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Ingestion and *In Vivo* Digestibility of a Concentrated Granulated Feed Containing Seeds of *Moringa oleifera* Associated with *Pennisetum purpureum* in Guinea Pigs

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Abstract

In order to contribute to the improvement of guinea pig nutrition through the use of unconventional vegetable protein sources (*Moringa oleifera* seeds), experimental trials were carried out at the Animal Production and Nutrition Research Unit (URPRONAN) of the University of Dschang in April 2018. *Moringa oleifera* seeds from North Cameroon were divided into three treatments: the first consisted of whole *Moringa oleifera* seeds (MO-whole), the second of *Moringa oleifera* seeds soaked in cold water for 24 hours (MO-water) and the third of peeled *Moringa oleifera* seeds (MO-peeled). The seeds belonging to these three treatments were analyzed for the determination of the chemical composition before being incorporated into the different concentrates. Each concentrate was then granulated and combined with *Pennisetum purpureum* to make up the different rations. 40 English Guinea pigs with an average weight of 350 ± 50 g were used to evaluate the ingestion and digestibility of each ration. During the digestibility test which lasted 17 days (10 days of adaptation and 07 days of data collection), each ration was repeated on 10 Guinea pigs. The main results showed that the total tannin and phenol contents decreased significantly ($p < 0.05$) in *M. oleifera* seeds soaked for 24 hours (8.2% DM; 2884 mg/100gMS) and pulped (13.6% DM; 3156 mg/100gMS). The intake of crude cellulose (10.26 gMS/d) and crude protein (3.21 gMS/d) in rations containing whole *M. oleifera* seeds was significantly ($p < 0.05$) lower than in other rations. Similarly, crude protein digestibility (77.04%) of rations

containing whole *M. oleifera* seeds was significantly ($p < 0.05$) lower than other rations. This study shows that *Moringa oleifera* seeds soaked for 24 hours or pulped can be used in guinea pig feed as an alternative source of protein.

Keywords

Chemical Composition, Digestibility, Ingestion, Guinea Pigs, *Moringa oleifera*, *Pennisetum purpureum*

1. Introduction

In Cameroon, there is a deficit of about 8 kg/inhabitant/year of protein of animal origin in the population [1]. To feed and satisfy these needs, caviar farming can be an alternative. Indeed, caviar farming is an important element in the rural landscape in Cameroon, in that it improves the rather meagre income of farmers [2]. However, one of the main handicaps to the development of livestock farming in this area is its diet. Indeed, the animals feed mainly on kitchen waste, crop residues and forage grasses, which are often deficient in essential nutrients such as proteins and minerals [3]. The result is low productivity.

Improving the productivity of Guinea pigs can be achieved, among other things, by improving their diet and, above all, by providing them with a balanced food ration [4]. Thus, one easily achievable solution is the use of fodder legumes and other non-conventional protein sources with an established nutritional value as a poor food supplement [5]. Among these unconventional protein sources are the seeds and leaves of *Moringa oliefera. Moringa oleifera* belongs to the mono-generic family Moringaceae [6]. It is a tree native to north-western India from Pakistan, which borders the Himalayas [7]. It has been introduced into all tropical and subtropical regions [8] and has become naturalized in many African countries [9]. A tree can produce an average of 15,000 to 25,000 seeds per year [10]. The leaves of this plant, in Cameroon as in most of the countries where it is produced, are used for food and feed. Notably the study by [11] showed that the leaves of *Moringa oleifera* improved growth and carcass yield in Guinea pigs. As for the seeds of *Moringa oleifera*, they are used to treat and purify milk, honey and water [12] [13], thanks to its richness in active cationic polyelectrolytes. In North Cameroon, the seeds are also pressed to extract the oil, and the resulting residues in the form of cake are used for fattening (cattle and small ruminants).

One of the major problems limiting the use of *Moringa oleifera* seeds in animal feed is their high concentration of anti-nutritional factors that reduce the ability of microorganisms to digest the nutrients they contain [14]. Consequently, detoxification methods (physical and chemical), more or less expensive, have been developed to control the negative effects of these anti-nutritional factors to a threshold that is harmless for both humans and animals [15]. Thus, the work of [16] showed that pulping reduced the levels of condensed tannins, fla-

vonoids and phytates contained in *M. oleifera* seeds. According to [17], soaking helps neutralize the enzyme inhibitors present in the seeds in order to improve the digestion of certain complex molecules. In Cameroon, very little work has been done so far on the use of *M. oleifera* seeds subjected to different treatments on the ingestion and digestibility of rations in Guinea pigs. It is therefore to fill this gap that the present work was initiated with the main objective of determining the effect of *M. oleifera* seeds treated and included in a concentrated granulated feed associated with *Pennisetum purpureum* on ingestion and digestibility in Guinea pigs.

2. Materials and Methods

2.1. Study Area

This study was conducted during the month of April 2018, in the Animal Production and Nutrition Research Unit (URPRONAN) of the Faculty of Agronomy and Agricultural Sciences (FASA) of the University of Dschang. The city of Dschang is located at the 15th degree of the East meridian, at latitude 5˚36' - 5˚44' North and longitude 09˚85' - 10˚06' East. The climate of the region is equatorial of Cameroonian type modified by altitude. Rainfall in the locality varies between 1500 and 2000 mm per year. The average annual temperature is around 20˚C, total annual insolation at 1800 hours and average relative humidity varies between 40% and 90%.

2.2. Plant Material

The plant material consisted of seeds of *Moringa oleifera* (whole, soaked and shelled) and *Pennisetum purpureum*. *Pennisetum purpureum* was mowed before flowering at the Dschang University Farm. As for the seeds of *Moringa oleifera*, they were bought in North Cameroon.

2.3. Animal Material and Housing

Forty English Guinea pigs (20 males and 20 females) aged approximately 4 to 5 months and with an average weight of 350 ± 50 g were used for the evaluation of ingestion and digestibility. The animals were placed in individual wire cages of 10.6 dm^3 (76 cm × 46.5 cm × 30 cm) each equipped with a 100 g plastic feeder and a plastic drinker.

2.4. Evaluation of the Chemical Composition of *Moringa oleifera* Seeds and Preparation of Rations

The chemical composition of *Moringa oleifera* seeds was carried out to determine the contents of dry matter, ash, organic matter, crude cellulose, crude protein, fat, condensed tannins, non-nitrogenous extractives and total phenols according to the methods described by [18]. The chemical composition of *Moringa oleifera* seeds was determined at the Laboratory of Animal Nutrition of the University of Agricultural Sciences and Veterinary Medicine of Cluj-napoca (Roma-

nia).

Four types of concentrates (Table 1) were manufactured, where three containers 7% of *Moringa oleifera* seeds, constituted according to the different treatments applied to the seeds.

Each concentrate was then granulated and combined with *Pennisetum purpureum* to make up the various rations as follows:

- **TEM + Pp**: concentrate without Moringa seed + 105 g of *P. purpureum*/Animal/Day;
- **MO-whole + Pp**: concentrate containing whole Moringa seed + 105 g of *P. purpureum*/Animal/Day;

Table 1. Centesimal composition of granulated concentrates.

Ingredients (%)	Different rations			
	TEM	MO-whole	MO-water	MO-peeled
Corn	31	28	28	28
Manioc	08	08	08	08
Wheat bran	25	25	25	25
Whole Moringa	-	07	-	-
Shelled Moringa	-	-	-	07
Soaked Moringa	-	-	07	-
Palm kernel cake	14	16	16	16
Soybean cake	03	-	-	-
Cotton cakes	04	-	-	-
Fish meal	07	08	08	08
Bone meal	01	01	01	01
Shell	01	01	01	01
10% concentrate	02	02	02	02
Molasses	04	04	04	04
TOTAL	**100**	**100**	**100**	**100**
Chemical composition				
Dry matter (%)	86.75	87.75	88	87.75
Metabolizable energy (Kcal/KgDM)	2308.67	2335.10	2335.10	2345.2
%DM				
Organic Matter	73.9	76.5	73.95	77.75
Crude Protein	17.49	17.25	17.57	17.66
Lipids	2.86	4.99	5.17	5.99
Raw cellulose	7.8	7.51	7.61	7.31
Cendre	12.85	11.25	14.05	10

TEM: control concentrate without *Moringa oleifera* seeds; MO-whole: concentrate containing whole *Moringa oleifera* seeds; MO-water: concentrate containing *Moringa oleifera* seeds soaked for 24 hours and MO-peeled: concentrate containing peeled *Moringa oleifera* seeds.

- **MO-water + Pp:** concentrate containing peeled Moringa seeds + 105 g of *P. purpureum*/Animal/Day;
- **MO-peeled + Pp**: concentrate containing Moringa seeds soaked for 24 h + 105 g of *P. purpureum*/Animal/Day.

2.5. Evaluation of Ingestion and *In Vivo* Digestibility of Rations

For each of the treatments, 5 males and 5 females were used. These animals were randomly assigned to individual cages, and feed was served only once each day between 8 and 9 hours. For intake assessment, the amounts of feed served were recorded, and refusals were collected daily and weighed before any further distribution. Refusals were quantified to determine the amounts of food ingested. Food ingestion or consumption was calculated according to the formula opposite:

Food ingestion
= Daily amount of food served − Amount not consumed (refusal)

The digestibility trial was preceded by a period of adaptation of the animals to the digestibility cage and the pelleted compound feed, which lasted 10 days. During this period, the amounts of granulated concentrate associated with the *Pennisetum purpureum* served were adjusted to the animal's consumption. During the data collection period which lasted 7 days, each morning before the feed was distributed, the faces were collected, weighed and dried at 60˚C in the laboratory in a ventilated oven. Subsequently, the analysis of their dry matter (DM), organic matter (OM), crude protein (CP) and crude fiber (CF) content was done according to the method described by [18]. The apparent digestive utilization coefficients of Dry Matter (CUDaDM), Organic Matter (CUDaOM), Crude Protein (CUDaCP), and Crude fiber (CUDaCF) were calculated according to the formula of [19]:

$$\text{CUDaDM (\%)} = (\text{DM ingested} - \text{fecal DM})/(\text{DM ingested}) \times 100$$

$$\text{CUDaMO (\%)} = (\text{OM ingested} - \text{OM fecal})/(\text{OM ingested}) \times 100$$

$$\text{CUDaPB (\%)} = (\text{CP ingested} - \text{CP fecal})/(\text{CP ingested}) \times 100$$

$$\text{CUDaCB (\%)} = (\text{CF ingested} - \text{CF fecal})/(\text{CF ingested}) \times 100$$

2.6. Statistical Analysis

Food intake and nutrient digestibility data were subjected to analysis of variance (ANOVA) at a factor following the general linear model (GLM). When significant differences existed between treatments, the separation of the means was done by the Waller Duncan test at the 5% significance level [20]. SPSS 21.0 analysis software was used.

3. Results

3.1. Effect of Different Treatments on the Chemical Composition of *M. oleifera* Seeds

The different treatments carried out on *M. oleifera* seeds have had a variable in-

fluence on its chemical composition (Table 2). The DM content of peeled *M. oleifera* seeds was significantly ($p < 0.05$) higher than the DM content of whole *M. oleifera* seeds and that of seeds soaked for 24 hours.

The organic matter, protein and fat contents of whole *M. oleifera* seeds and *M. oleifera* seeds soaked for 24 hours were comparable ($p > 0.05$) and significantly ($p < 0.05$) lower than those of dehulled *M. oleifera* seeds. The content of non-nitrogenous extractives was significantly ($p < 0.05$) lower in *M. oleifera* hulled seeds. Condensed tannins and total phenols were significantly ($p < 0.05$) lower in *M. oleifera* seeds soaked for 24 hours. On the other hand, the different treatments carried out on *M. oleifera* seeds did not significantly ($p > 0.05$) affect the crude cellulose content.

3.2. Ingestion of Granulated Concentrate with Inclusion of *M. oleifera* Seeds Subjected to Different Treatments Associated with *P. purpureum* in Guinea Pigs

Ingestion of granulated concentrates with different treatments of *Moringa oleifera* seeds induced no significant effect ($p > 0.05$) on the ingestion of *P. purpureum* in Guinea pigs (Table 3). However, different treatments of *Moringa oleifera* seeds significantly ($p < 0.05$) influenced the ingestion of granulated concentrate in Guinea pigs. Indeed, the intake of granulated concentrate in the MO-whole + Pp, MO-water + Pp and MO-peeled + Pp rations was comparable ($p > 0.05$) on the one hand and significantly ($p < 0.05$) lower than the intake of the TEM + Pp ration.

The different treatments of *M. oleifera* seeds did not significantly influence ($p > 0.05$) the total ingestion of dry matter and organic matter in the Guinea pigs whatever the ration.

The crude cellulose intake of the MO-water + Pp ration was comparable ($p > 0.05$) to the TEM + Pp ration and significantly ($p < 0.05$) higher than the BC

Table 2. Chemical composition of *M. oleifera* seeds subjected to different treatments.

Chemical composition	*Moringa oleifera* seeds			SEM	p
	Whole	Hardened 24 h	Peeled		
DM (%)	91.1[b]	90.7[c]	93.4[a]	0.41	0.001
% DM					
Organic Matter	87.3[b]	87.4[b]	89.5[a]	0.35	0.001
Crude protein	26.0[b]	26.4[b]	31.3[a]	0.85	0.001
Fat	22.5[b]	23.0[b]	32.7[a]	1.65	0.001
Raw cellulose	10.2[a]	9.4[a]	9.9[a]	0.11	0.05
Non-nitrogenous extractives	28.5[a]	28.4[a]	15.5[b]	2.16	0.001
Condensed tannins	18.4[a]	8.2[c]	13.6[b]	1.47	0.001
Total phenols (mg/100g DM)	4185[a]	2884[c]	3156[b]	247	0.001

[a,b,c]: Averages with the same letters on the same line are not significantly different at the 5% threshold; MS: Dry Matter; SEM: Standard Error on the Mean; p: Probability.

Table 3. Ingestion of granulated concentrate with inclusion of *M. oleifera* seeds subjected to different treatments associated with *P. purpureum* in Guinea pigs.

Ingestions (g MS/j/animal)	Treatments				SEM	p
	TEM	MO-whole	MO-water	MO-peeled		
P. purpureum	21.9[a]	20.93[a]	22.97[a]	24.11[a]	0.68	0.42
Granular concentrate	35.34[a]	29.36[b]	29.16[b]	26.40[b]	1.12	0.02
Total (*P.p* + GC)	57.23[a]	50.29[a]	52.13[a]	50.51[a]	1.12	0.87
Total dry matter	50.43 [a]	44.66[a]	46.40[a]	44.93[a]	0.96	0.12
Raw cellulose	12.94[ab]	10.26[c]	13.54[a]	11.59[b]	0.39	0.00
Crude protein	5.10[a]	3.21[c]	3.97[b]	4.72[a]	0.18	0.00
Organic Matter	38.59[a]	34.94[a]	35.69[a]	35.54[a]	0.15	0.27

[a,b,c]: Averages with the same letters on the same line are not significantly different at $p < 0.05$.

intake of the MO-whole + Pp and MO-peeled + Pp rations. Moreover, the crude fiber intake data obtained with the TEM + Pp ration did not show a significant difference ($p > 0.05$) with those obtained with the MO-peeled + Pp ration. The lowest significantly ($p < 0.05$) value of crude fiber intake was obtained with the MO-whole + Pp ration.

Crude protein intake of the TEM + Pp and MO-whole- + Pp rations were comparable ($p > 0.05$) on the one hand, and significantly ($p < 0.05$) higher than the intake of crude protein of the MO-whole- + Pp and MO-water + Pp rations on the other hand. The crude protein intake of the MO-whole + Pp ration was significantly ($p < 0.05$) lower than that of the other rations.

3.3. Nutrient Digestibility of the Granulated Concentrate with Inclusion of Seeds of *M. oleifera* Subjected to Different Treatments Associated with *P. purpureum* in Guinea Pigs

In this trial, the digestive utilization factor of DM, OM and CF of rations containing differently treated *M. oleifera* seeds was comparable ($p > 0.05$) to the control ration (Table 4). Similarly, the crude protein digestibility of the MO-whole + Pp rations was comparable ($p > 0.05$) to that of the MO-water + Pp ration. On the other hand, the CP digestibilities of the TEM + Pp and peeled + Pp rations were comparable and significantly higher than those of the MO-whole + Pp ration.

4. Discussion

The fat and protein contents of whole *M. oleifera* seeds and 24-hour soaked seeds were comparable and significantly lower than those of dehulled seeds. This could be attributed to seed pulping. Indeed, these elements are concentrated in large quantities in the kernels compared to the shell. Soaking and pulping significantly decreased the levels of condensed tannins and total phenols in *M. oleifera* seeds, but this decrease was more pronounced in *M. oleifera* seeds soaked for 24 hours. This result is consistent with work by [16] which showed that pulping reduced the levels of condensed tannins, flavonoids and phytates in

Table 4. Digestibility of granulated concentrate with inclusion of *M. oleifera* seeds subjected to different treatments associated with *P. purpureum* in Guinea pigs.

CUDa(%)	Treatments				SEM	P
	TEM	MO-whole	MO-water	MO-peeled		
Dry matter	82.01[a]	82.88[a]	79.24[a]	81.74[a]	0.76	0.40
Crude cellulose	77.26[a]	74.88[a]	79.90[a]	79.08[a]	1.19	0.49
Crude protein	86.30[a]	77.04[c]	78.72[bc]	82.65[ab]	1.15	0.001
Organic Matter	82.18[a]	83.14[a]	78.63[a]	81.29[a]	0.87	0.31

[a,b,c]: Averages with the same letters on the same line are not significantly different at $p < 0.05$; CUDa: apparent digestive utilization factor.

M. oleifera seeds. In addition, [21] reported a decrease in tannins, phytates and phenols in legume seeds after soaking in water for 24 hours.

During this study, the inclusion of treated (soaked and dehulled) *Moringa oleifera* seeds in the rations significantly increased crude protein intake compared to the ration containing untreated (whole) *Moringa oleifera* seeds. This could be explained by the fact that the technological treatments carried out on the *Moringa oleifera* seeds attenuated their negative effects of condensed tannins and phytates thus increasing the palatability of these rations. This is consistent with the work of [5] who reported a decrease in crude protein intake with the inclusion in rations of unconventional protein sources rich in anti-nutritional factors (*Desmodium intortum* and *Arachis glabrata*).

In the present study, no significant difference was observed between the different treatments for CUDa of BC DM and OM with fresh *Pennisetum purpureum* associated with the rations. This would be related to the small variation the proportions between the ingredients of the different rations, which accounts for the narrowness of the ration. This would be related to the small variation the proportions between the ingredients of the different rations, which accounts for the narrowness of the ration. Indeed, the different rations were iso-nitrogenated. The same observation was made by [5] [22]; [4] and [23] who reported that the inclusion of unconventional protein sources (*Desmodium intortum, Arachis glabrata* and *Moringa oleifera*) did not significantly affect the quality of the diet the apparent digestive utilization factor of DM, OM and CF. On the other hand, the digestibilities of the CP of the TEM + Pp and peeled + Pp rations were comparable and significantly higher than that of the MO-whole + Pp ration. This could be due to the treatment carried out on *M. oleifera* seeds which reduced their tannin content. Indeed, according to [24] and [25] the tannins present in the feed can form insoluble complexes with dietary proteins and thus decrease their digestibility or bind to digestive enzymes and inactivate them. These results are superior to those obtained by [3].

5. Conclusion

The study showed that the lowest levels of condensed tannins (8.2% MS) and total phenols (2884 mg/100gMS) were obtained with *M. oleifera* seeds soaked in

water for 24 hours. Guinea pig ingestion of the granulated concentrate was higher (35.34 gMS) with the ration not containing *Moringa oleifera* seeds. In addition, the highest crude protein intake values were obtained respectively with the control ration (5.10% DM) and the ration containing peeled *Moringa oleifera* seeds (4.72% DM).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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