect of monensin and forage type on dry matter, crude protein and fibre degradation. The specific level of monensin inclusion at which reductions occurred in nutrient degradation varied between the two forages. While monensin reduced *Panicum maximum* degradation mostly at 30 and 45 mg/kg DM, the degradation of *Gmelina arborea* forage reduced at 45 mg/kg DM inclusion levels. This suggests that the response of individual forage type to monensin inclusion would vary depending on the nature of the forage.

Conclusion

Monensin has been implicated in causing a reduction in dry matter, crude protein and fibre degradation. The use of monensin in ruminant forage diets would probably increase rumen fill, feed retention time in the rumen and flow of undegradable protein to the lower gut. An increase in rumen fill might result to possible reduction in dry matter intake and this consequently could influence animal performance. Where the protection of dietary protein from microbial degradation is of importance in ruminant nutrition, monensin may be included in the diet to increase the amount of dietary protein reaching the lower tract for its efficient utilization. With and without monensin supplementation, *Gmelina arborea* would serve as better forage in terms of crude protein content and digestibility in the rumen. It could therefore be used as a protein supplement to poor quality grasses. Further studies need to be carried out to determine the optimum level of monensin supplementation beyond which further reduction in nutrients degraded from forages would have an adverse effect on the nutritional efficiency and productive performance of ruminants. Specific level of monensin supplementation for different forage types could also be determined.

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