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African Journal of Agricultural Research

Full Length Research Paper

Gender differences in the use of plant health information services: A case of plant clinics under Plantwise Program in Kenya

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A total of 8,699 female and 12,928 male farmers visited plant clinics in Kenya between 2012 and 2016. The lower clinic attendance by women farmers indicates they may lack information on plant health problems. This study aimed to understand the environment plant clinics operate in, identify the reasons for low clinic attendance by women, and possible strategies to reach more female farmers. Stratified random sampling was used to select 118 female and 119 male plant clinic users and, 138 male and 156 female farmers who had not used plant clinics. The study established there were significant differences ($p<0.01$) in use of different plant health information sources depending on region, gender and whether a farmer was a clinic user or not. Lack of awareness about plant clinics, services offered and who was supposed to attend were the main reasons for failure to attend plant clinics. Thus more awareness creation should be done. Limited access to plant clinics was reported by some farmers, suggesting that more plant clinics are needed. There were significant differences ($p<0.05$) in regional and gender access to plant clinics, highlighting the need for stratified plant health information dissemination methods. To extend the reach of plant clinics, training of plant nurses/lead farmers who are easily accessible to all farmers is warranted.

Key words: Clinic attendance, gender, plant clinic, plant doctor, plant health advice.

INTRODUCTION

The Plantwise programme launched its first pilot plant clinics in Kenya in 2010. The Plantwise programme works with national partners to strengthen countries' plant health systems, through establishing a network of plant clinics as well as supporting plant health system stakeholder linkages. There are currently 122 plant clinics in 14 counties distributed in 5 regions; Central, Eastern, Rift Valley, Western and Nyanza. The clinics are run by 222 plant doctors; 141 male and 81 female. Plant doctors are extension staff mainly from the ministry of agriculture, livestock and fisheries (MoALF) who have received training on how to diagnose plant health problems and run a plant clinic (Danielsen et al., 2013; Scheidegger and Graf, 2013). Most plant clinics are situated in market

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places and operated during market days where farmers frequently take their farm produce for sale and purchase farm inputs. The MoALF is the local implementing organisation for Plantwise since it takes the lead in provision of agricultural extension services in the country (Scheidegger and Graf, 2013; Muyanga and Jayne, 2006). According to clinic records, a total of 8,699 female and 12,928 male farmers visited plant clinics between 2012 and June 2016. This means that women are under-represented at plant clinics and the proportion of female queries is on average half that of males, which does not reflect the proportion of female to male input to agriculture (Surridge and Rufsana, 2015).

Plantwise gender-disaggregated data on the number of queries per crop indicates gender differences in the types of crops taken to the clinics with male farmers taking mostly cash crops while female farmers take food crops. Data from the Kenyan agriculture sector shows that women do 80% of the food production, 50% of cash crop production, 80% of the food storage and transport from farm to the home, 90% of the weeding, and 60% of the harvesting and marketing of crops (GoK, 2007). This is consistent with Africa's agricultural sector, where women are responsible for producing 80% of the food as opposed to men who tend to engage more in income generating activities such as cash crop production, perhaps because of their responsibility of availing food for the family (Doss, 2001; FAO, 1998). Surridge and Rufsana (2015) established that there were differences in gender relations and division of labour at the household level even though the clinics were located in the same region. Women are involved in reproductive, productive and community activities in the different plant clinic areas and also interact with other service providers within their communities. There are however likely to be differences in gender roles in relation to farming and access to and control of resources in different clinic areas since there are differences in crops grown, farming systems and activity seasonal calendars. The different genders; adult male/female and youth male/female are likely to have different priorities, interests and needs in terms of plant clinic services and their time and availability.

The lower clinic attendance by women indicates that they lack information on key plant health problems about crops they prefer to grow. This is because providing information to one spouse (usually the husband) does not mean that the other spouse also learns about options and opportunities that meet their needs (Bernier et al., 2015). Women will miss the opportunity to receive advisory services that would enable them use effective and safe plant health management strategies to increase crop yields and have enough food and income for their families. It is necessary to understand the environment plant clinics operate in and involve the women in these communities to identify the reasons for low clinic attendance and possible strategies to reach more female farmers.

This study used a gender lens to examine the differences in use of plant health information services with a specific focus on plant clinics. In this study, gender refers to the social attributes and opportunities associated with being male and female and the relationships between women and men and girls and boys as well as the relations between women and those between men (EIGE, 2017). These attributes, opportunities and relationships are socially constructed and are learned through socialization processes. They are context/ time-specific and changeable. Gender determines what is expected, allowed and valued in a woman or a man in a given context. In most societies there are differences and inequalities between women and men in responsibilities assigned, activities undertaken, access to and control over resources, as well as decision-making opportunities. Gender may also be conceived of as adult men, adult women and youth. The new constitution of Kenya defines youth as all individuals in the republic of Kenya who have attained the age of 18 years but have not attained the age of 35 years (GoK, 2010). Gender is part of the broader socio-cultural context. Other important criteria for socio-cultural analysis include class, race, poverty level, ethnic group and age (EIGE, 2017). Whereas it is acknowledged that there are many gender categories, this study concentrated on two main gender categories that are adult men and adult women. This is because very few youth are involved in agricultural production or seek plant health information. The study examined sources of plant health advice as well as drivers and barriers to the use of plant clinics by men and women. In addition, the study developed recommendations on possible ways to make clinic services more attractive to men and women

MATERIALS AND METHODS

Data entered in the Plantwise Online Management System (POMS) between January 2012 and June 2016 were analysed for the 122 plant clinics and the ratio of male to female farmers calculated. Purposive sampling was used to select sites for the study. Clinics with the highest total attendance but a low proportion of women were selected from different regions. Study areas were adequately spatially separated to account for differences in agricultural potential based on differences in agro-ecological zones and ethnic groups hence diversity in the crops grown by farmers. Three clinics, Matumbei in Western Kenya, Kibugu in Central Kenya and Kauti in Eastern Kenya were selected for the study.

Stratified random sampling was used to select farmers who were interviewed in the survey using questionnaires. A minimum of 120 male and female farmers that is 30 male and 30 female users and 30 male and 30 female non-users were interviewed from four to eight villages in each clinic area. This translated to a sample size of 118 and 119 women and men users of plant clinics. The non-users were 138 and 156 men and women farmers, respectively. The list used to select clinic users was generated from POMS while for the non-users farmer lists kept by the agricultural extension officers were used. A total of 12 focus group discussions (FGDs) were held,

Table 1. Percentage of farmers interviewed in each region by gender and age group.

Age category	Kauti		Kibugu		Matumbei	
	Men (N=89)	Women (N=99)	Men (N=91)	Women (N= 85)	Men (N=77)	Women (N=90)
<21	2.2	2.0	0.0	1.2	3.8	2.2
21-30	4.5	6.1	8.7	12.9	33.7	18.9
31-40	21.3	23.2	16.5	22.4	18.2	16.7
41-50	19.2	18.2	33.0	31.8	14.3	27.8
51-60	24.7	31.3	12.1	17.6	15.8	23.3
61-70	22.5	13.1	14.3	10.6	6.5	7.8
>70	5.6	6.1	15.4	3.5	7.7	3.3

Table 2. Average farm size (acres) by gender.

Plant Clinic Area	Men	Women	Both men and women
Kauti	2.5 (2.23)	2.0 (1.68)	2.2 (1.97)
Kibugu	1.6 (1.52)	1.2 (1.40)	1.4 (1.48)
Matumbei	1.9 (1.38)	2.1 (1.63)	2.0 (1.52)
All clinic areas	2.0 (1.80)	1.8 (1.63)	1.9 (1.72)

Values in parentheses are standard deviations.

four in each clinic area, using FGDs checklist. Each FGD had 10-25 members that is, male users and non-users, female users and non-users of plant clinics separately. Those interviewed included different age groups (Table 1). Data collected were analysed using descriptive (mean, percentages, standard deviations and frequencies) and inferential statistics (Chi-Square and F-tests) for quantitative data and thematic analysis for qualitative data from the FGDs.

RESULTS AND DISCUSSION

Most farmers had formal education that was mainly primary level (47 to 64%) and secondary level (31to 44%). Less than 5% had no formal education and 1 to 5% had either middle level college education or a university degree. Respondents from Matumbei had the lowest education level, with more than 63% indicating that they had only schooled up to the primary level. This means that the farmers had low levels of education, which is consistent with small scale farmer categories in the African context. More educated persons look for white collar jobs because farming is assumed to generate low incomes that take a long time to be forthcoming. Female farmers had on average relatively lower levels of education compared to their male counterparts. The differences in education levels was statistically significant (χ^2=22.80, p<0.01). The average farm size of those interviewed ranged between 1.38 acres to 2.23 acres. There were significant differences ($F_{528, 2}$=12.21, p<0.01) in farm size across the three areas studied, with Kauti having the highest mean acreage and Kibugu the lowest (Table 2). Male farmers owned larger pieces of land than female farmers, but the differences were not statistically significant ($F_{528, 1}$ = 8.83, p>0.05).

There were statistically significant differences in farm size between plant clinic users and non-users ($F_{528, 1}$=15.95, p<0.01) with clinic users having relatively larger parcels of land (Table 3). Focus group discussions revealed that the plant clinic users were more active in farming and sought avenues to increase farm size to assure increased agricultural production.

There were significant differences in farm area under crops across the three study areas ($F_{528, 2}$=7.25, p<0.01) with Matumbei and Kauti having larger average acreage than Kibugu. Male farmers had significantly more acreage of land under crops compared to female farmers ($F_{528, 1}$=5.75, p<0.05). In Kenya generally men are considered the land owners and this may explain why on average women have smaller land parcels. A study by Bernier et al. (2015) revealed that women rarely consider themselves land owners. Youths especially females below 30 years had the lowest land under crops (Table 4). The youth, irrespective of whether they are female or male are less interested in agricultural production. In the perspective of most youths, agricultural production has low and slow return to investment. In addition agricultural

Table 3. Average farm size (acres) among plant clinic users and non-users

Plant clinic area	Plant clinic use	Men	Women	Both men and women
Kauti	Users	2.9 (2.13)	2.3 (1.79)	2.6 (1.97)
	Non-users	2.2 (2.29)	1.7 (1.55)	1.9 (1.93)
Kibugu	Users	1.9 (1.73)	1.8 (1.67)	1.8 (1.69)
	Non-users	1.3 (1.30)	0.6 (0.72)	1.0 (1.12)
Matumbei	Users	2.1 (1.26)	2.3 (1.58)	2.2 (1.41)
	Non-users	1.8 (1.47)	2.0 (1.67)	1.9 (1.59)

Values in parentheses are standard deviations.

Table 4. Average farm area (acres) under crops by gender and age category (years).

Age category	Kauti		Kibugu		Matumbei	
	Men (N=89)	Women (N=99)	Men (N=91)	Women (N=85)	Men (N=77)	Women (N=90)
<21	1.00	0.38	0.00	0.5	1.00	1.00
21-30	0.88	1.25	0.79	0.48	1.24	1.19
31-40	1.49	1.28	0.91	0.56	1.63	1.23
41-50	1.25	1.74	1.47	1.07	1.98	2.13
51-60	2.44	1.79	1.12	1.92	1.67	1.65
61-70	2.86	2.18	1.63	1.47	3.45	1.36
>70	4.71	1.46	2.36	1.67	2.95	1.42
All Age categories	2.13	1.63	1.43	1.09	1.73	1.58

activities are presumed to be labour intensive.

Sources of plant health advice

Farmers received plant health advice from different sources which included plant clinics. There were significant differences in the usage of different plant health information sources across the three regions (χ^2=51.77, p<0.01). Plant clinics and government extension workers ranked highest among male and female farmers across the three regions. Farmers who cited government extension workers as sources of plant health advice interacted with them in their capacity as extension agents and not at the plant clinics. There were significant differences (χ^2=14.65, p<0.01) in the use of information sources by female and male farmers. More male than female farmers seek plant health advice from government extension workers in Kauti and Matumbei, while in Kibugu more females than males seek plant health advice from this source. More males than females in Matumbei reported that they sought plant health advice from agro-input dealers, while more women than men reported getting advice from this source in Kibugu. Farmer groups ranked highest in Kibugu, and radio highest among males in Matumbei (Table 5).

Use of different sources of information by men and women in the different clinic areas was cross checked using focus group discussions in all the areas. Findings from the FGDs were consistent with those from individual interviews. This in practice means that prioritization of the different sources in different areas according to gender can be effectively used as a basis for dissemination of crop protection advice.

There were however significant differences (χ^2=11.44, p<0.01) in the sources of agricultural information between plant clinic users and non-users. All farmers who sought plant health advice from the internet, Kibugu coffee factory or used their own knowledge were non-users of plant clinics. Further, more than 70% of the non-users reported receiving plant health advice from agro-input dealers, women groups and family (Table 6).

Plant clinic users had more lead farmers, government extension and farmer groups as sources of advice. This suggests that plant clinic users are more proactive farmers in terms of seeking plant health advice and

Table 5. Percentage of respondents receiving Plant health advice from different sources across the three study areas.

Sources of Plant health advice	Kauti		Kibugu		Matumbei		All study areas	
	Male (N=89)	Female (N=99)	Male (N=91)	Female (N=85)	Male (N=77)	Female (N=90)	Male (N=257)	Female (N=274)
Government extension worker	21.2	11.8	9.3	11.3	12.9	12.7	14.4	11.9
Plant clinic	13.2	17.5	16.6	20.2	19.3	14.7	16.3	17.4
Agro input dealer	14.6	17.9	15.4	16.5	12.8	11.7	14.4	15.5
NGO extension worker	4.7	1.7	4.0	2.6	0.6	2.0	3.3	2.2
Friends and neighbours	17.9	17.9	20.3	17.0	23.4	21.3	20.3	18.7
Family	7.1	9.2	6.2	8.2	12.3	14.2	8.3	10.5
Lead farmer	5.3	4.4	7.0	3.6	1.8	1.5	4.9	3.2
Farmer group	8.5	4.8	10.6	8.2	4.1	4.6	8.0	5.8
Women group	0.0	9.2	0.0	6.7	0.0	6.2	0.0	7.4
Radio	6.1	5.2	9.3	5.2	10.5	9.6	8.5	6.6
Internet	1.4	0.0	0.9	0.5	1.1	0.0	1.1	0.2
Local leader	0.0	0.4	0.4	0.0	1.2	1.5	0.5	0.6

Table 6. Percentage of users and non-users of Plant clinics receiving Plant health advice from different sources.

Sources of plant health advice	Plant clinic users (%)			Plant clinic non-users (%)		
	Men (N=119)	Women (N=118)	Both men & women (N=237)	Men (N=138)	Women (N=156)	Both men and women (N=294)
Family	17.6	14.4	16.0	21.0	30.8	26.2
Lead farmer	14.3	11.9	13.1	9.4	3.8	6.5
Women group	0.0	15.3	7.6	0.0	17.9	9.9
Govt. extension officer	44.5	42.4	43.5	25.4	15.4	20.1
Plant clinic	100.0	100.0	100.0	0.0	0.0	0.0
Radio	19.3	12.7	16.0	21.0	16.7	18.7
Friends and neighbours	38.7	35.6	37.1	56.5	47.4	51.7
Local leader	1.7	0.0	0.8	0.7	2.6	1.7
Farmer group	23.5	17.8	20.7	15.2	9.6	12.2
NGO extension worker	10.1	5.9	8.0	5.8	3.8	4.8
Agro-input dealer	33.6	36.4	35.0	34.8	34.0	34.4
Internet	2.5	0.8	1.7	2.9	0.0	1.4

association amongst themselves. There were similarities between plant clinic users and non-users with respect to accessing information from agro-dealers. This is expected because both users and non-users of plant clinics obtain crop protection chemicals from agro-dealers.

Farmers gave various reasons for their most preferred choice of plant health advice. Those who preferred government extension workers stated they were more accessible and could visit farms, conduct on-farm demonstrations and they were generally very knowledgeable. Farmer groups were preferred by some farmers because they provided a platform to share their knowledge and experiences with each other, organized education days and other fora where experts educate farmers and also facilitated farmers' access to farm inputs by providing credit facilities. Some farmers preferred agro-input dealers because of their good knowledge about agro chemicals; they are easily accessible and operate on a daily basis, stock a wide variety of agro-chemicals and are experts in agriculture. Sources preferred by women in the different locations suggest that women prefer to receive advice from other women, especially female farmers, or those that they know. In addition, advice provided should reflect the needs of women farmers in terms of capacities and access to resources including time and physical inputs used in the production processes.

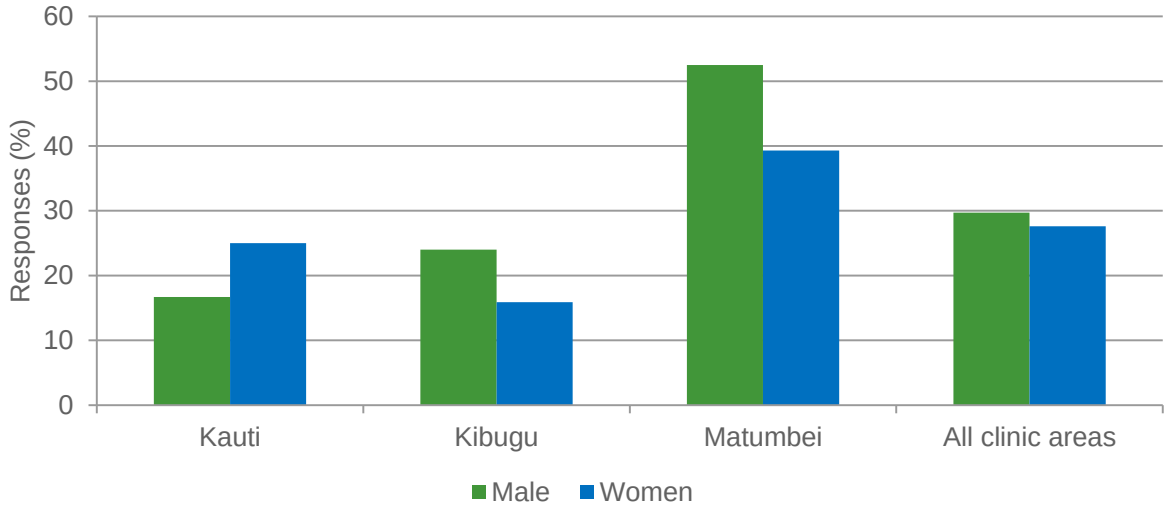

Figure 1. Percentage of respondents who knew about Plant clinics.

Awareness of plant clinics

Only 28.6% of the non-users knew about plant clinics, with slightly more male than female farmers (Figure 1). However, the difference between men and women was not statistically significant (χ^2=2.57, p>0.05) for all the plant clinic areas. These results suggest that one of the factors stopping the use of plant health advice from the plant clinics is lack of awareness. Women farmers were more disadvantaged in this regard because a relatively smaller number of women were aware of the plant clinics. Given the lack of statistical significance in difference between men and women who were aware of plant clinics the same awareness creation mechanisms could be used for both men and women.

Ten per cent of those who did not know about plant clinics prior to the interview reported that they would not attend the plant clinics even after they knew about them. The reasons they gave were that the plant clinics were too far from their homes; the time was not suitable, they were always very busy with other work or they did not need plant health advice. These reasons were similar for both men and female farmers.

Plant clinic attendance and number of visits

Focus group discussions revealed that on average, most of the plant clinic users had been to the clinic once or twice, mostly with different crops and crop health problems, or the same crops but different problems. Male farmers had been to the clinics more often than female farmers, with Kibugu having the highest number of average plant clinic visits by male farmers. More than 90% of respondents stated that gender of the plant doctor would not influence their visits to the clinic with the remainder stating that they preferred either a male or a female plant doctor. Knowledge about plant clinics was strongly positively correlated with plant clinic attendance.

Farmers reported that they took different lengths of time to reach the plant clinics because they come from different villages and the distances vary. On average, most farmers both male and female took 30 min to reach the plant clinic in Kauti and Kibugu with most farmers in Matumbei taking 10 min. This is because most plant clinic users in Matumbei live near the market where the clinic is usually held. In all regions there were some farmers who took 1 h or more to reach the plant clinics either due to distance or means of transport. Some farmers reported that they took longer time during the rainy season due to slippery soils. The association between frequency of plant clinic attendance and length of time it took to reach the clinic was however not statistically significant. The preferred time for clinic visits was not the same for men and women farmers. More farmers visited between 8 am and 11 am in Kauti, 8 am to 2 pm in Matumbei and 2 pm to 5 pm in Kibugu (Figure 2).

There are regional differences in patterns, as well as the gendered differences within the regions. This indicates that it is not accurate to make broad assumptions about gender patterns and to ensure Plantwise work is truly gender responsive or even transformative there is need to understand the local context in which the activities are undertaken. It is also necessary to take time to conduct analysis before the start of a project to ensure it is going to address the needs of both women and men in that particular community. Policies and an enabling environment should

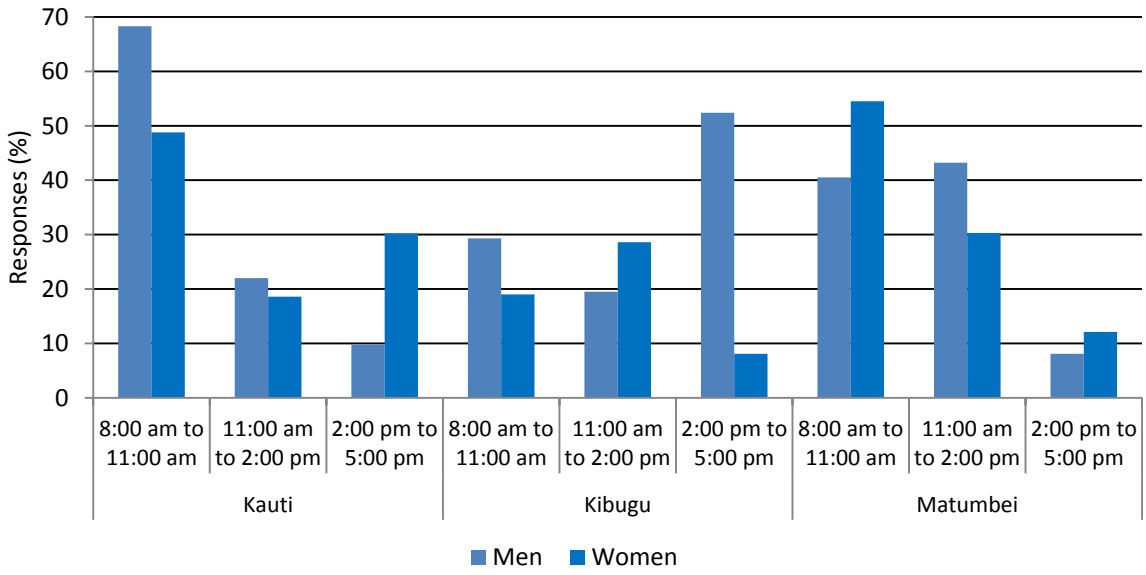

Figure 2. Percentage of respondents attending plant clinics at the specified times.

be in place, but action is necessary at the local level (Cathy et al., 2013). The appropriate plant clinic approach for reaching men and women farmers equitably will differ across regions. This assertion is consistent with the views of Cristina et al. (2013) who argue that it is necessary to adapt gender-responsive techniques and methods to local context. In this regard plant clinic operations need to take cognizance of gender and social norms that influence women's time, mobility and education.

Farmers across the study sites indicated the day clinics were usually held as Tuesday for Kauti, Friday for Kibugu and Monday for Matumbei. Some farmers, especially women would however like the clinics to be held on Saturdays since their children would be at home to look after the homestead while they were away. Most farmers, both male and female across the three study areas indicated that they attended the clinic once a month, with the least numbers in Kauti and Matumbei saying that they attended the clinic weekly. There were no major variations between the genders (Figure 3).

Thirty two per cent of the plant clinic users reported that they had sent someone to the plant clinic on their behalf (Figure 4). About 91% received prescriptions given at the plant clinic while 99% used the recommendations. All clinic users who sent somebody to the clinic found prescriptions effective and were willing to go back to the clinics. Sixty eight per cent of the plant clinic users had not sent anyone to the plant clinic on their behalf. Out of all those who did not sent anyone to the clinic, 96% found the recommendations worked and they would still go back to get services from the clinics. Four per cent said the recommendations given did not work but they were still willing to go to the plant clinics. More than 90% of the respondents said the gender of the plant doctor would not influence their visits to the clinic with a few saying they preferred either a male or a female plant doctor.

Reasons for farmers not visiting plant clinics

Distance from home/farm to the plant clinics posed a challenge to both male and female farmers, especially those who had to cover long distances to reach the clinic. This agrees with the work of Nambiro et al. (2005) that farmers who live close to a source of extension advice are more likely to seek its services. Where plant clinics are further away, women will be more disadvantaged. This is because men are generally more mobile than women and while they may not primarily travel to look for agricultural information they are more likely to access this information than the women who are less mobile (Jost et al., 2016). Ignorance, which was perceived as not knowing plant clinics as well as the benefits and functions of the clinic contributed to failure to visit plant clinics especially among the female farmers. Lack of awareness was attributed to lack of advertising of the clinic venue, time and date. There were instances of long queues at plant clinics and as a consequence farmers took too long to be served by the doctors. Frequency/regularity of clinic days was sometimes not suitable, especially when there was an outbreak requiring immediate attention on a non-clinic day. There were delayed results when a plant doctor was not able to diagnose the problem immediately and had to consult further.

Some women farmers had the view that clinics were

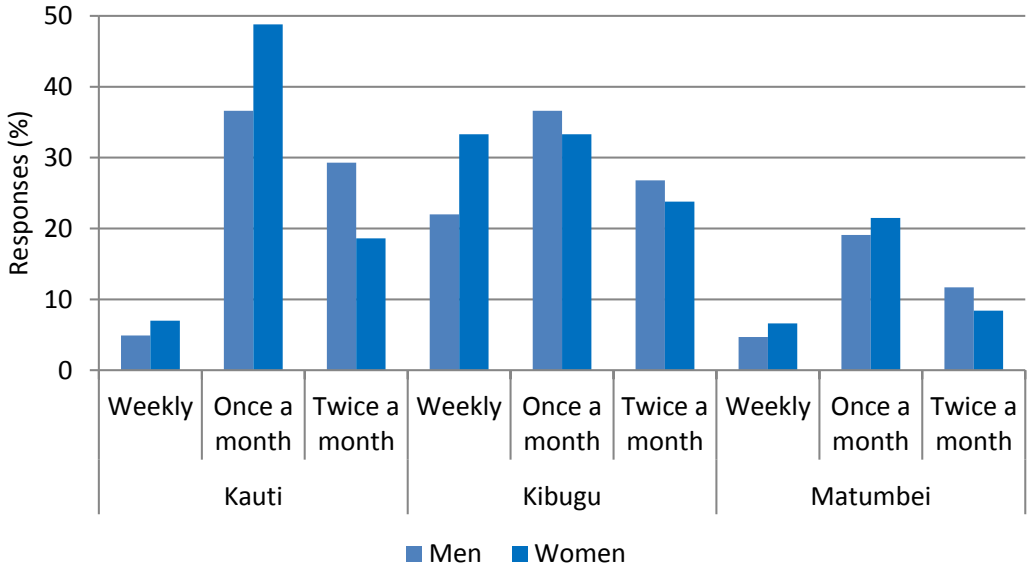

Figure 3. Frequency of clinic attendance across the three study regions by gender.

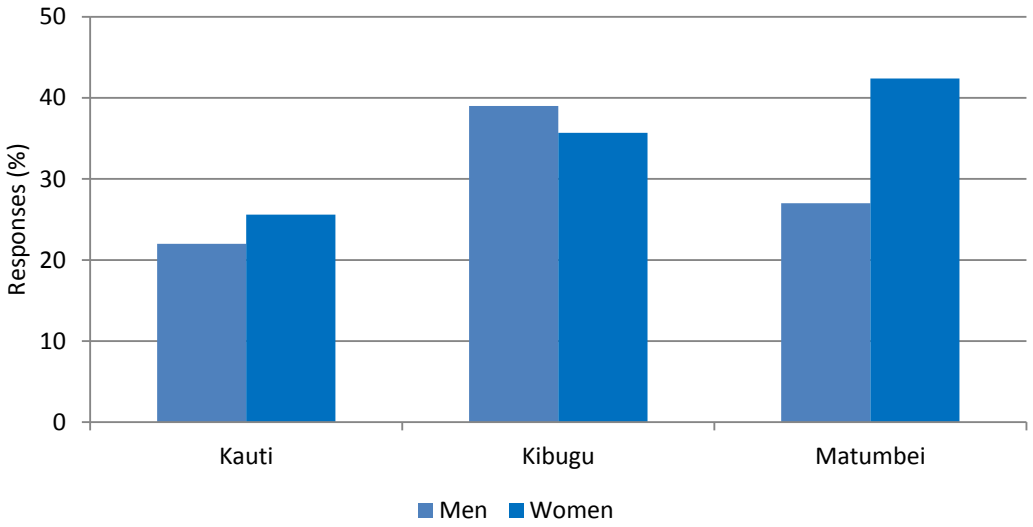

Figure 4. Percentage of respondents who sent someone to the Plant clinic on their behalf.

only for men, and for certain crops. The level of education was relatively low among the female farmers thereby compromising their capacity to understand plant clinic recommendations and discouraging them from attending. Female farmers reported that sometimes they could not make it to the clinic because they did not have someone to look after the homestead in their absence. Clinic time/duration, day and venue were not suitable to female farmers in some cases. In some instances the farmers noted that the venue was not permanent and was too open. The schedules were sometimes confusing especially in places where the clinic was held fortnightly as opposed to weekly.

The reproductive roles of women were in some cases incompatible with the timing of plant clinics. This finding agrees with Loagun (1998) who found that women in rural areas undertake many responsibilities concerning care and management of the family and farm animals. The burden of women was aggravated in certain instances by the need to obtain permission from

husbands before proceeding to attend plant clinics. Plant clinics were in an open environment that did not appear conducive to some women to present plant specimens in a cordial manner. This suggests that more direct action needs to be taken to site clinics where women can easily attend or to ensure that women realise that plant clinics are also meant for them. Some female farmers failed to visit the clinics because they felt that the advice given would need a lot of money to implement or would need a lot of physical strength which they may not have. Labour intensive and expensive technologies are less likely to be adopted by female farmers due to their limited access to labour and cash (Jost et al., 2016).

Conclusion

Farmers across the three regions received plant health advice from different sources. The main sources of plant health advice were plant clinics and government extension workers followed by agro-input dealers. Other sources were friends and neighbours, farmer groups, radio, women groups, lead farmers, family and own experience. There were significant differences in sources of information preferred by women and men, which calls for prioritization of sources depending on gender for effective information dissemination. In addition, various reasons were adduced for the preference of sources of information, reflecting the diversity in resource base and access options.

In order to increase plant clinic attendance by female farmers more awareness creation about plant clinics and services offered should be done through plant health rallies, branded t-shirts and caps, making announcements through radio, SMS and during chiefs' *barazas* and churches and other public places. More plant clinics need to be started to reach more farmers who might not be able to travel long distances to attend the plant clinics. In addition, more plant doctors should be trained to manage and cover more plant clinics. It would also be necessary to train plant nurses/lead farmers who would be easily accessible to all farmers. All these should be accompanied by an increase in the number of clinic sessions from twice a month to once per week. It is necessary to schedule different clinic dates in the areas so that farmers who fail to attend a clinic session in one area could visit other nearby clinics. Plant doctors should tailor their advice to circumstances of the farmer or provide a range of options which the farmer can choose from. Plant doctors should give reference materials at the plant clinic, as well as the prescription form.

It is also important to adopt a flexible approach to the clinic model to try and meet the needs of different farmers. Such approaches may include mobile clinics and plant nurses/lead farmers in order to reach farmers who live far away from the clinic area. It may also involve dealing with farmer (women) groups by explaining diagnosis and recommendations about different pest and diseases to the group. In this way plant doctors would be able to reach more farmers in a short time. These methods may also help to address obvious resource constraints in increasing the numbers of plant clinics as suggested above.

Plant clinics should make available reading materials such as manuals, booklets and posters specific to the area to be used as a point of reference by farmers to solve their problems. The materials made available should be in English, Kiswahili and the local language. Plant clinics should have latest technology and modern equipment to help in diagnosis of diseases e.g. soil analysis equipment, microscopes. They should pass new information on pest control options to farmers as soon as possible through sensitization. This would enable farmers to effectively control pests and diseases and hence improve farmer confidence in plant clinic services.

There were regional differences in patterns as well as gendered differences within the regions with respect to sources of plant health advice and use of plant clinic services. This means that it is not accurate to make broad assumptions about gender patterns. In order to ensure efficient and effective use of plant clinics as well as a have gender responsive and/or transformative plant clinics it is necessary to understand the local contexts in which the plant clinics are located. This would ensure that the plant clinics address the specific needs of both men and women in the different communities, and assure improved use by women farmers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Proximate, physical and chemical composition of leaves and seeds of Moringa (*Moringa oleifera*) from Central Malawi: A potential for increasing animal food supply in the 21st century

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The nutritive composition of Moringa (*Moringa oleifera* Lam) leaves and seeds were evaluated for their possible inclusion in livestock feed formulation because of the limited availability of conventional protein concentrates like soybean (*Glycine max)* seeds. Moringa seeds contained 50.70 g kernels and 19.03 g hulls per 100 seeds representing 72.71 and 27.29% as a fraction of the whole seed, respectively. The 100 seeds contained 28.48 and 20.71% oil as a fraction of kernels and seeds, respectively. Moringa leaves had 22.60±0.17% crude protein, 11.24±0.17% ash, 13.40± 0.25% crude fat, 8.07±0.17% crude fiber and 44.69±0.41% carbohydrates. The seeds revealed 28.56±0.41% crude protein, 5.37±0.11% ash, 34.92±0.17% crude fat, 7.90±0.27% crude fiber and 23.27±0.65% carbohydrates. Raw kernel recorded 37.86±0.38% crude protein, 4.60±0.13% ash, 41.18±0.06% crude fat, 4.80±0.23% crude fiber and 11.55±0.37% carbohydrate whereas roasted kernel registered 38.25±0.32% crude protein, 5.36±0.19% ash, 41.06±0.14% crude fat, 6.55±0.34% crude fiber and 8.78±0.60% carbohydrate. Raw kernel meal registered the highest calculated gross energy of 5.6±0.0 Mcal/kg DM and metabolisable energy of 4.4±0.0 Mcal/kg DM, compared to seed and leaves meals. The calculated fatty acid (g/kg DM) was the highest (329.5±0.5) in raw kernel compared to 107.2±2.0 in leaves, 279.3±1.4 in seed and 328.5±1.1 in roasted kernel meal. Titratable acidity (as oleic acid) ranged from 0.36±0.0 at pH 6.42±0.0 to 3.8±0.0 at pH 6.35±0.0 for raw kernel and leaves meal, respectively. Phosphorus concentration (mg/100 g DM) ranged from 427.6±0.0 to 873.9±0.0 for leaves and raw kernel meals. This research indicated that both seeds and leaves are rich in nutrients and could be potential replacements of conventional livestock feed ingredients to ease the feed/food crises in Malawi.

Key words: Moringa (*Moringa oleifera*), raw kernel, roasted kernel, crude protein, livestock production, Malawi.

INTRODUCTION

Global demand for livestock products is expected to increase due to escalating population growth, emerging economies and urbanization by 2050 (Thornton, 2010). Human population is expected to reach 9.6 billion with that of developing countries, Malawi inclusive, increasing five times by 2050. Human population in emerging worlds

would rise to 8.2 billion by 2050 reaching 9.6 billion by the 21st century (UNPD, 2012). The escalating human population coupled with urbanization and improved income may increase demand for food of animal origin such as meat (Thornton, 2010; Thewis and Galis, 2012) to 12 million metric tons (MMT) from 5 MMT in the duration of 17 years by 2020 in sub-Saharan Africa (SSA) (Delgado et al., 1999). The exploding demand for animal products will surge global livestock production (Thornton, 2010) with a consequent annual and total global increase of cereal animal feed to 292 and 928 MMT from 1993 to 2020 (Delgado et al., 1998; Delgado et al., 1999). Milk and meat consumption is universally projected to reach 880 and 452 MMT with that of developing countries projected to be 326 and 585 MMT by 2050 (FAO, 2006). Annual cereal consumption as animal feed in SSA by 2020 is estimated to be 4 MMT representing a 2.3% growth rate (Delgado et al., 1998) surging prices of cereals and legumes such as maize (*Zea mays* L.) and soybean (*Glycine max*) (Thornton, 2010; Thewis and Galis, 2012).

Furthermore, global warming is expected to both positively and negatively affect the production of crops resulting in 10 to 20% crop yields reduction in the tropics and sub-tropics by 2050 (Jones and Thornton, 2003). Cereal production, in SSA, would decline by 3.2% due to global warming resulting in 4.0% increase in maize price by 2050 (Ringler et al., 2010). Maize productivity which is a staple food for many SSA countries, Malawi inclusive, may decline by 10% (Hellin et al., 2012) and 22% (Schlenker and Lobell, 2010) reaching an average price of 4.0% by 2050 (Ringler et al., 2010). The scarcity and high prices of cereals and grain legumes like maize and soybean would be expected to increase animal products prices (Thewis and Galis, 2012).

Therefore, seeds and leaves of tropical and sub-Saharan plants could be investigated for the possibility of their inclusion in feed formulation to meet up with demand for animal products of the global surging population, urbanization and improved income. One of these tropical and SSA plants is Moringa (*Moringa oleifera* Lam). Moringa grows well in both acidic and alkaline soils and is drought resistant (Mughal et al., 1999). Moringa tree grows in humid and hot, dry tropical and subtropical regions (Sultana et al., 2015). It grows from about 5 to 15 m (Somali et al., 1984) with slender and droopy branches (Siddhuraju and Becker, 2003). *M. oleifera* is a fast growing tree with feathery foliage and innate leaves (Rolof et al., 2009). Moringa leaves, pods and seed are used as vegetables and oil extraction, respectively (Rebecca et al., 2006). It is reported that Moringa leaves have 23.61% crude protein (Abou-Elezz et al., 2011) and 4.50±0.10% crude fat observed in Nigeria (Offor et al., 2014). *M. oleifera* seeds contain 300 g/kg crude protein (Madubuike et al., 2015) and 348 g/kg oil (Anwar and Rashid, 2007). Research has been conducted to study the effects of Moringa leaf meal on the growth of layer chicks, productivity of layers and growth of broiler chicks (Olugbemi et al., 2010; Abou-Elezz et al., 2011; Melesse, 2011).

However, there is a paucity of information on the nutritive value of seeds and leaves of *M. oleifera* cultivated in Malawi for its inclusion in livestock feed formulation. Therefore, this study was conducted to analyze the proximate and chemical composition of *M. oleifera* seeds and leaves meal with an objective of addressing food/feed crisis in Malawi.

MATERIALS AND METHODS

M. oleifera sampling and sample preparation

M. oleifera L. leaves and seeds were collected from Mr. Goodson Dawa's house, who resides in the area surrounding Bunda in Lilongwe district. The leaves and seeds were sundried at temperatures above 37°C for 36 and 8 h, respectively (Brennand, 1994; Ahmed, 2013) and some of the seeds were dehulled for further analyses of kernels (Figure 1). Some of the kernels were roasted in the oven, at 130°C for 30 min (Adeyeye, 2010), to evaluate heat effects on the nutrient contents. The sundried leaves, raw kernels, roasted kernels and seeds were ground through a 1 mm sieve using a Thomas-WILEY model 4 Laboratory Mill before analyzing the chemical properties.

Moringa physical characteristics determination

The seeds were evaluated for weight and oil content whereby 100 seeds were randomly selected, thoroughly cleaned and weighed on a JP-2000 electronic balance to the nearest 0.01 g. The 100 clean seeds were dehulled and the kernels and hulls were weighed on the same JP-2000 electronic balance. *M. oleifera* dimensional axes were measured by an electronic digital Vernier Caliper with an accuracy of 0.02 mm. The arithmetic (Da) mean, geometric (Dg) mean and sphericity (Ø) of the kernel were calculated using the following equations (Mohsenin, 1986).

$$Da = L + W + T / 3 \quad (1)$$

$$Dg = (LWT)^{\frac{1}{3}} \quad (2)$$

$$\emptyset = (LWT)^{\frac{1}{3}}/L \quad (3)$$

where L is the length of the kernel, W is the width of the kernel, T is the thickness of the kernel, and Ø is the sphericity of the kernel.

Chemical determination

The ground samples were used to analyze for proximate

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Figure 1. A: *Moringa oleifera* seeds, B: *Moringa oleifera* hulls, C: *Moringa oleifera* kernels.

composition: dry matter (DM), ash, crude protein, crude fat and crude fiber using AOAC (1996) methods. Chemical analyses like titratable acidity as oleic acid, pH and phosphorus (P) were also analyzed from the ground samples.

Determination of dry matter content using oven drying method

Dry matter was determined by drying the samples in a laboratory drying oven at 105°C for 5 h. The crucibles were thoroughly washed, dried in the oven, cooled in a desiccator and weighed. 2.5 g of the sample was weighed into the crucible and dried to constant weight. The dry matter in percentage was calculated as the fraction of the original dry weight multiplied by 100 (AOAC, 1996).

Determination of ash content using muffle furnace

Ash content was determined by igniting 2.5 g of the samples, weighed in crucibles, in the muffle furnace at 550°C for 2 h. The amount of ash content in percentage was calculated as follows (AOAC, 1990):

$$\% \, Ash = \left[{}^{Wa \, - Wt}/_{Wo \, - Wt} \right] \times 100 \quad (4)$$

where W_0 is the weight of crucible and sample before igniting the sample, W_a is the weight of crucible and ash and W_t is the weight of crucible only.

Determination of crude protein using micro-Kjeldahl method

Nitrogen (N) content of the samples was analyzed by using micro-Kjeldahl method and the N content was converted to CP by multiplying by 6.25. The method involves digestion of the samples in concentrated (98%) sulphuric acid, distillation of the digests into weak acids (4% boric acid) and titration of the distillates with 0.1 M hydrochloric (HCl) acid using mixed indicator (Methyl and Bromocresol green) as an indicator (AOAC, 1990).

Determination of crude fat content

Crude fat was analyzed by extracting 2.5 g of the sample weighed in porous extraction thimbles by using petroleum ether in a soxhlet apparatus for 16 h. The soxhlet apparatus was equipped with a water cooled condenser fitted above the 250 ml flat bottomed flask containing petroleum ether as fat solvent. The solvent was boiled at 40°C and fat content was calculated as a percentage of the dry weight of the sample (AOAC, 1996).

Determination of crude fiber content

Crude fiber was determined by boiling 2.0 g of the samples in 200 ml of weak sulphuric acid (1.25%) and sodium hydroxide (1.25%), with few drops of anti-foaming agents being added, for 30 min. The residues were filtered and washed three times with hot water, then washed with 95% ethanol and dried at 105°C for 5 h to constant weight. The dried residues were ignited in a muffle furnace at 550°C for 2 h. The crude fiber in grams was calculated as the difference between the weight of the residues and ash and converted as a fraction of the sample weight in percentages (AOAC, 1990).

Determination of carbohydrate content by difference

Carbohydrates were calculated by difference using the following formulae (AOAC, 1990):

$$100 \, \% - (CP \, \% + CF \, \% + Crude \; fat \, \% + Ash \, \%) \quad (5)$$

Determination of titratable acidity

Titratable acidity (TA) was determined by dissolving 2.0 g of the sample in 100 ml of distilled water which was titrated with 0.1 M NaOH using phenolphthalein as an indicator. TA was calculated per 100 g of the sample and was converted to oleic acid per 100 g DM sample by multiplying by the molecular mass of 0.282 g as follows (AOAC, 1999, 1996):

$$TA \; (g \; 100^{-1} \; g \;) = \left[{}^{V_{NaOH} \; \times M_{NaOH} \; \times 0.282}/_{2.0} \right] \times 100 \quad (6)$$

where V_{NaoH} and M_{NaoH} are volume (titre volume) and molarity of sodium hydroxide, respectively.

Determination of pH using pH meter

In determination of pH, 2.0 g of the sample was dissolved in 100 ml of distilled water and the pH was measured by using GLP pH meter at 25°C (AOAC, 1999).

Table 1. Physical characteristics of *Moringa oleifera* Lam seed.

Parameter	Value
Weight of whole seed (kernel + shells) g/100 seeds	69.73
Weight of kernels g/100 seeds	50.7
Kernel fraction (% of whole seed)	72.71
Shell fraction (% of whole seed)	27.29
Oil fraction (%w/w of kernel)	28.48
Oil fraction (%w/w of seed)	20.71

Phosphorus determination using UV- Vis spectrophotometer

Phosphorus was determined by weighing 1.0 g of each sample in porcelain crucibles which were ignited in a muffle furnace at 550°C to constant weight. The ash was dissolved in 3 ml of 3 M hydrochloric (HCl) acid, filtered and diluted to the 100 ml mark in a volumetric flask (Ogungbenle and Atere, 2014). 1 ml of the diluted filtrate was pipette into a 20 ml vials, 2 ml of ammonium molybdate-ascorbic was added and diluted to 10 ml with distilled water. Standards were prepared by pipetting 0.0, 0.2, 0.3, 0.4 and 0.5 ml of the stock solution into the 20 ml vial, 2 ml of ammonium molybdate-ascorbic solution was added and was diluted to 10 ml with distilled water. The absorbance of the solutions were measured after 1 h of color development and absorbance was measured at 860 nm wavelength using DR 5000 WAGTECH projects ultra-violet visible spectrophotometer (AOAC, 2005; Habib et al., 2015).

Fatty acid calculation

Calculated fatty acid in mass per kg of the sample was computed by multiplying crude fat values by a factor of 0.8 (Aremu et al., 2013).

Gross energy calculation

Gross energy (GE) in kJ/100 g DM was calculated by multiplying the values of CP, crude fat and carbohydrate by the factors of 17, 37 and 16 kJ/100 g DM, respectively (Dalziel, 1955; Osborne and Voogt, 1978). The calculated GE (kJ/100 g) was converted to kcal/100 g DM by dividing by a factor of 4.184 (Butcher et al., 2006) and the Kcal/100 g DM was converted to Mcal/100 g DM dividing by 1000 and finally Mcal/100 g was converted to Mcal/kg DM by multiplying by a factor of 10.

Metabolisable energy calculation

Metabolisable energy (ME) in MJ/kg DM was calculated by using the regression equation of Ellis (1981):

$$ME\ (MJ\ kg^{-1}) = 1.549 + 0.0102\text{CP} + 0.0275\text{Oil (crude fat)} + 0.0148\ \text{Carbohydrate} - 0.0034\text{CF} \quad (7)$$

where CP = crude protein, CF = crude fiber. The calculated ME in MJ/kg DM was then converted to Mcal/kg DM by dividing by a factor of 4.184 (Butcher et al., 2006).

Chemical data statistical analysis

Laboratory chemical analyses were done in triplicate and the mean value of each chemical parameter was calculated using Microsoft excel. The data was statistically analyzed by using analysis of variance (ANOVA) in Microsoft Excel ToolPak. Two sample T-test with unequal variances was used to compare mean values and significance was accepted at $P \leq 0.05$ level.

RESULTS AND DISCUSSION

Physical characteristics of seeds and kernels

The physical characteristics of *M. oleifera* seeds and kernels are shown in Tables 1 and 2. 100 *M. oleifera* seeds weighed 69.73 g. The 100 seeds contained 50.70 g kernels and 19.03 g hulls representing 72.71 and 27.29% as a fraction of the whole seed, respectively. In Tanzania, the same amount of *M. oleifera* seeds weighed about 29.6 to 30.2 g with 72.5 and 27.5% representing the fractions of kernels and hulls, respectively (Proyecto, 1996) which was in close correlation with results reported in this study. The physical characterization revealed that 100 g of Moringa seeds and kernels contained 20.71 and 28.48 g of oil, respectively.

The oil content in seeds observed in this study was lower than 38.33±0.65 g oil per 100 g of seeds observed in *Moringa peregrina* (Salaheldeen et al., 2014). The variations in the oil content between *M. oleifera* and *M. peregrina* species could be attributed to species and climatic conditions of the area of their cultivation (Yang et al., 2006).

M. oleifera physical parameters are significant in designing oil expellers, harvesting, processing and transportation machines (Ajav and Fakayode, 2013; Adesina et al., 2013). Moringa kernel length, width and thickness were 7.39±0.40, 6.92±0.47 mm and 6.16±0.56 mm, respectively. The arithmetic and geometric mean diameters were 6.82±0.35 and 6.79±0.35 mm with sphericity mean percentage of 92.08±3.88 mm. Moringa seed kernels have been reported to be 9.22±0.76 mm long, 8.43±0.59 mm wide and 7.42±0.85 mm thick (Adesina et al., 2013). The arithmetic and geometric means of Moringa seeds have been reported to be 7.56±0.87 and 7.49±0.88 mm with mean sphericity value of 88.8±5.2% (Ajav and Fakayode, 2013). The sphericity value of92.08±3.88 mm was comparably similar to 90.37±5.69 mm (Adesina et al., 2013) reported in Nigeria. The proximate composition of *M. oleifera* L. leaves, seed, raw kernel and roasted kernel meals in percentages are shown in Table 3. The results revealed that Moringa products are good sources of crude protein, crude fat, crude fiber and minerals in the form of ash.

Moringa dry matter content

Moringa leaves meal dry matter (DM) content was lower ($P < 0.05$) than that of seed, raw kernel and roasted kernel meals. The DM content in leaves meal was in agreement with 94.0, 93.07 and 93.09% for early, mid

Table 2. Physical characteristics of *Moringa oleifera* Lam seed kernels.

Physical property	N	Minimum	Maximum	Mean
Length (mm)	100	6.54	8.26	7.39±0.40
Width (mm)	100	5.98	8.11	6.92±0.47
Thickness (mm)	100	4.80	7.42	6.16±0.56
Arithmetic mean diameter (mm)	100	5.96	7.62	6.82±0.35
Geometric mean diameter (mm)	100	5.90	7.61	6.79±0.35
Sphericity mean %	100	81.99	98.65	92.08±3.88

Table 3. Proximate composition of *Moringa oleifera* L.

Sample	DM (%)	CP (%)	Ash (%)	Crude fat (%)	CF (%)	Carbohydrate (%)
Leaves	93.78±0.07^{a}	22.60±0.17^{a}	11.24±0.15^{a}	13.40±025^{a}	8.07±0.17^{a}	44.69±0.41^{a}
Seed	96.86±0.30^{b}	28.54±0.41^{b}	5.37±0.11^{b}	34.91±0.16^{b}	7.90±0.27^{a}	23.27±0.65^{b}
Raw kernel	97.40±0.43^{b}	37.86±0.38^{c}	4.60±0.13^{c}	41.18±0.06^{b}	4.80±0.23^{b}	11.55±0.37^{c}
Roasted Kernel	99.72±0.07^{c}	38.25±0.32^{d}	5.36± 0.19^{b}	41.06±0.14^{b}	6.55±0.34^{c}	8.78±0.60^{d}

DM: Dry matter, CP: crude protein, CF: crude fibre. For each parameter, means with same superscript were not significantly different (P>0.05).

and late maturity stage Moringa leaves observed in the previous study (Bamishaiye et al., 2011), whereas the DM value for seed meal was lower than 97.40±0.43% for raw kernel meal in this study.

Ash content

The total ash content (% DM) ranged from 4.60±0.13 to 11.24±0.17% for raw kernel and leaves meal, respectively. In this study, ash content for leaves meal was almost twice (P< 0.05) that of seed, raw kernel and roasted kernel meals, respectively. The ash content for seed meal of 5.37±0.11% was comparable to 4.5% for oil extracted seed meal (Abbas, 2013) and 5.06±0.03% (Barakat and Ghazal, 2016) reported in Sudan and Egypt but higher than 4.10±0.14% from another study in Nigeria (Abiodun et al., 2012). Moringa leaves meal ash value of 11.24±0.15% was higher than 5.7, 8.00 and 9.25% for early, mid and late maturity stage leaves observed in Nigeria (Bamishaiye et al., 2011). The ash values for leaves meal, seed meal, raw kernel and roasted kernel meals were higher than those of pigeon peas (*Cajanus cajan*) (3.2%) observed in Sudan (Eltayeb et al., 2010) and 4.6 and 4.0% for NRC-35 and JS446 genotype *G. max* L. seeds (Jain and Jain, 2010). The high mineral ash content observed from *Moringa* species growing in Malawi means the leaves and seed meal could be used in formulating livestock feed for body tissues functioning and health.

Crude protein content

Crude protein values ranged from 22.60±0.17 to 38.25±0.32% for leaves and roasted kernel meals, respectively. The raw and roasted kernel meals, in this study, had the highest (P<0.05) crude protein content compared to leaves meal and seed meal. The crude protein content for seed meal of 28.54±0.41% was lower than 31.65±1.20% obtained in another study (Anwar and Rashid, 2007) but closely similar to 28.04±0.67% (Abiodun et al., 2012) and 30.06% (Madubuike et al., 2015) observed in another study in Nigeria. Moringa leaves meal revealed crude protein value of 22.67±0.09% which was closely similar to 23.61% (Abou-Elezz et al., 2011) and 24.20±0.90% (Offor et al., 2014) observed in previous studies. However, this value was lower than 26.5% (Kakengi el al., 2003) and 27.4% (Olugbemi et al., 2010) but higher than 10.71±0.81% (Amabye and Gebrihiwot, 2015) observed in Ethiopia.

The crude protein content for leaves meal was higher than 21.0% for *C. cajan* reported in Sudan (Eltayeb et al., 2010) but lower than 24.70±0.10 and 26.10±0.09% for cow pea and lentil (Iqbal et al., 2005). The crude protein values for seed meal were higher than that of cowpea and lentil (Iqbal et al., 2005) but closely similar to 32.18 and 32.81% for NRC-37 and JS-335 genotype *G. max* seed (Jain and Jain, 2010).

Raw kernel meal crude protein value of 37.86±0.38% was closely similar to 38.25±0.32% for roasted kernel meal in this study. Crude protein value for raw kernel meal was closely similar to 36.7% observed in another study in Sudan (Abbas, 2013) but lower than 51.8% observed in Sudan (Ochi et al., 2015). Both raw and roasted kernel meals had higher crude protein values than 36.6±0.70% for soybean (*G. max* L.) seed (Siulapwa and Mwambungu, 2014). The differences in crude protein values from those reported in previous studies could be

due to agro-climatic conditions of cultivation, Moringa trees ages and differences in the stage of maturity of the leaves and seeds. Mature leaves and seeds tend to contain higher crude protein values than young ones (Yang et al., 2006).

Crude fat content

Crude fat ranged from 13.40±0.25 to 41.18±0.06% for leaves and raw kernel meal, respectively. The roasted and raw kernel meals had comparably similar crude fat values which were higher than that of seed and leaves meals ($P<0.05$), respectively. The crude fat value of 13.40±0.25% for the leaves meal was higher than 4.50±0.10% observed in Nigeria (Offor et al., 2014). The crude fat content for raw kernel meal of 41.18±0.06% was closely similar to 41.7% reported in Sudan. The seed meal crude fat value of 34.92±0.17% was closely similar to 34.80% observed in Sudan (Anwar and Rashid, 2007) but lower than 40.0% reported in Nigeria (Aja et al., 2013). The variations in crude fat content could be attributed to the cultivated variety, climatic conditions and maturity stage (Yang et al., 2006). The observed crude fat values were all higher than that of *C. cajan* (4.8±0.07%), chickpea (*Cicer arietinum*, 5.2±0.01%) and lentils (*Lens culinaris*, 3.2±0.06%) (Iqbal et al., 2005). The high crude fat content in *M. oleifera* leaves, seed and kernel means that they could be used in livestock feed formulation as a source of lipids besides contributing to the energy value of the feed.

Crude fiber content

Crude fiber values ranged from 4.80±0.23 to 8.07±0.17% with seed meal recording the highest ($P<0.05$) and raw kernel meal the lowest value ($P<0.05$). The crude fiber content observed in seed meal was higher than 5.00±0.0% (Adegbe et al., 2016) and lower than 9.94% (Madubuike et al., 2015), 10.92±0.52%, 12.16±0.26% and 11.05±0.61% (Barakat and Ghazal, 2016) observed in Egypt but closely in agreement with 7.73±0.35% (Abiodun et al., 2012) and 6.84±0.42% (Siyanbola et al., 2015) for Moringa seed meal analyzed in Nigeria. Moringa leaves meal crude fiber value was lower than 17.3±0.20% reported in another study (Offor et al., 2014) but closely similar to 8.20±0.01% (Bamishaiye et al., 2011) and 8.51% (Melesse, 2011) for mid stage Moringa leaves grown in Nigeria and observed in Ethiopia. The observed crude fiber values were higher than 3.25 and 3.55% for NRC-37 and JS-335 genotype *G. max* seeds (Jain and Jain, 2010).

Carbohydrates content

Carbohydrate values ranged from 8.78±0.60 to 44.69±0.41% for roasted kernel and leaves meals, respectively (Table 3). The leaves had the highest ($P<0.05$) carbohydrate values compared to seed, raw kernel and roasted kernel meals. The carbohydrate value for seed meal was higher than 12.44±0.53% (Siyanbola et al., 2015), 3.93% (Madubuike et al., 2015) and 10.59±0.22% for Moringa flour (Abiodun et al., 2012) and lower than 56.42±0.72% (Orhevba et al., 2013) but closely similar to 20.03±1.56, 19.0±0.65 and 20.29±3.15% for *M. oleifera* seeds analyzed in Egypt (Barakat and Ghazal, 2016). Moringa raw kernel meal carbohydrate value of 11.55±0.37% was lower than 15.5% observed in Sudan (Ochi et al., 2015).

Moringa leaves meal carbohydrate value of 44.69±0.41% was lower than 64.87±0.18% (Adeyemi et al., 2014) and 57.61±2.19% (Amabye and Gebrehiwot, 2015) observed in Nigeria and Ethiopia but closely in agreement with 45.71% (Olugbemi et al., 2010). The differences in the nutrient values observed in this study and those of other previous studies with respect to region of growth could be attributed to environmental climate, soil fertility and plants strain (Chadare et al., 2009; Osman, 2004). The high nutrient content in *M. oleifera* means that it could be another source of protein, fat and minerals in livestock feed formulation in Malawi to ease the feed/food crisis and improve livestock production because of the lower cost of *M. oleifera* than that of conventional feeds like *G. max* meal. The crude fiber and carbohydrate values observed in this study would facilitate easy movement of food/feed bolus besides lowering constipation in the livestock intestines if they were included in feed formulation (Wasagu et al., 2013).

Energy and chemical content

The energy and chemical composition values of *M. oleifera* leaves, seed, raw kernel and roasted kernel meals are shown in Table 4.

Gross energy content

The calculated gross energy (GE) values in Mcal/kg DM ranged from 3.8±0.0 to 5.6±0.0 for leaves meal and raw kernel meal, respectively. Moringa raw kernel meal had 5.6±0.0 higher ($P<0.05$) gross energy value than 5.5±0.0, 5.1±0.0 and 3.8±0.0 for roasted kernel, seed and leaves meal, respectively. The gross energy value for leaves meal was higher than 3.0 observed in Tanzania (Olugbemi et al., 2010), but lower than 4.5 reported in Mexico (Abou-Elezz et al., 2011). However, the gross energy value for leaves meal was in close agreement with 3.7±0.04 (Amabye and Gebrehiwot, 2015). The gross energy for seed meal of 5.1.0±0.0 was closely similar to 4.5±0.4 observed in Egypt (Barakat and Ghazal, 2016). Moringa raw kernel meal and roasted kernel meal gross energy values were in close agreement

Table 4. Chemical composition of *Moringa oleifera* L.

Sample	Gross energy (Mca/kg DM)	ME (Mca/kg DM)	Fatty acids (g/kg DM)	pH	Titratable acidity (g/100 g oleic acid)	Phosphorus (mg/100 g DM)
Leaves	3.8±0.0[a]	3.3±0.0[a]	107.2±2.0[a]	6.35±0.0[a]	3.8±0.0[a]	427.6±33.9[a]
Seed	5.1±0.0[b]	4.1±0.0[b]	279.3±1.4[b]	6.26±0.0[b]	1.7±0.0[b]	599.5±4.5[b]
Raw kernel meal	5.6±0.0[c]	4.4±0.0[c]	329.5±0.5[c]	6.42±0.0[c]	0.4±0.0[c]	873.8±6.9[c]
Roasted kernel meal	5.5±0.0[d]	4.3±0.0[c]	328.5±1.1[c]	5.44±0.0[d]	1.2±0.0[d]	754.1±23.0[d]

ME: Metabolisable energy. For each parameter, means with same superscript were not significantly different (P>0.05).

with 6.4 observed in Khartoum, Sudan.

Metabolizable energy content

Metabolizable energy (ME), in Mcal/kg DM, ranged from 3.3±0.0 to 4.4±0.0 with raw kernel meal recording the highest and leaves meal the lowest values. Moringa raw and roasted kernel meals had 4.4±0.0 and 4.3±0.0 ME values higher (P< 0.05) than 3.3±0.0 and 4.1±0.0 for leaves meal and seed meal in this study. However, ME value for leaves meal was closely similar to 3.0 (Olugbemi et al., 2012) reported in Tanzania but higher than 2.2 observed in another study in Ethiopia (Melesse, 2011). The ME value for seed meal was higher than 1.8 observed in another study for Indian oil extracted seeds analyzed in Tunisia (Salem and Makkar, 2008). Moringa raw kernel meal ME value was higher than 3.4 reported by Ochi et al. (2015). ME values observed in this study were relatively higher than 2.7 for *G. max* L. (Siulapwa and Mwambugu, 2014) observed in Zambia. The relative high GE and ME values for kernel meals followed by seed meal indicated that *M. oleifera* products are concentrated sources of energy for livestock production and Moringa seeds and leaves could be potential alternatives of conventional livestock feed ingredients in Malawi.

Chemical composition

pH values

The pH values ranged from 5.44±0.0 to 6.42±0.0 for Moringa roasted kernel and raw kernel meal, respectively. The seed meal pH of 6.26±0.0 indicated that *M. oleifera* seeds are more acidic (P<0.05) than leaves and raw kernel meals with pH values of 6.35±0.0 and 6.42±0.0 in this study. The study indicated that roasted kernel meal had the highest acid content with relative to Moringa leaves, seeds and raw kernel meals.

Titratable acidity values

Titratable acidity, in g/100 g DM (as oleic acid), ranged from 0.36±0.0 to 3.8±0.0 for raw kernel and leaves meals, respectively. The titratable acidity of leaves meal of 3.8±0.0 was the highest (P<0.05) followed by seed meal, roasted kernel meal and raw kernel meal, respectively.

Fatty acids content

Calculated fatty acid, in g/kg DM, ranged from 107.2±2.0 to 329.5±0.5 for Moringa leaves and raw kernel meals, respectively. Moringa raw kernel meal had the highest (P<0.05) fatty acid followed by roasted kernel, seed and leaves meals, respectively.

Phosphorus content

Phosphorus (P) content, in mg/100 g DM, ranged from 427.6±0.0 to 873.9±0.0 for leaves and raw kernel meals respectively (Table 4). Moringa raw kernel meal had the highest (P<0.05) phosphorus content compared to leaves, seed and roasted kernel meals in that sequence. The seed meal revealed phosphorus content of 599.5±0.0 whereas roasted kernel meal registered a value of 754.1±0.0. The leaves meal phosphorus content of 427.6±0.0 was almost twice higher than 240 (Abou-Elezz et al., 2011) reported in previous studies but closely correlated to 430 (Melesse, 2011) analyzed in Ethiopia. Seed meal revealed phosphorus content of 599.5±0.0 lower than 738.15±9.71, 753.31±3.31 and 705.27±10.82 observed in Egypt (Barakat and Ghazal, 2016) but higher than 619 (Kawo et al., 2009) reported in Nigeria. Phosphorus content in raw kernel meal was 873.9±0.0 higher than 535 (Ochi et al., 2015) and 754.1±0.0 for roasted kernel meal observed in this study. Leaves and seed meals phosphorus values were lower than 960±1.0 for *G. max* L. (Siulapwa and Mwambugu, 2014). However, raw and roasted kernel meal phosphorus content was comparable to 960±1.0 for *G. max* L. (Siulapwa and Mwambugu, 2014) observed in Zambia.

Conclusion

The results showed that the species of *M. oleifera* grown

in central Malawi have nutritional potential for inclusion in animal feed because the leaves and seeds contained a high concentration of nutrients in the form of crude protein, crude fat, crude fiber, nitrogen free extracts, and total minerals and energy compared to conventional feed ingredients. Moringa kernels have higher concentration of nutrients than the whole seeds and leaves. The presence of more mineral elements in *M. oleifera* leaves and seed than in conventional feed ingredients such as *G. max* and *C. cajan* means Moringa products are suitable for livestock and human consumption. Therefore, smallholder livestock farmers in Malawi could be encouraged to use Moringa seeds and leaves in livestock feed formulation.

However, in the future Moringa plant samples from different districts in Malawi should be collected for *in vivo* digestibility trials to investigate the effect of including Moringa products in livestock feeds on growth, weight gain and productivity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest

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Full Length Research Paper

Phytochemicals (phenolic acids, flavonoids, and alkaloids) contribution to the feeding value of mulberry (*Morus* spp.) for rabbits

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The feeding value of mulberry leaves for rabbits was quantitatively evaluated based on a single-factor design with five levels in diets (0, 5, 10, 15 and 20%). Results showed that rabbits given mulberry at 20 and 15% had a relatively lower body weight gain, higher feed conversion ratio, and lower meat ether extract contents than that in the 0% group ($P < 0.05$). Increased activities of antioxidant enzymes and reduced formation of thiobarbituric acid-reactive substances were detected in the plasma of mulberry-treated rabbits. Mulberry reduced the production of trichloroacetic acid perceptible N and NH_3-N and increased total volatile fatty acids in rabbit cecum content through optimizing the intestinal micro-flora. Comparative analysis revealed that the content of phytochemicals in mulberry may be the main factor responsible for the feeding levels in rabbit diet, contributes to the effect of enhancing the antioxidant capacity of rabbit bodies and also optimizes intestinal micro-flora.

Key words: Animal nutrition, feed, rabbit, growth, health, blood, intestinal micro-flora.

INTRODUCTION

The use of alternative feed ingredients is an efficient means of reducing feed costs in animal production, and has attracted considerable attention among animal nutrition researchers in developing countries (Klinger, 2017). Mulberry (*Morus* spp.) species are widely distributed throughout Asia, Europe, Africa, and the Americas (Sánchez, 2002). The leaves of mulberries are highly palatable and easily digested (70-90%) by herbivores and can also be fed to monogastrics. The crude protein content of mulberry leaves and young stems range from 15 to 28%, the values of which are similar to those of most legume forages and superior to most grasses. Accordingly, mulberry is believed to have considerable potential and utility value as a non-conventional animal feed (Sánchez, 2000; Zhou et al., 2014). Mulberry leaf is non-toxic and can even protect human brain from pesticide toxicity (Yang et al., 2014; Smith, 2017). The Food and Agriculture Organization (FAO) strongly recommends that farmers use mulberry leaves as an animal feed source to replace edible grains consumed in animal production (Uribe and Sanchez, 2001). The nutritive value of mulberry leaves as a protein

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source has previously been estimated in different animals (Premalatha et al., 2012; Islam et al., 2015; Yupakarn et al., 2015).

The utilization of mulberry leaves as a nutrient source for rabbits has been previously studied. At first, it was advised that mulberry leaves could be used as a single feed for adult rabbits (Deshmukh et al., 1993). However, subsequent substitution experiments indicated that the use of mulberry leaves in feed should be appropriately limited; otherwise, the food intake and live-weight gain of rabbits would decrease, resulting in the production of lean meat. Bamikole et al. (2005) indicated that half of the concentrate in diets could be replaced by mulberry leaves; whereas Prasad et al. (2003) recommended that the proportion used in rabbit diets should be ≤45%, and that 15% could support a good productive performance. Nonetheless, the suitable proportion of mulberry leaves in rabbit diets remains controversial. It has been reported that the nutritional value and digestibility of mulberry leaves are as good as those of lucerne leaves; however, lucerne leaves constitute 48% of the matter in rabbit feed, whereas mulberry leaves are limited to a lower percentage (Premalatha et al., 2012). Consideration of the differences in usage amount should not only be based on difference in nutrient contents, as phytochemicals in mulberry leaves that have anti-obesity and antidiabetic effects may contribute to their inferior application. Previous studies have indicated that mulberry leaf extracts (rich in phenolic acids, flavonoids, and alkaloids) can regulate glycolipid metabolism and remove excess neutral fat and cholesterol from blood, tissues, and organs in humans (Zhang et al., 2014; Chang et al., 2016; Wu et al., 2017). Thus, long-term use and large doses may not fatten animals, thereby resulting in low productive performance. Therefore, as a non-conventional feed ingredient, the nutritive value of mulberry leaves should be assessed not only with respect to animal productivity, but also with regards to its effects on the health of animals, as well as on fat metabolism and mobilization that are critical to the meat type of animals. Numerous phytochemicals have been isolated from mulberry leaves; however, till date the effects of mulberry leaf constituents in feedstuffs on animal production are yet to be analyzed.

In this context, the feeding value of mulberry leaves in rabbits was evaluated. Specifically, the appropriate amount of mulberry leaves that should be used in feedstuff through providing a range of levels in formulated diets was assessed, and the effects of phytochemicals (phenolic acids, flavonoids, and alkaloids) contained in mulberry leaf-supplemented diets on rabbit production, health status, and cecum fermentation were examined.

MATERIALS AND METHODS

The research reported here was approved by the Ethics Committee of Zhenjiang Jiangbin Hospital, and all study procedures were conducted in accordance with national ethics regulations (GB/T 35823-2018 and GB/T 35892-2018). The rabbits slaughtered in this experiment were treated humanely.

Animals, housing and feeding

Eighty 2-months-old male New Zealand white rabbits with an initial body weight of 1.24 ± 0.20 kg were randomly distributed into five groups of eight animals each according to a single-factor design with five levels of mulberry leaf powder in the diet (0, 5, 10, 15, and 20%), which represented the following five treatments: control, ML5, ML10, ML15, and ML20. Eight replicates in each group and each replicate contain one animal. The mulberry leaf powder used was a commercial product processed from green leaves and new shoots, which were dried, ground, and passed through a 0.25-mm sieve.

The experiment was conducted for 42 days with a 14-day adaptive phase. During the trial period, animals were kept in galvanized wire batteries (45 × 45 × 40 cm) equipped with feeding hoppers and drinking nipples and maintained under the same managerial, hygienic, and environmental conditions in rooms with a natural temperature of 18–29°C, humidity of 43–70%, and photoperiod of 15L: 9D. All the rabbits were fed the respective diets allowing *ad libitum* intake and at least 10 g/day refusals. The chemical composition of the diets and mulberry leaf powder are shown in Table 1.

Samples collection and chemical analyses

Animals were weighed at the beginning of the trial and every week thereafter. Feed intake and uneaten feed were recorded weekly throughout the experimental period for determining growth performance. Daily weight gain and feed conversion ratio were calculated. At the end of the experiment, whole blood samples were collected from the marginal vein in the left ear of all rabbits and centrifuged at 3 000 × *g* for 10 min to collect plasma. Eight rabbits from each group were then deprived of feed for 12 h and slaughtered by jugular bleeding. Carcass parameters, including slaughter weight, dressing percentage, and organ percentage were determined according to Abu Hafsa et al. (2017); also, cecal contents were collected in a pre-warmed thermos and sampled for fermentation parameter and micro-flora population analyses. Subsequently, the right-side *longissimus dorsi* muscles were individually packed, weighed and frozen at -20°C until further analysis of chemical composition.

The chemical composition of mulberry leaf powder and meat samples, including dry matter, crude protein, crude fiber, ash, ether extract, nitrogen-free extract, and moisture content, were determined according to the Association of Official Analytical Chemists (AOAC, 2000). Total phenolic acids, flavonoids, and total alkaloids in mulberry leaf powder and diets were determined without fixation using the method described by Zhang et al. (2014).

Fasting glucose, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride total protein, albumin and globulin in plasma were determined using a Pentra 400 analyzer (HORIBA ABX). The content of thiobarbituric acid-reactive substances (TBARS) and the antioxidant activities of glutathione peroxidase (GPx), glutathione *S*-transferase (GST), catalase (CAT), and superoxide dismutase (SOD) were determined using commercial kits obtained from Jiancheng Bio. Tech., Nanjing, China, according to the procedures outlined by the manufacturer. TBARS was measured using ELISA method and examined at 450 nm wavelength; GPx and CAT were measured at 470 and 240 nm wavelength, respectively with a spectrophotometer; GST was measured using fluorescence method and examined at 340 nm wavelength; SOD was measured using hydroxylamine method and examined at the 560 nm wavelength.

Table 1. Ingredients and chemical composition of experimental diets.

Ingredients (%)	Control	ML5	ML10	ML15	ML20	Mulberry leaf powder
Mulberry leaf powder	0	5	10	15	20	
Alfalfa (14% CP)	32	30	29	28	26	
Corn grain (8.7% CP)	25.5	23.4	21.7	20.2	18.4	
Wheat bran (15.7% CP)	22	21.6	21	20.6	19.1	
Soybean meal (43% CP)	16.5	16	14.3	12.2	12.5	
Soybean oil	1	1	1	1	1	
Methionine	0.2	0.2	0.2	0.2	0.2	
Limestone	1	1	1	1	1	
Calcium phosphate	1	1	1	1	1	
Sodium chloride	0.3	0.3	0.3	0.3	0.3	
Vitamin-mineral premix*	0.5	0.5	0.5	0.5	0.5	
Chemical composition (%, DM basis except for DM)						
Dry Matter	84.58	84.79	84.97	85.14	85.36	90.90
Crude protein	17.26	17.31	16.99	16.53	16.80	16.20
Crude fiber	12.35	12.23	12.34	12.44	12.30	11.42
Ash	5.68	5.81	5.96	6.11	6.24	8.25
Ether extract	3.74	3.78	3.83	3.88	3.91	3.69
Nitrogen-free extract	45.84	45.26	44.86	44.59	43.83	39.03

ML5: rabbits received 5% mulberry replacement; ML10: rabbits received 10% mulberry replacement; ML15%: rabbits received 15% mulberry replacement; ML20%: rabbits received 20% mulberry replacement.
* Provided per kilogram of the diets: Vitamin A, 8 000 IU; Vitamin D3, 1 000 IU; Vitamin E, 50 mg; Vitamin K, 2 mg; Cu, 40 mg; Zn, 50 mg; Mn, 30 mg; Fe, 100 mg; I, 0.5 mg.

The pH of the cecal contents was determined by diluting 10 g of the contents with 15 ml distilled water and measured using a pH meter (PHSJ-4F; Shanghai Electric Science Instrument Company Limited). After centrifugation at 5 000 × *g* for 15 min, the supernatant was used for estimation of total N, trichloroacetic acid perceptible N (TCA-N), and total volatile fatty acids (VFAs) using a distillation method (Barnett and Reid, 1957). Aliquots of approximately 1 g of the cecal contents and 3 mL of a solution of 2% sulfuric acid or 2 mL of 2% ortho-phosphoric acid were mixed well for the analysis of ammonia nitrogen (NH_3-N) and VFAs, respectively. The NH_3-N concentration in the cecal contents was determined according to AOAC (2000). VFA analysis was conducted according to Alagón et al. (2014) using a gas chromatograph (Agilent 6890 GC) equipped with a 63 Ni electron capture detector (IECD: Agilent Technologies, Wilmington, DE, USA) and a 7694E automatic injector. Major bacterial species in the fresh cecal contents of rabbits were counted. In particular, aerobic and facultative anaerobic bacteria grown on tryptic glucose yeast agar, *Escherichia coli* on chromogenic coliform agar, *Lactobacillus* spp. on De Man-Rogosa-Sharpe agar, along with *Clostridium* spp., *Bacteroides* spp., and *Bacillus* spp. on PEA agar were determined and counted on plates using a Reichert Quebec® Darkfield Colony Counter according to Maturin and Peeler (2001).

Statistical analysis

Data were analyzed with one-way ANOVA using SPSS16.0 software (SPSS Inc., Chicago, IL, USA), followed by Duncan's multiple range test. Results were expressed as means and the standard error of the means (SEM). Differences were considered significant at $P \leq 0.05$. Dose-response curves were estimated using regression analysis available in SPSS (linear, quadratic, and cubic).

RESULTS

Chemical composition of diets

The chemical composition of the five levels of mulberry leaf inclusion diets were shown in Table 1. The nutritional composition of the dry matter was similar among the five treatments. In Table 2, the phenolic acid, flavonoid, and alkaloid contents of diets increased with an increase in the levels of supplemented mulberry leaves.

Growth performance, carcass, and meat quality analyses

Rabbits in the ML10, ML15, and ML20 groups had a lower ($P < 0.05$) weight gain and feed intake than those in the control and ML5 groups (Table 3). The feed conversion ratio of the ML20 group was higher ($P < 0.05$) than that of the control and other mulberry groups; whereas there were no significant differences among the control, ML5, ML10, and ML15 groups. The slaughter weight of rabbits in the ML20 group was significantly lower ($P < 0.05$) compared with that in the control and other mulberry groups. Mulberry inclusion diets (≤20%) had no significant ($P > 0.05$) effect on the dressing percentage, weight percentage of organs (liver, kidney, and heart), meat moisture, or protein and ash contents,

Table 2. Some phytochemicals in the experimental diets and mulberry leaf powder.

Ingredients (%)	Control	ML5	ML10	ML15	ML20	Mulberry leaf powder
Phenolic acids	0.86	1.27	1.68	2.09	2.50	9.08
Phenolic acids from mulberry[#]	0	0.45	0.91	1.36	1.81	
Flavonoids	0.40	0.43	0.46	0.50	0.52	1.00
Flavonoids from mulberry[#]	0	0.05	0.10	0.15	0.19	
Alkaloids	1.67	1.89	2.17	2.47	2.63	6.61
Alkaloids from mulberry[#]	0	0.30	0.66	1.05	1.30	

[#]Calculated value.

Table 3. Growth performance, carcass characteristics, and meat chemical composition of rabbits fed experimental diets.

Items	Control	ML5	ML10	ML15	ML20	SEM	*P*-value		
							Linear	Quadratic	Cubic
Growth performance									
Initial body weight, kg	1.25	1.21	1.26	1.15	1.34	0.031	0.538	0.373	0.388
Final body weight, kg	2.05^{a}	1.98^{ab}	1.97^{ab}	1.77^{c}	1.83^{bc}	0.052	0.003	0.012	0.021
Weight gain, kg	0.80^{a}	0.77^{a}	0.72^{b}	0.62^{bc}	0.49^{c}	0.058	< 0.001	< 0.001	< 0.001
Feed intake, kg	5.28^{a}	5.44^{a}	4.83^{b}	4.66^{b}	5.07^{ab}	0.142	0.046	0.007	0.001
Feed conversion ratio	6.82^{b}	7.10^{b}	7.02^{b}	8.38^{b}	10.87^{a}	0.759	< 0.001	< 0.001	< 0.001
Slaughter characteristic									
Live body weight, kg	1.92^{a}	1.91^{a}	1.82^{ab}	1.77^{ab}	1.68^{b}	0.073	0.060	0.118	0.227
Slaughter weight, kg	1.82^{a}	1.80^{a}	1.77^{a}	1.71^{a}	1.58^{b}	0.065	0.048	0.085	0.174
Dressing percentage, %	50.38	52.86	52.53	50.01	49.90	1.492	0.842	0.647	0.725
Liver, %	3.15	3.12	2.99	3.14	3.18	0.115	0.618	0.834	0.871
Kidney, %	0.86	0.78	0.82	0.77	0.77	0.061	0.653	0.869	0.964
Heart, %	0.30	0.32	0.31	0.33	0.35	0.044	0.206	0.423	0.595
Chemical composition of *longissimus dorsi* muscle, %									
Moisture	69.64	69.06	70.32	71.02	70.72	0.413	0.193	0.733	0.486
Protein	22.73	22.35	22.56	22.71	22.52	0.603	1.229	1.209	0.718
Ether extract	5.33^{a}	5.27^{a}	5.02^{a}	4.67^{b}	4.47^{b}	0.466	0.026	0.013	0.037
Ash	1.24	1.26	1.35	1.31	1.42	0.205	0.072	0.499	0.718

ML5: rabbits received 5% mulberry replacement; ML10: rabbits received 10% mulberry replacement; ML15%: rabbits received 15% mulberry replacement; ML20%: rabbits received 20% mulberry replacement.
SEM, standard error of means.
Different letters within the same row denote significant differences between treatments ($P < 0.05$, Duncan's test).

whereas the ether extract for ML15 and ML20 group rabbits decreased significantly ($P < 0.05$) compared with that of the other treatments.

Biochemical analysis of plasma

The results presented in Table 4 indicate that the plasma TBARS content in rabbits of the ML15 and ML20 groups was lower than that in rabbits in the control group, whereas the activities of SOD, GPx, GST, and CAT showed significant ($P < 0.05$) dose-dependent increases in rabbits treated with mulberry inclusion diets. However, there were no significant effects of mulberry leaf powder ($P > 0.05$) on the fasting glucose, cholesterol, HDL, LDL, triglycerides, protein, and albumin/globubin contents in the plasma of rabbits.

Cecal fermentation and micro-flora

As indicated in Table 5, increasing levels of mulberry leaf powder in diets did not result in substantial changes in the total N or pH of cecal content. The rabbits fed on the

Table 4. Effects of mulberry diets on the biochemical parameters of rabbit plasma.

Items	Control	ML5	ML10	ML15	ML20	SEM	*P*-value		
							Linear	Quadratic	Cubic
Fasting glucose, mg/dL	65.24	62.23	61.63	62.52	63.35	1.824	0.516	0.096	0.048
Total cholesterol, mg/dL	245.28	234.45	236.72	244.67	243.32	4.832	0.748	0.514	0.128
LDL, mg/dL	174.63	168.43	168.89	173.26	166.45	2.646	0.365	0.772	0.385
HDL, mg/dL	65.42	65.93	65.02	64.73	64.58	1.014	0.108	0.736	0.587
Triglyceride, mg/dL	66.82	65.12	65.57	66.44	65.76	1.668	0.816	0.850	0.048
TBARS, nmol/mL	0.47^{a}	0.36^{ab}	0.30^{ab}	0.27^{b}	0.19^{b}	0.093	0.009	0.057	0.094
GPx, U/ml	13.02^{c}	15.89^{bc}	17.28^{b}	20.32^{a}	21.35^{a}	1.735	0.007	0.051	0.513
GST, μmol/h/mL	1.35^{c}	1.58^{bc}	1.62^{b}	1.83^{a}	1.97^{a}	0.422	0.007	0.049	0.491
CAT, μmol H_2O_2	50.23^{d}	55.64^{c}	59.34^{b}	64.35^{ab}	70.63^{a}	2.534	0.002	0.008	0.052
SOD, U/mL	2.53^{b}	2.97^{b}	3.15^{ab}	3.63^{ab}	4.57^{a}	0.646	0.028	0.042	0.069
Total protein, g/dL	4.77	4.79	4.80	4.78	4.81	0.993	0.388	0.835	0.947
Albumin/Globubin	1.17	1.15	1.23	1.24	1.13	1.747	0.359	0.953	0.618

ML5: rabbits received 5% mulberry replacement; ML10: rabbits received 10% mulberry replacement; ML15%: rabbits received 15% mulberry replacement; ML20%: rabbits received 20% mulberry replacement.
SEM, standard error of means.
LDL, low-density lipoprotein; HDL, high-density lipoprotein; TBARS, thiobarbituric acid-reactive substances; GPx, glutathione peroxidase; GST, glutathione *S*-transferase; CAT, catalase; SOD, superoxide dismutase.
Different letters within the same row denote significant differences between treatments ($P < 0.05$, Duncan's test).

Table 5. Effects of mulberry diets on cecum fermentation parameters and microflora populations of rabbits.

Items	Control	ML5	ML10	ML15	ML20	SEM	*P*-value		
							Linear	Quadratic	Cubic
Total N, mg/g	10.89	10.53	11.01	10.64	10.36	0.824	0.713	0.855	0.174
pH value	6.12	6.25	6.06	5.93	5.90	1.052	0.097	0.623	0.318
TCA N, mg/dL	264.63^{a}	238.24^{b}	229.53^{bc}	210.37^{bc}	200.73^{c}	22.546	0.008	0.051	0.416
NH_3-N, mmol/L	19.87^{a}	17.10^{ab}	14.18^{b}	12.08^{bc}	10.59^{c}	0.174	0.004	0.005	0.042
Total VFA, mmol/L	46.84^{b}	49.28^{ab}	55.92^{a}	55.60^{a}	53.69^{a}	5.177	0.251	0.211	0.503
Acetic acid, % VFA	84.63^{b}	82.45^{b}	81.09^{b}	87.36^{ab}	89.32^{a}	4.623	0.522	0.416	0.741
Propionic acid, % VFA	1.56^{c}	1.74^{bc}	1.98^{b}	2.31^{a}	1.42^{c}	0.222	0.833	0.638	0.486
Butyric acid, % VFA	8.86	9.02	9.18	8.78	9.04	0.636	0.472	0.193	0.988
Valeric acid, % VFA	0.52	0.56	0.49	0.47	0.53	0.212	0.865	0.978	0.528
Aerobic and facultative anaerobic bacteria, log UFC/g	5.73^{c}	5.84^{c}	6.49^{b}	7.14^{ab}	7.43^{a}	0.427	0.008	0.053	0.048
Bacteroides spp., log UFC/g	2.57^{c}	2.87^{c}	3.63^{b}	4.13^{ab}	4.58^{a}	0.743	0.006	0.041	0.108
Escherichia coli, log UFC/g	5.23^{a}	4.15^{b}	3.34^{bc}	2.85^{c}	2.51^{c}	0.692	0.010	< 0.001	0.035
Clostridium spp., log UFC/g	4.45^{a}	3.62^{b}	2.92^{c}	2.47^{c}	2.36^{c}	0.424	0.018	0.005	0.008
Lactobacillus spp., log UFC/g	2.21^{b}	2.63^{b}	3.15^{ab}	3.61^{a}	3.84^{a}	0.352	0.006	0.010	0.006
Bacillus spp., log UFC/g	5.12^{b}	5.32^{b}	5.74^{ab}	6.21^{a}	6.46^{a}	0.525	0.005	0.051	0.031

SEM, standard error of means.
TCA-N, trichloroacetic acid perceptible N; VFA, volatile fatty acids.
Different letters within the same row denote significant differences between treatments ($P < 0.05$, Duncan's test).

mulberry inclusion diets did, however, show a marked reduction in the TCA-N level in cecum liquor at the end of the 42-day experimental period. A similar result was also obtained for the NH_3-N level, which, as expected, decreased significantly ($P < 0.01$). In contrast, total VFA in the cecum liquor of rabbits in the ML10, ML15, and ML20 groups increased significantly ($P < 0.05$) compared with the control group. In particular, animals fed the ML20 diet showed a significantly ($P < 0.05$) higher acetic acid concentration compared with those fed low mulberry leaf powder diets (ML5 and ML10) and the control. An increasing proportion of mulberry leaf powder in diets did

not result in a similar trend in prop ionic acid concentration in cecal contents, as the data indicated a sudden fall in the high mulberry diet group (ML20). However, butyric acid and valeric acid did not differ significantly between the control and mulberry inclusion diet groups. The data obtained from bacteriological analysis showed high variability, which tended to obscure differences among the experimental groups. However, the *E. coli* and *Clostridium* spp. counts were statistically lower in the mulberry leaf powder-treated groups than in the control group. Furthermore, an increase in the proportion of dietary mulberry leaf powder significantly ($P < 0.05$) increased *Bacteroides* spp., *Lactobacillus* spp., and *Bacillus* spp. in the high mulberry leaf powder inclusion groups (ML20 and/or ML15).

DISCUSSION

The selection of the percentages of mulberry leaves in rabbit diets (5, 10, 15 and 20%) used in the present study was based on reference to previous results. The data obtained indicated that the utilization of mulberry leaf in rabbit feed at levels of 5 and 10% in the ML5 and ML10 groups maintained growth performance normally during the entire experimental period, whereas levels of 15 and 20% in the ML15 and ML20 groups resulted in a significant reduction in growth. However, although the final weight and average daily gain of the rabbits were lower in rabbits fed the ML15 diet compared with those fed the control and ML5 diets, the feed conversion ratio and carcass yield were still satisfactory from a production point of view. Rabbits in mulberry treated groups were all healthy, which was indicated by the little variation in selected health markers (organ weight, plasma total protein, and albumin/globubin) between the control group and the mulberry treated groups (Huang et al., 2017), and through this it can be inferred that mulberry leaf is non-toxic, which is in accordance with Yang et al. (2014), who examined the toxicity of mulberry leaf ethanol extract and found no toxic effect on adipocytes. Hence, the level of mulberry leaf powder included in the diet of growing rabbits can be up to 15% without having an adverse effect. Prasad et al. (2003) reported that mulberry leaves can completely replace lucerne hay in the diet of growing rabbits, that was 15%, and that even higher levels (that is, 30 and 45% of the diet) could also promote growth of rabbits. Bamikole et al. (2005) suggested that 50% of concentrate could be replaced by mulberry leaves. The study results do not recommend the dosage of more than 15% of mulberry, for the feed conversion ratio may rise.

The chemical compositions were similar among treatments, indicating energy supply did not differ substantially among the control and mulberry leaf inclusion diets, and thus are probably not the main cause of the observed differences in growth rates. The primary difference among the different levels of mulberry inclusion diets may have been the contents of phytochemicals. Mulberry leaf ethanol extracts contain polyphenols and alkaloids (Jeszka-Skowron et al., 2014). It has been reported that mulberry leaf ethanol extracts inhibit fat accumulation in adipocytes by altering the protein expression levels of adipogenesis-related factors (Yang et al., 2014). Mulberry leaf polyphenols have been shown to inhibit hepatic lipogenesis, promote lipolysis, and lower high-fat diet-induced body weight in hamsters (Peng et al., 2011). The anti-lipogenesis and lipolysis-enhancement effect of mulberry leaf phytochemicals may result in lean meat and less weight gain in non-obese animals fed with normal feed, which was confirmed by the results obtained for the high mulberry inclusion treatment ML20. To analyze the dose-response effect of the mulberry leaf phytochemicals in detail, the content of supplemented phenolic acids, flavonoids, and alkaloids in the ML15 diet were calculated (1.36, 0.15 and 1.05%, respectively), and in terms of animal weight ratio, the data were 1034, 114, and 798 mg/kg of body weight [calculated using the middle weight (1.46 kg) and feed intake (111 g/day) in the ML15 group], respectively. It has previously been reported that an intake of 25 mg/L mulberry leaf phenolic-rich water extract reduces fatty acid storage in *Caenorhabditis elegans in vivo* (Zheng et al., 2014), and that intake of 1.0% mulberry leaf polyphenols (phenolic acids + flavonoids) or 2 mg/kg of body weight of alkaloids from curry leaves per day results in markedly less weight gain in murines and provided a high-fat diet (Peng et al., 2011; Jagtap et al., 2017). Although the influence of phytochemicals from different sources varies in different animals, their efficacy may be the same. The dosages of phenolic acids, flavonoids, and alkaloids in the ML15 treatment of the present study were higher than those used in the above-mentioned studies, and in this regard, the anti-lipogenesis and lipolysis-enhancement effect of the ML15 diet may be stronger, indicating that the content of phytochemicals (phenolic acids, flavonoids, and alkaloids) in mulberry leaves may be the main factor responsible for the low growth of rabbits.

To determine the health status of the experimental rabbits, biochemical indices in plasma were examined. On the basis of the data presented in Table 3, there was no significant change in fasting glucose, cholesterol, HDL, LDL, or triglycerides of rabbits in the mulberry leaf treatment groups. Mulberry leaves are used to prevent postprandial hyperglycemia and hyperlipidemia in type-2 diabetes mellitus treatment to promote glycolipid metabolism (Andallu et al., 2001; Tsuduki et al., 2013). For disease-free animals, glycolipid metabolism improves, and the levels of glucose and lipid metabolites can return to the normal levels a short time after feeding. Mulberry leaf ethanol extract has the effect of accelerating the reduction rate of postprandial hyperglycemia and hyperlipidemia in normal rats (Miyahara et al., 2004), illustrating that mulberry leaves can maintain blood

glucose concentrations at normal levels, which prevents excessive amounts of glucose from circulating in the blood or converts it into fat distributed throughout the body.

In the present study, plasma thiobarbituric acid-reactive substances (TBARS), which are considered early biomarkers of oxidative damage, were discovered in all rabbits groups, thereby indicating that the experimental animals were suffering from oxidative stress (Ghani et al., 2017). For animals, many factors, including environmental change, negative energy balance, consumption of oxidized diets, weaning, and infection, can lead to oxidative stress (Yin et al., 2013; Celi and Gabai, 2015). Mulberry leaves appear to have a significant inhibitory effect against the formation of TBARS, as the content of TBARS in rabbits fed on the ML15 and ML20 mulberry leaf diets were lower than in the control group animals, which in turn indicates that oxidative damage was reduced by consuming mulberry leaves. A reduction in oxidative damage was mediated through an increase in the activity of the antioxidant enzymes SOD, GPx, GST, and CAT in plasma (Table 3). These results are consistent with the findings of Cheong et al. (2012) obtained from the muscle of beef cattle fed by mulberry leaf silage. Mulberry leaf phytochemicals such as phenolic acids, flavonoids, and 1-deoxynojirimycin (DNJ, an important alkaloid in mulberry leaf) are strong antioxidants (Jeszka-Skowron et al., 2014; Pham et al., 2017). The increased activity of antioxidant enzymes in rabbits was stimulated by these antioxidants, which can be inferred from the study of Andallu and Nch (2003), in which it was demonstrated that the activities of antioxidant enzymes in uncontrolled diabetes were improved efficiently by mulberry leaf powder treatment in streptozotocin-induced diabetic rats.

In the present study, the use of mulberry leaves altered the concentrations of cecal metabolites in rabbits. The increase in total VFAs and the slight (non-significant) decrease in pH suggests that fermentation activity in the cecum was higher in the mulberry leaf-supplemented groups ML10, ML15, and ML20 (Table 3), which is consistent with the observations of Prasad et al. (2003), who reported a significant decrease in NH_3-N in the cecum of rabbits fed with 15% and 20% mulberry leaves. Studies on finishing steers revealed similar NH_3-N and VFA concentration changes in rumen fluid, although no differences were detected in pH values between mulberry leaf and non-mulberry leaf groups (Zhou et al., 2014). Different levels of NH_3-N and VFAs could be related to changes in the composition of commensal cecal and intestinal micro-flora, as some of the microbes in the cecum originate from gastrointestinal tract. An effect of mulberry leaf on modulating intestinal micro-flora was also identified in the cure of intestinal flora disorder in streptozotocin-induced diabetic rats (Sheng et al., 2017).

In ewes, supplementation of mulberry flavonoids has been shown to improve the digestibility of organic matter and reduce CH_4 output by inhibiting the populations of microbes involved in methanogenesis (Ma et al., 2016). As indicated by the findings of the present and previous studies, 10~20% mulberry leaves in the diet can effectively optimize the cecum micro-flora as well as the intestinal micro-flora of rabbits, and this capacity of mulberry leaf can be attributed to the constituent phytochemicals.

Conclusion

The results of this study indicate that inclusion of mulberry leaves in the diet was suitable for raising rabbits. Inclusion of ≤15% mulberry leaves in the diet could promote performance from a production point of view. Phytochemicals (phenolic acids, flavonoids, and alkaloids), which may be the main factor restricting the acceptable amount of mulberry leaves that can effectively be included in rabbit feed, also contribute to the enhancement of antioxidant activity in rabbit blood and optimization of the micro-flora population in the rabbit cecum. For the future research, how to reduce the restricting factors would be a significant direction, and that would be helpful to increase the usage of mulberry in animal feed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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The efficacy of jatropha (*Jatropha curcas* L.*)* seed cake as an organic fertilizer

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Studies to evaluate the efficacy of *Jatropha curcas* L. (Jatropha) seed cake as an organic fertilizer were carried out at Bunda College of Agriculture during the 2010/2011 growing season using an on-station experiment. The major objective was to evaluate the efficacy of Jatropha seed cake as an organic fertiliser. The field experiment was laid in a 3 × 3 design with 3 levels of Jatropha (0, 92 and 184 kg N/ha corresponding to 0, 2875 and 5750 kg DM of Jatropha seed cake, respectively) and 3 levels of inorganic fertilizer (0, 23 and 46 kg N/ha). Field results showed that maize yields responded to the amount of Jatropha seed cake while inorganic fertilizer rate and the combination of Jatropha seed cake and inorganic fertilizer (at the same level of Jatropha seed cake) did not affect the grain yield of maize. The best performer in terms of grain yield was a treatment combination of 5750 kg/ha of Jatropha seed cake and 23 kg N/ha of inorganic fertilizer which produced a grain yield of 2483 kg/ha. However, this was comparable to 2331 kg/ha produced by the combination of 2785 kg/ha of Jatropha seed cake and 46 kg N/ha of inorganic fertilizer. The grain yield of sole Jatropha 5750 kg/ha was 2126 kg/ha. The grain yield of full rate of inorganic fertilizer (23:21:0+4S + urea) application was 2853 kg/ha. It can be concluded from this study that sole application of Jatropha seed cake has a potential of producing grain yield comparable to full rate inorganic fertlizer application.

Key words: Jatropha seed cake, organic fertiliser, inorganic fertiliser.

INTRODUCTION

The term soil fertility is generally defined as the quality of a soil that enables it to provide nutrients in adequate amounts and in proper balance for the growth of specified plants when other growth factors, such as light, moisture, temperature, and the physical condition of the soil, are favorable (NAL, 2015). The inherent soil fertility decreases with an increase in soil cropping; especially when the essential nutrients taken up by crops are not replenished (Ilex EnvironSciences, 2018). Zingore et al (2015) reported that land degradation associated with poor soil fertility leading to decreasing agricultural productivity is a problem in sub-Saharan Africa (SSA) whereas Mungai

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et al. (2016) indicated that low soil fertility was among the factors that limits sustainable agricultural production in Central Malawi.

A decline in soil fertility leads to a number of consequences. These include a marked decline in crop productivity and food insecurity as the main consequence. The other consequences include less fodder for cattle, less fuel wood for cooking, and less crop residues and cattle manure to recycle nutrients. These effects often increase runoff and erosion losses because there is less plant cover to protect the soil (liu et al., 2018). Soil fertility depletion also decreases above and below ground biodiversity and increases the encroachment of forests and woodlands in response to the need for additional agricultural land (Gomiero, 2016). In sustainable agricultural and horticultural enterprises, efforts must always be made to maintain not only inherent soil fertility but also soil productivity. Soil fertility is the status of a soil with respect to its ability to supply the nutrients essential to plant growth while soil productivity is the capacity of a soil, in its normal environment, for producing a plant or crop sequence under a specified system of management (Singh, 2017). The maintenance of inherent soil fertility without maintaining soil productivity does not assure sustainable agricultural production. For soil productivity to be maintained, the soil must, *inter alia*: (a) be in a position to furnish plant nutrients, air and water in suitable proportions; (b) have a suitable reaction in the chemical sense; (c) contain no substances that are phytotoxic; (d) be physically permeable to cultivation and resistant to soil erosion; and (e) serve as a suitable culture medium for the micro–flora and micro-fauna to be of character able to ameliorate the general chemical and physical properties of the soil and soil-plant relationship. Any substance which, when added to the soil, brings about an improvement in any of these directions can legitimately be considered as fertilizer or manure using the terms in their widest sense (Maida, 2015).

Traditional methods of soil-fertility management range from recurring fertilizer applications to low external input agriculture based on organic sources of nutrients. In Malawi the external input agriculture based on organic inputs include the use of organic fertilizers, crop rotations, intercropping, biological nitrogen fixations, agroforestry techniques and fallowing (Nalivata et al., 2017).

Concurrent with the efforts of using inorganic fertilisers to address the problem of low and declining soil fertility are those initiatives aimed at the use of organic fertilisers in the form of compost manure, surface mulch, crop residues farmyard manure and green manure (Nalivata et al., 2017). When incorporated into the soil, organic fertilisers improve both chemical and physical properties of the soil (Hossain et al, 2017). The complementary use of organic and inorganic fertilisers is, therefore, of paramount importance in any agricultural production programme designed to maintain soil productivity. Among the soil fertility management technologies outlined above, the use of organic fertilisers ranks highly. In their study on farmer perceptions, choice and adoption of soil management technologies in maize-based farming systems in Malawi, Kabuli and Phiri, (2004) concluded that the use of organic matter technologies offer practical solutions to sustainable soil fertility management in Malawi so long as crop residues and green manures are returned to the soil. This therefore calls for increased research in other forms of organic fertilisers in addition to the already existing alternatives so that the smallholder farmers may have a wider range of choice. An alternative seems to have risen in the name of a crop called *Jatropha curcas* L. (Jatropha). There is a growing interest in Jatropha as an oil “miracle tree” to help alleviate the energy crisis and generate income in rural areas of developing countries. Jatropha is becoming a foster child among some proponents of renewable energy and appropriate technology, especially as an oil-bearing, drought resistant tree for marginal lands for small farmers. If this technology is fully adopted in Malawi there is likelihood that there is going to be a problem of managing the residues that will be left after the extraction of oil from the Jatropha. However, according to Massoud et al. (2017). *J. curcas* seed cake (J.S.C.) is a useful organic byproduct containing considerable amounts of nitrogen, phosphorus, potassium and micronutrients. It can be considered as a green economy soil amendment.

MATERIALS AND METHODS

Study site description and Jatropha seed cake analysis

The on - station, and researcher designed experiment was conducted at Bunda College, Department of Crop and Soil Sciences Research Farm in the 2010/2011 growing season. The farm is situated at latitude 14°11'S and longitude 33°46'E and it is at 1184 m above sea level. The total amount of rainfall in the 2010/2011 growing season was 932 mm. The mean rainfall for the recent five-year period (2010/2011 inclusive) is 892 mm. Thus the 2010/2011 season had better rainfall. Soils at Bunda have been classified as Chromic Luvisols in the World Reference Base System (Typic Hapludalfs in the USDA Soil Taxonomy) (Mutegi et al., 2015). The area is dominated by acidic to strongly acid sandy clay and sandy clay loam soils. Mean total N % was 0.472 and 0.495 in the depth ranges of 0-20 and 20- 40 cm respectively. Soil organic matter values were 1.5 and 1.1% in the depth ranges of 0- 20 and 20- 40 cm.

Treatments, experimental plot size and layout

The field experiment included exclusive application of Jatropha seed cake and a combination of Jatropha seed cake and inorganic sources of N in the soil. The experiment was set out in a 3 × 3 complete factorial arrangement in a completely randomised block design replicated three times. The Jatropha seed cake (JSC) was applied in the soil as follows:

JSC_0 = 0 kg seed cake/ha (supplying equivalent of 0 kg N/ha)
JSC_1= 2875 kg seed cake/ ha (supplying equivalent of 92 kg N/ha)

Table 1. Schematic lay out of the treatment plots.

Plot number	Rep 1	Rep 2	Rep 3
1	JSC_2IF_2	JSC_2IF_1	JSC_1IF_0
2	JSC_1IF_0	JSC_0IF_1	JSC_1IF_2
3	JSC_0IF_1	JSC_2IF_2	JSC_2IF_0
4	JSC_0IF_0	JSC_0IF_0	JSC_0IF_2
5	JSC_0IF_2	JSC_2IF_0	JSC_0IF_0
6	JSC_1IF_2	JSC_1IF_0	JSC_2IF_1
7	JSC_1IF_1	JSC_1IF_1	JSC_1IF_1
8	JSC_2IF_1	JSC_0IF_2	JSC_0IF_1
9	JSC_2IF_0	JSC_1IF_2	JSC_2IF_2

JSC_2= 5750 kg seed cake/ha (supplying equivalent of 184 kg N/ha) Three levels of inorganic N fertilizer (IF), urea (46% N), were applied as follows:

IF_0 = 0 kg N/ha
IF_1 = 23 kg N/ha
IF_2 = 46 kg N/ha

There was also an external plot for full rate of inorganic N fertiliser (92 kg N/ha). This was added to be used as a basis for comparison with the treatments under study. The schematic presentation of the treatment plots is shown in Table 1. The gross plot size was 4 ridges each measuring 5 m long spaced at 0.75 m. The net plot was the central 2 ridges measuring 4 m long, thus leaving at both ends 0.5 m. The maize was planted on the ridge at 0.25 m apart and 1 plant per planting station.

Research processes and procedures

The test crop was maize (*Zea mays*) variety SC403 which matures within 100 to 125 days and its potential yield is 5000 kg/ha. Before planting, the Jatropha seed cake was applied at a depth of 20 cm on the ridge to all the plots that required Jatropha seed cake as part of the treatment combinations. Germination took seven to eight days after planting and thinning was done on 28th February 2011. The same day 23:21:0 + 4S fertiliser as a basal dressing fertiliser was applied in the plots with full rate of inorganic fertiliser. Top dressing with urea (46% nitrogen) fertiliser was applied on 16th March 2011 in all the plots according to the treatment combinations. All the other agronomic practices like weeding, banking and pest control were done according to Guide to Agricultural Production (MoAI, 2014). During the maize growing season, we experienced a midseason dry spell. The plants were irrigated according to water requirements to avoid water stress. Harvesting of all the plots was done on 25th June 2011. Only the net plot was harvested to avoid border effects.

Growth, yield and yield components data

The growth data collected in this research were plant height and chlorophyll levels. Grain yield, stover yield (fresh and dry weight), 100-seed weight, and total biomass comprised the yield and yield components data that were collected. Maize plant heights were collected starting 27 days after planting and this was done every 2 weeks until time of harvesting. Ten plants were selected randomly from the net plot. The mean height was calculated accordingly. The height was measured using a meter rule from the top of the ridge up to the highest leaf. The crop growth rate was then calculated accordingly.

Chlorophyll levels were collected after 41, 55 and 69 days after planting. Ten plants were selected randomly from the net plot. From the ten plants, the mean chlorophyll level was calculated. The chlorophyll levels were measured using a chlorophyll meter (Minolta SPAD-502). Care was taken to make sure that the same leaf position was taken on each of the plants sampled for consistency sake. The mean chlorophyll level was calculated accordingly. The stover yield was determined by weighing all the stover in the net plot after removing the cobs without the husks. A sample of the stover was collected and oven dried at 80°C for 24-48 h for moisture determination. From the moisture determination the total dry stover yield for each plot was calculated. This was letter converted to stover yield in kilograms per hectare.

The 100 seed fresh weight was determined by randomly sampling 100 seeds from each net plot and weighing them while fresh. This was oven dried at 80°C for 24-48 h for moisture content determination. The seeds were reweighed after drying. The weight was recorded representing the dry weight of 100 seeds. The grain yield was determined by weighing the entire grain yield from each net plot while fresh. Then from the moisture content determined from the 100 seed weight, the dry weight of the grain yield for each net plot was calculated and this was later converted to yield in kilograms per hectare. The total biomass was determined by the addition of the dry weights of total stover, total grain, and total cores for each net plot. This was again converted to total biomass yield in kilograms per hectare.

Data analysis

The data from the two experiments was analysed using Genstat, 12th edition, computer package for analysis of variance (ANOVA). Means which were significantly different were separated by Fisher's protected least significance differences (LSD) at P = 0.05 level.

RESULTS AND DISCUSSION

Evaluating the effect of Jatropha seed cake and inorganic fertilizer on maize growth and yield

Chlorophyll in maize plant leaves

The results on the effect of Jatropha seed cake on

Table 2. Mean chlorophyll levels in maize leaves with time for the field experiment involving the use of jatropha seed cake and inorganic fertiliser at bunda college research farm during the 2010/2011 growing season.

Jatropha seed cake (kg/ha)	Inorganic fertilizer (kg N/ha)	Sampling time (days after planting)			
		41	55	69	Mean
0	0	30.44	35.70	35.77	33.97[d]
	23	33.01	38.35	45.12	38.83[c]
	46	31.14	42.90	45.23	39.76[c]
	Mean	31.53[c]	38.98[c]	42.04[c]	
2875	0	45.03	37.85	39.04	40.64[c]
	23	48.16	48.90	48.76	48.61[b]
	46	51.96	51.75	50.91	51.54[ab]
	Mean	48.38[b]	46.17[b]	46.24[b]	
5750	0	50.02	53.60	50.84	51.49[ab]
	23	54.32	54.65	52.37	53.78[a]
	46	50.36	54.85	51.82	52.34[ab]
	Mean	51.57[a]	54.37[a]	51.68[a]	
	Grand mean	43.83[b]	46.51[a]	46.65[a]	
Inorganic fertilizer (23:21:0+4S + urea)		46.01	54.00	53.68	
				$LSD_{0.05}$	**F prob**
Jatropha seed cake				2.459	<0.001
Inorganic fertilizer				2.459	<0.001
Sampling time				2.459	0.044
Jatropha seed cake × inorganic fertilizer				4.258	0.038
% CV		7.9			

* Means with different superscripts in the same column and row are significantly different (P<0.05).

chlorophyll in maize plant leaves are given in Table 2. The results indicate that there were significant differences (P<0.001) in chlorophyll index among the Jatropha seed cake rates. The levels of chlorophyll increased with increase in Jatropha seed cake rates. The rates of inorganic fertilizer also significantly affected (P<0.001) the index of chlorophyll. This increased with increase in inorganic fertilizer level. A closer scrutiny of Table 3 indicates that the inorganic fertilizer influenced more chlorophyll development than the *Jatropha* seed cake. The stage of crop growth also significantly affected the index of chlorophyll (P = 0.044). The index of chlorophyll increased as the crop was developing. However, there were no significant differences in chlorophyll index between 55 and 69 days after planting. The index of chlorophyll in the maize leaves were also significantly (P = 0.038) affected by the combination of Jatropha seed cake and inorganic fertilizer. At all levels of Jatropha seed cake rates, the chlorophyll index were increasing with increase in inorganic fertilizer rates. However, at all these levels of Jatropha seed cake, the difference between adding 23 or 46 kg N/ha of inorganic fertilizer did not bring significant differences. The highest synergy was recorded in the treatment combination of 5750 kg /ha of Jatropha seed cake and 23 kg N/ha of inorganic fertilizer (53.78) but this was comparable to 51.54 recorded in the treatment combination of 2875 kg/ha of Jatropha seed cake and 46 kg N/ha of inorganic fertilizer.

The increase in chlorophyll index with increase in Jatropha seed cake could be attributed to more nitrogen available where there was more Jatropha seed cake applied. The same could apply to increase in chlorophyll index with increase in amount of inorganic fertilizer. Schlichting et al. (2015), reported that chlorophyll content is usually strongly related to N concentration. Similar results were reported by Akhter et al. (2016). Nitrogen is part of the enzymes associated with chlorophyll synthesis (Chapman and Barreto, 1995) and the chlorophyll concentration reflects relative crop N status and yield level (Blackmer and Schepers, 1995). The observed change in chlorophyll content with the stage of crop growth coincided with Argenta et al. (2004) findings, who reported that the chlorophyll reading, mainly in initial stages, varied more compared to the later stages. Sunderman et al. (1997) also found greater increases in reading values in early vegetative stages (six to seven and 10 to 11 expanded leaves) than during reproductive stages (R1 and R6).

Table 3. Mean maize crop growth rate (cm/day) with time for the field experiment involving the use of jatropha seed cake and inorganic fertiliser at bunda college research farm during the 2010/2011 growing season.

Jatropha seed cake (kg/ha)	Inorganic fertilizer (kg N/ha)	Sampling time (days after planting)					
		27 – 41	41- 55	55 – 69	69 - 83	Mean	
0	0	0.630	0.608	1.448	2.177	1.215^{d}	
	23	1.051	1.428	2.458	1.995	1.733^{c}	
	46	0.981	1.117	2.491	2.877	1.867^{bc}	
	Mean	0.887^{b}	1.051^{b}	2.132^{b}	2.350^{a}		
2875	0	2.657	1.568	3.170	2.139	2.383^{a}	
	23	2.325	2.115	2.575	1.669	2.171^{abc}	
	46	2.583	2.147	3.121	1.357	2.302^{ab}	
	Mean	2.522^{a}	1.943^{a}	2.955^{a}	1.722^{b}		
5750	0	2.080	2.035	2.670	2.084	2.217^{ab}	
	23	3.012	2.546	2.916	1.512	2.497^{a}	
	46	2.594	1.832	3.201	0.712	2.087^{ab}	
	Mean	2.562^{a}	2.138^{a}	2.929^{a}	1.436^{c}		
	Grand mean	1.990^{b}	1.710^{b}	2.672^{a}	1.837^{b}		
Inorganic fertilizer (23:21:0+4S + urea)		2.044	2.076	3.161	1.440	2.180	
						$LSD_{0.05}$	**F prob**
Jatropha seed cake						0.256	<0.001
Inorganic fertilizer						0.256	0.287
Sampling time						0.296	<0.001
Jatropha seed cake × inorganic fertilizer						0.443	0.032
% CV						21.3	

*Means with different superscripts in the same column and row are significantly different (P<0.001).

Effect of Jatropha seed cake and inorganic fertilizer on maize crop growth rate

The results on the effect of Jatropha seed cake and inorganic fertilizer on maize crop growth rate are shown in Table 3. The results indicate that crop growth rate was significantly (P<0.001) affected by the amount of Jatropha seed cake applied. For sole Jatropha seed cake, the highest mean growth rate (2.383 cm/day) was observed where 2875 kg/ha of Jatropha seed cake was applied and this was significantly different from that of 5750 kg/ha of Jatropha seed cake (2.217 cm/day). The lowest (1.215 cm/day) crop growth rate was where no Jatropha seed cake was applied. The mean of crop growth rate for full rate inorganic fertilizer was 2.180 cm/day. The crop growth was however not significantly affected by the amount of inorganic fertilizer applied. The combination of Jatropha seed cake and inorganic fertilizer had also a significant (P = 0.032) effect on the crop growth rate. The mean crop growth rate was highest (2.497 cm/day) in the combination of 5750 kg/ha of Jatropha seed cake and 23 kg N/ha of inorganic fertilizer. This value was however not significantly different from 2.302 cm/day observed in the combination of 2875 kg/ha of Jatropha seed cake and 46 kg N/ha of inorganic fertilizer. These two values were comparable to 2.218 cm/day observed in full rate inorganic fertilizer. The time of sampling also significantly (P<0.001) affected the crop growth rate. The crop growth rate was high in the early stages of growth and dropped between 41 and 55 days after planting. The highest crop growth rate was experienced between 55 and 69 days and then dropped again towards harvest time.

The plant height which is used in determining the growth rate is among the most important biomass yield components of maize crop. Besides being a genetic trait, it is also a reflection of nutrient availability, management and favorable prevailing weather. The fact that a combination of 5750 kg/ha of Jatropha seed cake and 46 kg N/ha of inorganic fertilizer gave the highest growth rate which also means that it gave the tallest plants is an indication that abundant nutrient supply is directly correlated to growth. This might be due to the availability of N required for plant growth and development. Similar results were reported by Imran et al. (2015) who stated that application of high N rates had significant effect on plant height of maize and therefore on growth rate. The observed trend in the growth rate with time could be explained by the availability of water which is one the most essential requirements for plant growth. The high growth rate during the early stages could be due to availability of moisture with respect to the time the maize was planted. This growth rate was observed around the months of February and March when the area received a

Table 4. Mean maize 100-seed weight (g) for the field experiment involving the use of Jatropha seed cake and inorganic fertiliser at Bunda College research farm during the 2010/2011 growing season.

Jatropha seed cake (kg /ha)	Inorganic fertilizer (kg N/ha)			
	0	**23**	**46**	**Mean**
0	16.46	19.84	19.24	18.52[b]
2875	18.24	24.18	22.90	21.77[a]
5750	24.22	22.42	23.59	23.41[a]
Mean	19.64	22.15	21.91	
100-seed weight of inorganic fertilizer (23:21:0+4S + urea)				25.60
			$LSD_{0.05}$	**F prob**
Jatropha seed cake			2.854	0.011
Inorganic fertilizer			2.854	0.145
Jatropha seed cake × inorganic fertilizer			4.942	0.234
% CV			10.3	

* Means with different superscripts in the same column are significantly different (P<0.05).

lot of rainfall. The other reason could be the fact that the plants were still young and growth was fast. Another contributing factor would be the plant nutrients released with the fast decomposition of Jatropha in the first 14/28 days. The drop in growth rate between 41 and 55 days after planting could be attributed to low rainfall which fell in the month of April. A sudden increase in the growth rate thereafter could be attributed to more moisture content availability since the plants were irrigated after the rains stopped. The drop in growth at the end could be attributed to the fact the plants were approaching maturity and therefore growth was slow.

Effect of Jatropha seed cake and inorganic fertilizer on maize 100-seed weight

The results on the effect of Jatropha seed cake and inorganic fertilizer are given in Table 4. The results indicate that maize 100 seed weight was significantly (P = 0.011) affected by the amount of Jatropha seed cake applied. There was an increase in 100 seed weight with increase in Jatropha seed cake. Inorganic fertilizer and the combination of Jatropha seed cake and inorganic fertilizer did not significantly affect maize 100 seed weight. It is clear from Table 4 that the seed weights were higher between Jatropha seed cake rates than between inorganic fertilizer. For sole Jatropha the highest weight (24.22 g) was produced where 5750 kg of Jatropha seed cake was applied and this was comparable to 25.60 g produced where full rate of inorganic fertilizer was applied. In the combinations between Jatropha seed cake and inorganic fertilizer, the highest weight (24.18 g) was produced where 2850 kg of Jatropha seed cake was combined with 23 kg N/ha of inorganic fertilizer and this was once again comparable again to the seed weight for full rate of inorganic fertilizer.

The increase in seed weights with Jatropha seed cake and not with increase in inorganic fertilizer could be attributed to the availability of macro nutrients throughout the plant life especially at the time of flowering and seed setting. The three macro elements, N, P, and K were available in the Jatropha seed cake and only N was available in the inorganic fertilizer used. One of the macro nutrients worth mentioning is K which is one of 12 nutrient elements required for normal corn growth and development. Basically, K is associated with movement of water, nutrients, and carbohydrates within the plant. These functions will stimulate early growth, increase protein production, and improve the efficiency of water use and resistance to diseases and insects. As reported by Anarson (2015) the maize grain is composed mainly of starch (80%), and protein (10-15%). The other components are oil (5-6%), fiber (9-15%), sugar (1-3%), and ash (1.7%).

Effect of Jatropha seed cake and inorganic fertilizer on maize plant biomass

The results on maize plant biomass are given in Table 5. The results show that maize plant biomass was significantly affected (P = 0.004) by the amount of Jatropha seed cake. The biomass increased with increase in Jatropha seed cake. The maize plant biomass was also significantly affected (P = 0.011) by the amount of inorganic fertilizer. Here also the maize plant biomass increased with increase in inorganic fertilizer. However, the combination of Jatropha seed cake and inorganic fertilizer did not significantly affect the maize plant biomass. It is shown that the maize plant biomass was highest between the Jatropha seed cake as compared to values between the inorganic fertilizer. The highest value (5206 kg/ha) for sole Jatropha was where 5750 kg /ha of Jatropha seed cake was applied and this was comparable to 5959 kg/ha for full rate of inorganic

Table 5. Mean maize plant biomass (kg/ha) for the field experiment involving the use of Jatropha seed cake and inorganic fertiliser at Bunda College research farm during the 2010/2011 growing season.

Jatropha seed cake (kg /ha)	Inorganic fertilizer (kg N/ha)			
	0	**23**	**46**	**Mean**
0	763	2211	2959	1978[c]
2875	4399	4589	5615	4868[b]
5750	5206	5647	6297	5717[a]
Mean	3456	4149	4957	
Maize biomass weight of inorganic fertilizer (23:21:0+4S + urea)				5959
		$LSD_{0.05}$		**F prob**
Jatropha seed cake		1769.2		0.002
Inorganic fertilizer		1769.2		0.213
Jatropha seed cake × inorganic fertilizer		3064.3		0.956
% CV		32.4		

*Means with different superscripts in the same column are significantly different (P<0.05).

fertilizer. For the combinations of Jatropha seed cake the best performer was 5750 kg/ha of Jatropha seed with 46 kg N/ha which produced 6297 kg/ha. However, the one produced by a combination of 5750 kg/ha of Jatropha seed cake with 23 kg N/ha of inorganic fertilizer (5647 kg/ha) was comparable to the one produced where there was a full rate of inorganic fertilizer applied.

The observed significant performance in plant biomass with the application of Jatropha seed cake could be attributed to the essential nutrient elements contained in the Jatropha seed cake that are associated with increased photo-synthetic efficiency. This finding corroborates the report of Kareem et al. (2017) who observed significant increases in dry matter accumulation in maize with successive increases in organic manure rates. This could be due to the ability of the organic manure to supply the nutrient elements necessary to promote more vigorous growth, improve meristematic and physiological activities in the plants, as well as improve the soil properties; thereby resulting in the synthesis of increased photo-assimilates that enhanced maize yielding ability. The better biomass produced by a combination of Jatropha seed cake and inorganic fertilizer is consistent with other researchers' findings. Combination of organic and mineral fertilizer sources have been shown to result in synergistic effects and improved synchronization of nutrient release and uptake by crop, leading to higher yields. The use of organic fertilizer combined with NPK fertilizer can be applied to increase the biomass production, and maize grain yield in sustainable ways (Maswar and Soelaeman, 2015).

Effect of Jatropha seed cake and inorganic fertilizer on maize stover and grain yields

The results of the effect of Jatropha seed cake and inorganic fertilizer on maize stover and grain yield are given in Tables 6 and 7, respectively. The results indicate that both the grain and stover yield were significantly affected (P = 0.007 and P = 0.018 for grain) by the amount of Jatropha seed cake applied. There was an increase in stover and grain yield with increase in Jatropha seed cake. Both the stover and grain yield were not significantly affected by the inorganic fertilizer rates and neither were these two significantly affected by the combination of Jatropha seed cake and inorganic fertilizer. Once again here the Jatropha seed cake is showing to have more effect on the stover and grain yield as compared to the inorganic fertilizer. With respect to sole Jatropha seed cake, the highest stover yield (2774 kg/ha) was produced where 5750 kg/ha of Jatropha seed cake was applied. However, even the stover yield for 2875 kg/ha of Jatropha seed cake (2508 kg /ha) was comparable to the one produced by full rate of inorganic fertilizer which was 2572 kg/ha. For the combinations, the highest stover yield was produced in the treatment combination of 5750 kg/ha of Jatropha seed cake and 46 kg N/ha of inorganic fertilizer. However, it can also be noted from Table 6 that the treatment combination of 5750 kg/ha of Jatropha seed cake performed well. This produced 2807 kg/ha compared to 2572 kg/ha for the full rate inorganic fertilizer.

In terms of grain yield, the highest yield for sole Jatropha was realized from 5750 kg/ha of Jatropha seed cake while that for full rate inorganic fertilizer was 2853 kg/ha. As for the combinations, the highest grain yield (2418 kg/ha) was realized from the treatment combination of 5750 kg/ha Jatropha seed cake and 23 kg N/ha of inorganic fertilizer. Once again the observed significant performance in stover and grain yield with the application of Jatropha seed cake could be attributed to the essential nutrient elements contained in the Jatropha seed cake that are associated with increased photo-synthetic efficiency. This finding corroborates the report of Kareem et al. (2017) as already alluded to in this

Table 6. Mean maize stover yield (kg/ha) for the field experiment involving the use of Jatropha seed cake and inorganic fertiliser at Bunda College research farm during the 2010/2011 growing season.

Jatropha seed cake (kg /ha)	Inorganic fertilizer (kg N/ha)			
	0	23	46	Mean
0	404	1332	1255	997[b]
2875	2508	2484	2807	2598[a]
5750	2774	2757	3924	3152[a]
Mean	1894	2191	2662	
Stover yield of inorganic fertilizer (23:21:0+4S + urea)				2572
			$LSD_{0.05}$	**F prob**
Jatropha seed cake			1192.7	0.007
Inorganic fertilizer			1192.7	0.380
Jatropha seed cake × inorganic fertilizer			2065.8	0.835
% CV			40.6	

*Means with different superscripts in the same column are significantly different (P<0.05).

Table 7. Mean maize grain yield (kg/ha) for the field experiment involving the use of Jatropha seed cake and inorganic fertiliser at Bunda College research farm during the 2010/2011 growing season.

Jatropha seed cake (kg /ha)	Inorganic fertiliser (kg N/ha)			
	0	23	46	Mean
0	281	677	1375	778[c]
2875	1530	1729	2331	1863[b]
5750	1993	2418	1966	2126[a]
Mean	1268	1608	1891	
Maize grain yield of inorganic fertilizer (23:21:0+4S + urea)				2853
			$LSD_{0.05}$	**F prob**
Jatropha seed cake			896.6	0.018
Inorganic fertilizer			896.6	0.335
Jatropha seed cake × inorganic fertilizer			1552.9	0.712
% CV			43.2	

*Means with different superscripts in the same column are significantly different (P<0.05).

study. The better stover and grain yield produced by a combination of Jatropha seed cake and inorganic fertilizer is consistent with what other researchers found ((Maswar and Soelaeman, 2015), as already pointed out in this study. The observed significant effect on grain yield with increases in Jatropha seed cake application could also be attributed to biomass and 100-seed weight which were also significantly increased with application of Jatropha seed cake which resulted in an overall increase in grain yield per hectare. Ogbonna and Obi (2005) reported similar results where increases in organic manure application resulted in high dry matter partitioning towards increased grain yield and higher harvest index. The other contributing factor to the observed yield differences could be the plant height. It was observed from the study that the plant height increased with increased levels of Jatropha seed cake. The height of plant is an important growth character directly linked with the productive potential of plants in terms of grains. An optimum plant height is claimed to be positively correlated with productivity of plant (Saeed et al., 2001).

The observed trend in maize grain yield also corresponded with the increase in chlorophyll content in maize leaves which increased with increase in Jatropha seed cake rates. The results showed that there is direct relationship between the leaf chlorophyll and maize yield corroborating (Mehasen and Al-Fageh Mehasen, 2004). The lack of interaction between the Jatropha seed cake and the inorganic fertlizer is indicative of the fact that organic fertilizer alone was capable of providing enough of the nutrient elements. This may be due to the fact that the soil of the experimental site was found to be rich in total N and organic carbon content.

Conclusions

Base on the study findings and the objectives that were

investigated using the field experiment, the following conclusions are made. Sole Jatropha of about 6 tonnes per hectare has a potential of producing yields comparable to full rate of inorganic fertiliser. For smallholder farmers, Jatropha seed cake will be a favourable source of nutrients as it is cost effective, locally available and also effective in increasing the availability of essential plant nutrients. The use of Jatropha seed cake as an organic fertiliser could offer smallholder farmers a better source of organic fertiliser with manageable rates of residues as compared to other sources of organic fertiliser. For instance, the rates used in this research were between 3 and 6 tons/ha as compared to about 15 tons/ha for farmyard manure.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Physiologically meaningful moisture content determination for Brazil nut (*Bertholletia excelsa* Bonpl.) seeds

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Brazil nut (*Bertholletia excelsa* Bonpl.; Lecythidaceae) seeds are large, and the oily embryo (edible part) is covered by a thick, woody seed coat. Difficulties in seed storage may be due to desiccation sensitivity of the seeds, and prior variable results may be related to procedures for seed moisture content (MC) determination. This study first compared 3 methods of preparation of whole seeds, prior to MC determination: (i) intact seeds; (ii) chopped (<7 mm) and (iii) cut lengthwise. Drying at 105°C for 24 h was sufficient to remove free water in all three procedures, with results within tolerance limits. Secondly, seeds in equilibrium with relative humidity had a high variation in MC. This could be explained to some extent by the increased proportion of seed coat in smaller seeds, with 48% of seed weight average total. Seed coat MC was always higher than embryo MC. Linear regression revealed an increasing overestimation of embryo MC with desiccation. Considering 10% MC of the whole seed, embryo MC was 5.3% (a 1.9-fold overestimation). In this respect, it is physiologically meaningful to determine embryo MC and not whole seed MC. The approach may be applied to other species with similar seed morphology.

Key words: *Bertholletia excelsa*, drying, Lecythidaceae, recalcitrant seeds, seed moisture content, seed testing.

INTRODUCTION

Bertholletia excelsa Bonpl. (Lecythidaceae), popularly known as Brazil nut, is native to the Amazon region (Flora do Brasil, 2018). Seeds are the main product of this multiple-use tree of great socioeconomic importance for the Amazon region (Calvi and Ferraz, 2014). In 2014, about 37,500 tons of seeds were produced in Brazil, generating approximately USD 33.8 million on the national market or for export (IBGE, 2014). Seed

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collections are done primarily in native populations (IBGE, 2014) which reduce the demand for plantations. However, some studies indicate that this species is suitable for plantations (Ferreira and Tonini, 2009), or as restoration of degraded areas (Costa et al., 2009), and presents higher production in stands with clayey to very clayey soil texture (Guerreiro et al., 2017).

Research on seed technology of Brazil nut is scarce, which can be observed based on the lack of publications in recent years. The main studies were published in books and technical reports during the 1980s and 1990s. Brazil nut seeds measure 4 to 7 cm, are triangular in cross section and weigh 6.7 to 16.7 g (Müller et al., 1995; Ferreira et al., 2006). The seed coat is lignified, hard, rough, permeable to water (Müller et al., 1995), and offers mechanical restriction to germination (Müller and Freire, 1979). The embryo is mainly a large, reserve-storing hypocotyl, no distinction of cotyledons is visible with an ellipsoid shape being the shoot pole is sharper than the root pole on the opposite end (Prance and Mori, 1978).

Brazil nut seeds are not viable when stored for a long time (Figueirêdo et al., 1990a, b; Figueiredo and Carvalho, 1994; Camargo et al., 1997). This may be due to desiccation sensitivity of the seeds. However, the relation between seed moisture content (MC) and germination gives no conclusive results so far. Seeds had been classified as desiccation sensitive (recalcitrant), because germinability was reduced when dried to values lower than 14% seed MC (Figueirêdo et al., 1990c). However, seeds were also considered as intermediate, based on 42% germination with 5% MC (Figueirêdo and Carvalho, 1994) or 16% viability (tetrazolium test) with 4.5% MC (Camargo et al., 1997). As seed storage behaviour is based on seed tolerance to desiccation, the method used to determine seed MC is extremely important and can be the reason for these differences.

According to seed testing prescription of that time (Brasil, 1976, 1992), seed MC was determined with whole seeds, dried under 3 possible conditions (105°C for 24 h, 103°C for 17 h or 130°C for 1 h). Comparing these conditions, no difference was found with whole Brazil nut seeds (Camargo, 1997). However, due to the large seed size, the prescribed time might not have been enough for complete loss of free water. In the current edition for seed testing, the 3 temperature conditions continue to be valid and, in addition, large tree seeds (weight of 1,000 seeds > 200 g), with very hard integument and/or high oil content, should be cut in pieces smaller than 7 mm (Brasil, 2009; ISTA, 2015).

In the special case of Brazil nut, the woody seed coat may contribute around 50% of the whole seed weight (Müller et al., 1995; Ferreira et al., 2006). Seed MC determination is necessary for deciding adequate procedures to maintain seed quality during seed handling and commercialization. If the objective of seed MC determination is related to viability, as during seed development, maturation and storage, the analysis should determine MC of the physiologically active part (embryo and its seed reserves), and exclude all other seed components, such as dispersal aids, protective coats, etc.; in this way, the aim of this study was to evaluate the contribution of seed coat to seed weight and its effect on MC values and to propose a new protocol for seed MC determination of Brazil nut, in comparison with seed testing procedures of the last decades.

MATERIALS AND METHODS

Fruits of *B. excelsa* were collected in 2 municipalities of Amazonas State (Brazil), in Itacoatiara and Autazes, about 176 and 108 km from Manaus, respectively. Climate is tropical wet, type Am Köppen classification, with an annual average of 28.2°C; 82.7% relative humidity (RH) and 2,240 mm precipitation (INMET, 2016). Fruits were collected during natural dispersal between January and February. In the Seed Laboratory in Manaus, the indehiscent woody fruits were opened with a machete. Seeds were rinsed in running water to remove impurities and empty seeds eliminated after floating. Subsequently, seeds were superficially dried for 40 min under ventilation at room temperature (27 ± 3°C and 70 ± 3% RH). Until the beginning of the experiments, whole seeds were kept in double polyethylene bags (130 µm thick) in a cold chamber (15 ± 1°C, 96 ± 3.5% RH) to equilibrate moisture.

The Itacoatiara collection was used to compare 3 preparation methods of the seeds prior to moisture determination: (i) cutting into pieces smaller than 7 mm, following the most recent recommendations for seed testing (Brasil, 2009; ISTA 2015), (ii) using whole seeds according to Brazilian Seed Testing Rules of 1992 (Brasil, 1992), and (iii) cutting lengthwise with a knife and the help of a hammer. For each preparation, 60 seeds were used, considering each seed as a repetition. Following the Seed Testing Rules (Brasil, 1992; 2009), the samples were weighed using a balance with 0.001 g precision scale and dried in an oven at 105 ± 3ºC for 24 h. To verify if all free water was evaporated, the samples returned to the oven and were weighed every 24 h for another 6 days. Seed MC was calculated as percent of fresh seed weight (Brasil, 1992, 2009; ISTA, 2015).

The Autazes collection was used (i) to describe variation in seed size and the contribution of seed coat and embryo to the whole seed weight based on 1,100 seeds; (ii) to compare whole seed MC with the MC of the seed parts, that is, the embryo and the seed coat; thereafter, 1,700 seeds were tested through a desiccation gradient. Desiccation was done by spreading out the seeds in one layer in an air-conditioned room at 25 ± 3 °C and 68 ± 3% RH. After different periods of drying, seeds were packed in polyethylene bags (130 µm thick) and maintained at 15 ± 1 °C for at least a month in order to equilibrate moisture between seed coat and embryo and throughout each desiccation level. Seed MC was determined for each individual seed, separating the seed coat from the embryo with a longitudinal cut. Samples were dried at 105 ± 3°C for 24 h and seed MC was expressed as a percentage of fresh weight. Whole seed MC was obtained by summing the weight of the seed parts.

Differences in MC between preparation methods and between values during the 7 days of drying were evaluated according to Seed Testing Rules, based on the allowed levels of tolerance for seed moisture (Bonner, 1984; Brasil, 2009) and analysed by one-way ANOVA (Sisvar® version 5.3). The comparison between whole seed mass with embryo and seed coat mass was shown with linear regressions (SigmaPlot® version 11.0). Relation between MC of whole seeds and embryo and seed coat MC through a desiccation gradient was also determined with linear regressions.

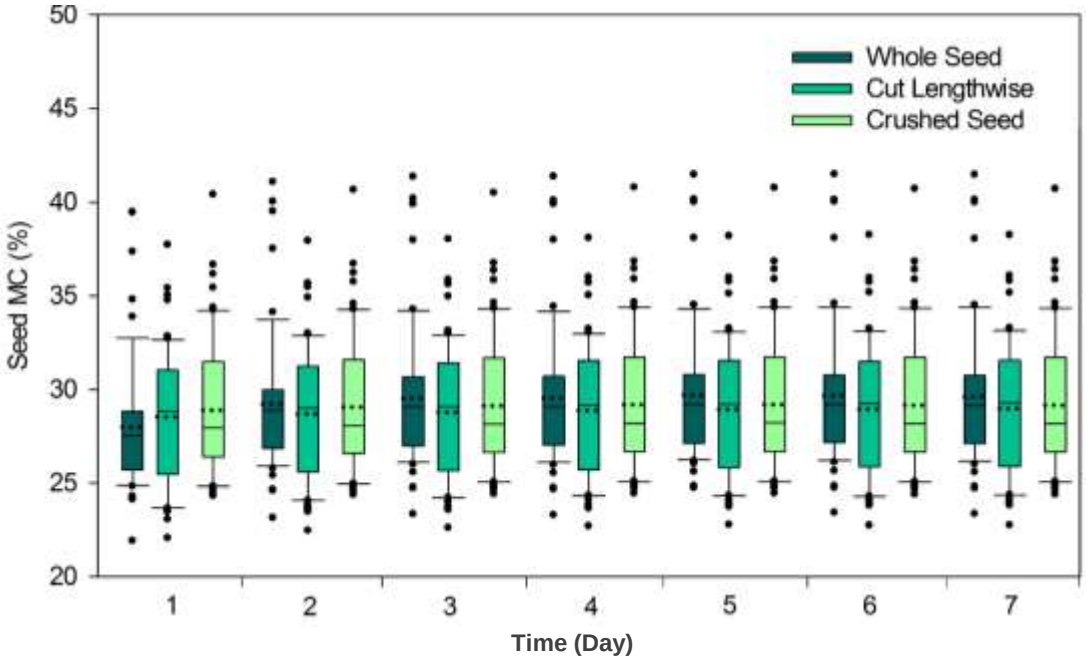

Figure 1. Boxplot of Seed Moisture Content (MC) of *Bertholletia excelsa* Bonpl. comparing three preparation methods: whole seeds, seeds cut lengthwise and crushed seeds (small pieces of ≤ 7 mm). Median (solid line) and mean value (dotted line) of 60 seeds for each treatment. Samples were dried at 105 ± 3°C and reweighed every 24 h, during seven days.

RESULTS

Average seed MC determined by the 3 methods was 28.0% (whole seed), 28.5% (cut lengthwise) and 28.9% (crushed) all with the same standard deviation (σ = 3.5). Seven days of drying increased these values slightly to 29.6, 29.0 and 29.2%, respectively (Figure 1). However, no statistical difference was detected between the methods and the length of drying time (P = 0.724). The findings are also consistent with the standards for seed quality analysis, as up to 2.5% difference between the results of 2 replicates are tolerated for large tree seeds with a high MC (> 25%) (Brasil, 2009). In this respect, all 3 methods can be used for MC determination of *B. excelsa* seeds, and 24 h at 105°C gave satisfactory results for the evaporation of free water. However, in all 3 seed preparations, MC between the individual seeds showed a high variation (Figure 1). The range between the extremes was 25 to 35% (excluding outliers). Considering that all seeds had been maintained under the same environmental humidity for at least 30 days before the MC determination, factors other than equilibrium moisture content may have affected these results; therefore, seed size and the percentage seed coat *vs.* embryo were measured.

Individual seed weight ranged from 3.76 to 20.35 g, an average of 8.80 g. The majority (66.6%) had between 6.55 and 11.05 g (Figure 2). The relation between seed coat and embryo mass in relation to whole seed mass is shown in Figure 3. It is possible to determine that, in smaller seeds, the percentage of seed coat and embryo is quite similar and, with an increase in size, seed coat proportion is reduced (Figure 3). On average, the woody seed coat in fresh seeds of *B. excelsa* accounts for 42.3% of the seed weight, complemented by 57.7% for the embryo. This relation changes to 40.4% for the seed coat and 59.6% for the embryo, by considering only dry matter.

Brazil nut embryos are primarily oily and the seed coat is woody and fibrous. The correlation between whole seed MC with embryo MC and seed coat MC was linear as attested by R^2 of 0.97 for the embryo and R^2 = 0.91 for the seed coat (Figure 4). Embryo MC was always lower and seed coat MC higher than MC determined with the whole seed. Consequently, if MC is determined using whole seeds, the MC of the living part is overestimated (Figure 4). Linear regression reveals that overestimation of embryo MC is augmented in drier seeds. Considering 10% MC of whole seeds, embryo MC will be 5.3% (a 1.9-fold overestimation), while seed coat MC will be 16.5% (a 0.6-fold underestimation). Values for embryo and seed coat MC will be similar only when whole seed MC reaches 43% (Figure 4).

DISCUSSION

Determination of seed MC aims to evaluate free water in

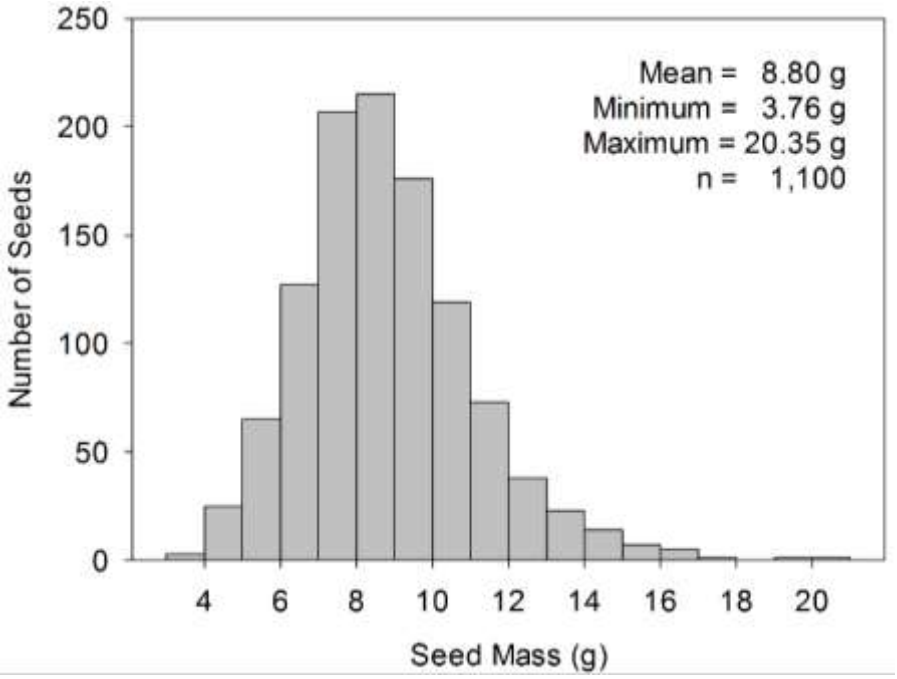

Figure 2. Variation in fresh seed weight of *Bertholletia excelsa* Bonpl.

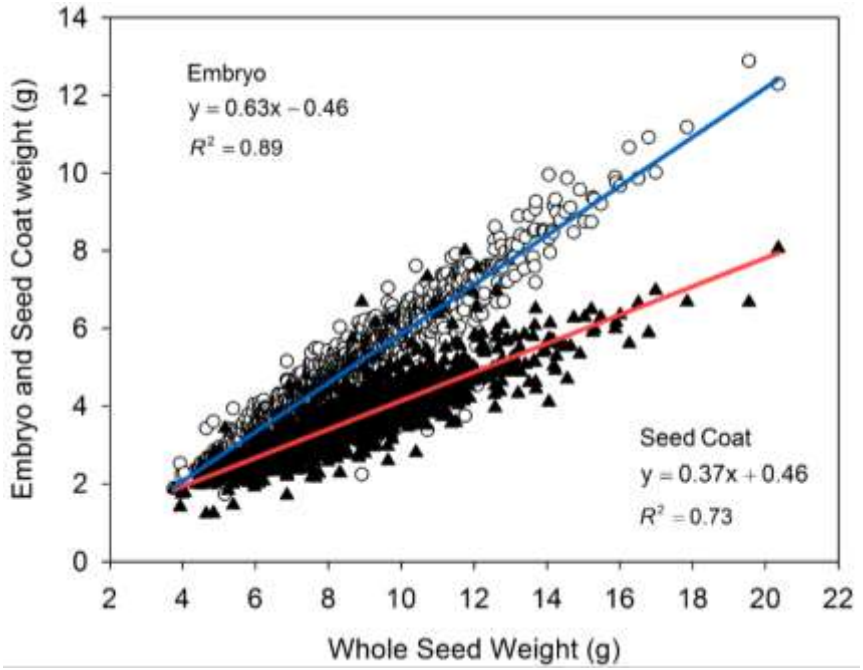

Figure 3. Relation between weight of whole seeds and the weight of seed components: embryo (open circles; blue line) and seed coat (closed triangles; red line) of *Bertholletia excelsa* Bonpl. (n = 1,100).

the seeds. According to Seed Testing Rules (Brasil, 2009), the drying period at 105°C should be completed in 24 h. In the special case of Brazil nut, the seeds are very large and contain about 67% lipids (Ferreira et al., 2006; Balbi et al., 2014); thus, the drying period should permit evaporation of free water and avoid or minimize

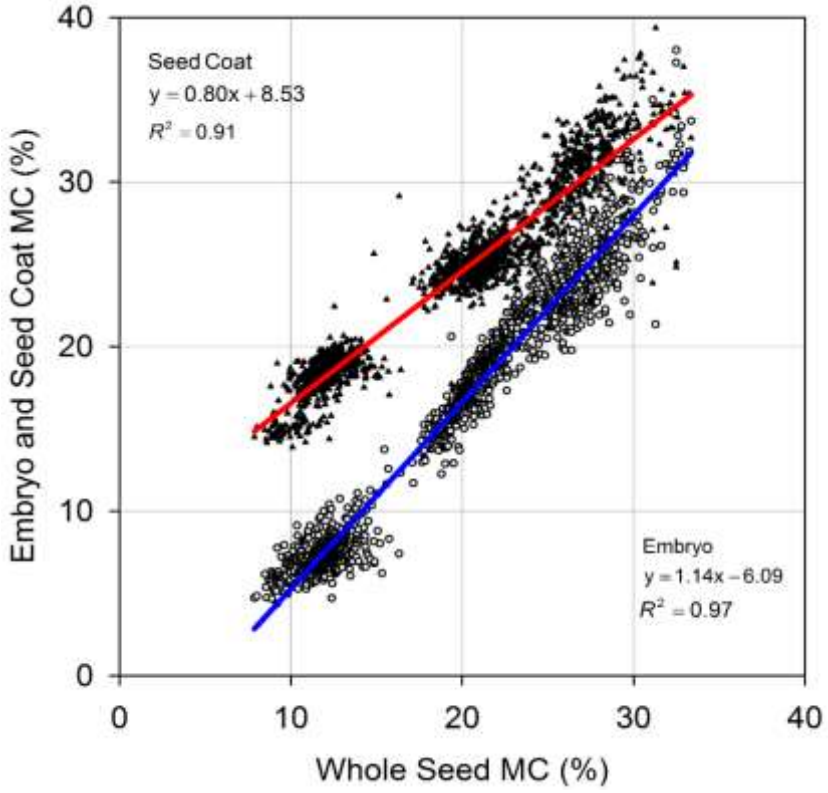

Figure 4. Relation between whole seed Moisture Content (MC) and the MC of seed components: embryo (open circles; blue line) and seed coat (closed triangles; red line) through a desiccation study of *B. excelsa* seeds. Data represent individual seed measurements (n = 1,700).

evaporation of volatile compounds and oils. This study showed that 24 h of drying provide results within the tolerance levels given for seed testing, which recommended whole seeds (Brasil, 1992), recently crushed seeds (Brasil, 2009; ISTA, 2015), and the lengthwise cut tested here. Grinding seeds cannot be recommended as the procedure resulted in significantly reduced values in comparison to whole seeds (Camargo, 1997). The extended drying period of up to seven days kept MC constant in all three preparation methods. In this way, 24 h of drying is an adequate desiccation time at 105°C for Brazil nut seeds and it can be assumed that the loss of volatile substances or oils under this condition is not significant for seed quality testing.

On the other hand, a large variation in MC between seeds in equilibrium with RH was detected. Variation could be explained to some extent by increasing percentage of the seed coat in smaller seeds, and by variation in seed coat weight of seeds with the same embryo size and shape. As Brazil nuts are collected after natural fruit release, further studies are suggested to evaluate embryo MC of the same fruit and between fruits, and if seed moisture is further affected during the transport of the indehiscent woody fruits.

Divergent results of Brazil nut seeds considering desiccation tolerance were probably based on the procedures for MC determination. As the protective seed coat holds a considerable percentage of seed weight, MC determination with the whole seed overestimates embryo MC. Differences of MC between the seed coat and the embryo was observed earlier in a seed storage study of Brazil nut by Kainer et al. (1999). These authors treated the seeds as desiccation-sensitive and stored them in moist sand. After six months, seed coat MC had increased from 29 to 40%, while embryo MC increased only from 22 to 28%. This result is contrary to what usually happens in most species, where the seed coat contains less moisture than in the reserves and/or embryo (Schmidt, 2000).

A linear regression to estimate embryo MC with MC of whole seeds is presented in this study, using seeds from Autazes. Average seed size (8.8 g) was slightly smaller and had a greater range (3.8 to 20.4 g) than seeds from other regions. Brazil nut seeds from Amapá State (Brazil) had an average weight of 10.5 g (ranging from 6.7 to 16.7 g) (Ferreira et al., 2006) and from Pará State (Brazil), had a weight of 11.9 g (Müller et al., 1995). These authors reported an even higher percentage of the seed coat in total seed weight, with 52% (Ferreira et al., 2006), and 51% of total (Müller et al., 1995), compared to 42.3% in

this study. Differences in the percentage of woody seed coat to whole seed mass, due to provenance, will hinder the direct application of the regressions presented here.

Conclusions

Seed viability of Brazil nut can only be guaranteed if embryo MC is maintained above a tolerable level. Considering this aspect, it is physiologically meaningful to determine embryo MC and not whole seed MC. Seed coat removal is easier using a longitudinal seed cut and, if desired, both parts can be evaluated. Although this is a case study of one species, the approach may be applied to other species with similar seed morphology.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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