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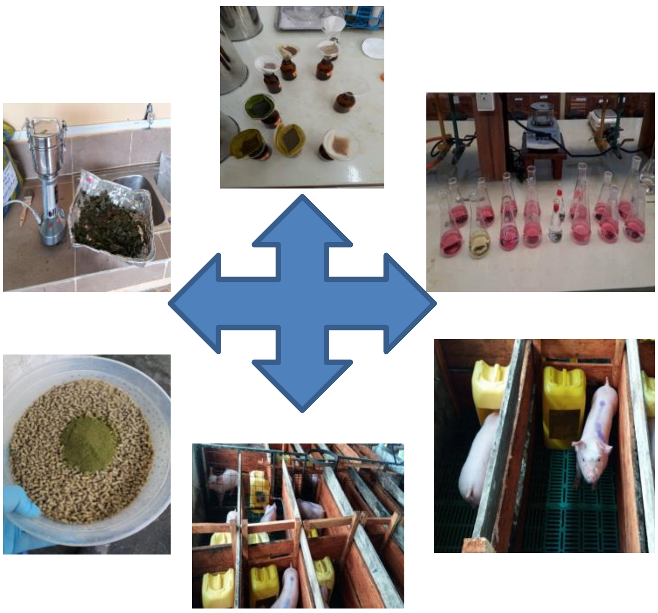
Content of phenolic compounds in powders of six Amazonian vegetables and its effect as a phytobiotic additive for pigs

W. Caicedo (orlando.caicedo@yahoo.es)[a,b], A. Flores (agr20140025@uea.edu.ec)[a], M. Pérez (mperez@uea.edu.ec)[a]

[a] *Universidad Estatal Amazónica, Departamento de Ciencias de la Tierra, Paso Lateral S/N Km 2 ½ Vía a Napo. Puyo, Pastaza, Ecuador.*

[b] *Granja Agropecuaria Caicedo, km 3 ½Vía a Madre Tierra. Puyo, Pastaza, Ecuador.*

Graphical Abstract



Abstract.
The content of phenolic compounds was evaluated in powders of six Amazonian vegetables and their effect as a phytobiotic additive for post-weaning pigs. We used 18 castrated male piglets from the commercial crossing (Landrace x Duroc x Pietrain) of 25 days of age with an initial average live weight of 9.17 ± 1.89 kg, which were distributed according to a completely randomized design in three treatments with six piglets each. The treatments consisted in T0: Basal Diet (BD) without Growth Promoter Antibiotic (GPA); T1: BD without GPA + inclusion of 0.5% foliage powder of guava; and T2: BD without GPA + inclusion of 1% foliage powder of guava. The piglets fed the T2 treatment had the highest final weight ($P<0.05$), and the lowest incidence of diarrhea ($P<0.0001$), respectively. The foliage of guava, wild anise, wild garlic, sacha inchi seeds, Chinese potato tubers and orito banana fruit had an appreciable content of phenolic compounds. The inclusion of 1% guava foliage powder in the diet improved the final weight and reduced the incidence of diarrhea in post-weaning pigs.

Key words: *antioxidant, foliage, piglets, health.*

Introduction

After weaning the piglets are exposed to the influence of the liquid feed change to a solid one, this causes stress in the animals, favoring the appearance of enteric pictures in the animals. In Ecuador, the balanced production factories use antibiotics in their formulations to inhibit the growth of pathogenic microorganisms in the gastrointestinal tract (GIT) of animals, so as not to affect the productive performance. On the other hand, there are reports that the prolonged use of antibiotics causes resistance of pathogens and generates residues in meat (Briceño-Fereira *et al*., 2015).

The European Union banned the use of antibiotics and growth promoters for pigs from the year 2006. This environment led to new research with live microorganisms (probiotics) and through the use of plant extracts and powders (phytobiotics) that have the ability to act as promoters of natural growth, they prevent diseases by better immune development, and several studies have obtained improvements in the productive indicators of animals (Liu *et al*., 2013).

In the Ecuadorian Amazon Region (EAR), there is no information regarding the use of phytobiotic additives for feeding pigs. However, it has an excellent range of plants with medicinal potential, among which we can highlight: guava (*Psidium guajaba*), wild anise (*Piper auritum*), wild garlic (*Mansoa alliacea*), orito banana (*Musa acuminata*), Chinese potato (*Colocasia esculenta*) and sacha inchi (*Plukenetia volubilis*). There are reports that the use of phytobiotic agents can be used to treat digestive, antibacterial, anti-inflammatory, antioxidant, antiviral conditions, with improvements in the productive performance and intestinal health of weaned pigs (Manzanilla *et al*., 2004; Sökmen *et al*., 2004; Michiels *et al*., 2010).

The objective of this research was to evaluate the content of phenolic compounds in powders of six Amazonian vegetables and their effect as a phytobiotic additive for post-weaning pigs.

Materials and Methods

The samples of foliage (guava, wild anise, wild garlic), fruit (orito banana), tuber (Chinese potato) and seed (sacha inchi) were collected at Rancho Santa Rita, located between the geographical coordinates 01°32'00 " From South latitude and 78°00'00 " West, at km 3 ½, via Madre Tierra, Tarqui parish. The research site has a humid subtropical climate, with rainfall ranging between 4000 and 4500 mm per year, with average relative humidity of 87% and minimum and maximum average temperature of 20 to 28 ºC (IGM, 2016). Immediately after collection, the samples were transferred to the Chemistry Laboratory of the Amazon State University, washed, and placed in the Barnstead Model 3523 stove for 72 hours at 65 ºC. Subsequently, they were milled in a Thomas-Wiley Model 4 mill, with a 1 mm sieve, and placed in sterile ziploc sleeves until use.

Determination of total phenolic compounds

To determine the content of total phenolic compounds, 40 µL of Folin Ciocalteu Reagent (FCR) was used in a 1:1 dilution with distilled water, placed in a 10 ml graduated flask, shaken and allowed to stand, protected from light for 10 minutes. 500 µL of the 10% sodium carbonate solution was added, then it was made up to a volume of 10 mL with distilled water, the solution was homogenized by manually shaking the volumetric flask, and kept in the dark at room temperature for two hours, and the absorbance was measured at 765 nm (Stratil *et al*., 2006), all analyzes were done in duplicate.

Management of animals

We used 18 castrated male piglets from the commercial crossing (Landrace x Duroc x Pietrain) of 25 days of age with an initial average live weight of 9.17 ± 1.89 kg, which were randomly housed in individual pens of 0.80 mx 1.0 m (0.80 m^2) during 18 days (four of adaptation to the diets and 14 in experimentation), 6 pigs were used per treatment. The final weight (FW) Lezcano *et al*. (2014) and the incidence of diarrhea (ID) according to (Hampson, 1986).

Handling pig feed

The treatments were, T0: Basal Diet (BD) without Growth Promoter Antibiotic (GPA); T1: BD without GPA + inclusion of 0.5% foliage powder of guava; and T2: BD without GPA + inclusion of 1% foliage powder of guava, the BD was formulated according to the procedures of the NRC (2012), (Table 1). The food was supplied twice a day; 08:00 am and 15:00 pm divided into two equal parts, drinking water was available at will.

Table 1. *Formulation of diets for post-weaning pigs.*

Ingredients (%)	**Treatments**		
	T0	**T1**	**T2**
Yellow corn	41.281	41.281	41.281
Whole milk powder	2.000	2.000	2.000
Soybean meal 44%	23.181	23.181	23.181
Vegetable oil	2.437	2.437	2.437
Wheat flour	10.000	10.000	10.000
Wheat germ	10.000	10.000	10.000
Calcium carbonate	0.482	0.482	0.482
Monodialcium phosphate	2.147	2.147	2.147
Vitamin mineral premix	0.400	0.400	0.400
DL-methionine 99%	0.339	0.339	0.339
L-Lysine HCL 78%	0.672	0.672	0.672
Chloride of choline	0.210	0.210	0.210
Antifungal	0.054	0.054	0.054

Sodium chloride	0.500	0.500	0.500
Starch	6.298	6.298	6.298
Guava foliage powder	-	0.5	1
Calculated values			
Metabolizable Energy, kcal.kg.MS^{-1}	3290	3290	3290
Crude protein, %	19.24	19.24	19.24
Raw Fiber, %	2.87	2.87	2.87

To analyze the phenolic compounds, descriptive statistics were used and the mean and standard deviation were determined. The results of the productive behavior were processed by analysis of variance (ANOVA) of simple classification; before performing the ANOVA, we proceeded to verify the normality of the data, by the Kolmogorov Smirnov test, and for the uniformity of the variance, the Bartlett test, the means were purchased with the Tukey test with P<0,05. All the analyzes were carried out with the use of the statistical program INFOSTAT version 1.0 for Windows (Di Rienzo *et al*., 2012).

Results and Discussion

The highest concentration of total phenolic compounds was found in the foliage powder of guava, followed by the powders of wild garlic, wild anise, Chinese potato tubers, sacha inchi seeds and finally the fruit of orito banana, Table 2.

Table 2. *Content of total phenolic compounds in powders of guava, wild anise, wild garlic, orito banana, Chinese potato and sacha inchi.*

Raw material	**Medium content**	**SD**
Guava foliage, mg/ml	35.32	0.19
Wild anise foliage, mg/ml	7.92	0.14
Wild garlic foliage, mg/ml	11.73	0.60
Orito banana fruit, mg/ml	1.03	0.12
Chinese potato tuber, mg/ml	1.88	1.31
Sacha inchi seed, mg/ml	1.80	0.09

SD: Standard Desviation

The six raw materials they showed an appreciable content of phenolic compounds. In this respect, guava foliage contains: gallic acid, catechin, epicatechin gallate, syringe acid, ocumárico acid, resveratrol and quercetin (Simão *et al*., 2017); wild anise foliage: terpenes, flavonoids, coumarins, tannins and cardiotonic glycosides (Valdivia *et al*., 2018); wild garlic foliage: alliin, tocopherol, ascorbic acid (Dávila *et al*., 2010); sacha inchi seeds: carotenoids, tocopherols, fatty acids, sterols, alcohols, phenols and volatile compounds (Ramos, 2014); Chinese potato tubers: total phenols, flavonoids, tannins and phytic acid (Rodríguez-Miranda *et al*., 2011); orito banana fruit: gallic acid, cyanidin, dopamine (González-Montelongo *et al*., 2010).

The phenolic compounds and antioxidants that these vegetables possess have many health benefits, which include antibacterial, anti-inflammatory, antihyperglycemic, hepatoprotective, analgesic and anticancer effects, as well as protecting against cardiovascular diseases (Camarena-Tello *et al*., 2018).

Post-weaning pig fed the T2 diet that included 1% guava foliage powder had the highest final weight and differed significantly (P<0.05) from the T0 and T1 diets respectively, Table 3. Mas Toro *et al*. (2016) used diets composed of 1% guava and cashew foliage powder in post-weaning pigs and achieved improvements in weight gain, feed conversion, final weight and lower incidence of diarrhea, results that are consistent with the obtained in the present study.

Table 3. *Productive indicators in post-weaning pig fed with guava foliage powder.*

Indicators	Treatments			SEM ±	P-value
	T0	T1	T2		
Initial weight, kg	9.17	9.17	9.17	0.82	P=0.9999
Final weight, kg	9.83^{b}	10.00^{b}	13.25^{a}	0.91	P<0.0294

SEM: Standard Error Mean

Dietary supplementation with 1% guava foliage powder from treatment T2 significantly reduced (P<0.001) the incidence of post-weaning pig diarrhea in relation to treatments T0 and T1 during the 14 days of study, (Figure 1) . In this regard, it can be affirmed that the antidiarrheal effect of the guava foliage is similar to what is obtained with antibiotic growth promoters and mixed powders of medicinal plants (Aroche-Ginarte *et al*., 2017).

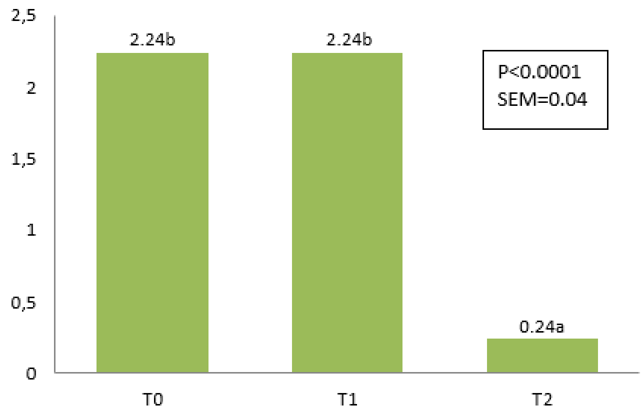


Figure 1. *Effect of dietary supplementation with guava powder on the incidence of post-weaning pig diarrhea.*

Conclusions

The foliage of guava, wild anise, wild garlic, sacha inchi seeds, Chinese potato tubers and orito banana fruit had an appreciable tenor of phenolic compounds. The inclusion of 1% guava foliage powder in the diet improved the final weight and reduced the incidence of diarrhea in post-weaning pigs.

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