Scientific Paper

Evaluation of secondary metabolites in the meal of *Stizolobium aterrimum* forage, for its use in animal feeding

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Abstract

The objective of the study was to evaluate the secondary metabolites present in the meal of *Stizolobium aterrimum* forage, for its use in animal feeding; the meal was elaborated from the leaves and fresh stems of plants which were cultivated on a Ferralitic Red soil, and the secondary metabolites were evaluated through phytochemical sieving. To determine the profile of phenolic compounds the content of total polyphenols (TP), free condensed tannins (FCT), protein-bound condensed tannins (PCT) and fiber-bound condensed tannins (FBCT), was quantified. The content of oligosaccharides was determined by high-performance liquid chromatography (HPLC), and they were expressed as raffinosaccharide equivalents. Tannins, alkaloids and reducing substances were found in higher quantity; while flavonoids, saponins, aminoacids, anthocyanidins and triterpenes/steroids were found in moderate and low quantities. The concentration of TP was 2,32 %. There were differences ($p < 0,001$) among the fractions of FCT (0,25 %), PCT (0,22 %) and FBCT (0,08 %). In the carbohydrate fraction only fructose was found (0,006 mg/kg DM) in low concentration. The qualitative presence of different secondary metabolites in the meal of *S. aterrimum* forage, in appropriate concentrations, allows to consider it a plant with potential to be used in animal feeding.

Keywords: flavonoids, polyphenols, saponins, tannins

Introduction

Stizolobium aterrimum is a legume that can be produced in sufficient quantities to satisfy the feeding needs of animals and humans (Castillo *et al*., 2015). According to Savón *et al*. (2004), as nutritional source it stands out for its high protein content (21,7 ± 0,7 % DM).

The foliage of this plant can be incorporated as meal up to 20 % in diets for rabbits, with good results in the productive performance (Dihigo *et al*., 2010; Caro *et al*., 2011). Nevertheless, some negative implications are ascribed to it related to the presence and concentration of secondary metabolites, such as alkaloids, tannins, terpenes, flavonoids and saponins (Scull, 2004; Tavares *et al*., 2015). These compounds can alter the efficient utilization of nutrients and, thus, decrease the productive potentialities of the animals (Erlwanger *et al*., 2001; Chaparro *et al*., 2009).

That is why the objective of this study was to evaluate the secondary metabolites present in the meal of *S. aterrimum* forage, for its use in animal feeding.

Materials and Methods

Plant material and meal elaboration. The *S. aterrimum* plants were sown on a Ferralitic Red soil of the experimental area of the Zaldívar farm, at the Institute of Animal Science –Mayabeque, Cuba.

The cutting was manually performed at 5 cm over the soil level, when 100 % of the plants were flowered (Díaz *et al*., 2003). The sampling was performed from five spots, randomly, on which approximately 1 kg of forage was collected.

The cut material was dried under the sunlight during 2-3 days, and dry matter was determined at different moments of the drying process until eliminating 20 % of moisture. Afterwards, it was ground in a hammer mill, with a 1-mm sieve; and the meal was stored in amber flasks, in a dark and dry place until its analysis.

Phytochemical analysis. Five grams of the forage meal were weighed in triplicate, 100 mL of 90 % ethanol v/v were added and it was refluxed during 4 h. With this alcoholic extract the essays of the phytochemical sieving were performed, according to the methodology proposed by Miranda and Cuellar (2000), with which the presence of twelve functional groups was determined: alkaloids, saponins, triterpenes/steroids, tannins, flavonoids, proanthocyanidins, coumarins, quinones, reducing sugars, amino groups, resins and cardenolides (cardiotonic steroids).

In the description of the essays the non-parametric cross system was used, to specify the presence or absence of metabolites (abundant presence +++, moderate presence ++, low presence +, absence -).

Chemical analysis

Determination of phenolic compounds. The concentration of total polyphenols in the samples (n = 5) was determined through the Folin Ciocalteu reagent, and was expressed in tannic acid equivalent, according to the method suggested by Makkar (2003). The tannin extraction was carried out following the technique proposed by Terrill *et al.* (1992).

The concentration of condensed tannins (CT) in the fractions was quantified with the reagent butanol/HCl (95 % of butanol and 5 % of concentrated 36 % HCl). The results were expressed in cathechin equivalent, for which the catechin calibration curves were made in water for free condensed tannins (FCT) and in sodium dodecyl sulfate (SDS) for protein bound condensed tannins (PCT) and fiber bound condensed tannins (FBCT).

Determination of oligosaccharides. The content of oligosaccharides was determined by high performance liquid chromatography (HPLC). For such purpose an isocratic system was used, with an LKB 2150 pump, the detection was performed with a refraction index detector (IR), Knauer). The Aminex HPX-87N column of 300 x 7,8 mm (BioRad) was used. As mobile phase a sodium sulfate 0,01 M was used, at a flow rate of 0,5 mL/min and an oven temperature of 85 ºC.

Statistical analysis. For the analysis of the data the statistical software Infostat version 1. (2001) was used. Descriptive analysis was made, and Duncan's (1955) test was used in the necessary cases.

Results and Discussion

The results of the phytochemical sieving in the meal of *S. aterrimum* forage are shown in table 1. From the 12 functional groups, there was presence of tannins, alkaloids, flavonoids, saponins, amino groups, terpenes, anthocyanidins and reducing sugars. These groups are important for animal nutrition, because of their biological activity and the effects they can cause (Stewart *et al*., 2000).

It cannot be categorically stated that the metabolites which were not identified do not exist, because as specific essays were conducted, it is possible that they were present in other structural variants that do not respond to such tests. In addition, interferences can occur due to the drying method, the solubility of other compounds in the solvent, the concentration of metabolites and the sensitivity of the essay (García, 2010). Nevertheless, the absence of coumarins and cardenolides is considered advantageous, because when they are found in remarkable concentrations in the diet, they cause nutritional disorders and low palatability of the feedstuff.

Table 1. Qualitative chemical composition of the meal of *S. aterrimum* forage.

Secondary compound	Presence/absence
Tannins	+++
Alkaloids	+++
Flavonoids	+
Saponins	++
Triterpenes	++
Anthocyanidins	+
Reducing sugars	++
Coumarins	-
Quinones	-
α-amino group	+++
Resins	-
Cardenolides	

Abundant presence +++, moderate presence ++, low presence +, absence -

The results of the chemical essays showed high levels of alkaloids. The main effect of these compounds is intake decrease, due to the bitter taste they confer to the feedstuffs (Ramos *et al*., 1998). They can also be toxic for the digestive system, by causing irritation in the gastric mucosae, with typical manifestations such as diarrhea and vomits.

The alcoholic extract of the forage meal developed an intense green color in the presence of ferric chloride, which suggests abundant presence of these compounds, results that coincide with the ones obtained by Delgado *et al.* (2010) and Scull *et al.* (2011).

The high concentration of functional groups α-amino is related to the presence of L Dopa, which is the main secondary compound in *S. aterrimum* (Sathiyanarayanan and Arulmozhi, 2007; Pulikkalpura *et al*., 2015).

The results of the phytochemical composition of the meal are fundamental elements to be taken into consideration for its incorporation to alternative feeding systems. Many of these compounds, when present in the diet, tend to originate digestive problems and to decrease feed intake.

TPs are considered one of the most abundant secondary metabolites in tropical plants. The concentration of TP is influenced by the phenological status, climate and geographical conditions, variety and analytical method, for which it is difficult to compare the results, although the obtained values (2,32 %) can be compared with the ones reported by Scull *et al*. (2015).

Figure 1 shows the values of free and protein- and fiber-bound condensed tannins, which showed significant differences ($p < 0,001$) among the fractions.

As for determining the TCT content the fraction of free tannins and the binding of other macromolecules were taken into consideration, the estimated quantities were in correspondence with the values shown by the legume.

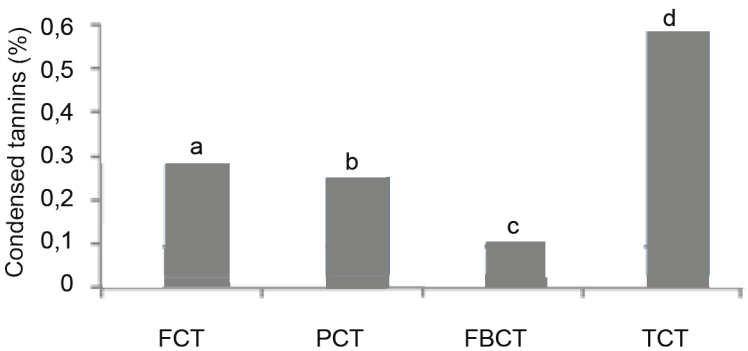
Among all the polyphenols, the CT are considered the main antinutrients, because they form complexes with proteins, starch and digestive enzymes, with the subsequent reduction of the nutritional value of feedstuffs, as well as effects on the digestive physiology of ruminants (Stewart *et al*., 2000).

The distribution of condensed tannins in the meal of *S. aterrimum* forage, in the three analytical fractions, is shown in figure 2. The highest percentage was that of free condensed tannins, similar to the result obtained by Cano *et al*. (1994) in other legumes.

The inclusion of permissible quantities of CT in the diets of monogastric animals is an aspect to be taken into consideration, because they are the most susceptible ones to the toxic effects of these metabolites.

Savón *et al*. (2004) found lesions in the cells of the intestinal mucosa of pigs which consumed diets of molasses B/soybean plus 20 % of meal of *Lablab purpureus* and *Canavalia ensiformis* forage, which were ascribed to the presence of antinutritional factors. Nevertheless, the inclusion of 20 % of the *S. aterrimum* meal did not cause these adverse effects and caused productive results similar to those of the control.

On the other hand, Mora *et al*. (2005) stated that with the inclusion of more than 25 % of *S. aterrimum* meal in diets for pigs similar results were obtained to those of the control treatment. Martínez *et al*. (2007), when including up to 10 % of another



a b c d different letters indicate different significance at $p \leq 0,05$ (Duncan, 1955)

Figure. 1. Content of condensed tannins in meal of *S. aterrimum* forage.

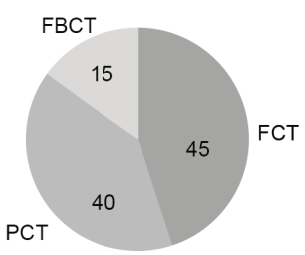


Figure. 2. Distribution of the fractions of condensed tannins (%) in the meal.

Stizolobium species (*Stizolobium deeringiana*) in the rations of birds in substitution of corn-soybean, found that the digestibility of the nutrients was affected.

In biological systems, the effects of phenolic compounds are influenced by the antinutritional composition as well as the species, without ignoring that there is a threshold over which each species manifests them (Addisu, 2016).

No concentrations of oligosaccharides were detected, expressed as raffinosaccharide equivalents, and of the sugars only fructose values were obtained (0,006 mg/kg DM). The non-identification of oligosaccharides in the meal of this legume is highly important, because these carbohydrates are considered the cause of flatulence in the animals that consume feedstuffs which contain them in remarkable quantities.

These results are considered to be linked to the evaluated variety, because they do not coincide with the report by Vijayakumari *et al*. (2002) for *Mucuna pruriens* in which verbascose was found as main oligosaccharide. They also differ from the results obtained by Kala and Mohan (2012) in seeds from *M. pruriens* var. *utilis*, in which raffinose was found in concentrations of 1,06 %.

It is important to state that the non-detection of sucrose and glucose could have been influenced by the extraction process, for which it is advisable to use other methods and solvents.

The fructose concentration was low (0,006 mg/kg DM of fructose), compared with other species, such as *Gliricidia sepium* (Fonte *et al*., 2013). The concentration of sugars in the plants can influence voluntary intake and feed acceptance by the animals (Castro and Martínez, 2015).

The results of the study showed the presence of secondary metabolites with different chemical nature in the meal of *S. aterrimum* forage. This meal can be considered a feedstuff with potentialities to be used for animal feeding, if it is compared with other species that are used for such purposes. It is recommended to continue the studies that quantify the secondary compounds identified in this plant.

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