

# Effect of aqueous and hydroethanolic extracts of avocado seeds (*Persea Americana*) on nutrient digestibility in guinea pigs (*Cavia Porcellus*)

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## Abstract

Feeding is the aspect with the greatest cost in livestock breeding. This work was launched in an attempt to find natural growth promoters in the breeding of domestic animals. This work was carried out in the city of Dschang in the western part of Cameroon. It focuses on the evaluation of two extraction solvents (water and water-ethanol mixture) on the phytochemistry of avocado seed extracts and their effect on feed digestibility in guinea pigs. To this end, three experimental rations (without extracts; 200 g aqueous extracts /100 kg rations and 200 g hydroethanolic extracts /100 kg rations) were tested on 30 guinea pigs divided into three groups of 10 (5 males and 5 females) similar in terms of body weight. The main results revealed the presence of secondary metabolites such as phenols and tannins in all extracts. However, the aqueous extracts had higher phenol content (1337.18 mg/100 g) as compared to the hydroethanolic extracts (561.5 mg/100 g). Moreover, adding extracts to the ration resulted in an improvement in nutrient intake compared to the control (without extracts). In effect, the aqueous extract induced an improvement in feed intake, cellulose digestion ( $51.85 \pm 5.35$ ) and in digestibility ( $93.27 \pm 0.72$ ) compared to other rations. In short, aqueous extracts can be used to improve feeding efficiency of guinea pigs. Nevertheless, a study on the inclusion rate is still necessary to better control its effect.

## Introduction

Feed represents the component with the largest pocket of expenditure in breeding. This work was initiated to search for natural growth promoters in domestic animal husbandry. The work was carried out in the town of Dschang in West Cameroon and focused on the evaluation of two extraction solvents (water and water ethanol mixture) on the phytochemistry of avocado kernel extracts and their effect on the food digestibility in guinea pigs. For this purpose, three experimental rations (without extracts; 200 g of aqueous extracts/100 kg of food and 200 g of hydroethanolic extracts/100 kg of food) were tested on 30 guinea pigs divided into three of 10 (5 males and 5 females). ) comparable in terms of live weight. The main results revealed the presence of secondary metabolites such as phenols and tannins regardless of the extract. However, the aqueous extracts recorded a higher phenol content (1337.18 mg/100 g) compared to the hydroethanolic extracts (561.5 mg/100 g). In addition, the introduction of the extracts in the ration induced an improvement in the intake of nutrients compared to the control (without extracts). Indeed, the aqueous extract induced an improvement in ingestion; the digestive utilization of cellulose ( $51.85 \pm 5.35$ ) and the digestive utilization coefficient ( $93.27 \pm 0.72$ ) compared to other rations. In short, the aqueous extract can be used to improve feed efficiency in guinea pigs. However, a study on the inclusion rate is still necessary to better control its effect.

Since the ban on antibiotics, Phytobiotics have been a constant source of interest among researchers. Phytobiotics are plants or plant extracts with ability to regulate the nutritional metabolism and immune response in low doses, thus contributing to the intestinal health of domestic animals [1]. They also contribute in stimulating the digestive tract and improving digestive utilization and animal performance [2,3]. According to Malayoglu *et al.* [4] the ingestion

of *Forsythia suspensa*, oregano essential oil, a mixture of several essential oils (oregano, cinnamon and pepper) or extracts (thyme and rosemary sage) in the feed improves proteins and starch digestibility. Despite these good results, competition with human food that spices could raise due to their industrial use is now leading to the search for phytobiotics from the inedible parts of plants. Thus, the avocado tree is well known in Cameroon and is grown for its fruits. Similarly, its leaves and bark which have undergone studies are in high demand in the area of alternative medicine for their antihelmintic and anti-diabetic properties [5]. Nevertheless, there is an ongoing debate around the various uses of its seed. According to Arukwe *et al.* avocado seeds are rich in tannins, flavoid, alkaloids, phenols, steroid and saponin. This could justify the use of its extracts as an additive in animal feed [6]. It is in this context that this trial was launched with the aim of improving on the productivity of guinea pigs using avocado seeds.

## Material and method

### Study area

This study was carried out in the Animal Production and Nutrition Laboratory (LAPRONAN) based in the Dschang University.

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LAPRONAN is situated at latitude 05°26' of north, and longitude 10°26' east at an altitude of 1420 m in the agro-ecological area of the Western Highlands in Cameroon. Its climate is equatorial, typical of Cameroon and has two seasons; a rainy season that runs from mid-March to mid-November and a dry season that runs from mid-November to mid-March. Annual rainfall stands at 2000 mm while average temperature stands at 21°C. Average annual insolation stands at 1873 hours and average relative humidity stand at 76.8%.

### Plant material

Plant material was made up of extracts (aqueous and hydroethanolic) from *Persea americana* and *Pennisetum purpureum* avocado seeds. Seeds of the Hass variety of avocado were obtained from a producer in the town of Penka-Michel with the same agro-ecological conditions as LAPRONAN. These were then dried in the shade until they reached a constant weight and then crushed. Two types of extracts were prepared: the hydroethanolic and aqueous extracts.

For the hydroethanolic extract, a maceration of avocado powder in a 2000 ml mixture consisting of 1000 ml ethanol and 1000 ml distilled water was prepared. The mixture was stirred six times a day for two days and then the resulting macerate was filtered using whatman paper No. 1. The resulting filtrate was evaporated under vacuum using a rotary evaporator (BUCHI R200) at 40°C. The residual solvent was evaporated by drying in an oven at 40°C and the crude extract was stored in the refrigerator.

The aqueous extract was prepared by adding 400 g of avocado seed powder in 2000 ml of distilled water and the mixture was boiled for 15 minutes. The resulting decoction was filtered using coffee filter paper and the resulting filtrate was evaporated by drying in an oven at 40°C. The crude extract was stored in a refrigerator.

*Pennisetum purpureum* was harvested around LAPRONAN and served fresh to the animals.

### Animal material

Thirty (30) local species guinea pigs (15 female and 15 males) were used for this test. Prior to the study the animals were subjected to 10 (ten) days of adaptation to the feed and digestibility cages, after which data was collected over five (5) days.

### Feed composition

From a main feed that was prepared to meet the feeding needs of guinea pigs as described by Nzokou et al (2015), two other feeds were prepared with 200 g/100 kg of aqueous extracts and 200 g/100 kg of hydroethanolic extracts, respectively (Table 1).

T0= main feed +0% extract

TA=T0+ 2% aqueous extract of avocado seed

Thy=T0+2% hydro-ethanolic extract of avocado seed

### Experimental arrangement and data collection

Three experimental rations were randomly divided into three batches of 10 animals (5 male and 5 female) each. The animals were distributed according to a random arrangement following two factors (ration and sex) with three treatments five repetitions of which five animals per ration and sex. The animals were kept in individual metabolism cages.

The rations were served once every morning between 6 am and 8 am. The distribution was done in two phases: a phase of adaptation

**Table 1.** Percentage composition of feed

Ingredients	Quantities
Corn	25
Cassava	8
Remolding	23
Soya meal	8
Cotton meal	5
Palm kernel meal	20
Fish meal	5
Bone meal	2
Palm oil	2
Premix 2%	2
<b>Total</b>	<b>100</b>

to the digestibility cage and the 10-day experimental ration during which each animal received vitamin-enriched water at will and the experimental ration corresponding to its batch. This phase was followed by a second one known as the five-day 'digestibility' that corresponds to the data collection phase. During these five days, each animal also received vitamin-enriched water at will and the experimental ration corresponding to its batch, as indicated above. Before distribution, each ration was weighed and the leftover was equally weighed each morning before the new service. Thanks to this, it was possible to evaluate the daily dietary intake in each experimental unit. The feces of each animal were collected and weighed. Representative samples of these feces and experimental feed were collected and dried at 60 °C in a ventilated oven till a constant weight was reached. These were later crushed and kept in plastic sachets to determine their chemical content (dry matter, organic matter, crude protein and crude fiber) as required by AOAC (1990).

Digestibility was assessed according to the guidelines of the European reference method for in vivo digestibility measurement established for rabbits.

### Statistical analysis

The data was subjected to a two-factor analysis of variance (extract type and sex). In case of any significant difference between treatments, averages were separated using the Duncan test at a 5% threshold. The software used for its analyses was SPSS 20.0.

### Results

The effect of the extraction solvent on the output and the presence of some secondary metabolites in avocado seed extracts are summarized in Table 2.

In general, it appears secondary metabolites were present in the extracts regardless of the extraction solvent. However, saponins were absent in hydroethanolic extracts and present in aqueous extracts. On the other hand, the yield of the hydroethanolic extract is higher than that of the aqueous extract.

Moreover, quantitative analysis revealed that the total phenol content was higher in the aqueous extracts compared to hydroethanolic extracts (Figure 1).

### Effect of Avocado seed extraction solvent on Guinea pig nutrient intake

Table 3 is a summary of the effect of the extraction type on guinea nutrient intake. Regardless of its sex or nutrient, higher values were recorded among animals that received aqueous avocado extracts in their ration.

In addition, the amount of forage consumed was higher than that of the concentrated feed. More, adding extracts significantly ( $p < 0.05$ ) increased the females' ration consumption.

When, regardless of sex (Figure 2) the aqueous extract produced a significant increase ( $p < 0.05$ ) in the intake of all nutrients studied.

The digestive use of nutrients by guinea pigs as presented in Table 4 did not vary significantly depending on the extract and sex type. In general, extract type did not have a significant effect on the digestive use of nutrients except in the case of crude fiber. In effect, the digestibility of crude fiber was higher ( $p < 0.05$ ) in guinea pigs receiving aqueous extract from avocado seed (AC) compared to other rations.

The digestibility utilization of nutrients regardless of sex is summarized in Figure 3. Adding extracts in the rations tends to

increase digestive utilization of nutrients. However, with the exception of the (AC) group which had the highest level of fiber utilization, all other nutrients were the same ( $p \geq 0.05$ ).

## Discussion

The extraction yield was higher with the water and ethanol (Thy) mixture. This result corresponds with who obtained a higher extraction yield with alcoholic solvents [7]. Aqueous extracts produced tannin content similar to hydroethanolic extracts ( $p \geq 0.05$ ) but the total phenol content was significantly higher in aqueous extracts. This result is consistent with those of Nguessan *et al.* who obtained a higher content of catechetal tannins and polyphenols in aqueous extracts than in alcoholic extracts [8].

**Table 2.** The effect of the extraction solvent on the output and the presence of some secondary metabolites in avocado seed. + =present, - =absent, Yd =extractions yield of (%)

Extracts	Yd (%)	Alkaloids	Phenols	Flavonoides	Sterols	Terpenoids	Tannins	Saponins	Anto cyanins	anthrachinones
Aqueous	16.28	+	+	+	-	-	+	+	+	+
Hydroethanolic	23.29	+	+	+	-	-	+	-	+	+

**Table 3.** Effect of avocado seed extract type on guinea pig nutrient intake

Nutrients	Sex	Treatment			p
		C	AC	Thy	
Fodder ( <i>p. purpureum</i> )	M	67.139 ± 1.20	65.070 ± 1.20	67.284 ± 1.20	
	F	64.464 ± 0.53	67.954 ± 0.53	67.284 ± 0.53	
Concentrated	M	13.990 ± 3.12	15.971 ± 3.12	11.062 ± 3.12	
	F	11.185 ± 2.96	20.185 ± 2.96	15.8 ± 2.96	
DM	M	81.13 ± 6.05 <sup>aA</sup>	81.04 ± 5.04 <sup>aA</sup>	78.35 ± 6.70 <sup>aA</sup>	0.711
	F	75.65 ± 7.34 <sup>aA</sup>	88.14 ± 8.29 <sup>abA</sup>	81.52 ± 1.44 <sup>aA</sup>	0.031
OM	MF	78.39 ± 2.80 <sup>a</sup>	84.59 ± 3.98 <sup>b</sup>	79.93 ± 2.80 <sup>a</sup>	0.027
	M	76.59 ± 5.14 <sup>aA</sup>	76.70 ± 4.60 <sup>aA</sup>	74.33 ± 5.85 <sup>aA</sup>	0.726
CP	F	71.58 ± 6.43 <sup>aA</sup>	83.24 ± 7.37 <sup>baA</sup>	77.13 ± 1.33 <sup>abA</sup>	0.023
	MF	74.08 ± 2.39 <sup>a</sup>	79.97 ± 3.48 <sup>b</sup>	75.73 ± 2.41 <sup>a</sup>	0.017
CF	M	15.92 ± 0.90 <sup>aA</sup>	15.87 ± 0.88 <sup>aA</sup>	15.37 ± 0.94 <sup>aA</sup>	0.588
	F	14.93 ± 1.18 <sup>aA</sup>	17.14 ± 1.32 <sup>ba</sup>	15.84 ± 0.26 <sup>abA</sup>	0.018
CF	MF	15.43 ± 0.42 <sup>a</sup>	16.50 ± 0.60 <sup>b</sup>	15.61 ± 0.37 <sup>a</sup>	0.008
	M	33.88 ± 0.54 <sup>ab</sup>	48.82 ± 5.37 <sup>ba</sup>	33.46 ± 0.62 <sup>aA</sup>	0.000
CF	F	32.23 ± 1.12 <sup>aA</sup>	54.88 ± 9.63 <sup>ba</sup>	33.84 ± 0.61 <sup>aA</sup>	0.000
	MF	33.06 ± 0.43 <sup>a</sup>	51.85 ± 5.35 <sup>b</sup>	33.65 ± 0.39 <sup>a</sup>	0.000

A, b: averages with the same letters on the same line are not significantly different by 5% A, b: averages with the same letters on the same column are not significantly different p =probability. C=control; AC=aqueous extract; Thy=hydroethanolic extract; M=male; F=female; MF=mixed DM=dry matter; OM=organic matter; CP=crude protein; CB=Crude fiber. PP=*pennisetum purpureum*

**Table 4.** Effect of the type of avocado seed extract on guinea pig digestive use of nutrients

Characteristics	Sex	Treatments			p
		C	AC	Thy	
DMaDU	M	86.97 ± 2.78 <sup>a</sup>	85.84 ± 2.76 <sup>a</sup>	89.21 ± 2.50 <sup>a</sup>	0.172
	F	85.49 ± 4.69 <sup>a</sup>	88.58 ± 3.05 <sup>a</sup>	87.08 ± 0.93 <sup>a</sup>	0.361
	MF	86.23 ± 2.28 <sup>a</sup>	87.21 ± 1.60 <sup>a</sup>	88.15 ± 1.37 <sup>a</sup>	0.278
OMaDU	M	88.09 ± 2.62 <sup>a</sup>	87.18 ± 2.52 <sup>a</sup>	90.17 ± 2.26 <sup>a</sup>	0.189
	F	86.72 ± 4.26 <sup>a</sup>	90.02 ± 2.66 <sup>a</sup>	88.44 ± 0.82 <sup>a</sup>	0.248
	MF	87.41 ± 2.08 <sup>a</sup>	88.60 ± 1.41 <sup>a</sup>	89.31 ± 1.25 <sup>a</sup>	0.215
CPaDU	M	92.96 ± 1.60 <sup>a</sup>	92.38 ± 1.56 <sup>a</sup>	93.64 ± 1.47 <sup>a</sup>	0.460
	F	88.39 ± 1.95 <sup>a</sup>	93.46 ± 1.68 <sup>a</sup>	93.55 ± 0.61 <sup>a</sup>	0.529
	MF	90.67 ± 1.00 <sup>a</sup>	92.96 ± 0.87 <sup>a</sup>	93.09 ± 0.83 <sup>a</sup>	0.914
CFaDU	M	89.95 ± 2.82 <sup>aA</sup>	91.61 ± 1.39 <sup>aA</sup>	92.26 ± 1.64 <sup>ab</sup>	0.225
	F	89.03 ± 3.35 <sup>aA</sup>	94.93 ± 1.12 <sup>bb</sup>	91.19 ± 0.54 <sup>aA</sup>	0.002
	MF	89.49 ± 1.64 <sup>a</sup>	93.27 ± 0.72 <sup>b</sup>	91.72 ± 0.90 <sup>b</sup>	0.001

a, b: averages with the same letters on the same column are not significantly different p =probability. C=control; AC=aqueous extract; Thy=hydroethanolic extract; M=male; F=female; MF=mixed aDU=Digestibility Utilization; DM=dry matter; OM=organic Matter; CP=crude protein; CF=CB=Crude fiber

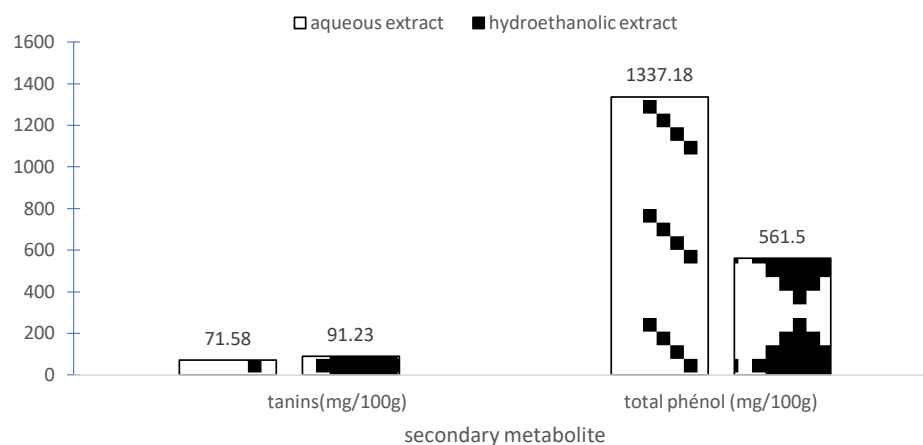


Figure 1. Effect of the extraction solvent on the tannin and phenol content

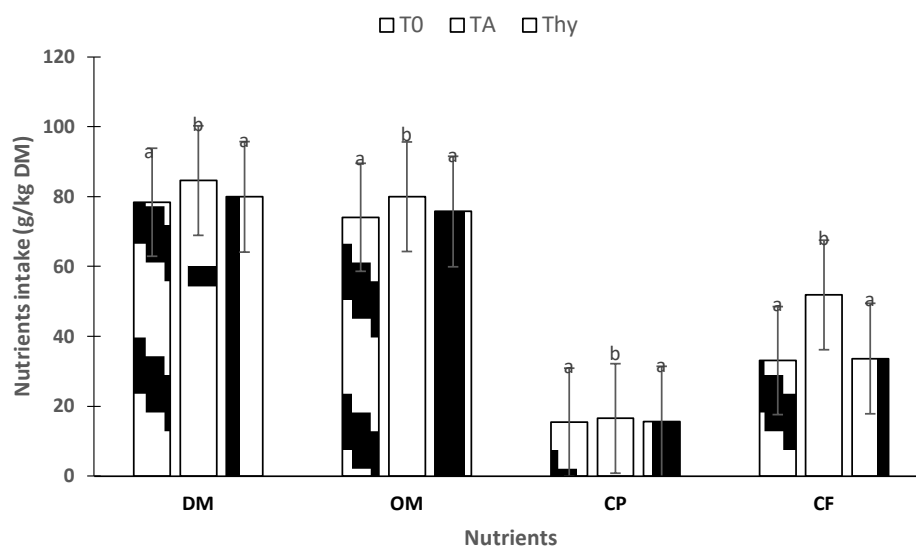


Figure 2. Effect of avocado seed extraction solvent on guinea pig nutrient intake regardless of sex

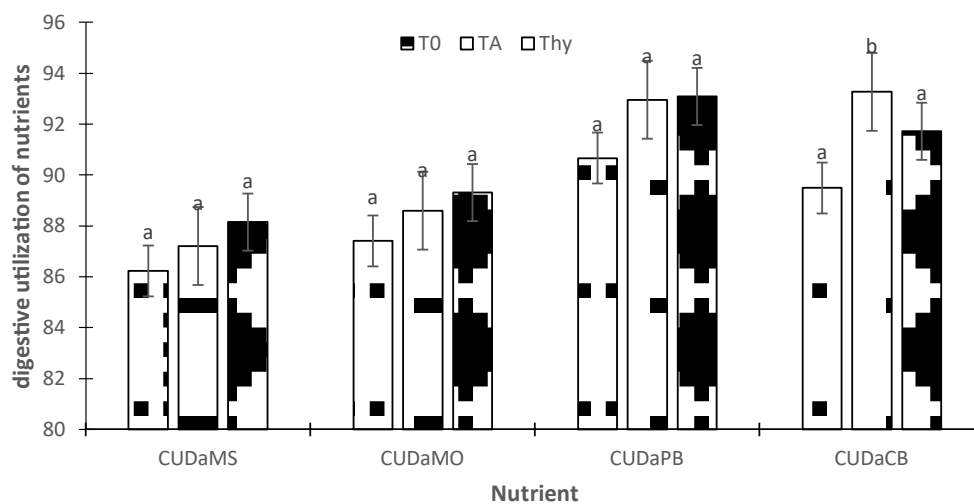


Figure 3. Effect of the type of avocado seed extract type on guinea pig nutrient digestive utilization

Adding extracts significantly ( $p<0.05$ ) increased ration consumption in females, suggesting that females are more responsive to extracts than males. Regardless of sex, extracts increased the consumption of the ration. This finding corroborates the work of Tendongkeng *et al.* who obtained a significant increase ( $p<0.05$ ) in dry matter, organic matter and cell wall (NDF) intakes of rations by small ruminants using the essential oil of the leaves of *C. viminalis* [9].

Nutrient intake increased significantly ( $p<0.05$ ) with the adding of aqueous extracts in the ration. These findings are in line with the work of Tendongkeng *et al.* who obtained an improvement in feed intake with the adding of phytobiotics (essential oils) in the ration of ruminants [10]. These findings could be explained by the higher phenol content in aqueous extracts. In effect, since the extraction yield is higher with the hydroethanolic solvent and the phenol content lower with the same solvent, these extracts would therefore be richer in other compounds such as alkaloids which are known to obstruct the DNA of eukaryotic cells, which damages the health of animals. They are therefore not good phytobiotics (Sarah 2011). This allows us to suppose aqueous extracts in guinea pigs compared to hydroethanolic extracts.

Adding extracts has increased the digestive utilization of all nutrients in terms of numbers. This supposes that a change in the addition rate could produce more remarkable effects. Furthermore, as concerns crude fiber, adding aqueous extracts produced a digestive utilization significantly ( $p<0.05$ ) higher among guinea pigs irrespective of their sexes. This finding corroborates the work of [11-13] Hernández *et al.* who noted an improvement in the digestibility of proteins or starch following the intake of *Forsythia suspensa*, oregano essential oil, and mixtures containing several essential oils (oregano, cinnamon and pepper) or extracts (thyme and rosemary sage) in the ration. This could be explained by the varied contents of the different phenol extracts, given the use of these in digestion [14-16].

## Conclusion

This trial focused on comparing the effect of aqueous and hydroethanolic extracts on guinea pig digestibility. Findings reveal that, regardless of the type of extract considered, this is an improvement in the intake of nutrients compared to the control (without extracts). The aqueous extract significantly improved the intake and digestive utilization of fiber. This situation supposes the use of aqueous avocado seed extracts in guinea pig feed. A further study on dose and effect on growth performance would be necessary.

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