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Roughage digestion evaluation in horses with total feces collection and mobile nylon bags[1]

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ABSTRACT - This study aimed to evaluate the nutrient digestibility of roughages in horses with total feces collection and mobile bags. Two trials were carried out simultaneously. The first trial evaluated the digestibility of nutrients of coastcross hay (*Cynodon dactylon* cv. coastcross) with total feces collection. The second trial assessed the digestibility of nutrients of alfalfa hay (*Medicago sativa*), peanut (*Arachis pintoi*) and coastcross hay with mobile bags. This trial was conducted with gastric insertions of nylon bags every 12 hours, and each bag contained 663 mg of feed samples in a proportion of 17 mg DM/cm^2. Feces and bags were collected directly from the stall floor immediately after excretion. There was no difference between the digestibility of dry matter, crude protein, carbohydrates and hydrolysable carbohydrates of coastcross hay estimated with feces collection and mobile bags. Forage peanut showed high nutrients digestibility, with values close to those observed with alfalfa, indicating potential for use in diets for horses.

Key Words: alfafa hay, coastcross hay, digestibility, equine, forage peanut

Introduction

Knowledge of the nutritional value of forages as well as the digestive physiology of horses promote the formulation of more effective and safe diets, ensuring better performance of athletes horses.

The collection of total feces is still the most used method for the evaluation of horse feed. However, some limitations of this technique, such as the use of expensive equipments, large feces production by horses, control of feed intake and fecal excretion and use of few animals due to the handling have stimulated the replacement of this technique by others that are faster and less expensive (Pereira et al., 1995).

The *in situ* digestion method with nylon mobile bags was developed as an alternative to total feces collection, as a faster and easily applied method. The advantage of this technique is that various feeds can be evaluated in a simple protocol, where the feed samples are placed in polyester bags, inserted in the stomach of the animal and recovered with the feces. Araújo et al. (2000) reported that the mobile nylon bags technique can be used in replacement of the total collection of feces in horses.

This study aimed to evaluate the digestibility of nutrients of coastcross hay with total collection of feces and the mobile nylon bag techniques, as well as to evaluate the digestibility of nutrients of alfafa hay, coastcross hay and forage peanut with the mobile nylon bag technique.

Material and Methods

The experiment was carried out at the Laboratório de Pesquisas em Saúde Equina in the Instituto de Veterinária of Universidade Federal Rural do Rio de Janeiro. Two digestion trials were conducted: one to evaluate the apparent total nutrient digestibility of coastcross hay (*Cynodon dactilon* cv. coastcross) with total feces collection and another to assess the digestibility of nutrients of alfalfa hay (*Medicago sativa*), peanut (*Arachis pintoi*) and coastcross hay with mobile bags. The trials were conducted simultaneously, with 10 days of adaptation of the horses to coastcross hay as exclusive feed. From the 11th to the 15th day of the assay, a nasogastric tube was inserted into the stomach of the horses every 12 hours. The nylon bags were inserted into the tube and bags excreted in the feces were collected until the 19th day.

Four mature half breed horses with body weight average of 270 kg were used. The animals were maintained in individual pens and exercised for 30 minutes daily. At the beginning of the assay the horses were weighted to adjust the diet. The diet was exclusive of coastcross hay, with daily intake as 2% of body weight, according to NRC (2007)

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recommendations of horses with 300 kg at maintenance. During the trial, the diet was fractioned in equal parts and offered at 7:00, 13:00 and 19:00 h.

Nylon bags (Tenyl®) with internal area of 6.5 × 3.0 cm and pore size of 45 μ were used. After the confection of the bags, they were identified, oven-dried (55-60 ºC, 24h) and weighed. In each bag, a sample of 633 mg of each feed, grounded at 1 mm was inserted in a relation of 17 mg DM/cm^2 (Silva et al., 2009). Then, bags were sealed with heat with an automatic sealer (Araújo et al., 1996). In each nasogastric tube, 25 bags were inserted, 8 of each feed and one empty bag was also inserted to estimate the impregnation. Two gastric insertions were performed each day, one at 7:00 am and another at 7:00 pm, during five days.

To optimize the passage of the bags through the tube, bags were previously inserted in the tubes and then the tube inserted into the stomach. About 750 to 1,000 mL of water were pumped in by pressure, so the bags could reach the stomach of the horses (Moore-Colyer et al., 2002).

The recovery of the bags took place simultaneously to the collection of feces. Feces were collected from the floor of the pens immediately after excretion, during the 24 hours of the collection period. Bags were separated manually from the feces, and data such as time of excretion were recorded. Bags were maintained at -18 ºC until the washing. Feces were kept in separate plastic bags and at each interval of 6 hours they were weighed, homogenized, and a sample with 10% of weight was taken and stored at -18 ºC. Each day, the samples were homogenized and a composite sample of each animal was taken and stored at -18 ºC. At the end of the digestion trial, daily samples of each animal were defrosted and a composite sample was made and then oven-dried (55 ºC, 72 hours), and ground at 1 mm.

The nylon bags were defrosted, washed gently with cold water in a washing machine for about 15 minutes (Macheboeuf et al., 2003). Then, bags were oven-dried (55 ºC, 48 h). After that, each bag was weighed to estimate dry matter loss. A composite sample of each feed with the same animal was made from the collected bags. The bags without samples were used to determine impregnations and correction of nutrients digestibility estimative.

Ten bags of each feed were used to determine the particle loss through the bags pores. So, the bags with samples of feed were weighed, sealed and washed, then they were dried and weighed for determination of dry matter loss.

Dry matter (DM), mineral matter (MM), crude protein (CP), ether extract (EE), gross energy (GE) hemicelluloses (HEM), cellulose (CEL), lignin (LIG), neutral detergent fiber (NDF) and acid detergent fiber (ADF) were quantified according to the procedures described by Silva & Queiroz (2006).

The carbohydrate contents were estimated according to Hoffman et al. (2001). The content of non-fiber carbohydrates (NFC) was calculated by the formula: NFC = 100 – CP – EE – MM – NDF. The contents of rapidly fermentable carbohydrates (CHO-RF) were calculated by the diference between non-fiber carbohydrates (NFC) and hidrolizable carbohydrates (CHO-H), according to Hoffman et al. (2001): CHO-RF = NFC – CHO-H. Total carbohydrates (CHO-T) were calculated through the formula: CHO-T = CHO-H + CHO-RF + NDF.

The apparent digestibility coefficient of nutrients with total feces collection was estimated according to formula proposed by Schneider & Flatt (1975).

Nutrient loss from the bags was expressed as apparent digestibility coefficient determined by the residues of the composite samples, and estimated using the formula proposed by Moore-Colyer et al. (2002): Digestibility coefficient (%) = ((I-F)/I) × 100, where I is the amount of feed (mg) inserted in each bag, and F is the feed residue of the feed (mg) after the recovery of the bags in the feces.

The kinetics of passage of the nylon bags in the digestive tract was estimated. The transit time (TT) of the bags in the digestive tract was determined by interval between the insertion of the bags into the stomach of the horses and first recovery of bags in feces.

The mean retention time (MRT) of the bags in the digestive tract was calculated by the formula proposed by Faichney (1975): MRT (hours) = $\Sigma mi \times ti / \Sigma mi$, mi = number of bags recovered in feces in i interval ti = time interval between the insertion of the gastric bags in i collection time in the feces. The transit rate (TR) of the bags in the digestive tract was estimated as the reciprocal to MRT: TR (/ h) = 1 / MRT.

The percentage recovery was estimated using the total bags collected until 96 hours after gastric insertion of bags. The percentage of collected bags was calculated cumulatively. The bags recovered outside of these periods were discarded, since they are not representative of the digestive process.

The results of the digestibility of nutrients, transit time, the mean retention time, passage rate and percentage of recovery of nylon bags until 96 hours were subjected to analysis of variance and mean values were compared by Tukey test with Statistics and Genetics Analysis Software – SAEG.

Results and Discussion

The daily intake of dry matter of costcross hay was 2.40±0.04% of body weight, values within the range proposed by the NRC (1989), from 2.0 to 2.5% of body weight to horses at maintenance. Almeida et al. (1999) observed voluntary intake of 1.39% of body weight in horses fed coastcross hay. The intake of coastcross hay reported by these authors was lower than that was observed in this experiment, and this variation may be due to intake regulation by horses in relation to the quality of the forage.

The coastcross hay presented CP values similar to the observed by Araújo et al. (2000), Perali et al. (2001) and Silva et al. (2009): 6.4; 4.7 and 6.3% respectively. However, these were lower than the observed by Pimentel et al. (2009), 12.2% CP. Despite the low level of crude protein, the values of NDF and ADF were not very high: 60.9% and 32.2%, respectively, when compared with values cited by Araújo et al. (2000), of 84.6% and 40.15%; Perali et al. (2001), 79.3% and 41.8% and by Pimentel et al. (2009), 82.9% and 39.8 %, respectively.

According to Almeida et al. (1999), crude protein contents of alfafa range from 9.1 to 24.7%; NDF, from 30.9 to 60% and ADF, from 23.9 to 48.2%, on a dry matter basis. Therefore, the alfafa composition in this assay is within the expected values, with 17.1% of CP, 31.6% of NDF and 21.0% of ADF (Table 1).

The lower levels of fiber of the alfafa hay are related to the degree of maturity, and according to Queiroz Filho et al. (2000), with aging of the plant, the proportion of potentially digestible components tend to be lower and the fiber contents tend to increase, so it is possible that the sample evaluated in this essay was younger than others mentioned in the literature.

The forage peanut presented values of NDF of 39.0%, ADF of 24.5% and lignin of 7.2%, values lower than described by Ladeira et al. (2002), of 52.5; 35.8; 11.2% and by Silva et al. (2009), of 46.8; 30.7 and 12.4% respectively, indicating better quality in relation to the ones used by these authors.

There was no difference between the apparent digestibility of DM, CP, CHO-H and CHO-T of coastcross estimated from the two techniques (Table 2). The mean values of digestibility were 51.2 and 53.2% of dry matter, 69.7 and 70.1% of crude protein, 99.5 and 95.5% of CHO-H, and 48.8 and 51.3% of CHO-T with the feces collection and the mobile bags, respectively.

The digestibility coefficient of the organic matter, ether extract, gross energy, neutral detergent fiber and acid detergent fiber of coastcross hay were higher with feces collection, with mean values of 75.1; 68.0; 71.6; 72.4 and 68.9% in relation to the values observed with the mobile bags technique, of 52.2; 44.9; 43.9; 42.5 and 41.6% respectively. The values of rapidly fermentable carbohydrates were higher with mobile bag technique: 75.1%, compared with the feces collection, of 67.5%.

The coefficient digestibility of organic matter estimated by feces collection is above the levels cited by LaCasha et al. (1999), of 60%, suggesting that high values of digestibility coefficient of organic matter can be justified by the presence of soil waste. However, the coefficient of digestibility of organic matter through the technique of mobile nylon bag is within the range cited in the literature, of 48.2% (Santos et al., 2008) and 60% (LaCasha et al., 1999), indicating that the soil waste may have been eliminated in the making of the bags.

The values of dry matter digestibility of coastcross hay with feces collection and the mobile bags were 51.2 and

Table 1 - Chemical composition of coastcross hay, alfalfa hay and forage peanut, on dry matter basis

Item	Coastcross hay	Alfafa hay	Forage peanut
Dry matter (%)	90.60	88.90	28.70
Organic matter (%)	94.42	92.27	92.70
Crude protein (%)	5.49	17.08	15.43
Ether extract (%)	1.34	1.94	1.80
Gross energy (%)[1]	3.62	3.49	3.65
NDF (%)[2]	60.91	31.63	39.01
ADF (%)	32.19	21.01	24.52
Hemicellulose (%)	28.71	10.65	14.51
Celullose (%)	30.32	20.74	23.90
Lignin (%)	5.74	5.82	7.24
CHO-H (%)	2.23	1.61	3.23
CHO-RF (%)	15.74	29.90	26.11
CHO-T (%)	73.02	59.60	65.32

ADF - acid detergent fiber; CHO-H - hydrolysable carbohydrates; CHO-RF - carbohydrates rapidly fermentable; CHOT - total carbohydrates.
[1] Mcal/kgDM.
[2] Carbohydrates slowly fermentable represented by neutral detergent fiber (NDF).

Table 2 - Coefficient of digestibility of nutrients of coastcross hay with feces collection and mobile bags techniques

Nutrients	Digestibility coefficient (%)		CV (%)
	Feces collection	Mobile bags	
Dry matter	51.19a	53.21a	8.1
Organic matter	75.15a	52.18b	4.3
Crude protein	69.73a	70.12a	4.6
Ether extract	68.02a	44.87b	14.5
Gross energy	71.64a	43.89b	4.3
NDF[1]	72.35a	42.54b	2.1
ADF	68.91a	41.63b	1.9
CHO-H	99.52a	95.50a	7.8
CHO-RF	67.51b	75.11a	2.8
CHO-T	48.75a	51.32a	9.5

CV - coefficient of variation; ADF - acid detergent fiber; CHO-H - hydrolysable carbohydrates; CHO-RF - rapidly fermentable carbohydrates; CHO-T - total carbohydrates.
Different letters in the line differ according to F test (P<0.05).
[1] Carbohydrates slowly fermentable represented by NDF (neutral detergent fiber).

53.2%, respectively; lower than the described by Pimentel et al. (2009), of 60.3%. This variation may be due to the difference in the composition of the hay, which presented lower quality in this experiment.

Araújo et al. (2000), comparing the techniques of total feces collection and mobile nylon bags to evaluate dry matter digestibility of hay, observed values of 44.7, 40.9 and 40.3% to the samples size of 1, 3 and 5 mm, respectively; values lower than 53.2% observed in this assay. However, in accordance with the results, these authors did not observe differences between digestibility coefficients of the dry matter estimated by total feces collection and the mobile nylon bags when the samples were chopped at 1 mm.

Values of digestibility coefficient of NDF of 72.4%, ADF of 68.9% and gross energy of 71.6%, were overestimated by total feces collection. Santos et al. (2008), evaluating the digestibility of coastcross hay by total feces collection, observed values of digestibility of NDF and gross energy of 47.9% and 45.5% respectively, and these values were similar to the observed in this assay with the mobile nylon bags technique, of 42.5% and 44.9%,. Similarly, Silva et al. (2009) observed values of 47.5%; 39.4% and 48.7% of the digestibility coefficients of NDF, ADF and gross energy with the mobile nylon bags technique.

Results from the literature cited values of dry matter and crude protein digestibility of alfafa hay of 55,2 and 71,2% (Almeida et al., 1999). Silva et al. (2009), using the nylon bag technique, observed values of 63.5; 64.3; 77.0 and 61.0% digestibility coefficient of dry matter, organic matter, crude protein and gross energy of alfafa, respectively. Takagi et al. (2002) observed values of 80.2% of the digestibility coefficient of crude protein of roughages with feces collection. High values of digestibility coefficient of crude protein of roughages observed at the present study may be related to the loss of nutrients by the nylon bag pores.

The digestibility coefficients of forage peanut are below the values mentioned by Silva et al (2009), of 83.7% of dry matter digestibility, 72.2% of NDF digestibility and 70.9% of ADF digestibility, and this variation can be explained by differences in the composition of the feed.

Coastcross hay presented the greatest uniformity of the coefficients of digestibility in relation to the data from the literature. The digestibility coefficients of dry matter, crude protein and NDF were 53.2; 70.1 and 42.5%%; values close to the results observed by Araújo et al. (2000) and Silva et al. (2009), of 44.7, 68.2 and 36.8%; and 52.4, 69.8 and 47.5%, respectively.

Among the forages, the alfafa hay and forage peanut presented highest coefficients of the digestibility (Table 3), which was expected, according to Singer et al. (1999), that legumes present higher nutrients digestibility when compared to grass.

Any differences were observed (P>0.05) in transit time (TT), mean retention time (MRT), transit rate (TR) of nylon bags along the digestive tract of the horses and the total recovery of bags until 96 hours with mean values of 23.6h, 52.2h, 0.019/h and 94.8%, respectively (Table 4).

The transit time of 23.6 h observed for nylon bags was similar to the described by Silva et al. (2009), of 23.3h. Oliveira et al. (2003), evaluating the kinetics of passage in horses fed diets with different levels of forage, observed transit time of 27 hours of the solid fase of the digesta in horses fed only forage. Supporting these values, Oliveira et al. (2007) observed a mean retention time of 28.9 h in horses fed mixed diets with different substitution levels of corn grain for wheat dry grain or silage.

The mean retention time of the bags was of 52.2 h, higher than the value observed by Silva et al (2009), of 48.6 h, also with bags in horse's digestion studies. The difference on mean retention time values may be related to the difference in the size of the bags. The bags in this study were bigger,

Table 3 - Coefficients of apparent nutrient digestibility of coastcross hay, alfafa hay and forage peanut, with the mobile nylon bag technique

Nutrients	Digestibility coefficient (%)			CV (%)
	Alfafa hay	Forage peanut	Coastcross hay	
Dry matter	76.81a	75.90a	53.23b	1.6
Organic matter	76.71a	75.44a	52.25b	1.7
Crude protein	94.05a	82.62b	70.12c	1.1
Ether extract	83.54a	75.87a	49.17b	9.7
Gross energy	72.28a	67.75a	43.94b	4.8
NDF[1]	47.19b	59.17a	42.47b	3.9
ADF	46.54b	55.36a	41.57b	4.8
CHO-H	98.56a	99.41a	95.54a	1.9
CHO-RF	92.74a	90.18a	75.12b	1.2
CHO-T	70.43b	74.82a	51.30c	1.4

CV - coefficient of variation; ADF - acid detergent fiber; CHO-H - hydrolysable carbohydrates; CHO-RF - rapidly fermentable carbohydrates; CHO-T - total carbohydrates.
[1] Carbohydrates slowly fermentable represented by NDF (neutral detergent fiber).

Table 4 - Kinetics of the mobile nylon bags through the digestive tract of horses

Feed	TT (hours)	MRT (hours)	TR (/h)	Recovery until 96 h (%)
Alfafa hay	23.0±6.1	51.3±4.1	0.019±0.002	95.9±3.6
Forage peanut	25.9±3.0	52.1±2.6	0.019±0.001	96.3±2.9
Coastcross hay	21.8±8.3	53.3±4.5	0.019±0.002	92.0±6.5
Mean	23.6	52.2	0.019	94.8
CV (%)	8.9	1.9	1.8	2.5

TT - transit time; MRT - mean retention time; TR - transit rate; CV - coefficient of variation.

with 6.5 × 3.0 cm, and the bags used by Silva et al. (2009) measured 7.5 × 2 cm, and this could result in a slower time of passage through the large intestine. Oliveira et al. (2007) observed a mean retention time of 47.86 h of digesta in the digestive tract of horses fed mixed diets, and these values are close to the observed in this study.

It should be noted that there is variation in the transit time of the bags according to the diet content and frequency in feeding (De Fombelle et al., 2004). Although the kinetics of the passage of the bags present their own characteristics, it is important that the parameter presents itself the closest to reality and so that there are no interferences in the digestion.

The diets were fractioned in three meals through the day allowing intestinal motility to be regularly maintained. The recovery of the bags until 96 hours in this study was 94.8%, higher than the observed by Araújo et al. (2000), with mean value of 85.7% with bags containing samples of coastcross hay with different granulometry. This difference is due to the way the bags are collected; for the authors mentioned, it was done with the collection of feces in collector bags that were emptied three times a day, while in this study the bags were collected immediately after excretion, 24 hours a day, during the experimental period.

Rosenfeld & Austbø (2009) recovered about 61% of the bags in the feces 72 h after gastric insertion. De Fombele et. al. (2004), observed that the number of bags recovered and the time of recovery can vary according to the diet, indicating alterations in the retention time of the bags. The efficient recovery of the bags is essential to the success of the technique. Araújo et al. (2000) suggest a minimum recovery of 80% of the bags in the feces for the viability of the technique.

The particle losses through the pores were measured by weighing bags after washing without digestion. The losses were 26.5, 27.1 and 16.7% of dry matter and, 27.2, 26.2 and 1.6% crude protein in the bags containing alfalfa hay, peanut forage and coastcross hay, respectively. The highest values of crude protein losses through the bags were proportional to the crude protein of the roughage and could explain the highest values of protein digestibility coefficients of alfafa and peanut forage. Araújo et al. (2000) observed about 14% loss of coastcross dry matter in the bags with 60-μ pores, these were bigger than the pores used in this study,with 45-μ size.

According to Hyslop (2006), loss of particles from bags is one of the major flaws of the mobile nylon bags technique, since it is not possible to assess the location and extent of degradation and absorption of lost particles. Particle losses may occur in two stages: the finely ground particles in contact with the gastric juices are diluted and leak out of the bags or losses may occur during washing procedures of the bags, whose function is to remove impregnations, endogenous enzymes, microbial biomass and food residues (Moore-Colyer et al., 2002).

The finely ground particles that are soluble in the stomach can also be considered part of the digested material in the technique of mobile bags, because after they leave the bags they have the opportunity to be digested along the digestive tract.

Conclusions

The mobile nylon bag technique was efficient in relation to the total collection of feces to evaluate the apparent coefficient of digestibility of dry matter, crude protein, hydrolyzable carbohydrates and total carbohydrates of coastcross hay. However, the losses due to particle size used in this experiment, of 1 mm, can cause overestimation of the digestibility of other nutrients of the diet. Therefore, further studies are necessary to evaluate and correct possible losses occurred within the mobile nylon bags technique. Among the forages studied, forage peanut presented digestibility coefficient similar to the alfafa, indicating potential for use in diets for horses.

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