Trop Anim Health Prod (2009) 41:935–942
DOI 10.1007/s11250-008-9282-7

ORIGINAL PAPER

Chemical composition of Mopane worm sampled at three sites in Botswana and subjected to different processing

O. R. Madibela · K. K. Mokwena · S. J. Nsoso · T. F. Thema

Accepted: 25 November 2008 / Published online: 6 December 2008

Abstract Effects of site and degutting on chemical composition of mopane worm were investigated. Samples were cooked for 30 min in either brine or in plain water. Samples from Moreomabele were high in acid detergent fibre (ADF) ($P<0.05$) and acid detergent insoluble nitrogen (ADIN) ($P<0.01$) while samples from Sefophe had least ADF and ADIN concentrations. *In vitro* true dry matter digestibility (IVTDMD) tended ($P=0.06$) to be high in samples from Maunatlala than those from Moreomabele and Sefophe. No site difference ($P>0.05$) was observed on the rest of the variables. Degutted samples had high crude protein (CP), ADF and IVTDMD ($P<0.05$) but lower ($P<0.001$) concentrations of ash, acid detergent lignin (ADL) and condensed tannins. Acid detergent insoluble nitrogen was significantly higher ($P<0.001$) in degutted than whole samples. Salting did not ($P>0.05$) change concentrations of all but ADF which tended ($P=0.09$) to be high in salted samples. Samples which were degutted and salted had higher ($P<0.01$) ADF and ADIN than degutted and salted or those left whole and salted or left unsalted. Leaving samples as whole diluted the concentration of CP but increased the fibre components and condensed tannins content. However, mopane worm destined for livestock feeding may be left whole and salt added.

Keywords Condensed tannins · Crude protein · Degutting · Livestock · Mopane worm

Abbreviations

BMC	Botswana Meat Commission
DM	dry matter
DNA	deoxyribonucleic acid
GLM	General Linear Model
LSD	Least Significance Difference

O. R. Madibela · S. J. Nsoso · T. F. Thema
Department of Animal Science & Production,
Botswana College of Agriculture,
P/Bag 0027,
Gaborone, Botswana

K. K. Mokwena
Department of Agricultural Engineering and Land Planning, Botswana College of Agriculture,
P/Bag 0027,
Gaborone, Botswana

O. R. Madibela (✉)
Agriculture and Life Science Division, Lincoln University,
P.O. Box 84, Lincoln 7647,
Canterbury, New Zealand
e-mail: madibeo2@lincoln.ac.nz
e-mail: omadibel@bca.bw

Introduction

Imbrasia belina (mopane worm; commonly known as *phane* in Botswana) is the larva of emperor moths (Lepidoptera) (Ditlhogo 1996). This species feeds on mophane trees (*Colophospemum mopane*), hence the worm is confined to areas of mopane woodland (Ditlhogo 1996; Moruakgomo 1996) in the semi-arid environments of Botswana, Namibia, South Africa and Zimbabwe. There is a continuing interest in mopane as a food resource for human (Motshegwe et al. 1998; Illgner and Nel 2000; Mpuchane et al. 2000; Ghazoul 2006) or as a feed resource for livestock (Madibela et al. 2007) and as well as its biology (Frears et al. 1997; Frears et al. 1999). Mopane worm is an important natural resource for many people in Southern Africa and it is a source of protein and cash, especially among the poor, hunger-averse rural dwellers. The chemical quality of mopane worm; protein content (van Voorthuizen 1976; Sekhwela 1989; Ohiokpehai et al. 1996; Madibela et al. 2007), amino acids (Ohiokpehai et al. 1996) and fatty acids (Zinzombe and George 1994; Motshegwe et al. 1998) has been investigated and is irrefutable. In a good year, mopane worm is estimated to be a \$3.3 million industry and is able to create employment for 10 000 people (Ghazoul 2006). During mopane worm seasons which occurs during months of December/ January and April/May (Mpuchane et al. 2000), women who constitutes 96% of harvesters (Stack et al. 2003) and children (an economically vulnerable group) are mostly engaged in harvesting and selling mopane worm. However, the drawback of these advantages is that, the availability of the worm relies on the amount and timing of rainfall (which in most seasons is unreliable and erratic) and hence the vegetative production of mopane trees, relative to the hatching of its eggs from the emperor moth (Madibela et al. 2007). Mopane worm also faces a threat of being overexploited by harvesters because of lack of regulatory and monitoring policy (Arntzen and Fidzani 1998). These threats further strengthen a case for research into finding existence of any difference in mopane worm population with a view of using genetic biology and breeding to enhance its productivity.

In Botswana there appears to exist two variant/ biotype or sub-populations of mopane worm. In the north east, Nata (20° 12′ 38″ S, 26° 11′ 13″ E and 915 asl), the dominant variant/biotype of mopane worm has a green colouration and does not have spikes whereas the one predominantly found in the eastern parts of Botswana (between 26.5° E and 29.5° E, and 23.2° S and 20.1° S) has yellowish stripes against a black background with conspicuous spikes. This could imply two species. The question of whether mopane worm from different ecological zones differs in its genetic make-up and indeed in its chemical composition still remains. Finding an answer to these questions has many implications; from enhancing the productivity of mopane worm and to developing better strategies of utilization of this important natural resource. Any variation in mopane worm would in itself be an impetus for research in breeding programs and later in the possibility of mopane worm farming (Larviculture), which will promote agroforestry based on mopane trees. However, the initial step is to find if there is any difference in sub-populations of mopane worm. The objectives of this study were to find if there is a difference in the chemical composition of mopane worm from different parts of the country. Another objective was to investigate if samples of mopane worm left as whole contain condensed tannins which are present in mopane leaves. Traditionally, salt is added when mopane worm is processed and hence the effect of salting on chemical composition was also investigated.

Materials and methods

Sites

Initial sampling schedules included obtaining samples from Maun in the north and Nata in North east. Maun (19° 59′ 18″ S and 23° 25′ 59″ E, and 937 m asl) has vegetation consisting of *Colophospermum mopane*, *Terminalia sericea* associated with *Cochocarpus nelsii* (Bekker and De Wit 1991) and it is described as almost flat to gently undulating delta floodplain (De Wit and Bekker 1990). Nata (20° 12′38″ S and 26° 11′ 13″ E, and 915 m als) has vegetation which consists of *C. mopane, T. sericea* associated with *Combretum imberbe* (Bekker and De Wit 1991) and is described as flat to almost flat plain with associated shorelines and major pans (De Wit and Bekker 1990). However, surveillances of these areas showed no prevalence of *phane* and therefore samples were obtained only from three sites in the Central District; Moreomabele (22° 02′ 59″ S and 27° 13′ 48″ E, and 1014 m asl), which is described by De Wit and Bekker (1990) as alluvial flat with a vegetation consisting of *Colophospermum mopane* with a mixture of *Acacia nigrescens*, *Combretum apiculatum* and *A. tortilis* (Bekker and De Wit 1991); Maunatlala (22° 36′ 12″ S and 27° 37′ 55″ E, and 872 m als), located in the Tswapong hills and whose land system is described as a hilly dissected plateau with pediments and associated alluvium (De Wit and Bekker 1990); Sefophe (22° 11′ 36″ S and 27° 58′ 06″ E, and 849 m asl) has undulating to rolling plain that has frequent kopjes and almost flat pediments (De Wit and Bekker 1990). The vegetation of Maunatla and Sefophe is similar to that in Moreomabele. Annual rainfall of the area where the sampling sites are located range from 400 to 450 mm/year.

Sampling and processing

Live worms were picked from trees using plastic hand gloves. Traditionally the 4th and 5th instars which are still feeding on the tree are harvested. A handful of worms were harvested from one tree, chosen at random, and a composite sample from four trees was mixed and portioned into two subsamples. One sample was degutted and the other was left with gut contents intact (whole). Degutting was achieved by forcibly squeezing the gut contents from the body by pushing the head towards the anal region (Siame et al. 1996). The samples were then washed in clean water. Two subsamples of the degutted and whole were subjected to cooking with salt or without salt by holding them in boiling water for 30 min. Salt is used by harvesters to preserve the worms and give them taste. Course salted was added at 1% of fresh weight of the sample. After cooking, the samples were sun dried for 4 hours and were taken to laboratory within 72 hrs for further oven drying at 105°C for 48 hrs. This resulted into 2×2 factorial arranged complete randomized design. Factors were degutting with two levels; degutted and whole; and salting; salted and unsalted.

Chemical analysis

Oven dried samples were ground to pass through a 2 mm screen. Chemical analysis was done in duplicate except for *in vitro* true dry matter digestibility (IVTDMD), in which one sample was analysed. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed with the ANKOM fibre analyzer using reagents described by van Soest et al. (1991). Acid detergent lignin (ADL) was determined by digesting the ADF residue in 72% sulphuric acid and burning the insoluble material in a muffle furnace. Sodium sulphite and amylase were used during the NDF determination. Crude protein (CP) was determined using the Kjeldahl method (AOAC, 976.06, 1996). IVTDMD was determined by a Daisy^{II} Incubator (ANKOM Technology Corp) using multi-layered polyethylene cloth bags, (F57 filter bags; ANKOM, Technology Corp). The rumen fluid samples for IVTDMD were collected from three ruminally cannulated steers and pooled together. The steers were fed Bana grass *ad lib*. At the end of the incubation, the bags were rinsed four times with distilled water, dried, weighed and placed in an ANKOM fiber analyzer and boiled in neutral detergent solution for 60 minutes. IVTDMD was calculated as the difference between dry matter (DM) incubated and the residue after NDF analyses. In our previous study (Madibela et al. 2007) it was hypothesized that the leafy material in whole samples may have anti-nutritional compounds, therefore condensed tannins in mopane worm were analysed in the present study. Soluble condensed tannins were extracted in duplicate from finely ground samples, with 10 ml of aqueous acetone (70:30; v/v). Condensed tannins were estimated according to Makkar (1995). The amount of condensed tannins (g/kg DM) is presented as leucocyanidin equivalent. For comparisons, samples of mopane leaves collected at Maunatlala, blood-meal and carcass-meal obtained from Botswana Meat Commission (BMC) were analysed for chemical composition but were not subjected to statistical analysis.

Statistical analysis

All data were analysed using the General Linear Model (GLM) of SAS (1990) to find the effect of the site, degutting and salting on chemical composition. The effects of degutting x salting interaction was determined. Any differences in means between sites or between treatments were tested for significance by least significance difference (LSD) at which those with $P<0.05$ were considered significant (Sokal and Rohlf 1969). Results are reported as mean with standard error of the mean.

Results

Preliminary data on mopane leaves, carcass and blood meals is shown in Table 1. Blood meal had more CP than mopane leaves and carcass meal. But carcass

Table 1 Chemical composition of mopane leaves sampled in Maunatlala, carcass meal and blood meal obtained from Botswana Meat Commission (g/kg DM) [1]

Parameters	n	Mopane leaves	Blood meal	Carcass meal
Ash	2	44.26 (3.53)	16.22 (0.05)	349.42(16.25)
CP[2]	2	154.70 (16.22)	794.65(24.07)	475.32(23.38)
NDF	2	231.38 (1.22)	72.47(13.02)	478.19(27.00)
ADF	2	187.23 (0.32)	12.94(0.01)	33.39(2.02)
ADL	2	162.09 (4.55)	272.18 (9.62)	11.29(2.12)
ADIN	2	6.13 (0.08)	1.75(0.01)	0.45(0.05)
IVTDMD	1	587.18	710.40	710.64
Condensed tannins	2	23.38 (1.19)	-	-

[1] Standard deviation in brackets

[2] CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre;

ADL = acid detergent lignin; ADIN = acid detergent insoluble nitrogen; IVTDMD = in vitro true dry matter digestibility.

meal had higher ash content, probably due to minerals in the bone component of the carcass.

There was a significant difference due to site on ash, with samples from Maunatlala having high ash ($P<0.05$) and those from Moreomabele having high ADF ($P<0.05$) and ADIN ($P<0.01$). Samples from Sefophe had the least of these variables. *In vitro* true dry matter digestibility tended ($P=0.06$) to be high in samples obtained from Maunatlala than those from Moreomabele and Sefophe. Other than that no differences ($P>0.05$) were observed on the rest of the variables tested (Table 2).

Whole samples had significantly higher ash, ADL and condensed tannins than degutted samples. Only NDF was similar ($P>0.05$) between whole and degutted samples (Table 3). However, degutted samples had high ($P<0.05$) concentrations of CP, ADF and IVTDMD than samples left intact. The difference in ADIN was significantly higher ($P<0.001$) in degutted than whole samples. Salting did not have any effect ($P>0.05$) on all but ADF, which tended ($P=0.09$) to be high in salted than unsalted samples (Table 4).

There was no degutting × salting interactions for ash, CP, NDF, ADL, IVTDMD and condensed tannins. Only samples which were degutted and salted had higher ($P<0.01$) ADF and ADIN (Table 5). No correlations were observed between CP and

Table 2 The effects of site on the chemical composition and *in vitro* dry matter digestibility of mopane worm (g/kg DM) sampled from Maunatlala, Moreomabele and Sefophe in Botswana

Parameters	Maunatlala	Moreomabele	Sefophe	SEM[1]	SL
Ash	54.94^{a}	48.43^{ab}	44.60^{b}	2.58	*
CP[2]	571.99	529.35	548.3	17.16	NS
NDF	278.97	256.70	327.42	31.92	NS
ADF	175.07^{ab}	230.90^{a}	155.46^{b}	20.09	*
ADL	38.24	37.55	37.02	3.54	NS
ADIN	12.20^{b}	17.99^{a}	11.76^{b}	1.20	**
IVTDMD	858.11^{a}	817.66^{ab}	802.34^{b}	13.27	0.06
Condensed Tannins	1.29	1.33	1.42	0.08	NS

[ab] Means in the same row with different superscripts in row are differ significantly; NS = P>0.05; * = P<0.05; ** = P<0.01.

[1] SEM = standard error of the mean; SL = significance level.

[2] CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre;

ADL = acid detergent lignin; ADIN = acid detergent insoluble nitrogen; IVTDMD = in vitro true dry matter digestibility

Table 3 The effects of degutting on chemical composition and in vitro true dry matter digestibility of Mopane worm (g/kg DM) sampled from Maunatlala, Moreomabele and Sefophe in Botswana

Parameters	Degutted	Whole sample	SEM[1]	SL
Ash	40.07[b]	58.57[a]	2.11	***
CP[2]	573.40[a]	521.38[b]	14.00	*
NDF	296.63	278.76	26.07	NS
ADF	213.4[a]	160.88[b]	16.40	*
ADL	22.72[b]	52.49[a]	2.89	***
ADIN	18.19[a]	9.78[b]	0.98	***
IVTDMD	852.82[a]	799.25[b]	10.84	*
Condensed tannins	0.59[b]	2.10[a]	0.06	***

[ab] Means in the same row with different superscripts in row are differ significantly; NS = P>0.05; * = P<0.05; ** = P<0.01; *** = P<0.001

[1] SEM = standard error of the mean; SL = significance level

[2] CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre;

ADL = acid detergent lignin; ADIN = acid detergent insoluble nitrogen; IVTDMD = in vitro true dry matter digestibility

IVTDMD ($r=0.28$; $P>0.05$) and between IVTDMD and NDF ($r=0.24$; $P>0.05$).

Discussion

The results of the present study confirm previous results from our laboratory (Madibela et al. 2007) that mopane worm has high protein content and that degutting improves on the concentration of CP of Mopane worm. Before degutting CP level of Mopane was 521.4 g/kg DM but upon degutting it was improved by about 10 percent. The undigested leafy material in the gut of the worm brought down the total CP of the worm by dilution effect since mopane leaves have low CP as indicated in Table 1. The level of protein in mopane worm is similar to that of carcass-meal but lower than blood-meal (see Table 1), which previously were used as protein ingredients in livestock diets in Botswana.

It was only ash, ADF, and ADIN which were affected by site, with samples from Maunatlala having a high concentration of these parameters. In addition, IVTDMD tended to be different and once again, samples from Maunatlala were more digestible. There was a concerted effort to sample worms of actively feeding 4th and 5th instars developmental stage to reduce variation due to growth stages. Besides the possibility of harvesting worms of different instars, which was remote, there seem to be no obvious reason for these differences, except for ash, which may be attributed to differences in soil type. Differences in soil between the sites may influence the inorganic content of mopane leaves on which the worm feeds, consequently affecting the amount of ash in the samples from different sites. Alternatively, the samples from Maunatlala may have been unintentionally contaminated with soil.

As far as ruminants' nutrition is concerned in Botswana, the most limiting nutrient is nitrogen, which in the present study was indexed by CP. For poor quality roughages such as grass and cereal straw which are used as feed in Botswana during the dry period, the supply of nitrogen to the rumen microbes is important for the fermentation of such diets. In addition, for high producing animals it is important that a fraction of the protein be protected from rumen fermentation, so that it supplies dietary amino acids to the small intestine. It may be tempting to suggest that since CP was similar between the three sites, there would be no need to explore the prospects of mopane worm breeding. However, there may be differences in the DNA (deoxyribonucleic acid) that codes for amino acids and the lack of difference in CP observed in the present study may just reflect the amount of nitrogen available but not necessarily the profile of amino acids. So it would still be worthwhile to test for

Table 4 The effects of salting on the chemical composition and *in vitro* true dry matter digestibility of mopane worm (g/kg DM) sampled from Maunatlala, Moreomabele and Sefophe in Botswana

Parameters	Salting	Unsalted	SEM[1]	SL
Ash	50.19	48.46	2.11	NS
CP[2]	552.50	542.28	14.00	NS
NDF	295.61	279.79	26.07	NS
ADF	208.29	166.00	16.40	0.09
ADL	36.26	38.95	2.89	NS
ADIN	15.20	12.77	0.98	NS
IVTDMD	829.2	822.68	10.84	NS
Condensed Tannins	1.36	1.32	0.06	NS

[1] SEM = standard error of the mean; SL = significance level

[2] CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre;

ADL = acid detergent lignin; ADIN = acid detergent insoluble nitrogen; IVTDMD = in vitro true dry matter digestibility

Table 5 Chemical composition and IVTDMD of degutted and whole of salted and unsalted Mopane worm samples (g/kg DM) Maunatlala, Moreomabele and Sefophe in Botswana

		Ash	CP[3]	NDF	ADF	ADL	ADIN	IVTDMD	CT
Degutted	salted	41.18[b]	567.54[a]	310.67	268.05[a]	22.78[b]	21.52[a]	867.27[a]	0.62[b]
	unsalted	38.97[b]	579.26[a]	282.60	158.76[b]	22.65[b]	14.85[b]	838.37[ab]	0.56[b]
Non-degutted	salted	59.21[a]	537.45[ab]	280.55	148.52[b]	49.75[a]	8.88[c]	791.52[b]	2.11[a]
	unsalted	57.95[a]	505.31[b]	276.97	173.24[b]	55.24[a]	10.69[c]	806.98[b]	2.10[a]
SEM[1]		2.98	19.81	36.86	23.20	4.09	1.39	15.32	0.09
Main effects	Site	*	NS	NS	*	NS	**	NS	NS
	Degutting	***	***	NS	*	***	***	*	***
	Salting	NS	NS	NS	NS	NS	NS	NS	NS
Interaction	Deg x salted[2]	NS	NS	NS	**	NS	**	NS	NS

[ab] Means in the same column with different superscripts differ significantly; NS = P>0.05; * = P<0.05; ** = P<0.01; *** = P<0.001

[1] SEM = standard error of the mean; SL = significance level.

[2] Deg x Salted = degutting x salting interaction.

[3] CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; ADIN = acid detergent insoluble nitrogen; IVTDMD = in vitro true dry matter digestibility;

CT = Condensed tannins.

the amount of DNA and indeed the amount of different amino acids from samples from different ecological zones. Using starch gel electrophoresis, Greyling et al. (2001) showed that genetic diversity of mopane populations is relatively high. In addition, a preliminary genetic study by Riddoch and Solomon (1996) found sufficient variation in gene expression of *Pgm* locus to warrant future investigation of population structuring. The *Pgm* enzyme is involved with glycogen synthesis and might play a role in energy metabolism in the moth (Greyling et al. 2001). To promote sustainable use of mopane worm, farming (larviculture) and agroforestry based on mopane trees should be considered. However, the cost implication of such selection/breeding would need to be assessed first.

The nutritive significance of insects as feed for fish, poultry, pigs and other farm animals is valuable where insect-derived diets can be cost-effective alternatives to more conventional fish meal diets (Gullan and Cranston 2005). This is true for mopane worm in Botswana, more especially that the country relies on imported fish meal as an ingredient for livestock feeds. There are reports (Mpuchane et al. 2000) that mopane worm exported out of Botswana to South Africa is used as animal feed but there is no literature highlighting performance of livestock offered mopane worm. The correct profile of amino acids is vital for supporting the immunity system in parasitized animals (Hoskin et al. 2002; Liu et al. 2005), for growth, foetal growth, milk production and it is achieved by supplying high quality undegraded protein to the small intestine. Amino acid profile of mopane worm (Ohiokpehai et al. 1996) has revealed that it is comparable to fish meal. However, since whole samples of mopane worm had low CP content, it would be interesting to investigate how the profile of amino acids would be affected by the degutting treatment.

In the current study, degutting was found to affect the chemical composition, IVTDMD and condensed tannins of mopane worm. Degutted samples had high CP and IVTDMD. In our previous study (Madibela et al. 2007) we suggested that the leafy material in the undegutted samples lower the concentrations of CP and the digestibility of mopane worm. The leafy material also increased the fiber contents of the sample. In that study (Madibela et al. 2007), it was also hypothesized that the leafy material in the undegutted samples may contribute anti-nutritional compounds to mopane worm samples since mopane leaves do have condensed tannins. Indeed, the present study revealed that undegutted mopane worm had more condensed tannins than degutted samples, reflecting the presence of condensed tannins compounds in the gut contents. It would have been interesting to examine the condensed tannin content of the gut contents after degutting.

Including salt during the cooking of mopane worm did not affect the nutritional attributes of the samples. This means that mopane worm destined for livestock can either be salted or left unsalted. But since addition of salt improves the self-life of the product and that, salt is relatively cheap in Botswana and has traditionally been added to ruminant salt/dicalcium phosphate lick, it would be advisable to add salt to mopane worm. There was no interaction between salting and degutting for all parameters except for ADF and ADIN, indicating salting treatments had the same effect on both degutted and whole samples in all the parameters except ADF and ADIN. A similar result was observed by Madibela et al. (2007) between interactions of cooking treatments with degutting of mopane worm. This shows that degutting has a major influence on the chemical attributes of mopane worm samples than salting or site.

Conclusions and implications

Degutting has a major influence on the chemical attributes of mopane worm samples than salting or site. However, without DNA evaluation it can not be concluded with confidence from chemical composition alone wether mopane worm samples used were from the same or different sub-variant. As for feeding ruminants, mopane worm from any of the sites would be sufficient to meet their nutrient requirements. However, there is a need for sustainable use of mopane worm for wealth creation and value addition by women harvesters. This entails putting in place strategies such as mopane farming and regulatory mechanism to monitor and preserve mopane. This entails mopane woodland agroforestry and protection of mopane vegetation from overgrazing.

Acknowledgements This paper was financed by Botswana College of Agriculture's Research and Publication Committee (Project # A621). The authors would like to thank M. Obopile (Botswana College of Agriculture) and G. Kabwe (Lincoln University, New Zealand) for their constructive critique and suggestions to the manuscript.

References

AOAC. 1996. Official Methods of Analysis. Association of Official Analytical Chemists. Washington, DC.

Arntzen, J and Fidzani, H., 1998. Incentives for sustainable natural resource management and economic diversification in Botswana. A Final Report of the environmental Economics, Sub-component in the BNCS Action plan. Available at: http://envirobotswana.gov.bw Accessed: 17/12/2005.

Bekker, R.P. and De Wit, P.V., 1991. Vegetation map of Republic of Botswana. Soil mapping and Advisory Services Project. AG:DP/BOT/85/011. Food and Agricultural Organisation and Government of Botswana.

De Wit, P. V. and Bekker, R. P., 1990. Land systems map of the republic of Botswana. Soil Mapping and Advisory Services Project AG:AD/BOT/85/011. Food and Agricultural Organisation and Government of Botswana.

Ditlhogo M., 1996. The ecology of Imbrasia belina (westwood in north-Eastern Botswana In; Phane. Gashe B. A. and Mpuchane S. F. (Eds) Proceedings of the multidisciplinary symposium on phane, held in Gaborone 18 June 1996. Department of Biological Sciences and Kalahari Conservation Society.

Frears, S.L., Chown, S.L. and Webb, P.I., 1999. Temperature dependence of feeding behaviour in mopane worm (Lepidoptera). Journal of Thermal Biology, 24, 241–244. doi:10.1016/S0306-4565(99)00017-0

Frears, S.L., Chown, S.L. and Webb, P.I., 1997. Behavioural thermoregulation in mopane worm (Lepidoptera). Journal of Thermal Biology, 22, 325–330. doi:10.1016/S0306-4565(97)00029-6

Ghazoul, J., 2006.(ed) Mopane woodlands and the Mpane worm: Enhancing rural livelihoods and resource sustainability. Fnal Tchnical Report. DFID Project R7822. Forestry Research Project. UK. Available at: http://www.frp.uk.com/dissemination_documents/R7822_-_FTR_-_30-01-06.pdf

Greyling, M., van der Bank, F. H., Grobler, J. P., Wessels, D.C. J., 2001. Allozyme variation in two populations of the Mopane worm, *Imbrasia belina* (Saturniidae), and the effect of developmental stage and staggered generations. South African Journal of Animal Science, 31, 15–24.

Gullan, P.J. and Cranston P.S., 2005. The Insects. An Outline of Entomology. 3rd Edition. Blackwell, Oxford.

Hoskin, S. O., Lobley, G. E., Coop, R. L. and Jackson, F., 2002. The effect of cysteine and glutamine supplementation on sheep infected with *Trichostrongylus columbriformis*. Proceedings of the New Zealand Society of Animal Science, 62, 72–76.

Illgner, S.L. and Nel, E., 2000. The geography of edible insects in sub-Saharan Africa: A study of the Mopane caterpillar. The Geography Journal, 166, 336–351. doi:10.1111/j.1475-4959.2000.tb00035.x

Liu, S. M., Smith, T. L., Plamer, D. G., Karlsson, L. J. E., Besier, R. B. and Greeff. J. C., 2005. Biochemical differences in Merino sheep selected for resistance against gastro-intestinal nematodes and genetic and nutritional effects on faecal worm egg output. Animal Science, 81, 149–157. doi:10.1079/ASC50180149

Madibela, O.R., Seitiso, T.K., Thema, T.F. and Letso, M., 2007. Effect of traditional processing methods on chemical composition and in vitro true dry matter digestibility of Mophane worm (*Imbrasia belina*). Journal of Arid Environments, 68, 492–500. doi:10.1016/j.jaridenv.2006.06.002

Makkar, H. P.S., 1995. Quantification of Tannins: A Laboratory Manual. International Centre for Agriculture Research in the Dry Areas. Aleppo, Syria, pp 1–24.

Moruakgomo M.B.W., 1996. Commercial utilization of Botswana's veld products - The economics of phane - the dimensions of phane trade. In; Phane. Gashe B. A. and Mpuchane S. F. (Eds) Proceedings of the multidisciplinary symposium on phane, held in Gaborone 18 June 1996. Department of Biological Sciences and Kalahari Conservation Society.

Motshegwe, S. M., Holmback, J and Yeboah, S. O., 1998. General properties and fatty acid composition of the oil from the Mophane caterpillar, *Imbrasia belina*. Journal of American Oil Chemical Society, 75, 725–728. doi:10.1007/s11746-998-0213-6

Mpuchane, S., Gashe, B.A., Allotey, J., Siame, B., Teferra, G. and Ditlhogo, M., 2000. Quality deterioration of phane, the edible caterpillar of an emperor moth *Imbrasia belina*. Food Control, 11, 453–458. doi:10.1016/S0956-7135(00)00010-4

Ohiokpehai, O., Bulawayo, B.T., Mpotokwane, S., Sekwati, B and Bertinuson, A., 1996. Expanding the use of phane, a nutritionally rich local food. In: In: Gashe B.A., Mpuchane S.F. (Eds), Phane, Proceedings of the multidisciplinary Symposium on Phane, Held in Gaborone 18 June 1996. Department of Biological Sciences and Kalahari Conservation Society.

Riddoch, B.J. and Solomon, J., 1996. Genetic variation in the Mophane moth, Imbrasia belina. In: Gashe B.A., Mpuchane S.F. (Eds), Phane, Proceedings of the multidisciplinary Symposium on Phane, Held in Gaborone 18 June 1996. Department of biological Sciences and Kalahari Conservation Society.

SAS Institute Inc., 1990. SAS User's Guide. Statistics Version 6. SAS Institute Inc., Cary, NC, USA.

Sekhwela M.B.M., 1989. The nutritive value of Mophane bread - Mophane insect secretion (maphote or Moaboti). Botswana Notes and Records, 20, 151–153.

Siame, A.B., Mpuchane, S.F., Gashe, B.A., Allotey, J. and Teferra, G., 1996. Nutritional quality of Mophane worms, Imbrasia belina (Westwood), and the microorganisms associated with the worms. In: In: Gashe B.A., Mpuchane S.F. (Eds), Phane, Proceedings of the multidisciplinary Symposium on Phane, Held in Gaborone 18 June 1996. Department of Biological Sciences and Kalahari Conservation Society.

Sokal, R.R. and Rohlf, F. J., 1969. Biometry. The Principles and Practice of Statistics in Biological research. 2nd Ed. WH Freedman. San Francisco.

Stack, J., Dorward, A., Gondo, T., Frost, P., Taylor, F and Kurebgaseka, N., 2003. Mopane worm utilization and rural livelihoods in Southern Africa. A presentation for International Conference on Rural Livelihoods, Forests and Biodiversity. 19–23rd May 2003, Bonn, Germany

van Soest, P.J., Robertson, J.B. and Lewis, B.A., 1991 Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583–3597.

van Voorthuizen, E.G., 1976. The Mophane tree. Botswana Notes and Records, 8, 223–261

Zinzombe, I.M., and George, S., 1994. Larval lipid quality of Lepidoptera, *Gonimbrasia belina*. Botswana Notes and Records, 26,167–173.