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Research Article

# Rumen degradation of cassava foliage meal supplemented with graded levels of monensin by N'Dama bulls in the humid zone of Nigeria

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**Abstract:** The effect of rumen degradation of cassava foliage meal (CFM) supplemented with graded levels of monensin was studied. Dried samples of CFM were mixed with monensin at four different levels of 0, 15, 30 and 45 mg/kg in the rumen of mature fistulated N'Dama bulls weighing averagely 345 ± 0.02kg. These levels of monensin supplementation represented treatments $T_1$, $T_2$, $T_3$ and $T_4$ respectively in a Completely Randomized Design (CRD) experiment. The nylon bag technique was used to determine the dry matter (DM) disappearance of CFM in response to monensin supplementation. The bags were firmly fitted unto slit plastic tubes and incubated in the rumen of N'Dama bull at 6, 12, 24, 48, 72 and 96 hours after which they were withdrawn from each animal and washed in cold running tap water for 25 minutes to terminate further fermentation and dried to constant weight at $60^0$C for 48 hours in a forced air oven and weighed to determine DM loss. Nylon bags filled with samples not subjected to rumen incubation (representing the zero hour time) were soaked in water for 30 minutes and washed in the same way as those incubated and dried at $60^0$C. Results showed that monensin at 45 mg/kg DM level had significant ($P< 0.05$) reductions in the degradation of DM, crude protein and neutral detergent fibre. Ruminal degradation of cellulose and its subsequent reduction was however observed at 15, 30 and 45 mg/DM of monensin supplementation relative to the control. The reduction in DM and fibre degradation led to increased rumen fill and a slower rate of ruminal outflow of digesta. Reduction in CP degradation could in the long run increase the supply of undegradable protein in the rumen. The study concluded that monensin at 45 mg/kg DM supplementation could be used to regulate feed intake and improve the supply of by-pass undegradable protein in ruminants.

**Keywords:** Foliage, ruminants, rumen, micro biota, nutrients.

## INTRODUCTION

Ruminant livestock are an essential component of most smallholder farming systems in sub-Saharan Africa. They account for more than 20% of the total assets of smallholder farmers while contributing substantially to their livelihoods and cash income. Inadequate feed supply (in terms of quantity, quality and accessibility) is recognized as a major constraint to increased livestock production in this zone. Traditional sources of feed are crop residues and unproductive natural grasses and weeds from waste areas, undeveloped grazing areas and forest land. These feeds are usually utilized by grazing animals but the challenge of quality (nutrient composition) is often the problem.

Nigeria is the largest producer of cassava (*Manihot esculenta*) producing about 50 million metric tonnes annually (FAOSTAT, 2008; Ogunjinmi *et al.*, 2010). Although, the principal economic products are its roots; leaves or foliage also have excellent potential as feed/food and are extensively used in Africa and Asia. Cassava foliage provides a high level of protein (about 21%) which can be conveniently used for feeding ruminants. Due to the high quantity and quality of protein and particularly rich in minerals, carotene and Vitamin C, fresh cassava foliage resembles conventional legumes and is suitable as forage for ruminants (Buitrago *et al.*, 2012).

Efficient utilization of this enormous feed resource should therefore be encouraged particularly by ruminant animals because of the significant role played by microbes in the rumen in the digestion of high fibrous feedstuffs. Several attempts have been made to manipulate the rumen environment in ways that would alter digestion pathways to improve nutritional

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efficiency, reduce methane emissions and maximize available feed resources for increased livestock production. According to Bello and Escobar (1997); Aderinboye and Onwuka (2008) modification of the rumen microbial composition and microbial dynamics can be achieved by means of feed additives that selectively affect rumen symbionts by manipulating rumen microorganisms. Gram positive bacteria are generally acetate and butyrate producing bacteria while gram negative bacteria are generally propionate producing (Stewart, 1991; Benchaar and Greathead, 2011). One of the important feed additives includes ionophores such as monensin, lasalocid, salinomycin (laidlomycin, tetronasin, narasin, lysocellin etc.).

Monensin is considered as a growth promoter due to its favourable effects on rumen fermentation including methane reduction, propionate enhancement and ammonia reduction with preventive effects on coccidiosis, bloat and lactic acidosis (Callaway *et al.*, 2003). Monensin acts strictly within the rumen (Macgregor, 1988; Aderinboye and Onwuka, 2008) and exerts its effects primarily by selectively altering the balance of ruminal biota (Bergen and Bates, 1984; Cox, 2016). Ionophores such as monensin have been extensively investigated for their ability to reduce methane ($CH_4$) production in ruminants (Beauchemin *et al.*, 2009). Enteric $CH_4$ is a loss of productive energy typically between 2 and 12% of gross energy in ruminants depending on level of feed intake and diet composition (Boadi *et al.*, 2004). Thus, the use of monensin is beneficial from the environmental perspective because of reduced methane production but more important is the nutritional benefits of improved feed efficiency and animal productivity (Callaway *et al.*, 2003; Benchaar and Greathead, 2011).

Ionophores have multiple modes of action by inhibiting gram-positive bacteria that produce hydrogen, ammonia or lactic acid and counteract the decrease in grain-dependent rumen pH that can be harmful to fibre degrading microorganisms (Russel and Rychlic, 2001; Benchaar and Greathead, 2011; Loor *et al.*, 2016).

The use of monensin could probably have an influence on the digestion of cassava (*Manihot esculenta*) foliage in the rumen. This study was therefore designed to examine the effect of monensin supplementation on the degradation within the rumen of the nutrients in cassava foliage meal.

## MATERIALS AND METHODS

### Study location

The study was carried out at the Cattle Unit of the Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike – Abia State, Nigeria. Umudike is situated within the tropical rainforest zone and has an annual rainfall averaging 2177mm in 148 – 155 rain days. Its relative humidity is over 72% during the rainy season with an average ambient temperature of $25.5^0$C within a range of 22 – $32^0$C (Ahamefule, 2005).

### Animal management

Two fistulated N'Dama bulls weighing averagely 345 ± 0.02kg were selected from the cattle herd and used in this study. The bulls were kept intensively, maintained on cassava foliage meal and concentrates throughout the period of this study. Portable drinking water was provided *ad-libitum.*

### Experimental samples and design

Cassava (*Manihot esculenta*) foliage of TMS 30555 variety were used for the degradation study. Fresh samples of Cassava leaves were collected from the garri processing unit of the National Root Crop Research Institute Umudike. The leaves were weighed and oven dried in a forced air laboratory oven at $60^0$C for 48 hours and used for the dry matter (DM) degradation studies. Dried cassava foliage samples were milled to obtain cassava foliage meal (CFM) separately to pass through a 1mm screen sieve for determination of nutrient composition and 2.5mm mesh sieve size for rumen degradation studies. Dried samples of CFM were mixed with monensin at four different levels of 0, 15, 30 and 45 mg/kg. These levels of monensin supplementation formed treatments $T_1$, $T_2$, $T_3$ and $T_4$ respectively. The experiment followed a completely Randomized Design (CRD) with the following experimental model:

$$Yij = \mu + Ti + \sum ij$$

Where Yij = the jth observation in the ith treatment

μ = Overall mean

Ti = effect of monensin supplementation or treatment

∑ij = residual error

### Rumen degradation

Rumen degradation determination was by the nylon bag technique as described by Ørskov *et al.* (1980). This was used to determine the DM disappearance of CFM in response to monensin supplementation at varying levels of 0, 15, 30 and 45mg/kg DM. Nylon bags (16 × 7cm, 40 micron porosity) of known weights were used. Exactly 5g of each sample per treatment were weighed into the nylon bags in triplicates and repeated or replicated for the second bull. The bags were then firmly fitted unto slit plastic tubes (this system simplifies withdrawal of the bags since bags with individual nylon cords can become tangled and difficult to withdraw from the rumen) and incubated in the rumen of N'dama bull at 6, 12, 24, 48, 72 and 96 hours after which they were withdrawn from each animal and washed in cold running water for 25 minutes to terminate further fermentation and dried to constant weight at $60^0$C for 48 hours in a forced air laboratory oven and weighed to determine DM loss. Nylon bags filled with samples not subjected to rumen

incubation (representing the zero hour time) were soaked in water for 30 minutes and washed in the same way as those incubated and dried at $60^0$C for 48 hours to a constant weight to determine DM loss. To determine degradation characteristic constants, the results of DM disappearance were fitted to the exponential equation

$P = a + b (1 - e^{-ct})$ (Órskov and McDonald, 1979).

Where a, b and c are constants

a= intercept or rapidly degradable fraction at zero hour
b= insoluble but potentially degradable fraction at time t
c= fractional rate constant at which the fraction described by b will be degraded per hour
e= the natural logarithm
t= time of incubation
p= level of degradation at time t

The cell wall disappearance was estimated by refluxing the nylon bag residue with neutral detergent solution and acid detergent solution for NDF and ADF determinant.

**Chemical and statistical analysis**

Chemical composition of incubated samples and residues evacuated from the rumen at specific incubation time were determined by the methods of AOAC (2006). The NDF, ADF and lignin were determined by the methods of Van Soest (1982). Data generated were subjected to one-way analysis of variance for CRD using the general linear model procedure of statistical analysis (SAS, 2008). Duncan's multiple range test (Duncan, 1955) as outline in Obi (1991) was used to separate significant means.

**RESULTS**

The crude protein and cell wall constituents of cassava (*Manihot esculenta*) foliage meal supplemented with varying levels of monensin are presented in Table 1. Monensin supplementation did not affect (P> 0.05) the nutrient composition of cassava foliage meal. Crude protein contents across the treatments were within the range of 18.28 – 18.44% while the neutral detergent fibre (NDF) ranged between 63.54 and 65.71%. The acid detergent fibre (ADF) fraction ranged from 53.29 – 54.64 %. The lignin fractions were however low but ranged between 15.25 and 15.71%. The effect of monensin supplementation on nutrient degradation of cassava foliage is presented in Table 2. The rapidly soluble fraction of (a) dry matter, crude protein and fibre fraction were not affected significantly (P> 0.05) by monensin supplementation. Values were within the same range across the various treatments. The insoluble but potentially degradable fraction (b) of dry matter, crude protein and neutral detergent fibre was reduced significantly (P< 0.05) at 45mg/kg level of monensin supplementation compared with the control. Dry matter degradation value reduced significantly (P< 0.05), the crude protein degradation also had a significant (P< 0.05) reduction in value while neutral detergent fibre was also affected significantly (P< 0.05) and reduced from the insoluble but potentially degradable fraction (b) of cellulose was affected and reduced (P< 0.05) at monensin supplementation levels of 15, 30 and 45mg/kg DM relative to the control. There was no significant difference (P> 0.05) between the monensin supplemented treatments.

**Table 1: Nutrient composition (% DM) of cassava foliage meal (CFM) used for rumen degradation**

| Composition | Treatments | | | | SEM |
|---|---|---|---|---|---|
| (%) | $T_1$ | $T_2$ | $T_3$ | $T_4$ | |
| CP | 18.44 | 18.38 | 18.35 | 18.28 | 0.02 |
| NDF | 65.12 | 63.54 | 63.71 | 65.02 | 0.42 |
| ADF | 54.41 | 53.29 | 53.31 | 54.64 | 0.35 |
| Lignin | 15.71 | 15.25 | 15.40 | 15.38 | 0.09 |

Note: $T_1$, $T_2$, $T_3$ and $T_4$ represent monensin supplementation levels at 0, 15, 30 and 45 mg/kg DM. CP: Crude protein, NDF: Neutral detergent fibre, ADF: Acid detergent fibre

**Table 2: Effect of monensin supplementation on nutrient degradation characteristics**

| Degradation characteristics (%) | $T_1$ | $T_2$ | $T_3$ | $T_4$ | SEM |
|---|---|---|---|---|---|
| Dry matter | | | | | |
| a | 18.44 | 18.95 | 19.35 | 18.85 | 0.19 |
| b | 60.71[a] | 58.16[b] | 56.55[b] | 52.14[c] | 1.80 |
| p | 79.15 | 77.11 | 75.90 | 70.99 | 1.74 |
| c | 0.03 | 0.02 | 0.02 | 0.02 | 0.00 |
| Crude protein | | | | | |
| a | 29.27 | 29.21 | 29.29 | 28.28 | 0.02 |
| b | 50.60 | 49.49 | 48.50 | 43.11 | 1.66 |
| c | 79.87 | 78.70 | 77.79 | 71.49 | 1.87 |
| C | 0.04 | 0.04 | 0.04 | 0.04 | 0.00 |
| NDF | | | | | |
| a | 20.71 | 20.65 | 20.61 | 21.06 | 0.03 |
| b | 55.21 | 52.10 | 52.12 | 48.73 | 1.32 |
| p | 75.92 | 72.75 | 72.73 | 69.79 | 1.25 |
| c | 0.03 | 0.03 | 0.03 | 0.03 | 0.00 |
| ADF | | | | | |
| a | 19.94 | 19.65 | 19.50 | 18.75 | 0.25 |
| b | 58.04 | 57.71 | 57.55 | 56.25 | 0.39 |
| p | 77.98[a] | 77.36[ab] | 77.05[ab] | 75.00[b] | 0.65 |
| c | 0.03 | 0.03 | 0.03 | 0.03 | 0.00 |
| Cellulose | | | | | |
| a | 19.80 | 19.48 | 19.15 | 19.05 | 0.17 |
| b | 49.33[a] | 40.51[b] | 40.81[b] | 40.90[b] | 2.15 |
| p | 69.13[a] | 59.99[b] | 59.96[b] | 59.95[b] | 2.29 |
| c | 0.04 | 0.04 | 0.04 | 0.04 | 0.00 |

[a,b, ab] Means on the same row with different superscripts are significantly different (P< 0.05).

**Note:** $T_1$, $T_2$, $T_3$ and $T_4$ represent Monensin supplementation levels at 0, 15, 30 and 45mg/kg DM

NDF = Neutral detergent fibre

ADF = Acid detergent fibre

- a = Intercept or rapidly degradable (soluble) fraction
- b = Insoluble but potentially degradable fraction
- c = Fractional or degradation rate constant at which b will be degraded per hour
- p = Potential degradation at time T
- e = natural logarithm

## DISCUSSION

Monensin is known to exert its action site within the rumen environment (Macgregor, 1988). This probably explains why its inclusion in the foliage samples did not affect or alter the crude protein of *Manihot esculenta.* Therefore, one can opine that the existing micro biota were insensitive to monensin supplementation. The crude protein content of cassava foliage meal across the various treatments was appreciably high, suggesting that cassava leaves could serve as a good source of protein resembling some conventional forage legumes. The crude protein value of cassava foliage was within the range of 10 – 21.5% reported for *Manihot esculenta* foliage in the tropics (Buitrago *et al.,* 2012). The rapidly degradable soluble fraction of dry matter was not affected by monensin supplementation levels which imply that the proportion of feed particle size leaving the rumen and escaping microbial attack (degradation) were similar with or without monensin supplementation. This position was reported by Aderinboye and Onwuka (2008) in their work with *Gmelina arborea* foliage/monensin inclusion. The reductive effect of monensin supplementation at 45 mg/kg DM level on the dry matter degradation of cassava foliage could however be considered to be due to a possible inhibitory activity that slows down the rate of microbial activity to break down the forage particle size. This observed reduction in DM degradation could possibly lead to depression in feed DM intake due to increased rate of feed retention within the reticulo-rumen and a subsequent increase in rumen fill. Several workers (Chalup 1988; MaCgregor 1988; Fajuke *et al.,* 2006; Casamiglia *et al.,* 2007; Callaway et *al.,* 2003) have reported reduction in feed intake with monensin inclusion in diets. Kobayashi *et al.* (1992) in their studies with salinomycin, an ionophore like monensin reported that these products have a special mode of action of reducing ruminal

motility, thereby providing a physiological basis for the increased ruminal fill and reduced feed intake with monensin and or salinomycin. The reductive effect of monensin on crude protein degradation is directly linked to a reduction in microbial degradation of dietary protein. This however, could imply reduction in microbial growth which affects the overall rate of microbial protein synthesis. The reduction in crude protein digestion in the rumen would probably increase the amount of dietary protein (by-pass protein) entering the lower gut. Kobayashi *et al.* (1992) reported that monensin and salinomycin (representative ionophores) are known to shift to some extent the digestion site of protein from the rumen to the lower gut. Ruminal digestion of protein and the flow of microbial protein to the lower gut (small intestine) and the efficiency of microbial growth have been reported to decrease with ionophores (Chalupa, 1988; Callaway *et al*., 2003; Aderinboye and Onwuka, 2008).

The observed reduction in neutral detergent fibre and cellulose degradation implies a reduction in ruminal degradation of fibre. Monensin is known for its inhibitory effect on protozoa, gram positive bacteria including ruminococci, streptococci and lactobacillus species (Russel and Strobel, 1989). This could explain the rumen fill and reduction in protein/fibre degradation in the rumen. Ruminococcus species are considered as the major fibre degrading micro biota in the rumen, an inhibitory/toxic effect of monensin on this micro biota and the entire micro biome in the rumen would definitely reduce their degradation capability and or efficiency.

## CONCLUSION

The observation of reduction in dry matter and fibre degradation in the rumen with monensin supplementation in *Manihot esculenta* foliage could have an effect on the feed intake pattern of ruminants by physically reducing the quantity of feed consumed by these animals. Reduced ruminal degradation of DM would be of benefit in directly reducing feed cost, provided the productive performance of the animal, particularly in terms of feed conversion ratio is not jeopardized or negatively affected. Reduction in crude protein degradation observed in this study implies increased proportion of dietary protein would by-pass microbial degradation in the rumen, and reaching the lower gut. This has implication for improved utilization of undegradable protein reaching the lower gut. Monensin at 45mg/kg DM supplementation level could be used to regulate feed intake and improve the supply of by-pass undegradable protein to the lower gut of ruminants. Monensin is considered as a growth promoter due to its favourable effects on rumen fermentation. However, its use has been banned within the European Union but its utilization has been on the increase in other climes.

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