Original Article

CHARACTERIZATION OF *Urochloa humidicola* METHANOL EXTRACT AS A PHYTOGENIC ADDITIVE FOR RUMINANTS

CARACTERIZAÇÃO DO EXTRATO METANÓLICO DE *Urochloa humidicola* COMO ADITIVO FITOGÊNICO PARA RUMINANTES

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ABSTRACT: This survey aimed to characterize a *Urochloa humidicola* methanol extract regarding the presence of secondary metabolites classes and to determine its bromatological composition. *U. humidicola* samples were dried under shade, milled on a 2-mm sieve by a Willey mill. The solution obtained was filtered using filter paper and concentrated in a rotary evaporator under reduced pressure; the concentrated residue was then placed in an open vessel to complete solvent removal using continuous air flow dryers. Phytochemical prospection tests and bromatological composition analyses were performed on the dry methanol extract, and the results were compared to *in natura U. humidicola*. The methanol extract had 10.2% CP and 35% EE and *in natura U. humidicola* had 5.17% CP and 1.57% EE, with a difference ($P < 0.05$) of 5% by Fisher's test. *In natura U. humidicola* had 75.59% NDF, 40.77% ADF, 38.82% HEM, 29.93% CEL, and 7.19% LIG. Methanol extraction by cold maceration reduced the LIG (0.17%) and CEL (0.21%) contents as only soluble constituents were extracted. A phytochemical assay was positive for the presence of saponins, tannins, alkaloids, non-protein amino acids, carbohydrates, cardiac glycosides, steroids, tripernoids, catechins, and saccharides and was negative for the presence of flavonoids and purines. The *U. humidicola* methanol extract possesses traits that allow its use as a phytogenic and natural additive.

KEYWORDS: Plant extract. Secondary metabolites. Nutrition.

INTRODUCTION

World population growth has resulted in more food production and a greater demand for agricultural products (WANAPAT et al., 2013). Responding to international and national demands, Brazilian livestock has been targeted for investments in production development, as well as in biotechnology, to consolidate Brazil as the main exporter of meat products; this caused an increase in tests on natural products for use in animal feed, such as nutraceutical food or phytogenic additives.

Secondary metabolites are present in plant extracts in large amounts and possess several functions including chemical defense (SLIWINSKI et al., 2002). Some metabolites have specific functions such as protection against herbaria and infection by pathogenic microorganisms (NEPOMUCENO et al., 2013) and have antimicrobial traits, thus allowing their application as ruminal fermentation inducers by the selective inhibition of ruminal microorganisms (KAMRA et al., 2006) and consequent mitigation of methane production (SANTRA et al., 2012).

According to Wanapat et al. (2013), plant extracts with condensed tannins and saponins have been used as additives in ruminant feed in contrast to ionophores for the mitigation of methane production.

Plant extracts from garlic (*Allium sativum*), pepper (*Capsicum annuum*), cinnamon (*Cinnamomum cassia*), oregano (*Origanum vulgare*), and fennel (*Pimpinella anisum*) have been used as *in vitro* ruminal fermentation inducers in beef cattle feed with a high-concentrate quantity (CARDOZO et al., 2005); on the other hand, when used in human feed, some conflicts might exist related to the increase in product prices.

Forage legumes and grass have active components such as saponins, tannins, and phenolic compounds (SIROHI et al., 2014), allowing the use of the forage itself as a method for modifying ruminal fermentation. *U. humidicola* contains saponins that allow its application in ruminant feed to transform fermentative patterns.

The present study evaluated *U. humidicola* methanol extract production and characterization to

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study its chemical–bromatological composition and secondary metabolite classes.

MATERIAL AND METHODS

The experiment was performed at the Animal Science Institute in UFRRJ, Seropédica, Rio de Janeiro State, Brazil (22°46′59″ S, 43°40′45″ W and 33 m altitude). Experimental soil was classified as Haplic Planosol (EMBRAPA, 1999). The region climate was classified as Aw according to Köppen.

U. humidicola samples were harvested from the section 0.05 m above the soil surface, in particular, in October 2013. The sample was dried under shade for 7 days and was subsequently milled on a 2-mm sieve by a Willey mill (Tecnal TE 680 model). A portion of the sample was stored in glass flasks containing methanol. The solution obtained was filtered through filter paper and concentrated under reduced pressure using a rotary evaporator. The concentrated residue was placed on an open container for solvent removal by employing continuous air flux dryers.

The *U. humidicola* crude methanol extract (*Uh*ME) was subjected to several chemical reactions for the detection of saponins, tannins, alkaloids, flavonoids, non-protein amino acids, carbohydrates, cardiac glycosides, saccharides, steroids, tripernoids, catechins, and purines (BARBOSA FILHO, 2001; MATOS, 1998).

In natura U. humidicola and the *U. humidicola* crude methanol extract were subjected to bromatological analysis for determining the dry matter (DM), crude protein (CP), mineral matter (MM), and ether extract (EE) contents, according to methods 934.01, 984.13, 924.05, and 960.39, respectively (AOAC, 1990). The non-fibrous carbohydrate (NFC) content was determined according to the following equation, NFC content = 100 − (%NDF + %CP + %EE + %MM), described in NRC (2001); the neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (LIG), and cellulose (CEL) contents were determined according to the method by Van Soest et al. (1991), and the hemicellulose (HEM) content was determined as the difference between the NDF and ADF contents. The determination of the EE content from the *U. humidicola* crude methanol extract was performed according to the methodology by Bligh & Dyer (1959), where chloroform, methanol, distilled water, and 1.5% Na_2SO_4 were used at ratios of 1:1:0.8:0.5, respectively. The *U. humidicola* samples were harvested from three different plots located at UFRRJ goats research facility; each sample was divided into two subsamples: one for performing bromatological analysis and another for obtaining the crude methanol extract. The crude methanol extract was sampled during bromatological analyses, and the results were compared with those of *in natura U. humidicola*.

The results of bromatological analyses were subjected to ANOVA by comparing averages by Fisher's test using the 9.1 Saeg software (UFV, 2007). The results of the phytochemical prospecting tests were characterized as in terms of the intensity of the presence of each class of metabolites and represented via the cross system, where (+++) denotes a large presence, (++) denotes a considerable presence, (+) denotes a moderate presence, and (0) denotes no presence or inconclusive presence for each secondary metabolite class, the result was the average number of crosses given by two evaluators for each repetition of the extracts.

RESULTS AND DISCUSSION

There was a significant difference ($P < 0.05$) between the CP, EE, MM, and NFC contents, in the *U. humidicola* extract that showed higher values than those in *in natura U. humidicola*. For the NDF, ADF, HEM, CEL, and LIG contents, *in natura U. humidicola* had higher values than the *U. humidicola* extract (Table 1).

The *U. humidicola* extract had 5.17% CP, which was lower than the values of 11.74% and 7.89% reported by Pereira et al. (2011) from cuts performed in two periods: December 2006 to March 2007 and November 2007 to March 2008 at Alto Vale do Jequitinhonha, Minas Gerais, Brazil.

The difference in protein content might be related to the time of the year (cutting performed in October) and plant phenological period (four months following the last grazing period), in addition to soil traits and the absence of fertilization. The *U. humidicola* methanol extract protein content (10.2%) and the EE content (35%) were higher than those in *in natura U. humidicola* ($P < 0.05$). Thus, the increase of these constituents might be explained by the extraction method which carries out only the methanol soluble constituents, due to the LIG, CEL, and hemicellulose constituents are not solubilized by methanol.

In natura U. humidicola and the *U. humidicola* methanol extract had 9.59% and 39.92% NFCs, respectively ($P < 0.05$). NFCs are represented by fractions (soluble sugars, pectin, and starch) that are soluble in water (OLIVEIRA et al., 2016). Using methanol as the solvent, NFC fractions were concentrated in the *U. humidicola* extract.

Table 1. Chemical–bromatological composition of *Urochloa humidicola* and the *U. humidicola* methanol extract (*Uh*ME*)* based on dry matter

Constituents	*U. humidicola*	*Uh*ME	CV %
DM %	89.36 A	81.42 B	0.66
CP	5.17 B	10.20 A	3.86
EE	1.57 B	35.00 A	1.18
MM	8.14 B	16.14 A	4.51
NFC	9.59 B	39.92 A	7.20
NDF	75.59 A	0.14 B	3.11
ADF	40.77 A	0.18 B	1.23
HEM	34.82 A	0.00 B	5.38
CEL	29.73 A	0.21 B	0.63
LIG	7.19 A	0.17 B	2.19

DM: dry matter, CP: crude protein, EE: ether extract, MM: mineral matter, NFC: non-fibrous carbohydrate, NDF: neutral detergent fiber, ADF: acid detergent fiber, HEM: hemicellulose, CEL: cellulose, LIG: lignin. CV: coefficient of variation. Values followed by different letters in the same column differ by 5% according to Fisher's test ($P < 0.05$).

NDF, ADF, and LIG contents were 75.59%, 40.77%, and 7.19%, respectively, which were in contrast to the values reported by Pereira et al. (2011), which were 68.10%, 43.91%, and 5.10%, respectively. *In natura U. humidicola* had 34.82% and 29.73% HEM and CEL, respectively. This difference may have occurred due to the time when forage cutting was done as the work by Pereira et al. (2011) was performed during the rainy season with 42-day-old plants, with the correction of soil pH in the experimental area with limestone and fertilization with nitrogen and phosphate sources, which did not occur in the present study.

LIG (0.17%) and CEL (0.21%) contents in the methanol extract were low due to cold maceration by methanol extracting soluble constituents such as proteins, lipids, and MM.

The phytochemical prospection qualitative assays provided general information on the *U. humidicola* chemical profile and presented several metabolites classes; however, no presence of flavonoids and purines was reported (Table 2).

Table 2. Phytochemical prospection qualitative assays of the *U. humidicola* methanol extract

Secondary Metabolite Classes	Results
Saponins	+++
Tannins	+++
Alkaloids	+++
Flavonoids	0
Non-protein amino acids	+++
Carbohydrates	+++
Cardiac glycosides	+++
Steroids and terpenoids	+++
Catechins	+++
Saccharides	+++
Purines	0

(+++) large presence and (0) no presence or inconclusive presence

Phytochemical prospection has lower accuracy than other identification methods such as chromatographic analysis; phytochemical prospection might interfere with the interpretation of some results due to staining (GRANATO et al., 2013) as it provides an overview of the various classes of chemical constituents that may be present in the plant analyzed. However, the same authors addressed the importance of performing these tests as a mechanism to direct the fractionation of crude extracts and to identify active components to allow biological assays to be performed. This was corroborated by Bessa et al. (2013) who mentioned that this easy to perform, fast, and inexpensive technique is important in preliminary studies on plants considering that the phytochemical profile is still not widely studied.

Age and plant development may influence the presence and amount of metabolites (GOBBO-NETO; LOPES, 2007). Brum et al. (2009) observed high protodioscin levels in *U. decumbens* and *U. brizantha* at the maturation period. In animal nutrition, secondary metabolites such as tannins and saponins are generally mentioned in the literature

because of their action on ruminal a microorganism, it may justify the use of plant extracts containing them as inducers of ruminal fermentation in order to optimize the use of nutrients in foods (TADESSE 2014; WENCELOVÁ et al., 2014). To promote an increase in the performance of animals and reduce the negative effects on the environment, there should be a greater use of food energy and lower production of ruminal methane (ANANTOSOOK et al., 2014).

Alkaloids act on ruminal microbiota and promote improvement in ruminal fermentation patterns and the better use of nitrogenous constituents of food when present in cattle diets (AGUILAR-HERNANDEZ et al., 2016). To date, other secondary metabolites such as non-protein amino acids, carbohydrates, cardiac glycosides, steroids, terpenoids, catechins, and saccharides have not been mentioned as promoters of ruminal fermentation by the modification of ruminal microbiota to promote increased animal performance. However, cardiac glycosides, steroids, and terpenoids bring about negative effects when present in animal diets (NEPOMUCENO et al., 2013).

CONCLUSION

The *U. humidicola* methanol extract possesses traits that allow its use as a phytogenic or natural additive for inducing ruminal fermentation.

RESUMO: Objetivou-se neste estudo caracterizar o extrato metanólico de *Urochloa humidicola*, quanto à presença de classes de metabólitos secundários presentes bem como determinar a sua composição bromatológica. Para isto, amostras da parte aérea de *U. humidicola* foram secas à sombra, moídas em moinho tipo Willey em partículas de 2 mm, submetidas à extração por maceração a frio com metanol, a solução obtida foi concentrada em rotaevaporador e posto para termina a secagem sob fluxo de ar contínuo. O extrato metanólico seco foi submetido aos testes de prospecção fitoquímica e análises de composição bromatológica comparado com a *U. humidicola in natura*. O extrato apresentou 10,2% de PB e 35% de EE e *U. humidicola in natura* apresentou 5,17 % de PB e 1,57% de EE, diferindo entre si (P<0,05) pelo teste de Fisher a 5% de significância. A *U. humidicola in natura* apresentou teores de FDN (75,59%), FDA (40,77%), hemicelulose (38,82%), celulose (29,93%) e lignina (7,19%). O método de extração por maceração a frio com metanol contribuiu para a diminuição dos teores de lignina (0,17%) e celulose (0,21%), por extrair somente os constituintes solúveis. O ensaio fitoquímico apresentou presença positiva para saponina, tanino, alcaloides, aminoácidos não proteicos, carboidratos, glicosídeos cardioativos, esteroides e tripernoides, catequinas e sacarídeos, e negativa para a presença de flavonoides e purinas. O extrato metanólico de *Urochloa humidicola* apresenta características que permitem seu uso como aditivo natural ou fitogênico.

PALAVRAS-CHAVE: Extrato de plantas. Metabólitos secundários. Nutrição.

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