Animal (2014), **8:2**, pp 245–249 © The Animal Consortium 2013
doi:10.1017/S175173111300205X

Apparent digestibility of broken rice in horses using *in vivo* and *in vitro* methods

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(Received 23 November 2012; Accepted 16 July 2013; First published online 21 November 2013)

The aim of this study was to assess the apparent digestibility of broken rice using total collection of feces and the pepsin-cellulase in vitro *technique to provide updated and more accurate digestion coefficients for this by-product when fed to horses. The* in vivo *digestibility trial was consecutively performed, using five adult geldings, weighing 555.6 kg on average. First, hay was given as the only feedstuff, while second, the experimental diet consisted of the same hay plus broken rice at a forage-to-concentrate ratio of 70/30 (on dry matter (DM) basis). Feces were collected over 6 days preceded by a 14-day adaptation period. The digestibility trial was carried out to determine the digestion coefficients for DM, organic matter (OM), CP and gross energy in both diets, while apparent digestion coefficients for the same parameters were calculated for broken rice alone, using the difference between the two sets of results. At the same time, an* in vitro *trial was carried out using pepsin-cellulase technique on the samples of hay and broken rice tested during the* in vivo *trial. As expected, supplementation with broken rice increased digestibility according to all the parameters used. The high OM digestion coefficients of broken rice were confirmed both by the calculated* in vivo *method and by the predicted results of pepsin-cellulase technique (92.6% and 87.1%, respectively), underlining the high digestibility of this by-product when fed to horses.*

Keywords: horse, broken rice, apparent digestibility, pepsin-cellulase technique

Implications

Mixed feed incorporating broken rice is used for horses but estimates of its nutritive value are based on determinations using rice by-products in pig, ruminant and poultry feeds. The importance and the novelty of this experiment is that similar studies carried out on horses in Europe are not available in the scientific literature, but only dated works performed in Australia with different rice cultivars on different livestock species. The present study updates the chemical composition and digestion coefficients of Italian broken rice for horses. Our results should enable companies to produce well-rationed mixed feeds for horses.

Introduction

Rice (*Oryza sativa*) is the seed of an annual plant belonging to the *Gramineae* family, one of the most widespread grain crops in the world. Indeed, in 2010, rice was the grain with the second-highest worldwide production, behind maize. Italy produces large amounts of rice, especially in the North. Consequently, many by-products of the rice cleaning process are available for livestock use. Among these, broken rice separated out after the polishing stage has the same chemical composition as polished rice; even though the quantities available are not very high, this by-product is a palatable, energy-rich and easily utilized feed. Another characteristic of this by-product is that it has a low-protein content but of high quality: its protein is richer in lysine than that of other major cereals.

In literature, studies on broken rice digestibility in horses are very limited and also dated, so very few data are available. Rice-based pellets incorporating rice pollard and broken rice are fed to horses, and the energy values are based on determinations using rice by-products in pig, ruminant and poultry feeds (Farrell and Warren, 1982). McCarthy *et al.* (1989) pointed out that rice milling by-products are high in energy for horses and that broken rice is highly digestible.

Broken rice has been widely used as a high-energy horse feed in Australia for many years and ground rice hulls have

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been used successfully in horse feeds with low energy content at 25% inclusion in the pelleted part of the ration. Previous research carried out in Australia (McMeniman *et al.*, 1990) determined the chemical composition, digestibility and digestible energy (DE) content of cracked rice and rice pollard included in horse rations. No similar studies have been carried out in Europe.

Determination of apparent digestibility in horses by *in vivo* trial based on total collection of feces is still considered the best method due to its accuracy (De Marco *et al.*, 2012). Nevertheless, it does have its limits, concerning the direct use of animals, the planning of the experimental procedure and considerable experimentation costs.

In vitro digestibility techniques using enzymes and incubation lengths that simulate *in vivo* digestion can be used to predict *in vivo* dry matter (DM) and organic matter (OM) digestibility with reasonable accuracy, thus avoiding the need to perform trials with animals and providing results in less time. Among the various *in vitro* methods, the pepsin-cellulase technique is one of the most tested, in particular by INRA researchers, highlighting its suitability both in ruminants (Aufrère and Michalet-Doreau, 1988) and horses (Miraglia and Tisserand, 1985). These authors reported that OM digestibility with the pepsin-cellulase method was highly correlated ($R = 0.978$ and 0.943, respectively for ruminants and horses) with *in vivo* digestibility values either for forages and concentrates.

This study was undertaken to investigate the apparent digestibility of broken rice in horses using total collection of feces and *in vitro* pepsin-cellulase technique and to provide updated and more accurate digestion coefficients for this by-product when fed to horses.

Material and methods

Animals, diets and experimental design

The *in vivo* digestibility trial was performed using five adult geldings, weighing 555.6 kg on average (Table 1). The horses were fed at a feeding level of 1.2 according to INRA requirements (Martin-Rosset, 2012), since the horses performed light exercise made up of a daily 20 min walk. The horses were dewormed (Equalan; Merck, Rahway, New Jersey, USA) before the experiment and were individually stabled in $4 \times 3\ m^2$ boxes with shavings as litter and with free access to water using an anti-waste corner feeder (120 cm long and 70 cm deep) in each box. First, hay was given as the only feedstuff; second, the experimental diet consisted of 70% of the same hay plus 30% of broken rice on a DM basis. The two experimental period lasted 20 days, made up of 14 days of adaptation to the diet, followed by 6 days of total collection of feces. A suitable device (horse diaper) was used, allowing feces to be collected separately from urine and allowing the horses to roam around freely in digestibility boxes.

Sampling and analytical determinations

Total collection of feces was carried out daily at 0800 and at 1600 h during the last 6 days. Total individual daily feces were weighed, mixed, and then a 10% daily sample was kept at −30°C pending analysis. The 6-day cumulative feces samples from each horse were dried to constant weight for at least 2 days in a forced-draft oven at 65°C and then pooled to obtain representative samples. The horses were daily supplied with half of their rations at 0900 h and the remaining half at 1700 h. The hay and broken rice were sampled daily immediately before being fed to the horses and then pooled to obtain representative samples. Individual diet quantities were weighed daily during the 6-day collection period.

The feed samples were dried for 8 h at 105°C to determine the DM content. All the samples were ground in a 1 mm screen Cyclotec mill. Dried feed and feces samples were analyzed for CP, ether extract and ash, according to Association of Official Analytical Chemists (AOAC) (2004). The OM content was calculated using the sample ash content. The gross energy (GE) was measured using an adiabatic calorimeter bomb (IKA C7000; Staufen, Germany). NDF, ADF and ADL were determined using a fiber analyzer (Fibersac 24; Ankom Technology Corp., Macedon, New York, USA), following the Ankom Technology Method and corrected for residual ash. The NDF of broken rice was analyzed with α-amylase. The *in vitro* determination was conducted on the same feed samples (hay and broken rice) collected during the *in vivo* trial using the pepsin-cellulase technique, applying the method proposed by Aufrère and Michalet-Doreau (1988) for both feeds (forages and concentrates). All analytical measurements were replicated three times.

Calculations and statistical analyses

The *in vivo* digestion coefficients for the two experimental diets (hay alone and hay plus broken rice) were determined for DM, OM, CP and GE on the basis of total collection of feces for each horse and for each diet according to Martin-Rosset (2012). Assuming no associative effects, the digestibility of broken rice was calculated for DM, OM, GE, CP according to Martin-Rosset and Dulphy (1987):

$$\mathrm{dC} = (\mathrm{dR} - 0.7\mathrm{dH})/0.3$$

where dC is the concentrate digestibility in percentage, dR the ration digestibility, dH the hay digestibility, f the percentage of forage compared with DM in the ration and c the percentage of the concentrate compared with DM in the ration.

The *in vitro* DM and OM digestion coefficients of hay and broken rice were determined as proposed by Aufrère and Michalet-Doreau (1988), while the digestion coefficients of Diet 2 (hay + broken rice) for the same parameters were calculated according to the DM proportion in the diet (70/30). The individual horse was considered as the experimental unit. The *in vivo* apparent digestion coefficients of the two diets were analyzed by means of a within-subjects one-way ANOVA, in order to underline the effect of concentrate supplementation on the same horse, using R open source programming language (R Development Core Team, 2012). Significance was accepted for P-values < 0.05.

Results and discussion

The intake levels of the trials are reported in Table 1. The intake of horses fed the diet with hay alone was higher than that of horses fed hay plus broken rice. No refusal was observed during the trials. Table 2 shows the chemical composition of the two experimental diets and of broken rice. The hay used was a lowland first-cut meadow hay, typical of the hay that can be found in Northern Italian stud farms. The CP and NDF content of the hay were 7.5% and 56.1%, respectively. The chemical composition of the broken rice was characterized by a CP content of 9.6%, low fibrous fraction content and a GE content of 17.8 MJ/kg DM. In literature, the GE content of broken rice ranges from 15.6 to 18.4 MJ/kg DM (McCarthy *et al.*, 1989; McMeniman *et al.*, 1990; INRA, 2012). This high energy variability may certainly be attributed to environmental influences, fertilizer treatments, degree of milling and storage conditions. Furthermore, another important factor that could affect the chemical and nutritional composition of this by-product is breeding variety. The main source of energy in broken rice is starch; its content reported in INRA tables (INRA, 2012) is 88.2% DM for broken rice and 86.8% DM for cargo rice. The amount of starch as well as the enzymatic degradability of the starch are important parameters to evaluate the inclusion of broken rice into horse diets, because the starch in white rice appears to be largely resistant to enzyme attack (Richards *et al.*, 2003). Starch not digested by enzymes in the small intestine could enter the cecum and undergo bacterial fermentation leading to a reduction in energy utilization (Ellis and Hill, 2005). The fermentation of starch in the hindgut can lead to changes in the microbial profiles and increase the volatile fatty acid and lactic acid concentrations, a situation that may lead to hindgut acidosis (Julliand *et al.*, 2006). The problem associated with the high levels of starch in the concentrate can be managed by both the imposition of a maximum starch inclusion rate and a recommended minimum fiber intake per day (Ellis and Hill, 2005).

Tables 3 and 4 show the *in vivo* and *in vitro* digestion coefficients and the DE content of experimental diets and

Table 1 *Live weight (kg) of horses fed experimental diets and intake level (g DM/kg $W^{0.75}$)*

	Diet 1 (hay)	Diet 2 (hay + broken rice)
Horses (n)	5	5
Live weight	550.8	560.4
Forage/concentrate	100/0	70/30
DM intake	94.5	67.7

DM = dry matter.

Table 2 *Chemical composition (% DM) and GE content of the diets and broken rice*

	Diet 1 (hay)	Diet 2 (hay + broken rice)	Broken rice
DM (% as fed)	89.3	88.6	86.8
OM	92.2	94.3	99.1
CP	7.5	8.1	9.6
NDF	56.1	40.2	3.2
ADF	32.0	22.6	0.6
ADL	4.4	3.1	0.1
EE	2.2	1.9	1.1
GE (MJ/kg DM)	16.2	16.7	17.8

GE = gross energy; DM = dry matter; OM = organic matter; EE = ether extract.

Table 3 In vivo *digestion coefficients (%), DE (MJ/kg DM) and* in vitro *digestion coefficients (%) of experimental diets*

	Diet 1 (hay)	Diet 2 (hay + broken rice)	RSD	Diet effect
In vivo				
DM	57.2	66.8	2.2	0.002
OM	62.4	72.1	1.6	0.001
CP	63.3	70.8	4.1	0.045
GE	58.1	69.2	1.9	0.001
DE	9.4#	11.6#	–	–
In vitro				
d-cell DM	63.4	74.4#	–	–
d-cell OM	61.4	72.7#	–	–

DE = digestible energy; DM = dry matter; OM = organic matter; GE = gross energy; d-cell DM = pepsin-cellulase digestibility of DM; d-cell OM = pepsin-cellulase digestibility of OM.
#Calculated from the rate of each ingredient in the diet as reported by Martin-Rosset and Dulphy (1987).

Table 4 In vivo *digestion coefficients (%), DE (MJ/kg DM), NE (MJ/kg DM) and* in vitro *digestion coefficients (%) of broken rice alone*

	Broken rice
In vivo	
DM	89.5[#]
OM	92.6[#]
CP	83.8[#]
GE	90.2[#]
DE	16.1[#]
NE	12.7[§]
OMD	87.1*
In vitro	
d-cell DM	100.0
d-cell OM	99.0

DE = digestible energy; DM = dry matter; OM = organic matter; GE = gross energy; NE = net energy; OMD = organic matter digestibility; d-cell DM = pepsin-cellulase digestibility of DM; d-cell OM = pepsin-cellulase digestibility of OM.
[#]As calculated and reported by Martin-Rosset and Dulphy (1987) for *in vivo* digestion coefficient.
[§]As calculated and reported by Martin-Rosset (2012) for NE value.
*Predicted by Martin-Rosset (2012) equation for simple concentrates.

broken rice alone, respectively. As expected, the use of broken rice added to the hay in Diet 2 improves the ration digestibility for all the parameters studied compared with Diet 1; indeed, the digestibility of Diet 2 was significantly higher respectively for DM ($P = 0.002$), OM ($P = 0.001$), CP ($P = 0.045$) and GE ($P = 0.001$). The DM and OM digestibility of broken rice was 89.5% and 92.6%, respectively, showing that this by-product is highly digestible. McCarthy *et al.* (1989) tested various types of rice by-products on horses, including broken rice and found similar values to those obtained in the present experimentation, 88.7% and 90.1% for DM and OM, respectively.

The high digestibility of broken rice was confirmed by the pepsin-cellulase method, in which the *in vitro* digestion coefficients for DM and OM were 100% and 99%, respectively. Slightly higher values for DM digestibility than for OM digestibility using the pepsin-cellulase method are reported in literature (Miraglia and Tisserand, 1985). Nevertheless, these digestion values are 10.5% and 6.5% higher than those calculated *in vivo* for DM and OM, respectively. Norman *et al.* (2008) used a method with bags that overestimates the OM digestibility results of 10%. This overestimation may arise from the method itself or from the particle size due to the pore size of the bag. Aufrère and Michalet-Doreau (1988) pointed out an overestimation of the digestion coefficients by the pepsin-cellulase technique for feeds rich in high digestible fiber. The work of Opatpatanakit *et al.* (1995) suggests that in cereal grain–forage mixtures, the nature of associative effects (synergistic *v.* antagonistic) depends on the ratio of incorporation, the species of cereal, and the species of forage in the mixture.

DE values for rice pollard and broken rice have also been determined for horses at the University of Queensland, where it was found that broken rice was almost completely digestible and had a DE content of 18.2 MJ/kg DM (McMeniman *et al.*, 1990). This latter value was considerably higher than that reported by McCarthy *et al.* (1989) for the same product. The low value (13.3 MJ/kg DM) found by the latter authors was associated with poor palatability and acceptance of the trial diets by the horses and by coprophagia (McCarthy *et al.*, 1989). The broken rice tested in our work showed a DE content of 16.1 MJ/kg DM that was close to the value of 15.7 MJ/kg DM given in INRA tables (INRA, 2012) for this by-product. These differences in DE content could be attributed to the different chemical composition and energy content of this by-product. The *in vivo* OM digestibility of this by-product was predicted using the equation proposed by Martin-Rosset (2012) for treated simple concentrates. The value obtained is 87.1%, that is lower than the value observed (92.6%). The equation proposed for cereals used in ruminants (Aufrère and Michalet-Doreau, 1988) gives an even closer value of 91.8%.

In conclusion, the addition of broken rice to hay increases ration digestibility in horses. In order to optimize activities of microorganisms and then prevent any digestive disturbances in the large intestine, further investigations on the apparent digestibility of rations supplemented with different levels of broken rice are needed to determine the optimum threshold of rice to be incorporated in the diet.

Acknowledgment

The authors wish to thank Dr. J. Aufrère and A. Le Morvan (INRA, Saint-Genès Champanelle, France) for their assistance during the trial with the pepsin-cellulase technique and Prof. W. Martin-Rosset for paper revision.

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