

## Research Article

# Intake and *in vivo* digestibility of *Pennisetum purpureum* K. Schum supplemented with graded level of cassava leaf meal (*Manihot esculenta* Crantz) in guinea pigs (*Cavia porcellus* L.)

Mweugang Ngouopo Nathalie<sup>1\*</sup>, Miegoue Emile<sup>2</sup>, Lemoufouet Jules<sup>2</sup>, Nkana Kontchiachou Gwladys J.<sup>3</sup>, Fonteh Anyangwe Florence<sup>2</sup>, and Pamo Tedonkeng Etienne<sup>2</sup>

<sup>1</sup> Department of Biological Sciences, Faculty of Science, University of Ngaoundere, P.O. Box. 454, Ngaoundere, Cameroon

<sup>2</sup> Department of Animal Sciences, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box: 222, Dschang Cameroon

<sup>3</sup> Agronomic Research Institute for Development (ARID), Mankon-Bamenda, PO. Box. 125, Bamenda, Cameroon

\*Correspondence should be addressed to Nathalie Mweugang Ngouopo: nathaliemweugang@yahoo.fr, nathalie.ngouopo@univ-ndere.cm, Tel: + 237 699094589/ 670394168

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### Abstract

Fresh cassava leaves are a satisfactory protein supplement, but they could be dried before feeding and used prudently for good results. The intake and *in vivo* digestibility of *Pennisetum purpureum* associated with cassava leaf meal (CLM) as protein source in the diet of guinea pigs was investigated. 24 adult guinea pigs (12 males and 12 females) of local breed, aged 6 months old and weighing averagely 614.25 ± 04.03 g were placed in a factorial design (diet and sex) in 4 batches of 6 animals (3 males and 3 females) each and randomly distributed to 4 dietary treatments RC0, RC8, RC10 and RC12 which contained no CLM (Control), 8, 10 and 12% CLM respectively. Each treatment was replicated 6 times in a completely randomized design. All animals received 250 g of *P. purpureum* + 60 g of concentrate of the corresponded dietary treatment. Independently of sex and according to sex, complementation did not (P>0.05) increase intake of nutrients. In females alone and independently of sex, nutrient digestibility (DM, OM and CP) was significantly (P<0.05) higher than the control. Complementation did not significantly (P>0.05) influence the nutrient digestibility between males and females. Dietary intake of CLM did not significantly (P> 0.05) improve the ADG regardless of the sex and considering the sex. CLM can therefore be used as source of protein in the feeding of guinea pigs without reduction of ingestion and digestibility. However, taking into account its fiber content, the level of incorporation of these leaves should not exceed 10%.

**Key words:** intake, *in vivo* digestibility, *Pennisetum purpureum*, cassava leaf meal, soybean meal, guinea pig.

### 1. Introduction

In animal husbandry, meeting the nutrient and especially protein requirements is the most important

component of the cost of production (Mweugang et al., 2014; Sourokou, 2014). The scarcity and high cost of conventional protein sources such as fish meal, soybean

meal and groundnut cakes have led to the development of non-conventional resources that are inexpensive locally and readily available in livestock feed (Obun *et al.*, 2008; Sourokou, 2014; Mweugang, 2016). Cassava leaves considered as by-products at root harvest (Vongsamphanh, 2003; Phengvilaysouk and Wanapat, 2008; Dung *et al.*, 2010) have the advantage of providing the proteins (Dada and Oworu, 2010; Udo and John, 2015) that are lacking in the diet of animals. The use of cassava leaves has been limited in monogastric feeding due to antinutritional factors such as cyanogenic glucosides, tannins and phytic acid

(Ayodeji, 2004; Dada and Oworu, 2010) Studies on the use of leaves and other vegetative parts of plants in monogastrics have shown that pretreatment through sun drying improves palatability, ingestion, growth and renders antinutritional factors inactive (Leterme *et al.*, 2005; Dahouda *et al.*, 2009; Ayssiwede *et al.*, 2012).

Accurate information on the nutritional value of a novel ingredient is very important before it is incorporated into compound feed (Adeyemi *et al.*, 2001; Obun *et al.*, 2008). The analysis of chemical composition alone does not give an indication on the palatability or on the precise nutritive value of the raw materials but only gives a quantitative estimate of the nutrients in an ingredient (Obun *et al.*, 2008). Thus, the proportion of food consumed by animals is very important since it affects the total ingestion of nutrients, digestibility and therefore the performance of the animal.

Several works on cassava leaf meal (CLM) supplementation have shown that cassava leaf meal has been used with good results up to 35% incorporation in cattle concentrate (FAO, 2003). Khang and Wiktorsson (2004) showed that CLM incorporated at 18% level in urea-treated rice straw feed improves ingestion and digestion of the whole feed by improving the ecology of the rumen. In a study conducted by Phuc *et al.* (1996) on

pigs fed increasing levels of silage and dried cassava leaves (0, 15, 30 and 45%) in substitution of soybean meal in a diet of cassava root, the latter observed up to 30% substitution, an improvement in ingestion, digestibility and thus growth, but also in food conversion and nitrogen retention. Onibi *et al.* (2008) reported that an incorporation rate of 10% of CLM as a replacement of soybean meal results in good performance and reduced feed costs in broilers as well as in guinea pigs as also reported by Mweugang *et al.* (2014, 2016). On the other hand, Tandzong *et al.* (2015) confirmed that 10% cassava leaf is considered as the optimum cassava-leaf level to achieve optimum growth and to produce good quality meat, compared with other meat such as rabbit and pork. However in guinea fowl, Dahouda *et al.* (2009) reported that incorporating 6 and 8% dried cassava leaves into the diet had no effect on carcass composition and meat quality. In the same way, Mweugang *et al.* (2016) documented that guinea pigs fed with 8% inclusion of CLM in diet exhibit good reproduction performances. Thus, the use of dried cassava leaves could enhance ingestion and digestibility and consequently growth in animals such as guinea pigs. Hence, the present investigation has been carried out to assess the effect of increasing levels of cassava leaf meal in the diet on the ingestion and *in vivo* digestibility of *Pennisetum purpureum* in guinea pigs.

## 2. Material and Methods

### 2.1. Experimental site

The study took place at the Research and Application Farm (R.A.F.) of the Faculty of Agronomy and Agricultural Science of the University of Dschang, Cameroon. Dschang is located at an average altitude of 1410 m and between 10° 26' East longitude and 5°26' North latitude. It receives between 1500 and 2000 mm of rain fall per year and the temperatures ranges between

10 °C (July-August) and 25°C (February). The climate of the locality is an altitude equatorial Cameroonian-type with a dry season that runs from mid-March to mid-November and a rainy season from mid-November to mid-March.

## 2.2. Animal material and housing

Twenty-four guinea-pigs (*Cavia Porcellus*) of which 12 males and 12 females purchased from surrounding villages, with an average weight of  $614.25 \pm 04.03$  g and aged between 5-6 months. These animals were housed individually in metabolic cages measuring 80 cm x 50 cm x 30 cm. Each cage was provided with a feeder and a drinker and equipped with a feces collecting device consisting of a mesh net of 1 mm mesh. To counter a possible deficiency in vitamin C, the latter (1 tablet of 240 mg in 1.5 L of water) was distributed daily to all animals via drinking water served *ad libitum*.

## 2.3. Plant material

*P. purpureum* was harvested before flowering every morning in the farm of the University of Dchang (Cameroon) and its neighborhoods and left to dry about in the shade before being served the next day to the animals. Branches of cassava leaves were cut off from plants of cassava before flowering in the farms twice a week. Their leaves were cut off from branches and sun dried (2–3 days) until they became crumbly, then they were milled using a hammer grinding machine to produce leaf meal and the meal obtained was stored until use.

## 2.4. Diet formulation

Four iso-nitrogenous diets (RC0, RC8, RC10 and RC12) in which CLM was incorporated at the rates of 0, 8, 10 and 12% respectively as a vegetable protein source in replacement of soybean meal were formulated. The

chemical composition of the formulated diets is presented in table 1 (AOAC, 1990).

## 2.5. Ingestion and *in vivo* digestibility test

Twenty four (24) guinea pigs of comparable weight ( $614.25 \pm 04.03$  g) were divided into 4 batches of 6 (3 males and 3 females) corresponding to the four diets in a completely randomized design and kept in individual metabolic cages. This test lasted 14 days, made up of an adaptation period of 10 days and a data collection period of 05 days (Pérez *et al.*, 1995). The aim of adaptation period was allow the animals to get familiar with the digestibility cages and the new diet that will be served to them. During the digestibility period (05 days), food was served daily in the morning (7-8h), refusals and fresh feces were collected daily and weighed before a new distribution. Each animal received a daily diet consisting of 250 g of *P. purpureum* + 60 g of formulated diets (RC0, RC8, RC10 and RC12) (Miégoué *et al.* 2016).

**Ingestion:** the ingestions of each diet were calculated by difference between the quantities served the day before and the refusals collected the next day.

**Average daily gain:** At the start and end of each of the two test periods, the animals were weighed on an empty stomach for the determination of their weight gain. The mean daily gain was obtained as the difference between the live weight of the animal at the start and at the end of the data collection period.

**Digestibility:** A sample of fresh feces (100 g) collected from each animal daily was weighed and oven-dried at 60 °C, weighed again and ground to 1mm and kept in plastic bag for evaluation of their chemical composition (AOAC, 1990) in order to estimate the amounts excreted in the feces and then the apparent digestibility coefficient (ADC) of the dry matter (DM), organic matter (OM), crude protein (CP) and crude fiber (CF) was evaluated (Roberge and Toutain, 1999).

These parameters above were calculated using the  $\text{Ingestion} = \text{Diet dayN} - \text{Refusal dayN} + 1$  following formulas below:

$$\text{Average daily gain(ADG)} = \frac{\text{Final body weight(g)} - \text{Initial body weight(g)}}{\text{Experimental period (Number of days)}}$$

$$\text{ADC DM(\%)} = \frac{(\text{DM ingested} - \text{DM excreted})}{\text{DM ingested}} \times 100$$

$$\text{ADC OM(\%)} = \frac{(\text{OM ingested} - \text{OM excreted})}{\text{OM ingested}} \times 100$$

$$\text{ADC CP(\%)} = \frac{(\text{CP ingested} - \text{CP excreted})}{\text{CP ingested}} \times 100$$

$$\text{ADC CF(\%)} = \frac{(\text{CF ingested} - \text{CF excreted})}{\text{CF ingested}} \times 100$$

An electronic balance of capacity 3 kg and precision 1 g was used for the different weightings.

**Table 1. Chemical composition of the experimental diets and of *P. purpureum***

Parameters	Diets				
	RC0	RC8	RC10	RC12	<i>P. purpureum</i>
Dry Matter (%)	90.85	90.38	91.50	90.61	90.30
Organic Matter (% MS)	89.52	86.95	87.69	86.46	86.32
Crude Protéins (% MS)	19.25	19.19	19.16	19.13	7.89
Fats (% MS)	4.66	4.27	6.19	6.13	2.20
Crude Fiber (% MS)	7.74	8.51	9.71	10.69	33.46
Ash (% MS)	10.48	13.05	12.31	13.54	9.68
Metabolizable Energy (Kcal/KgMS)	2915.65	2896.00	2837.29	3045.51	407.18

RC0: Diet containing no CLM ; RC8: Diet containing 8% CLM; RC10: Diet containing 10% CLM; RC12: Diet containing 12% CLM.

## 2.6. Statistical analyzes

The data were subjected to 2-factor variance analysis (food intake and sex) using the general linear model (GLM). When significant differences existed between treatments the means were separated by the Waller Duncan test at 5% threshold (Steel and Torrie, 1980). The comparison between the sexes was made by Student's "t" test at the 5% threshold.

## 3. Results

### 3.1. Effect of inclusion level of cassava leaf meal in the diet on intake and digestibility of experimental diets in guinea pigs

#### 3.1.1. Ingestion of *P. purpureum* and of the concentrate with graded levels of CLM

The ingestion of the forage or the concentrate did not result in a significant difference ( $P > 0.05$ ) between the sexes or within the sexes (Table 2).

#### 3.1.2. Ingestion of DM, OM, CP and CF of guinea pigs fed with graded level of CLM

The inclusion of CLM in the diet did not significantly ( $P > 0.05$ ) improved ingestion of DM, OM and CF independently of sex and regardless of sex (Table 2).

### 3.2. Comparative ingestion of nutrients between males and females based on inclusion level of CLM in the diet in guinea pigs

The ingestion of nutrients (DM, OM, CP and CF) was comparable ( $P > 0.05$ ) both in males and in females of supplemented groups (Figure 1). In contrast, ingestion of DM, OM, and CP of the RC0 diet was significantly ( $P < 0.05$ ) higher in males.

**3.3. Effect of inclusion level of CLM in the diet on apparent digestive coefficient (ADC) of nutrients in guinea pigs**

The inclusion of CLM in the diet significantly ( $P < 0.05$ ) improved the digestibility of DM, OM, and CF in females

and in all animals (Table 3). On the other hand, in males, dietary intake of CLM did not influence ( $P > 0.05$ ) the digestibility of all the nutrients (DM, OM, CP and CF) nor that of CP regardless of sex and gender.

**Table 2. Food intake according to experimental diets and sex in guinea pigs**

Ingestions (gDM/day/animal)		Rations				SEM	P
		RC0	RC8	RC10	RC12		
<b>Experimental Diets</b>							
<i>P. purpureum</i> *	♂	24.67	25.80	25.67	27.37	0.19	0.67
	♀	22.90	28.19	24.29	26.90	0.03	0.12
	♂♀	23.78	27.00	24.98	27.14	0.01	0.08
<b>Concentrate*</b>	♂	18.90	17.96	16.96	17.52	0.12	0.42
	♀	16.53	15.49	17.32	17.28	0.15	0.54
	♂♀	17.72	16.72	17.14	17.40	0.15	0.77
<b>Nutrients</b>							
<b>Dry Matter* (DM)</b>	♂	43.56	43.76	42.62	44.89	0.25	0.89
	♀	39.43	43.68	41.61	44.18	0.03	0.12
	♂♀	41.50	43.72	42.12	44.54	0.06	0.28
<b>Organic Matter*(OM)</b>	♂	42.20	41.94	40.79	42.88	0.25	0.89
	♀	38.18	41.85	39.82	42.20	0.05	0.18
	♂♀	40.19	41.89	40.31	42.54	0.08	0.40
<b>Crude Protein* (CP)</b>	♂	8.37	8.25	8.09	8.23	0.27	0.95
	♀	7.56	8.26	7.91	8.10	0.08	0.30
	♂♀	7.97	8.26	8.00	8.16	0.15	0.77
<b>Crude fiber* (CF)</b>	♂	61.79	64.22	64.18	67.83	0.21	0.73
	♀	57.25	69.78	60.89	66.67	0.03	0.13
	♂♀	59.52	67.00	62.53	67.25	0.02	0.11

\*: No significant difference was observed between the different rations at 5% threshold; SEM : standard error of mean ; P: Probability; RC0: Diet containing no CLM ; RC8: Diet containing 8% CLM; RC10: Diet containing 10% CLM; RC12: Diet containing 12% CLM.

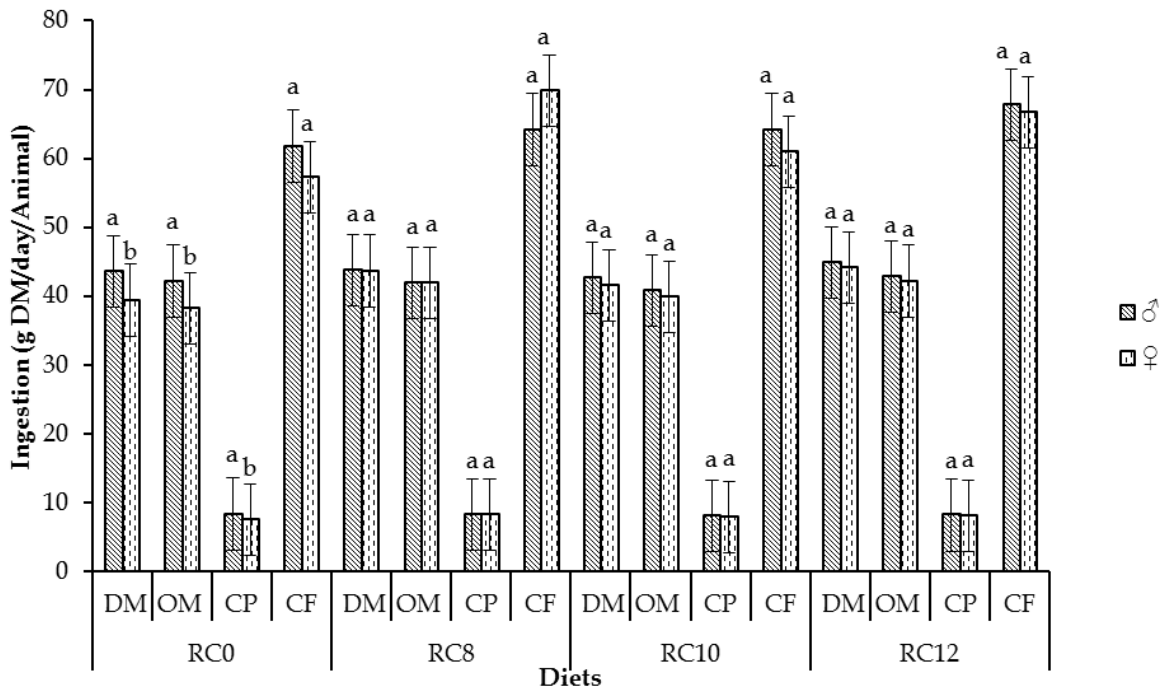
**3.4. Compared apparent digestibility coefficient (ADC) of nutrients between males and females based on inclusion level of CLM in the diets in guinea pigs**

The DM digestibility of the RC8 diet and that of the DM and OM of the RC12 diet were significantly ( $P < 0.05$ ) higher with the females (Figure 2). On the other hand, the digestibility of all the nutrients (DM, OM, CP and CF) was comparable ( $P > 0.05$ ) between males and females of RC0 and RC10 rations. Similarly, the digestibility of the OM, CP and CF of the RC8 ration,

as well as that of the CP and CF of the ration RC12 was comparable ( $P > 0.05$ ) between the two sexes.

**3.5. Effect of the inclusion level of CLM in the diet on average daily gain (ADG) in guinea pigs during the *in vivo* digestibility test**

The dietary intake of graded levels of CLM did not significantly ( $P > 0.05$ ) improve the ADG regardless of sex and gender (Table 4)



**Figure 1. Comparative ingestion of DM, OM, CP and CF between males and females fed experimental diets.** *a,b:* Averages with the same letter on the same line are not significantly different at 5% threshold; DM: dry matter; OM: organic matter; CP: crude protein; CF: crude fiber; RC0: Diet containing no CLM ; RC8: Diet containing 8% CLM; RC10: Diet containing 10% CLM; RC12: Diet containing 12% CLM.

**Table 3. Apparent digestibility coefficients (ADC) of nutrients in guinea pigs depending on the level of complementation**

ADC (%)		Rations				SEM	P
		RC0	RC8	RC10	RC12		
ADC DM	♂	55.22 <sup>a</sup>	57.31 <sup>a</sup>	61.34 <sup>a</sup>	60.04 <sup>a</sup>	0.03	0.12
	♀	56.86 <sup>b</sup>	66.44 <sup>a</sup>	64.25 <sup>a</sup>	63.42 <sup>a</sup>	0.00	0.02
	♂♀	56.04 <sup>b</sup>	61.87 <sup>a</sup>	62.79 <sup>a</sup>	61.73 <sup>a</sup>	0.00	0.02
ADC OM	♂	56.34 <sup>a</sup>	58.39 <sup>a</sup>	61.28 <sup>a</sup>	60.28 <sup>a</sup>	0.05	0.18
	♀	57.85 <sup>b</sup>	67.10 <sup>a</sup>	65.15 <sup>a</sup>	64.70 <sup>a</sup>	0.00	0.01
	♂♀	57.10 <sup>b</sup>	62.74 <sup>a</sup>	63.22 <sup>a</sup>	62.49 <sup>a</sup>	0.00	0.03
ADC CP	♂	64.12 <sup>a</sup>	64.72 <sup>a</sup>	67.72 <sup>a</sup>	64.75 <sup>a</sup>	0.11	0.41
	♀	66.55 <sup>a</sup>	71.06 <sup>a</sup>	70.81 <sup>a</sup>	68.90 <sup>a</sup>	0.17	0.61
	♂♀	65.33 <sup>a</sup>	67.89 <sup>a</sup>	69.27 <sup>a</sup>	66.83 <sup>a</sup>	0.08	0.41
ADC CF	♂	91.45 <sup>a</sup>	91.89 <sup>a</sup>	92.55 <sup>a</sup>	92.28 <sup>a</sup>	0.13	0.46
	♀	91.19 <sup>b</sup>	94.26 <sup>a</sup>	93.61 <sup>a</sup>	93.45 <sup>a</sup>	0.00	0.02
	♂♀	91.32 <sup>b</sup>	93.07 <sup>a</sup>	93.08 <sup>a</sup>	92.87 <sup>a</sup>	0.00	0.04

*a,b:* Averages with the same letters on the same line are not significantly different at 5% threshold; DM: dry matter; OM: organic matter; CP: crude protein; CF: crude fiber; SEM : standard error of mean; P: Probability. RC0: Diet containing no CLM ; RC8: Diet containing 8% CLM; RC10: Diet containing 10% CLM; RC12: Diet containing 12% CLM.

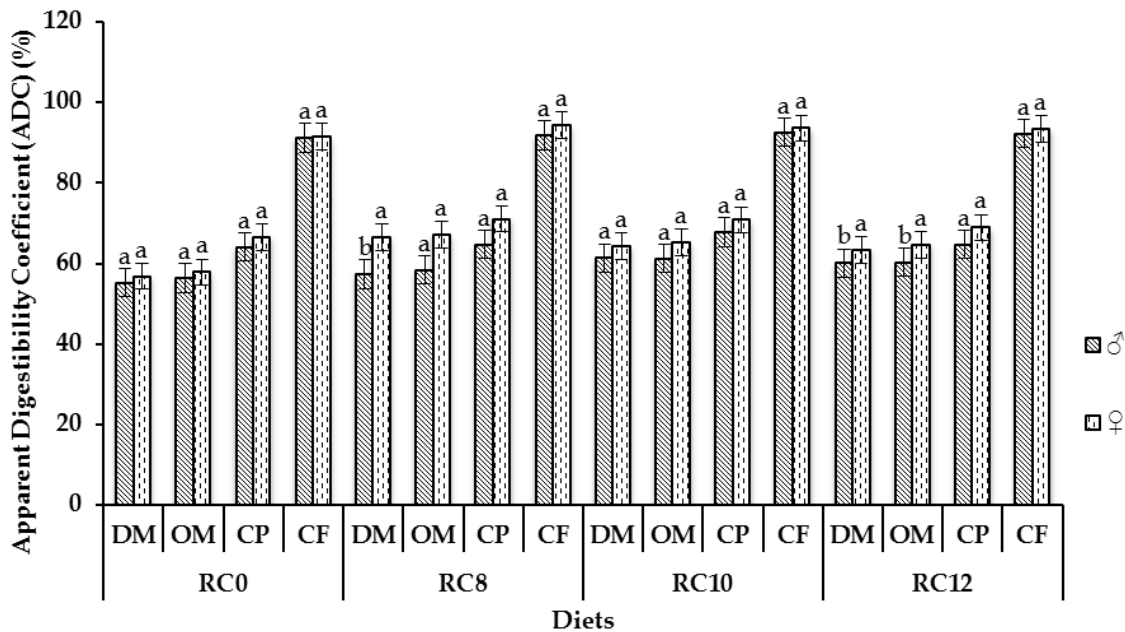


Figure 2. Compared Apparent Digestibility Coefficients of DM, OM, CP and CF between males and females guinea pigs fed experimental diets. <sup>a,b</sup>: Averages with the same letter on the same line are not significantly different at 5% threshold; DM: dry matter; OM: organic matter; CP: crude protein; CF: crude fiber. RC0: Diet containing no CLM; RC8: Diet containing 8% CLM; RC10: Diet containing 10% CLM; RC12: Diet containing 12% CLM.

Table 4. Average daily gain (ADG) in guinea pigs fed experimental diets during digestibility test

	Diets				SEM	P	
	Sexe	RC0	RC8	RC10			RC12
ADG (g/day)	♂*	5.80	3.13	4.13	5.20	0.09	0.32
	♀*	4.27	6.47	4.53	3.33	0.07	0.26
	♂♀*	5.03	4.80	4.33	4.27	0.18	0.88

\*: No significant difference was observed between the different rations; SEM: standard error of mean; P: Probability. RC0: Diet containing no CLM; RC8: Diet containing 8% CLM; RC10: Diet containing 10% CLM; RC12: Diet containing 12% CLM.

#### 4. Discussion

Guinea pigs are basically herbivorous monogastrics and from the consumption of forages their basal diet, they obtain the necessary fibers essential for the proper functioning of their digestive system (Fuss, 2002). This assertion is confirmed in this study with the high consumption of *P. purpureum* (forage) compared to that of the concentrate containing graded levels of CLM observed in all guinea pigs independently of the sex (Picron, 2007). The values of the dietary intake of *P. purpureum* and of the concentrate of this study are close

to those reported by Miégoué *et al.* (2016) in guinea pigs fed *P. purpureum* associated with forage legumes (*Arachis glabrata*, *Calliandra calothyrsus* or *Desmodium intortum*). Incorporation levels of CLM did not significantly influence ( $P > 0.05$ ) ingestion of nutrients irrespective of sex and gender. This result can be attributed to the treatment applied to cassava leaves. Indeed, several authors (Leterme *et al.*, 2005; Bindelle *et al.*, 2007; Ayssiwede *et al.*, 2012) showed that sun drying improves palatability. On the other hand, this result shows that cassava leaves, although of low biological value

compared to soybean meal, were valued by guinea pigs. In this study, the greatest DM ingestion value recorded in males of RC12 diet (44.89 gDM/animal/day) is lower than the values 58.12; 74.3 and 95.67 gDM/animal/day reported by Noubbissi *et al.* (2014), Kouakou *et al.* (2010) and Egena *et al.* (2010) respectively, but greater than 21.32 g MS/animal/day observed by (Niba *et al.*, 2004). These differences are probably related to the experimental foods used in each study.

The complementation did not significantly ( $P > 0.05$ ) influence the ADG in animals and between the sexes. In general, these gains have been mitigated between the sexes. Indeed, the best digestive users have sometimes been males (RC0 and RC12 diets), sometimes females (RC8 and RC10 diets). This suggests that males as well as females digest with the same efficiency diets containing high fiber contents. Louembé *et al.* (2003) reported that cassava leaves are a fibrous commodity (16-26%) and their incorporation into the diet increases the fibrous nature of the diet. This remark is contrary to the observations made by Noubbissi *et al.* (2014), Niba *et al.* (2004) and (Manjeli *et al.*, 1998) who showed that males better digest the fibers contained in diets than females. Given the variability of these observations, other factors other than sex and diet would influence the digestive use in animals. The highest ADG observed in the present 5.80 g/day (RC0 diet) and 6.47 g/day (RC8 diet) are greater than the 3.64 and 1.37 g/day values obtained by (Noubbissi *et al.*, 2014) as well as the values 5.11 and 5.38 g / d recorded by Pamo *et al.*(2005) respectively in males and females.

The digestibility of DM, OM, and CF of supplemented diets was significantly ( $P < 0.05$ ) greater than that of the control diet. These results are consistent with those of previous works (Vongsamphanh and Wanapat, 2004; Kouakou *et al.*, 2010; Noubbissi *et al.*, 2013; Miégoué *et al.*, 2016, 2019). Improving the efficiency of digestive use

through cassava leaf flour supplementation has allowed the animal to make better use of *P. purpureum* and thus ensure coverage of nutritional requirements. On the other hand, the fact that the supplementation did not significantly affect ( $P > 0.05$ ) the digestibility of PB regardless of the ration reflects the beneficial effect of drying cassava leaves, which would have mitigated the effect of antinutritional factors such as cyanogenic glycosides or tannins that may complex enzymes responsible for the digestion of the proteins contained in these leaves (Dada and Oworu, 2010). Our results confirm the statements of Vongsamphanh and Wanapat (2004) and Ffoulkes and Preston (1978) who have testified that cassava leaves are highly digestible; although of low average protein value, they have been digested in the same way as high protein soybean meal.

However, in general, nutrient digestion was comparable ( $P > 0.05$ ) between sexes and in all batches, although females were the best transformers compared to males. This observation is contrary to the observations of Niba *et al.* (2004), Noubbissi *et al.* (2014), Miégoué *et al.* (2016, 2019) who noted that males were better food processors than females. The highest DM ADC obtained in females fed RC8 diet (66.44% DM) and males fed RC10 diet (61.34% DM) in this study, were greater and lower respectively than the values 64.40% in females and 64.42% in males reported by Noubbissi *et al.* (2014). The experimental diets used in each of the studies could explain the differences.

## 5. Conclusion

This study revealed that the inclusion of cassava leaf meal in the diet of guinea pig fed *P. purpureum* did not significantly improve ( $P > 0.05$ ) ingestion of nutrients irrespective of gender and sex but improved significantly ( $P < 0.05$ ) the digestive use of DM, OM and CF of supplemented diets with the exception of CP. On an

overall point of view, this study also revealed that females are much better food users than males although no significant difference ( $P > 0.05$ ) was observed between the two sexes and that 10% inclusion level of CLM resulted in high digestibility parameters. Hence, dried cassava leaves represent a good source of proteins and nutrients for animal nutrition which improves digestibility and promote growth. Since considerable losses of vitamins and minerals occur during storage and processing, then contribution of CLM on the chemical composition especially the mineral content of guinea pig's meat need to be investigated.

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