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Comparison between two methods for estimating the digestibility of starch and other nutrients in high moisture sorghum grain silage in horses[1]

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ABSTRACT - It was carried out two assays with the purpose of evaluating the mobile nylon bag technique and the method of total feces collection method in the estimative of the apparent digestibility of starch and other nutrients in high moisture sorghum grain silages in horses. Four adult horses of mixed origin at the age of 7 years and average body weight of 330 kg were used. In the first assay, the digestibility of the nutrients in the silage of high moisture sorghum grain with low level of tannin was evaluated by utilizing the total feces collection and the mobile nylon bag technique with silage samples ground in three particle sizes. In the second assay, the two methods were used for estimating the digestibility of the nutrients of the silage of high moisture sorghum grain with high level of tannin. A randomized block experimental design was used, considering each animal as a block. The disappearance of the nutrients, in the naylon bag, of the samples of the high moisture sorghum grain silages ground in three particle sizes of 1, 8 and 13 mm was also evaluated in laboratory. Daily dry matter intake was 2.0% of the body weight and test diets were made up of 30% of high moisture sorghum grain silages and 70% of coast-cross hay. The coefficients of dry matter digestibility, energy and protein of both silages were similar among the total feces collection method and the mobile nylon bag technique when 8-mm feed samples were used. The coefficient of starch digestibility was similar among the methods when sorghum samples were ground at 1 mm in the mobile nylon bag technique. Reduced particle size increased the nutrient losses of the nylon bags after washing in water. The mobile nylon bag technique allows to estimate the digestibility coefficient of nutrients, however, further studies for evaluation of particle size of the samples should be conducted.

Key Words: horse, mobile nylon bag technique, total feces collection

Comparação entre dois métodos para estimativa da digestibilidade do amido e de outros nutrientes em silagem de grãos úmidos de sorgo em equinos

RESUMO - Foram realizados dois ensaios com o objetivo de avaliar a técnica do saco de náilon móvel e o método de coleta total de fezes na estimativa da digestibilidade aparente do amido e de outros nutrientes em silagens de grãos úmidos de sorgo em equinos. Foram utilizados quatro cavalos adultos sem raça definida, com 7 anos de idade e peso corporal médio de 330 kg. No primeiro ensaio, avaliou-se a digestibilidade dos nutrientes da silagem de grãos úmidos de sorgo com baixo teor de tanino utilizando a coleta total de fezes e a técnica do saco de náilon móvel com amostras da silagem moídas em três granulometrias. No segundo ensaio, foram utilizados os dois métodos na estimativa da digestibilidade dos nutrientes da silagem de grãos úmidos de sorgo alto tanino. O delineamento experimental foi o de blocos casualizados, considerando cada animal um bloco. Também foi avaliado em laboratório o desaparecimento, nos sacos de náilon, dos nutrientes das amostras das silagens de grãos de sorgo úmidos moídas em três granulometrias (1, 8 e 13 mm). A ingestão diária de matéria seca foi de 2,0% do peso corporal, e as dietas-teste foram compostas de 30% de silagem de grãos úmidos de sorgo e 70% de feno de coast-cross. Os coeficientes de digestibilidade da matéria seca, energia e proteína de ambas as silagens foram semelhantes entre as metodologias de coleta total de fezes e a técnica de sacos de náilon móveis quando utilizadas amostras de alimento de 8 mm. O coeficiente de digestibilidade do amido foi similar entre os métodos quando as amostras de sorgo foram moídas a 1 mm na técnica de sacos de náilon móveis. A redução da granulometria aumentou as perdas dos nutrientes dos sacos de náilon após lavagem em água. A técnica dos sacos de náilon móveis permite estimar o coeficiente de digestibilidade dos nutrientes, entretanto devem ser realizados mais estudos para avaliação da granulometria das amostras.

Palavras-chave: cavalo, coleta total de fezes, técnica do saco de náilon móvel

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Introduction

Both digestibility and nutritional value of the values of feeds for horses are evaluated through the total feces collection, a traditional technique that demands the use of large amounts of the feed-tests and the accurate control of intake and fecal production of the animals. That procedure is very stressful especially for large herbivorous animals because of their total confinement in metabolism cages. However, the mobile nylon bag technique, in spite of still being little investigated in horses, is feasible since it demands small samples of feeds which makes possible the evaluation of the digestibility of nutrients of several feeds at the same time in a simple and fast way.

The supply of concentrate feeds is important to keep the good health and to meet the nutrient requirements of the several horse categories. Among the most used energy-supply feeds, corn and oats stand out, but the high prices of these cereals restrict their inclusion in ration formulation. Sorghum grain can be an alternative grain because of its similar nutrient composition to that of corn as well as its lower cost/unit of supplemented energy.

The use of high moisture grain silages in equine production reduces the costs with feeding and acquisition of processed grains (Santos et al., 2002; Oliveira et al., 2007). The unprocessed dry sorghum grain presents a high degree of organization in its subcell components, but, when it is ensiled, there is a loss of this structure with the rupture of the endosperm (protein matrix), releasing free starch granules and protein bodies (Rooney & Pflugfelder, 1986; Lopes et al., 2001).

There are few works on the use of grain silage for horse nutrition. Oliveira et al. (2007) evaluated the use of high and low tannin high moisture sorghum grain silages in feeding of mares at maintenance and found similarity among the digestibility coefficients of dry matter, crude protein, starch and neutral detergent fiber among the diets of ensiled sorghum and low tannin dry sorghum.

It is worthwhile to emphasize that experiment with the purpose of validating the mobile nylon bag technique for horses are scarce and there are no works evaluating cereal grain silages using this methodology. This work was carried out aiming at estimating, in horses, the apparent digestibility of high and low tannin sorghum grain silages by means of the total feces collection and mobile nylon bag technique in horses with samples ground into three particle sizes.

Material and Methods

The assays were developed in the Sala de Metabolismo de Equinos belonging to the Departamento de Zootecnia/ Universidade Federal de Lavras. Four geldings of mixed origin at the age of 6 years and average body weight of 330 kg were used. Before the experiment, a broad-spectrum vermifuge was applied on the animals.

It was conducted two experimental assays to evaluate the two methods used for estimating the apparent digestibility, which were total feces collection and the mobile nylon bag technique. Three degrees of grinding of the samples of high and low tannin silages were used.

The sorghum varieties (*Sorghum bicolor* L. Moench) used for making the high moisture grain silages were Sara and BRS-701 for low and high tannin contents, respectively. The harvest was performed during the physiological maturation phase, when grains presented 29.32 and 30.73% of moisture in the low and high tannin contents, respectively. During harvest, the equipment intended for filling of "bag" type silo was used (Boelter, model OB 20) for the grain grinding and, by altering the setting of this equipment, the pressing to reduce the wholeness of the grains was performed, resulting into their opening. After being processed, the material was packed in 200 L experimental barrels and they were duly sealed for a 45-day period.

Because sieves were unfeasible due to the moisture present in the grains at the moment of ensiling, the particle size of the silage was monitored by determining the average geometric diameter according to the methodology reported by Zanotto & Bellaver (1996) obtaining the average geometric diameter of 8 mm. When silos were opened, samplings for analysis of the chemical composition of the silage were done, according to the methodology reported by Silva & Queiroz (2002).

Previously, the coefficients of apparent digestibility of the nutrients of *coast-cross* hay (*Cynodon dactylon*) were determined by using the same animals of the experimental phase. The daily dry matter intake in kg/animal was 2.0% of live weight on exclusive diet of *coast-cross* hay, therefore characterizing the reference-diet That phase lasted 20 days in which 15 days were for adaptation to the diet and five days for total feces collection.

During the experimental phase, the two methods were simultaneously evaluated in horses housed in metabolism cages. The total feces collection method was applied as a control, estimating the digestibility coefficient of starch and other nutrients of the high moisture sorghum grain in horses based on feed intake and fecal production. The

animals were fed *coast-cross* hay (*Cynodon dactylon*) and sorghum grain silage with high and low moisture tannin (*Sorghum bicolor* L. Moench.).

The amount of feeds given to the animals was established according to the recommendations by the National Research Council (NRC, 2007), aiming to meet the nutrient requirements of animals at maintenance. The diets were composed by 70% of *coast-cross* hay and 30% of ensiled high or low tannin sorghum in particles of average geometric diameter of 8 mm and fed in three daily meals (at 8 a.m., 12 p.m., and 5 p.m.) with removal and weighing of the orts 15 minutes before each meal. Mineral salt was fed in the amount of 50 g/day and the daily total dry matter intake was equivalent to 2.0% of live weight.

The analyses of *coast-cross* hay and the silage were done in the Laboratório de Nutrição Animal of the Universidade Federal de Lavras and in the Laboratório de Bromatologia of Escola Superior de Agricultura "Luiz de Queiroz" of the Universidade de São Paulo (ESALQ-USP) (Table 1).

Each digestion assay lasted 20 days in which 15 days were of adaptation of the animals to the facilities, to the diets and to the management. During this period, the animals were housed in 2 × 3 m individual pens, with cement floor, a feeder and a drinker and without bed. After the adaptation period, the horses were housed in metabolism cages, fitted with plastic bucket for water and mineral salt, front feeder and feces collector, in which the total feces collection was performed for five days. The feces were collected four times a day at 6 a.m., 12 p.m., 6 p.m. and 0 a.m. After that they were weighted, homogenized, sampled in 10% and placed into identified plastic bags, which were stored at -15°C for further analyses. The daily samples of the feces constituted a compound sample at the end of the collection period.

The particle size of the feeds used in the mobile nylon bag technique was determined according to the methodology reported by Zanotto & Bellaver (1996) for the average geometric diameter, obtaining the values of 8 and 13 mm for grinding and pressing, respectively. The smallest particle size was obtained in laboratory with a 1-mm sieve mill, obtaining the three particle sizes (1mm, 8 mm, and 13 mm) for the mobile nylon bag technique. As experimental silos, it was used for both silages PVC containers (20 cm in diameter and 40 cm in height) with capacity of 22 kg and duly sealed with four replicates per particle size.

Table 1 - Chemical composition of the feeds

Nutrient (% DM)	Feeds		
	Coast-cross hay	Low tannin sorghum silage	High tannin sorghum silage
Dry matter	90.72	67.72	68.68
Organic matter	95.64	98.69	98.34
Starch	2.85	71.48	67.80
Crude protein	7.67	12.91	11.44
Neutral detergent fiber	88.89	3.98	8.29
Acid detergent fiber	42.45	3.53	6.53
Gross energy (Mcal/kgDM)	4.335	4.509	4.334

White polyester nylon bags with internal size of 3.5 × 6.5 cm and 60 µ of determined porosity, according to Araújo et al. (1996), were used. Each nylon bag was filled with 1 g of the test feed, that is, the 1, 8 and 13 mm samples of the sorghum grain silage, maintaining the ratio of 10 to 20 mg of dry matter of sample per cm^2 of surface of the bags, according to the recommendation by Nocek (1988). The insertion of the nylon bags in the stomachs of the animals was performed by using a naso-gastric probe in the four horses for five days, coinciding with the period of total feces collection. So, at 1 p.m. on the first day of passage of the naso-gastric probe, 16 nylon bags were inserted into each horse with five replicates for each test feed and one empty test-bag, which is called blank.

At the end of the feces collection period, 25 nylon bags per particle size of the test feed used for obtaining adequate residue for the laboratory analyses were counted. The nylon bags in the feces were collected four times a day at 6 a.m., 12 p.m., 6 p.m. and 0 a.m. and immediately frozen for further analyses. The bags which remained in the digestive tract of the horses for a period longer than 96 hours were disregarded according to Araújo et al. (1996).

The nylon bags were thawed at room temperature, washed in washing machine with running water for about 40 minutes untill the water became clean. Next, they were dried in ventilated oven for 72 hours at 60°C. The nylon bags were opened and the residues constituted a composed sample of each silage in each particle size and for each animal. The samples were ground in a 1mm-sieve, identified and used in the bromatologic analyses.

Bags with no feed (blanks) were used to evaluate the impregnation rate. Those bags were washed together with the bags coming from the digestion assays, therefore, undergoing the same washing procedure in washing machine. Next, the bags were taken to the air forced oven for 72 hours at 60°C.

The analyses of dry matter and crude protein of the feeds, feces and residues were performed according to the methodology reported by Silva & Queiroz (2002); the analyses of cell wall components (neutral detergent fiber and acid detergent fiber) according to Van Soest et al. (1991)

and gross energy were determined in adiabatic calorimeter (*Parr-1261 Instruments Co*). The determination of starch was performed according to the methodology reported by MacRae & Armstrong (1968).

The digestibility coefficients of apparent digestibility of dry mater, starch and other nutrients of the sorghum grain silages estimated in the total feces collection were obtained by the difference of the nutrient digestibility of the reference diet using the equations proposed by Matterson et al. (1965).

The apparent digestibility coefficients of dry matter, starch and other nutrients of the sorghum silage obtained by the mobile nylon bag technique were calculated according the formula:

$$CDAN\ (\%) = \frac{(Pa \times Na) - (Pr \times Nr)}{(Pa \times Na)} \text{ x } 100$$

in which CDAN = apparent digestibility coefficient of nutrient; Pa = weight of the sample dried at 60ºC; Na = percentage of the nutrient in the sample Pr = weight of the residue dried at 60 ºC; Nr = percentage of the nutrient in the residue.

In the two digestibility assays, the randomized block design was used to control the effects of the animals, considering each animal as a replicate. The results were submitted to the analysis of variance in SISVAR program, version 4.6., and the means were compared by Tukey's test at 5% of significance.

A third study was conducted in the Laboratório de Nutrição Animal of the Departamento de Zootecnia of the Escola Superior de Agricultura "Luiz de Queiroz" of the Universidade de São Paulo (ESALQ-USP), with the purpose of evaluating the disappearance of nutrients of the nylon bags after washing in water.

The completely randomized design in factorial scheme 3 × 2 with three degrees of grinding and particle sizes of 1 mm, 8 mm and 13 mm and two sorghum silages, of high and low tannin content, were used. The samples of sorghum ensiled with a high tannin content and ensiled with a low tannin content were ground at 1 mm and 8 mm and pressed at 13 mm, amounting to 24 plots with four replicates per particle size per silage.

The means were compared by the Tukey's test at 5% of probability and when there was a significant interaction among the particle sizes and the silages in the disappearance of nutrients, the means were compared by the F test at 5% of probability.

Results and Discussion

The average recovery rate of the nylon bags was approximately 90% of the total of inserted bags, ranging from 80 to 100%, greater than the values (55%) reported by Machebouef et al. (1995), obtained (80%) by Fombelle et al. (2004) and obtained (84%) by Silva et al, (2009). However, this rate was lower than that (96%) obtained by Araújo et al. (2000), differences which may be related to the characteristics of the diet fed to the horses, to the composition of the feeds and to the feeding management.

The results of apparent digestibility of dry matter, starch, crude protein and gross energy of low and high tannin silages at high moisture were similar among the estimate methods. There were no differences (P>0.05) among the estimates of apparent digestibility coefficient of starch (Table 2) obtained by the total feces collection method and by the mobile nylon bag technique with 1 mm ground samples. The apparent digestibility coefficients of dry matter, crude protein, and gross energy estimated by the mobile nylon bag technique with 1 mm ground samples were higher (P<0.05) than those determined by the total feces collection.

The digestibility coefficients of dry matter, crude protein and gross energy were similar between the total feces collection methods and the mobile nylon bag technique when samples of low tannin sorghum ensiled with 8 mm sample with average values of 85.81, 82.95 and 85.73%,

Table 2 - Digestibility of the nutrients of low tannin sorghum grain silage obtained by the mobile nylon bag technique in three particle sizes compared with the total feces collection in horses

Technique	Apparent digestibility coefficient (%)			
	Dry matter	Starch	Crude protein	Gros energy
Mobile nylon bag (1 mm)*	98.19a	99.92a	97.65a	98.03a
Mobile nylon bag (8 mm)*	83.93b	86.55b	83.54b	83.75b
Mobile nylon bag (13 mm)*	29.67c	27.17c	43.61c	30.53c
Total feces collection	87.70b	100.00a	82.36b	87.70b
CV (%)	2.93	3.06	4.14	2.89

Means with different letters in the same column differ (P<0.05) from one another by Tukey test.
*Average geometric diameter.

respectively, were used. Those results were close to the dry matter digestibility coefficient of 86.66% and higher than the digestibility coefficients of gross energy and crude protein of 81.96; 64.18%, respectively, found by Santos et al. (2002) in foals, which shows increased digestibility of sorghum grains.

Among the total collection and the mobile nylon bag technique, the digestibility coefficients of high tannin sorghum silage at the different particle sizes were similar (Table 3).

The mobile nylon bag technique by using samples of sorghum silage with 8 mm and 13 mm in average geometric diameter was inefficient to estimate starch digestibility ($P<0.05$), because it was lower to the total feces collection method. The difference may have occurred for the lack of chewing of the feeds deposited in the small bags. So, the granules of starch were not broken into smaller particles altering the crystalline shape of starch, which is likely to have occured in the silage fed directly to the animals.

Moron et al. (2000) reported that grinding and the break of sorghum grain increased starch degradability in the rumen, dismissing the application of heat, and that a possible explanation for that increase would be the separation of starch granules from the protein matrix, exposing them to the action of rumen microorganisms

The difference in starch digestibility among the particle sizes may also be related to the sort and distribution of starch in the endosperm of sorghum grain, because according to Rooney & Pflugfelder (1986), the peripheral region of the grain endosperm is extremely dense, hard and resistant to water penetration and digestion, turning into a physical barrier the starch-rich adjacent layer. That fact accounts for the use of samples of finely ground sorghum grain silages with the purpose of estimating the digestibility coefficient of starch.

Al Jassim (2006), when evaluating the effect of types of sorghum grain processings, found that starch digestibility was of 100% in horses, regardless of the processing, crushing or extrusion. According to Kotarski et al. (1992), cited by Costa et al. (1999), decreased sorghum degradation is accounted for the presence or the protein matrix which involves the starch granule which makes it difficult the action of the microbial enzymes and it is higher in sorghum than in corn. The lest intense degradation can also be accounted for the starch composition, by the ratio of amylose:amylopectin, the physical shape and cell integrity of the units of starch in grain processing.

The advantage of the mobile nylon bag technique is the possibility of evaluating several feeds simultaneously and in an isolated manner, without any interference of associative effects. However, this technique possess specifications which should be followed and/or controlled, such as nylon porosity, the density of the sample inserted into the bag and the particle size of the sample. Assays have been developed to solve these requirements. Nocek (1988) managed to standardize satisfactorily the tissue porosity advising, that it should be of 40 to 60 mm and the density of the sample, that is, amount o sample per surface of are of the bag should be of 10 to 20 mg/cm^2.

The washing of the nylon bags in water affected ($P<0.05$) the disappearance of nutrients, pointing out that disappearance for all nutrients, decreased as the sample particle size increased (Table 4). There were no effects of the interaction among the particle sizes and the types of silages on the disappearance of nutrients ($P>0.05$).

The range in the average performance of dry matter was of 7.93% (13 mm), 27.46% (8 mm) and 57.36% (1 mm). These losses are very superior to the values reported by Nocek (1985) in cattle, of 27% of the dry matter of the soybean meal ground to 2 mm and by Araújo et al. (2000) of 30.1% of the dry matter of corn grain ground to 1 mm. Those differences are related to the washing time of the small bags by the authors, of 15 minutes, whereas in this work, it was of 40 minutes.

Huntington & Givens (1995) by using the mobile nylon bag technique in ruminants, call the attention on the

Table 3 - Digestibility coefficient of the nutrients of the high tannin sorghum grain silage determined by the method of the mobile nylon bag technique in three particle sizes as compared with the total feces collection in horses

Techniques	Apparent digestibility coefficient (%)			
	Dry matter	Starch	Crude protein	Cross energy
Móbile nylon bag (1 mm)*	90.04a	99.68a	87.24a	89.20a
Móbile nylon bag (8 mm)*	67.93b	76.97b	70.11b	69.84b
Móbile nylon bag (13 mm)*	21.18c	26.28c	39.08c	21.57c
Total feces collection	73.65b	100.00a	71.33b	73.65b
CV (%)	8.07	4.63	5.48	5.20

Means with different letters in the same column differ ($P<0.05$) from one another by Tukey test.
*Average geometric diameter of the sample.

effective losses of dry matter from the bags in relation to the intense washing programs, overestimating the digestibility values. However, Molina et al. (2003) obtained for low and high tannin high moisture sorghum grain silages losses of 23.01% and 19.49% of dry matter, respectively, in the zero time for 20 minutes and found that the presence of tannin interferes on grain solubility.

Although the interaction was not significant, the disappearance of starch in the samples with particle size of 13 mm was lower by 50% of the other nutrients. This remark can be related to the type and distribution of starch in the sorghum grain endosperm.

Michalet-Doreau & Ould-Bah (1992) stated that it is reasonable to suppose that small particles which escape from the small bags during the washing process consist mainly of potentially degradable material and do not affect the final degradability of the incubated material.

The composition of the losses in the nylon bags is the material itself that escapes through the tissue pores (mechanical loss) as well as of water-soluble nutrients, starch and protein. Therefore, fermented feeds such as silages present significant loss of nitrogen (Nocek, 1988). However, the high percentages of nutrient disappearance in the samples of high moisture sorghum grain silages in the three evaluated particle sizes is not only due to the losses of soluble compounds, but also to the washing time of the used bags of 40 minutes**,** longer than the ones cited in the literature. Huntington & Givens (1995) warns on the severity of the effective losses of dry matter from the bags in relation to the intense washing programs, overestimating the digestibility values.

There is no agreement among researchers about the ideal geometry of the particle size of the samples used in the mobile nylon bag technique and the use of *in natura* samples, that is, as it is fed to the animals. However, reports confirm that when the degree of grinding of the sample is increased, the material becomes more susceptible to the high mechanical losses, resulting into unreal digestion values. In addition to that, previously grinding of the material inserted into the bags increases the losses of water-soluble compounds before incubation when compared to the use of *in natura* samples (Nocek, 1988). The results obtained in this assay confirm those statements, since the losses of nutrients of the samples of *in natura* sorghum grain silages with an average geometric diameter of 13 mm were lower (P<0,05) than those obtained with samples ground to 1 mm.

The standardization of the mobile nylon bag technique is indispensable for the spread of the technique in horse experimentation. The great draw of this procedure is the possibility of evaluating several feed at the same time and in an isolated manner, without any interference of associative effects.

The main limiting factor of the technique is related to the particle size of the sample, because there is no consensus among researchers on which guidance should be followed. Thus, the recommendation is divided in using *in natura* finely ground sample in the same way is fed to the animal or to try to simulate the particle size of the feed when it reaches the stomach after chewing. Therefore, before choosing on the sample processing options, decision must based upon the identification of the limitations and on the benefits of each kind of particle size, being aware of the feed to be tested and its behavior in the experimental unit and establish the objectives to be reached with the assay, as to obtain value of digestion either comparative or absolute. However, further research should be conducted to answer these questions and to standardize the mobile nylon bag technique in order to make it possible to accurately estimate the digestibility coefficients of nutrients in horses consuming high moisture cereal grain silages.

Table 4 - Percentage of disappearance of dry matter (DM), of starch, crude protein (CP) and gross energy (GE) in the nylon bags with high moisture low and high tannin sorghum silages in the three degrees of grinding after washing of the bags in water

	Disappearance of nutrients (%)			
Particle size*	Dry matter	Starch	Crude protein	Gross energy
	Low tannin sorghum silage			
1 mm	60.28a	58.27a	54.13a	58.86a
8 mm	30.49b	20.92b	22.97b	27.77b
13 mm	8.36c	3.06c	8.66c	8.02c
CV (%)	9.62	3.52	7.09	6.13
	High tannin sorghum silage			
1 mm	56.43a	63.33a	55.54a	57.52a
8 mm	24.42b	19.48b	21.89b	25.36b
13 mm	7.49c	3.01c	7.28c	7.84c
CV (%)	6.19	10.62	4.34	4.47

Means with different letters in the same row differ (P<0.05) from one another by Tukey test.
*Average geometric diameter of the sample.

Conclusions

The digestibility coefficients of dry matter, gross energy and crude protein of high moisture sorghum grain

silage can be precisely obtained through the mobile nylon bag technique containing 8 mm ground sample. For estimating the digestibility coefficient of starch of sorghum grain silage through the mobile nylon bag technique, the use of samples ground to 1 mm is recommended.

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