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Assessing the nutritional value of agroindustrial co-products and feed through chemical composition, *in vitro* digestibility, and gas production technique

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ABSTRACT. Agroindustrial co-products are a viable alternative for use in animal nutrition. Tests were conducted using eight different types of co-products and feed to evaluate the chemical composition, *in vitro* digestibility of dry matter, crude protein and neutral detergent fiber, and gas production by them. The co-products tested were: coffee hulls; pelleted citrus pulp; grape residue; soybean hulls; cottonseed; cassava foliage; and foods usually supplied to ruminants: corn silage and ground corn concentrate. Data of *in vitro* digestibility of dry matter, crude protein and neutral detergent fiber were tested by analysis of variance using the least square method; the results of gas production were interpreted by a non-linear regression by the Gauss-Newton method; and the effects of treatments were evaluated by the Tukey's test. The coefficients of *in vitro* digestibility of dry matter, crude protein and neutral detergent fiber of co-products were different. Gas production was also different between co-products and feeds evaluated for the volume of gas produced from the fast and slow degradation fractions, degradation rate, bacterial colonization time, and the total volume of gas produced. The evaluated co-products exhibited greater *in vitro* dry matter digestibility compared to corn silage, except for cottonseed, grape residue, and cassava foliage. Co-products showed higher values of *in vitro* crude protein digestibility compared to corn silage, and a reduced *in vitro* digestibility of neutral detergent fiber, except for pelleted citrus pulp and soybean hulls. Corn silage produced larger volume of gas from the fast degradation fraction compared to the co-products and corn concentrate. Co-products analyzed had appropriate nutritional characteristics according to the techniques applied and can be included in ruminant diets.

Keywords: alternative feed, degradation, fermentation, gas production.

Avaliação do valor nutricional de coprodutos da agroindústria e de alimentos por meio da composição bromatológica, da digestibilidade *in vitro* e da técnica de produção de gás

RESUMO. Os coprodutos da agroindústria constituem uma alternativa para a utilização na nutrição animal. Foram realizados ensaios com oito tipos de coprodutos e alimentos para avaliar a composição bromatológica, a digestibilidade *in vitro* da matéria seca, a proteína bruta e a fibra em detergente neutro, bem como a produção de gases gerados por eles. Os coprodutos utilizados neste estudo foram: casca de café; polpa cítrica peletizada; resíduo de uva; casca de grão de soja; caroço de algodão; rama de mandioca e alimentos tradicionalmente empregados na alimentação de ruminantes: silagem de milho e concentrado de grão de milho moído. Os dados de digestibilidade *in vitro* da matéria seca, da proteína bruta e da fibra em detergente neutro foram interpretados por uma análise de variância, utilizando o método de quadrados mínimos; os resultados da produção de gás foram interpretados por uma regressão não linear pelo método de Gauss-Newton; e os efeitos de tratamentos foram avaliados por teste de médias (Teste de Tukey). Os coeficientes da digestibilidade *in vitro* da matéria seca, da proteína bruta e da fibra em detergente neutro dos alimentos analisados foram distintos. A produção de gases também foi distinta para os coprodutos e alimentos avaliados em relação ao volume de gás produzido nas frações de degradação rápida e lenta, taxa de degradação e para o tempo de colonização das bactérias e para o volume de gás total produzido. Os coprodutos avaliados apresentaram maior digestibilidade *in vitro* da matéria seca em relação à silagem de milho, com exceção do caroço de algodão, do resíduo de uva e da rama de mandioca. Além disso, os coprodutos apresentaram maiores valores de digestibilidade *in vitro* da proteína bruta em relação à silagem de milho, e redução da digestibilidade *in vitro* da fibra em detergente neutro, exceto para a polpa cítrica peletizada e a casca de grão de soja. A silagem de milho apresentou o maior volume de gás produzido na fração de degradação rápida comparada aos coprodutos e ao concentrado de milho. Os coprodutos analisados apresentaram características nutricionais adequadas pelas técnicas utilizadas e podem ser adicionados à dieta de ruminantes.

Palavras-chave: alimentos alternativos, degradação, fermentação, produção de gás.

Introduction

Agroindustrial residues resulting from the processing of plant products, such as meals, seeds, pulps and peels, are generated in large quantities and have the potential to be used to feed animals of zootechnical interest (Prado & Moreira, 2002). Alternative sources of energy for dairy cattle, of low commercial value and that do not impair animal performance, such as agroindustrial co-products, may represent an increase in competitiveness in the zootechnical activity. However, the level of substitution of such foods has to be assessed, since each ingredient has intrinsic positive and/or negative characteristics.

The use of *in vitro* digestibility techniques is convenient in studies on animal nutrition to estimate the quality of food and co-products, since they are simple, fast methods and because they present physical-chemical uniformity of the fermentation site (Alcalde, Machado, Santos, Picolli, & Jobim, 2001). Techniques such as *in vivo* tests are the most accurate methods for determining the nutritional value of feeds used in animal diets. However, these techniques require the use of animals, food, labor and time, limiting their application (Oliveira et al., 2014). Because of this, several *in vitro* techniques and laboratory techniques have been developed, such as those proposed by Tilley and Terry (1963); Orskov and Mcdowell (1979); Weiss, Conrad, and Pierre, (1992), and Maurício, Mould, and Dhanoa (1999), with the function of enabling nutritional assessment of foods.

The methodology of Tilley and Terry (1963) simulates *in vitro* the digestion processes that take place in the gastrointestinal tract of animals. Nevertheless, the *in vitro* gas production technique allows to estimate the digestibility, the fermentation rate of the different food fractions and the ruminal microbial activity. This technique also allows the determination of the nutritional value of the food by the production of the gases CO₂ (carbon dioxide) and CH₄ (methane), and short chain fatty acids (SCFA), which are produced from the fermentation of the substrate (Oliveira et al., 2014). Such information provides a rapid estimate of digestibility of feed for cattle (Theodorou, Lowman, Davies, Cuddelford, & Owen, 1998). The *in vitro* gas production technique has advantages over other digestibility techniques, since it provides two distinct food data with only one incubation: the apparent digestibility of the food by the volume of gas obtained and the true digestibility of the food by means of the residue generated.

The *in vitro* gas production technique can be complemented by determining the sample residue to obtain the residual nutritional value of the food. The residue represents the amount of the substrate that was not used in the fermentation; contrary to what occurs in the measurement of gas production, which reflects only the production of SCFA and gases. In addition, the determination of produced gases is useful in predicting the voluntary intake of food for ruminants (Blummel & Becker, 1997).

In this context, this study aimed to determine the nutritional value of the co-products from agroindustry: coffee hulls; pelleted citrus pulp; grape residue; soybean hulls; cottonseed; cassava foliage; and the feeds: corn silage and ground corn concentrate; which are foods commonly used in ruminant feeding. Such products were subjected to chemical analysis, *in vitro* digestibility assays (adapted from Tilley and Terry method) and gas production technique.

Material and methods

The experiment was conducted at the Experimental Farm of Iguatemi, Laboratory of Food Analysis and Animal Nutrition, and at the Laboratory of *In vitro* Digestibility and Animal Metabolism, all located at the Animal Science Department, State University of Maringá, Maringá, state of Paraná.

Agroindustry residues were obtained in the North and Northwest regions of the state of Paraná, collected only once. The co-products used were the coffee hulls (CH); pelleted citrus pulp (PCP); grape residue (GR); soybean hulls (SH); cottonseed (CS); cassava foliage (CF); and conventional foods such as corn silage (CSIL); and ground corn grain concentrate (GCG). The samples of co-products and foods were ground in a knife mill with a sieve of 1mm and used for determination of dry matter (DM, method 934.01), crude protein (CP, method 990.03), ether extract (EE, method 920.39), gross energy (GE, method 962.09), lignin (LIG, method 921.91) and ash (CT; method 942.05), in accordance with Association of Official Analytical Chemists (AOAC, 1998) recommendations, in duplicate. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were performed in duplicate according to the methodology described by Van Soest, Robertson, and Lewis (1991). NDF and ADF values were corrected for ash. The chemical composition of the co-products evaluated is found in Table 1.

It was performed assays of *in vitro* digestibility of dry matter (DIVDM), crude protein (DIVCP) and NDF (DIVNDF), of the co-products (CH, PCP,

GR, SH, CS and CF) and foods (CSIL and GCG), according to the technique described by Tilley and Terry (1963) adapted to the artificial rumen (Daisy II®, ANKOM®, Macedon, NY, USA), as described by Holden (1999).

The inoculum for the incubation of the samples was the ruminal liquid of a multiparous P&B Holstein cow, weighing approximately 550 kg, which received a diet composed of forage concentrate in the 70:30 ratio. The ruminal fluid was collected before the morning feeding via the ruminal fistula. The liquid was taken with a vacuum pump (Tecnal, Piracicaba, São Paulo State, Brazil) and a 2,000 mL Kitasato flask, kept in a water bath at 39°C. The ruminal inoculum was then transferred to a thermos bottle preheated to 39°C and pre-purged with CO₂. After storage, CO₂ was added again for approximately 30 seconds, and the thermos bottle was subsequently sealed and transported immediately to the laboratory (Alcalde et al., 2001).

For incubation, 0.5 g sample was placed in F57 filter bags (ANK ANKOM®, Macedon, NY, USA) previously washed with acetone to remove surfactants that could inhibit microbial digestion. The filters were then placed in glass jars containing ruminal liquid and McDougall's (1948) buffer solution (artificial saliva) in the presence of CO₂. The buffer solution was composed of: 9.80 g NaHCO₃; 7 g Na₂HPO₄·7H₂O; 0.57 g KCl; 0.47 g NaCl; 0.12 g MgSO₄·7H₂O; 0.04 g CaCl₂; and 5.0% urea, which were solubilized in distilled water, completed to 1000 mL and stored in a refrigerator until the incubation time, adjusted to obtain a final pH of 6.8 at 39°C. Incubation occurred for 48 hours plus 24 hours of enzymatic digestion, according to ANKOM® adapted by Holden (1999). DIVDM, DIVCP and DIVNDF were calculated by the difference between the amount incubated and the residue remaining after incubation.

The kinetics of digestion through *in vitro* gas production in the fermentation process of each co-product and food was obtained using the methodologies described by Theodorou et al. (1998) and Pell, Pitt, Doane, and Schofield (1998) modified for wireless computerized system, equipped with pressure transducer with radio frequency communication (ANKOM® RF- Gas production system, ANKOM®, Macedon, NY, USA). In duplicate, 0.5 g sample was weighed and allocated in the modules. McDougall (1948) artificial saliva (120 mL) and ruminal fluid (25 mL) were added in the presence of CO₂, and incubated for 24 hours. Pressure data, in psi, were collected every 10 min., and transformed into mL gas 100⁻¹ mg dry matter. The degradability values corresponding to the analyzed fractions were obtained according to the bicompartimental logistic model: $y = A / \{1 + \exp[2 + 4 \cdot B \cdot (C - T)]\} + D / \{1 + \exp[2 + 4 \cdot E \cdot (C - T)]\}$, where y is the total volume of gas in time T; A and D are the gas volumes (mL) of the fast (soluble sugars and starch) and slow (cellulose and hemicellulose) degradation fractions, respectively; B and E are the degradation rates of the fast and slow digestion fractions in % h⁻¹, respectively; and C is the time of colonization of the bacteria, in hours. This model has a double compartment, since it assumes that the foods consist of nutrients whose nature is heterogeneous, presenting fractions of fast and slow digestion, which are digested differently by the ruminal microbiota (Schofield, Pitt, & Pell, 1994).

Data of DIDM, DIVCP and DIVNDF were tested by an analysis of variance using the least squares method ($p < 0.10$). However, the gas production results were interpreted by a non-linear regression by the Gauss-Newton method ($p < 0.10$). The effects of the treatments were evaluated by means test (Tukey's test) using the statistical package Statistical Analysis System (SAS, 2001).

Table 1. Chemical composition of the co-products and foods.

Variables	Co-products and Foods evaluated							
	CH	PCP	GR	SH	CS	CF	CSIL	GCG
DM (%)	85.94	86.10	90.02	88.66	91.72	66.07	31.20	88.95
MM (% DM)	6.24	4.91	2.98	5.03	3.91	6.65	4.67	7.78
OM (% DM)	93.76	95.09	97.02	94.97	96.09	93.35	95.33	92.22
PB (% DM)	14.14	6.27	21.48	13.47	18.06	12.62	7.88	9.83
CP (% DM)	0.78	1.47	9.18	2.28	20.24	2.13	2.45	2.77
NDF (% DM)	36.92	19.10	65.19	63.06	33.26	57.21	55.4	-
ADF (% DM)	31.91	14.59	55.49	39.61	37.31	48.44	31.78	6.67
LIG (% DM)	10.37	6.84	13.01	5.82	5.30	14.37	3.82	1.08
GE (% DM)*	3.81	3.59	4.96	3.78	5.22	4.06	4.56	3.81
TC (% DM)	78.84	87.35	66.36	79.22	57.79	78.60	85.00	59.62
NFC (% DM)	41.92	68.25	1.17	16.16	24.53	21.39	29.6	-

CH: coffee hulls; PCP: pelleted citrus pulp; GR: grape residue; SH: soybean hulls; CS: cottonseed; CF: cassava foliage; CSIL: corn silage; GCG: corn concentrate; *GE em Mcalkg⁻¹, DM: dry matter, MM: mineral matter, OM: organic matter, CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, LIG: lignin, GE: gross energy, TC: total carbohydrates; NFC: non-fiber carbohydrates.

Results and discussion

The knowledge of the digestibility of foods is an important factor to define the portion of nutrients that will be effectively used by the animal and to evaluate the quality of the diet (Oliveira et al., 2014). Pelleted citrus pulp and ground corn grain presented higher levels of DIVDM, 95.33 and 94.76%, respectively (Table 2). These values were higher than the soybean hulls and the coffee hulls (83.44 and 80.73%, respectively), followed by corn silage (72.67%). The lowest values of DIVDM were found for samples of cassava foliage, grape residue and cottonseed (53.17, 51.24 and 49.52%, respectively).

Table 2. Coefficients of *in vitro* digestibility of dry matter (DIVDM), crude protein (DI/CP) and neutral detergent fiber (DIVNDF) of different co-products and foods by the method of Santos (2001).

Co-products and foods	<i>in vitro</i> digestibility of nutrients (%)		
	DIVDM	DI/CP	DIVNDF
CH	80.73 ^b	74.46 ^{bc}	77.29 ^c
PCP	95.33 ^a	81.24 ^{ab}	96.12 ^a
GR	51.24 ^d	63.65 ^c	54.29 ^e
SH	83.44 ^b	70.64 ^{bc}	89.38 ^b
CS	49.52 ^d	78.69 ^{ab}	63.26 ^d
CF	53.17 ^d	66.58 ^{bc}	60.52 ^d
CSIL	72.67 ^c	61.28 ^c	78.46 ^c
GCG	94.76 ^a	89.96 ^a	98.02 ^a
P-value	< 0.0001	0.0011	< 0.0001

Means followed by different lowercase letters, in the same column, are significantly different by Tukey's test ($p < 0.10$); CH: coffee hulls; PCP: pelleted citric pulp; GR: grape residue; SH: soybean hulls; CS: cottonseed; CF: cassava foliage; CSIL: corn silage; GCG: ground corn concentrate; DIVDM: *in vitro* dry matter digestibility; DI/CP: *in vitro* crude protein digestibility; DIVNDF: *in vitro* NDF digestibility.

The observed value for DIVDM of the pelleted citrus pulp differed from that found by Fegeros, Zervas, Stamouli, and Apostolaki (1995) of 78.6%. The nutrient content in the pelleted citrus pulp can be influenced by a number of factors, including fruit (orange and etc), seed quantity and type of processing (Silva, Souza, Silva Neta, Inácio, & Muniz, 2013). The pelleted citrus pulp presents in its composition 25% DM as pectin. Pectin is considered soluble fiber and is a structural carbohydrate that is not covalently bound to the lignified portions, being 90-100% degradable in the rumen (Nocek & Tamminga, 1991), thus increasing the values of DIVDM.

The DIVDM of soybean hulls was 83.44%, differing from the results found by Zambom et al. (2001), who reported values of 94.96% for DIVDM and 95.69% for DIVNDF. Soybean hulls have a thin film rich in pectin, which comprises 30% of the structural carbohydrates (Gnanasambandam & Proctor, 1999). During the drying process of the grains, this film can detach from the hull, resulting in a lower amount of pectin in the sample. In general, increasing the pectin concentration in the food raises the digestibility of the food and the fiber fraction. The highest values of DIVDM and

DIVNDF reported by Zambom et al. (2001) may be related to a higher amount of pectin in soybean hull, which is variable and can be affected by soybean processing.

Cottonseed had the lowest DIVDM, 49.52%. This co-product is composed of three parts: the fiber (composed of the linters and leftovers of the lint); the shell; and the kernel (Silva & Queiroz, 1990). Cottonseed is a food with peculiar characteristics, because it contains high energy content, resembling concentrate foods, besides being rich in effective fiber, common to forage foods (National Research Council [NRC], 2001). Thus, the low value of DIVDM may be due to the lower digestibility of the fiber fraction, which is affected by the coating of fiber by wax from the food itself (20.24%). This process hinders microbial attack, while causing toxic effects directly on certain microorganisms, and reduces the availability of cations by combination with fatty acids (Palmquist & Jenkins, 1980).

The concentrate of ground corn grain presented high DIVCP (89.96%), and corn silage presented the lowest DIVCP (61.28%). The grape residue and the cassava foliage showed similar results of DIVCP to that found for corn silage, differing from the other co-products analyzed. The inclusion of grape residue in the ruminant diet may result in a decrease in protein digestibility because it is a food that contains tannins that can bind to proteins and form complexes, reducing its availability for digestion and absorption.

The corn grain concentrate (98.02%) and the pelleted citrus pulp (96.12%) exhibited high DIVNDF, higher than soybean hulls (89.38%), followed by corn silage (78.46%) and coffee hulls (77.29%), cottonseed (63.26%) and cassava foliage (60.52%). According to Souza et al. (2004), the presence of phenolic compounds, low CP values, and available energy in coffee hulls may be limiting factors for the addition of this co-product in the diet for ruminants at high levels or replacing forage foods.

In the present study, the DIVNDF of coffee hulls may have been affected by the high lignin content. The negative effect of lignin on the digestibility of the fiber fraction of the coffee hull can be confirmed by the high content of the indigestible acid detergent fiber of this residue, as observed by *in vitro* studies performed by Souza et al. (2001). In the study, the low digestibility of neutral detergent fiber (mean of 28.9%) differed from the value found in this study (77.29%).

The grape residue had the lowest value for DIVNDF (54.29%). This co-product is included in

the forage fraction of the diet because of the high fiber content. The lignin content reported in the literature is high, 22.87% (Tosto et al., 2007), 22% (Barroso, Araujo, Silva, & Medina, 2006), 26% (Baumgartel, Kluth, Epperlein, & Rodehutschord, 2007), possibly present in a greater proportion in the seed than in the peel, which causes the decrease in nutrient digestibility. Meanwhile, the lignin content of the grape residue in the present study was 13.01%.

In the gas production technique, the effects of the co-products were observed for the volume of gas produced in the fast degradation fraction (Fraction A), for degradation rate of the fast digestion fractions (Fraction B), for the time of colonization of bacteria (Fraction C) and for the sum of the volume of gas produced for the fast and slow degradation fractions (Fraction A + D; Table 3).

Table 3. Means of parameters estimated by *in vitro* gas production in the different co-products and foods evaluated.

Variables	Co-products and foods evaluated								p-value
	CH	PCP	GR	SH	CS	CF	CSIL	GCG	
A (mL)	4.48 ^b	2.17 ^{bc}	1.46 ^{bc}	2.60 ^{bc}	0.58 ^c	1.28 ^c	9.49 ^a	0.77 ^c	0.00001
B (% h ⁻¹)	0.15 ^{abc}	0.57 ^a	0.04 ^c	0.16 ^{abc}	0.08 ^{abc}	0.56 ^{ab}	0.06 ^{bc}	0.13 ^{abc}	0.01691
C (h)	4.08 ^{bc}	2.37 ^c	11.98 ^a	5.25 ^{bc}	5.26 ^{bc}	6.29 ^b	2.67 ^c	3.31 ^{bc}	0.00001
D (mL)	3.28	2.77	2.38	5.89	1.76	1.39	2.15	6.44	0.15896
E (% h ⁻¹)	0.23	0.09	0.79	0.06	0.30	0.55	0.59	0.12	0.17988
A+D (mL)	7.76 ^{abc}	4.94 ^{bcd}	3.84 ^{bcd}	8.49 ^{ab}	2.34 ^d	2.67 ^{cd}	11.64 ^a	7.21 ^{abc}	0.00091
R ² (%)	98.97	98.47	84.45	98.61	99.32	85.00	99.7	99.25	-

Means followed by different lowercase letters, in the same row, are significantly different by Tukey's test ($p < 0.10$); CH: coffee hulls; PCP: pelleted citric pulp; GR: grape residue; SH: soybean hulls; CS: cottonseed; CF: cassava foliage; CSIL: corn silage; GCG: ground corn concentrate; A and D: gas volumes (mL) of the fast and slow degradation fractions, respectively; B and E: degradation rates (% h⁻¹) of the fast and slow digestion fractions, respectively; C: time of colonization of bacteria; A+D: sum of the volume of gases (mL) produced for the fractions of fast and slow degradation. R²: coefficient of determination.

The corn silage presented the largest volume of gas produced in the fraction of fast degradation (soluble sugars and starch) produced per g OM (9.49 mL), differing from cottonseed (0.58 mL), cassava foliage (1.28 mL) and ground corn grain concentrate (0.77 mL). The coffee hulls differed from the other analyzed foods by producing in the fast degradation fraction a volume of 4.48 mL gas. The citrus pulp (2.17 mL), the grape residue (1.46 mL) and the soybean hull (2.60 mL) did not differ from any of the other co-products tested.

The citrus pulp obtained the highest degradation rate of the fast digestion fraction (% h⁻¹), presenting

a value of 0.57% hour⁻¹. This value is higher than the co-products coffee hulls (0.15% hour⁻¹), cottonseed (0.08% hour⁻¹), soybean hulls (0.16% hour⁻¹) and corn concentrate (0.13% per hour⁻¹), which did not differ from each other. Such foods had a similar behavior in the degradation of the fast digestion fraction.

Corn silage and pelleted citrus pulp had a shorter bacterial colonization time, with values of 2.67 and 2.37 hours, respectively, differing from the other co-products. The grape residue had the longest bacterial colonization time (11.98 hours), differing from the coffee hulls (4.08 hours), cottonseed (5.26 hours), soybean hulls (5.25 hours), and corn concentrate (3.31 hours).

The corn silage presented the largest total volume of gas produced (11.64 mL), differing from citrus pulp (4.94 mL), cassava foliage (2.67 mL), cottonseed (2.34 mL), and grape residue (3.84 mL). Rocha Filho, Machado, D'arce, and Francisco-Jr (1999) conducted a trial to evaluate the effects of citrus pulp and corn grain replacing corn silage on production of short chain fatty acids (SCFA) in the rumen and observed higher production of acetate and butyrate for the diet containing pulp compared to the diet with corn grain. While total SCFA yields were similar, ruminal acetate/ propionate ratios were different, being 3.04 and 2.59 for diets with citrus pulp and corn grain, respectively.

The analyzed co-product with the lowest total volume of gas was the cottonseed, which differed from the others analyzed. The cottonseed has linter in its composition, a very lignified part that protects the cottonseed, and so this is a difficult digestion food which causes more delay in the production of gases. Another factor that may have contributed to the low volume of gas produced may be related to the high content of ether extract (20.24%) found in the cottonseed.

Conclusion

All co-products evaluated here have nutritional characteristics that make them potential sources of energy and can be used in the formulation of ruminant diets. It is important to emphasize that it is necessary to know in detail the peculiar characteristics of each food and how to partially replace the foods conventionally used in the animal diet.

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