

Dry Matter and Crude Protein Degradability of Mopane Worm (*Imbrasia belina*) in Rumen of Steers

Research Article

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Received on: 5 Jan 2012

Revised on: 19 Mar 2012

Accepted on: 1 Apr 2012

Online Published on: Mar 2013

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Online version is available on: www.ijas.ir

ABSTRACT

Three cannulated Tswana steers were used to investigate the rumen degradability of mopane worm (*Imbrasia belina*) by measuring the amount of dry matter (DM) and crude protein (CP) disappearing at incubation periods up to 72 h. The effective degradability (ED) of DM and CP in the rumen was calculated at outflow rates of 0.03 / h (ED_{0.03}) and 0.05 / h (ED_{0.05}). Rumen degradable CP (RDP) was estimated as the amount of CP x ED_{0.05} and rumen undegraded protein (RUP) was calculated as CP minus RDP. The soluble DM, slowly degradable DM, ED_{0.03} and ED_{0.05}, and degradation rate of evacuated (i.e. fresh leaves and faecal material removed) mopane (EM) and intact mopane (IM) were similar (P>0.05). Water soluble protein fraction, slowly degradable protein, degradation rate and RDP were also similar (P>0.05) between EM and IM samples. However, EM had significantly more (P<0.01) RUP than IM samples. The current results support previously observations that removal of gut contents from mopane worms increased CP, consequently improving RUP. Further research is needed to evaluate the effects of roasting on degradability of EM and IM as well as the availability of amino acids post-ruminally.

KEY WORDS effective degradability, mopane worm, outflow rates, Tswana steers.

INTRODUCTION

Imbrasia belina (mopane worm, commonly known as *Phane* in Botswana) is the larva of the emperor moth (Ditlhogo, 1996). Besides being used as a protein source by people in semi-arid environments of Botswana, Namibia, South Africa and Zimbabwe (Marais, 1996; Moruakgomo, 1996; Styles, 1996), mopane exported to South Africa is used as animal feed (Mpuchane *et al.* 2000). However, there is limited literature highlighting the performance of livestock fed mopane worm. Use of mopane worm in livestock feeds is appealing in Botswana due to heavy reliance on imports of oil cake meals and fish meal. While Botswana produces blood and carcass meals, inclusion of these products in livestock feeds is prohibited (Ministry of Agri-

culture, 1998). Mopane worm is a high quality nutrient source (Ohiokpehai *et al.* 1996; Madibela *et al.* 2007). Comparing the amount of essential amino acids of mopane worm, in a study conducted by Ohiokpehai *et al.* (1996), with those of soyabean meal and fishmeal (Mcdonald *et al.* 2002) reveals that they are similar to fishmeal but dissimilar to soyabean meal (Table 1). In pursuing the evaluation of the suitability of mopane worm as livestock feed in Botswana, we have shown that crude protein (CP) concentrations of intact mopane samples were lowered by 9% (Madibela *et al.* 2009) and 26% (Madibela *et al.* 2007), while neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were increased by 46, 38 and 181%, respectively when the results are compared to evacuated mopane (gut contents removed) samples (Madibela *et al.*

2007). This suggests that the leafy material inside the gut of mopane worms 'dilutes' CP content but increases NDF, ADF and lignin concentrations.

Table 1 Essential amino acids (g/100 g) of mopane worm compared with other high quality livestock feeds

Amino Acid	Mopane worm*	Soya bean meal†	White fish meal†
Histidine	2.9	1.3	1.4
Threonine	4.6	1.9	2.5
Valine	4.1	2.2	3.1
Methionine	1.5	0.8	1.5
Isoleucine	3.0	2.0	2.6
Leucine	5.0	3.5	4.5
Phenylalanine	3.8	2.3	2.9
Lysine	4.7	3.0	4.8
Tryptophan	1.1	0.6	0.7

*: *Ohiokpehai et al. (1996)*; †: *McDonald et al. (2002)*.

It was also observed that intact mopane contain more condensed tannins than evacuated samples (*Madibela et al. 2009*), suggesting existence of secondary compounds in the leafy material in the gut of the worm. Since condensed tannins complex with protein and protect it from rumen fermentation (*Ben Salem et al. 2005*), intact mopane worm may supply more Rumen degradable crude protein (RUP) to the small intestine than evacuated mopane worm. Although chemical analysis is essential for understanding the nutritional potential of new feed resources, it is not sufficient to mimic processes and dynamics inside the rumen (*Hovell et al. 1986; El-hassan et al. 2000*). Therefore, the objective of this study was to determine the ruminal DM and CP degradability of intact mopane worm and mopane worm subjected to gut evacuation.

MATERIALS AND METHODS

Three steers weighing on average 367 ± 8.7 kg and fitted with a flexible rumen cannula were kept in an open pen (10 m x 15 m) with enough space for exercise. The protocol of care for the animals was according to the Council for the International Organization of Medical Sciences (*CIOMS, 1985*). Steers were fed, on a group basis, a diet consisting of grass / lucerne hay (about 11 kg per animal each morning) containing (g/kg DM) 115 CP, 8.4 Ca and 4.3 P offered at a rate of 3% of their body weight. Clean water was available at all times. Before incubation, the steers were adapted to the new diet for 5 days.

Experimental Feedstuff

Samples of mopane worm were sourced from three sites in Botswana as previous described by *Madibela et al. (2009)*. In that study, there were no differences in CP of mopane worms between sites and therefore samples in this study were pooled across sites.

For instance, CP for Maunatlala ($22^{\circ}36'12''S$ and $27^{\circ}37'55''E$), Moreomabele ($22^{\circ}02'59''S$ and $27^{\circ}13'48''E$) and Sefophe ($22^{\circ}11'36''S$ and $27^{\circ}58'06''E$) in Central District of Botswana were 572, 529 and 548 g/kg DM, respectively. Mopane worm samples of 0.5 kg from 5-6 trees were obtained and mixed thoroughly. Samples were divided equally into two portions of either evacuated (EM) or intact (IM) mopane.

Evacuation entails forcibly squeezing the gut contents from the body of the mopane worm by pushing the head towards the anal region (*Siame et al. 1996*). After evacuating and cleaning with water, both samples of EM and IM were placed in boiling water for 30 min to kill the worms, sun dried for 4 h and taken to the laboratory within 72 h for oven drying at $105^{\circ}C$ for 48 h. Samples were then ground to pass a 2 mm screen.

In situ incubation

Rumen incubation was according to the methods of *Ørskov et al. (1980)* without a lag time. Two grams of feed material was placed in nylon bags made of white polyester monofilament (10x20 cm, 53 μ m; ANKOM Technology Corp, Fairport NY, USA).

Each treatment group had 42 samples (6 replicates x 7 incubation periods x 3 steers) in individual nylon bags. Samples were incubated in the ventral sac of the rumen for 0, 3, 6, 12, 24, 48 and 72 h. For ease of withdrawal, the nylon bags were fastened and secured to the fistula with length of nylon string. After removal from the rumen, bags were immediately rinsed in cold tap water and immediately placed in a deep freezer at $-20^{\circ}C$ until the incubation period was completed. Thereafter the bags, together with the zero hour bags were washed under tap water until the water ran clear.

Analysis

Dry matter (DM) for mopane worm residues were determined by weighing samples after drying at $105^{\circ}C$ for 24 h. The DM degradability was expressed as the amount of DM that disappeared after weighing the residue. The CP of the experimental feed and residues used a Kjeldahl procedure according to *AOAC (1990)*. As variation of 3.3% between nylon bags is lower than variation of 4.9% between days (*Mehrez and Ørskov 1977*), two samples at each time point were pooled to increase sample weight for CP analysis, thus remaining with 3 replicates.

The CP degradability was estimated as the difference between concentration of CP in the initial sample and that of the residue. The RDP was estimated as the amount of CP multiplied by its effective degradability at assumed rumen outflow rates of 0.05/h while rumen undegraded protein (RUP) was calculated as CP minus RDP (*Marichal et al. 2000*).

Statistical analysis

Degradation constants (a , b , c ED_{0.03} ED_{0.05}) of DM and CP were estimated by general non-linear models (NLIN) procedures (SAS, 2004) using a SAS program written by the Rowett Research Institute (Osuji *et al.* 1993) and modified for the current data set by including the following derivatives between the model and the output statements:

der.a= 1; der.b= 1 – exp (c*time); der.c= b x time x exp (-c*time)

The calculations were without a time lag. The constants were generated by fitting the degradation data for both DM and CP at various stages of incubation to the Ørskov and McDonald' (1979) exponential model:

$$P = a + b (1 - e^{-ct})$$

Where:

P : DM or CP which has disappeared at time.

t , a : the zero-time intercept.

b : the slowly degradable fraction.

c : the rate of degradation.

The effective degradability (ED) of DM and CP was calculated using the equation:

$$ED = a + bc / (c+k) \text{ (Ørskov, 1992)}$$

Where:

k is the fractional outflow rate from the rumen, which was assumed to be 0.03/h or 0.05/h. These constants (a , b , c , ED_{0.03} and ED_{0.05}), RDP and RUP were analyzed using GLM in SAS (2004), as a randomized complete block design using steers as blocks.

Differences in means between treatments were tested for significance by least significance difference and those with $P > 0.05$ considered not significant (Sokal and Rohlf, 1996).

RESULTS AND DISCUSSION

The amount of the soluble fraction, the slowly degradable fraction, ED_{0.03} and ED_{0.05} of DM between evacuated mopane (EM) and intact mopane (IM) samples was similar (Table 1, $P > 0.05$). Additionally, there were no differences in degradation rates between DM of EM and IM samples ($P > 0.05$).

The amount of water soluble protein fraction, the slowly degradable protein fraction, protein ED_{0.03} and ED_{0.05} between EM and IM samples were similar ($P > 0.05$). Protein degradation rates and amount of rumen degradable protein also did not show any difference ($P > 0.05$) between EM and

IM samples (Table 3). But EM had significantly more ($P < 0.01$) RUP than IM samples.

Table 2 Dry matter degradability constants of evacuated and intact mopane worm samples

Parameters	Evacuated	Intact	SEM	P-value
a	23.1	23.8	0.43	0.324
b	43.6	43.7	0.10	0.376
c	0.06	0.05	0.002	0.099
ED _{0.03} (%)	51.5	51.3	0.37	0.749
ED _{0.05} (%)	46.2	45.8	0.37	0.519

a : water soluble fraction which is rapidly washed out of bags and assumed to be completely degradable; b : the slowly degradable fraction; c : the rate of degradation per hour.

ED_{0.03}: effective degradability at assume fractional outflow rate of 0.03/h.

ED_{0.05}: effective degradability at assumed fractional outflow rate of 0.05/h.

SEM: standard error of mean.

Table 3 Protein degradability constants, RDP and RUP of evacuated and intact mopane samples

Parameters	Evacuated	Intact	SEM	P-value
a	5.1	4.3	0.38	0.188
b	35.4	39.7	2.24	0.246
c	0.010	0.012	0.0013	0.136
ED _{0.03} (%)	13.3	15.5	1.10	0.240
ED _{0.05} (%)	10.6	12.0	0.827	0.304
RDP (g/kg)	60.9	62.4	4.70	0.827
RUP (g/kg)	512.1	457.8	4.70	0.001

a : water soluble fraction which is rapidly washed out of bags and assumed to be completely degradable; b : the slowly degradable fraction; c : the rate of degradation per hour.

ED_{0.03}: effective degradability at assume fractional outflow rate of 0.03/h.

ED_{0.05}: effective degradability at assumed fractional outflow rate of 0.05/h.

RDP: rumen degradable protein (CPxED_{0.05}).

RUP: rumen undegradable protein (CP-RDP).

The present study is the first to report protein degradability of mopane worm and there is currently no information on the attributes of mopane worm as an ingredient for live-stock feed. Proteins that are extensively degraded in the rumen can be protected from rumen degradation but it is important to evaluate the extent of that degradation before any intervention could be made to protect the protein from being extensively degraded.

Dry matter degradability

Evacuating of mopane worm did not have any effect on DM degradability constants. Rasengwatshe and Madibela (2005) found a higher water soluble fraction for mopane worm that was sourced from rural harvesters. Though mopane worm harvesters roast the worms on hot ash or cook it in brine, these processing methods may not be providing sufficient protection at the rumen. The potentially degraded fraction “ b ” of DM for EM and IM in the present study was found to be similar, implying that there was degraded to a similar extent. The values of “ b ” in the present study are comparable to that found by Rasengwatshe and Madibela (2005). Evaluating different proteins from Turkey,

Kamalak *et al.* (2005) observed similar degradation rates (0.05/h) for DM between blood meal and fish meal; this rate is similar to those of EM and IM samples in the present study. Degradation rates of DM for mopane samples were slower than those recorded by Rasengwatshe and Madibela (2005).

Crude Protein Degradability

Kamalak *et al.* (2005) found protein degradation rate (0.05/h) for blood meal and fishmeal which was higher than the rates of mopane worm. These results reported by Kamalak *et al.* (2005) indicate that protein from blood meal and fish meal would possibly be more available in the rumen than that from either EM or IM. Since fish meal is commercially used to provide undegraded protein, the mopane worm which has proven to have similar amino acids profile as fish meal (Table 1) would need protection to have similar value as fish meal post-ruminally. Rumen protein degradability of mopane worm was less by 46.2% of soya-bean meal evaluated by Erasmus *et al.* (1994), less by 59.3% of soya bean meal reported by Maiga *et al.* (1996) and even less by 28.3% of fish meal indicated by Erasmus *et al.* (1994). Botswana does not produce soya bean meal and fish meal, but import these as ingredients for livestock feed. Therefore, utilization of mopane worm coupled with its sustainable production to supply protein to livestock would reduce imports and save foreign currency. However, if mopane worm had to be used for ruminant feed, processing intervention would be needed. It is recognized that the value of proteins in improving ruminant performance is determined by their ability to supply specific amino acids to the small intestine and to supply nitrogen for use by the rumen microbes (Titgemeyer *et al.* 1989), but in case of mopane worm, it has still to be determined since roasting by mopane harvesters, may over-protect mopane protein, thus limiting rumen fermentation. Its determination is important because according to Erasmus *et al.* (1994), proteins may be too highly protected to allow synthesis of microbial protein or RUP may be undigestible and not absorbable in small intestine. Therefore, this calls for further research in evaluating the amino acid profile and intestinal digestibility of rumen residual components of mopane worm.

Apparently, in contrast to our hypothesis, condensed tannins observed by Madibela *et al.* (2009) in the IM samples neither affected degradability nor increase RUP of samples. It has been proposed that condensed tannins complex with proteins, rendering them undegraded in the rumen (Ben Salem *et al.* 2005) but a high proportion of free tannins from disintegration of such complex may prove detrimental (Barry, 1985).

However, at low concentration (20-40 g/kg DM), condensed tannins are considered safe and even beneficial to

the supply of protected protein to the small intestine (Barry, 1985).

The amount of condensed tannins in IM (2.1 g/kg DM), according to Madibela *et al.* (2009) results, would therefore be too small to cause deleterious effects to livestock. Interestingly, there was a high amount of RUP from evacuated mopane than in intact mopane worm, probably reflecting the high CP reported by Madibela *et al.* (2007) for evacuated mopane. The differences in CP between EM and IM worm observed by Madibela *et al.* (2009) and that of RUP between EM and IM worm in the present study were similar, at the rate of 9.1 and 10.6%, respectively.

CONCLUSION

Evacuating mopane worm had no effect on the DM and protein effective degradability of mopane worm at both 0.03/h and 0.05/h ruminal outflow rates. Evacuation of mopane worm increased amount of RUP. Future research should determine the quantity and intestinal digestibility of amino acids from mopane worm post-ruminally because increased supply of RUP to the duodenum does not directly translate into high digestibility and absorbability.

ACKNOWLEDGEMENT

This study is part of Botswana College of Agriculture's Research and Publication Committee (RPC) funded project A621. Part of this study was submitted by the second author as a dissertation in partial fulfillment of requirements of BSc at the University of Botswana / Botswana College of Agriculture. Mr. T.F. Thema is acknowledged for his technical input.

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