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Apparent digestibility of hays in horses determined by total collection of faeces and using internal marker methods

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Abstract

A remarkable subject in the field of horse nutrition concerns the reliability of different analytical methods for digestibility assessment of horse feeds and rations. The digestibility of horse hays can be determined using different techniques: calculations from the chemical composition of hays, *in vitro* or *in vivo* methods. For the determination of apparent digestibility coefficients, the total collection method, which is the reference method in the French system, could be replaced by easier internal marker method. The aim of this work was to compare indigestible internal markers, acid-insoluble ash (AIA) and acid detergent lignin (ADL), and total collection techniques for apparent digestibility estimation in horses. Analyses were carried out over three trials that differed according to the quality of hays, dry matter, organic matter, crude protein, crude fiber, ash, NDF, ADF, ADL, AIA, and the gross energy. The digestibility of all the parameters, with the exception of ADL, AIA and ash, was calculated on the basis of the total faeces collection and alternatively using AIA or ADL markers. The analysis of variance was performed to study the different digestibility coefficients. The digestibility coefficients obtained using AIA were very close to those obtained with the total collection method. Both methods can therefore be used for digestibility trials in horses. On the contrary apparent digestibility cannot be estimated using ADL as an internal marker.

Key words: Horse, apparent digestibility, acid insoluble ash, acid detergent lignin, forages.

Introduction

The digestibility of horse feeds and rations can be determined using different techniques: calculations based on the chemical composition of feeds[1], *in vivo* [2-4] or *in vitro* methods[5-7]. The *in vivo* methods involve the total collection of faeces and the marker methods; they allow digestibility to be studied in different metabolic conditions and thus give more detailed information than the other methods. The total collection of faeces method is considered to be the most accurate and it has been tested on a large number of feeds with a significant number of horses. Among the *in vivo* digestibility assessment methods the one based on total collection could be replaced by the easier indigestible markers methods. These methods overcome some difficulties that are encountered with the analysis of the ingesta/egesta method: discomfort for the animals, the necessity of closing the horses in narrow stalls, longer experimental times, and the total collection method cannot be used with nervous or working horses[8].

In recent years some indigestible markers have been used in digestibility trials. Some markers are naturally occurring substances such as n-alkanes [9, 10], acid insoluble ash (AIA)[11-13] and acid detergent lignin (ADL)[8] while others are added to feedstuffs as chromic oxide (Cr_2O_3)[14-18] or titanium dioxide (TiO_2)[19]. Many problems have been encountered concerning the use of Cr_2O_3 such as incomplete recovery and difficulty in the determination of the chromium concentration[20-22]. Furthermore, it cannot be added legally to feedstuffs, whereas titanium dioxide is free of pollution problems. In spite of this, Titgemeyer et al.[19] found that the cattle fecal recoveries of TiO_2 ranged from 90 to 95% and thus underestimated total tract dry matter digestibility by 1.1 to 5.5 percentage units.

In field conditions, when it is unpractical to add a marker to a diet, a naturally occurring indigestible marker (e.g. AIA or ADL) can be used with no changes in the normal ration, thus providing a very useful tool for digestibility trials. The aim of this work is to find the most reliable method to determine the apparent digestibility of hays in horses by comparing the AIA and ADL markers with the total collection of faeces method.

Materials and Methods

Three different first cut hays harvested from different natural meadows have been used for total collection digestibility trials. The horses were fed in all the trials at a feeding level that was near maintenance, and first cut meadow hay was given as the only feedstuff. The first trial was performed on 4 saddle horses, weighting 553.5 ± 28.9 kg, a fifth horse (mean weight 535.0 ± 39.1 kg) was added to the other two trials.

The experimental period lasted 20 days for each trial: 14 days of adaptation to the diet and 6 days of total faeces collection [2], using a suitable device (horse diaper) that allows the complete recovery of the faeces and avoids the forced seclusion of the horses in digestibility stalls thus allowing the horses to walk and roam around freely. The horses were individually stabled in boxes with free access to water.

The faecal samples were dried in a forced-draft oven at 100°C for one hour and after that at 60°C to constant weight and then ground in a Cyclotec mill (Tecator, Herndon, VA, USA) to pass a

1 mm screen. The hays were sampled each day during each trial and subsamples were dried for immediate determination of the dry matter content at 80°C, then they were air equilibrated, weighed, ground and stored for later analyses.

The faeces and dried hay samples were analysed to determine the crude protein (CP), crude fibre (CF) and ash, according to the AOAC methods [23]. The neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest et al.[24], the gross energy (GE) was measured with an adiabatic calorimeter bomb (IKA C7000, Staufen, Germany) and the AIA content was determined according to Van Keulen et al.[11]. The nitrogen-free extract plus ether extract (NFE + EE) and organic matter (OM) were calculated for each material on the basis of the previous data. The apparent digestibility coefficients of the hays were calculated for DM, OM, CP, CF NDF, ADF and GE both on the basis of the total collection and the two internal markers methods.

One way analysis of variance was used to study the differences between the DM, OM, CP, CF, NDF, ADF and GE coefficients that were obtained using the different methods. An LSD post hoc multiple comparison test was also used to split the groups. Linear regressions between apparent digestibility coefficients obtained by total collection and derived from the AIA method, according to the previously proposed equation[25] methods were calculated for DM, OM, CP and GE, using SPSS statistical software[26].

Results

The compositions of the studied forages are shown in Table 1. The fibre content ranged from 32.1 to 37.5%, whereas the crude protein content varied from 8.0 to 8.9%. The described forages can be considered medium or low quality; they are however typical hays that can be found in stables in Central and Southern Italy.

The digestibility coefficients obtained with the total collection method, the AIA method and the ADL method are shown in Tables 2-4, respectively. Statistically significant differences were found for the digestibility coefficients of all the studied parameters ($P<0.01$). The LSD test, in particular, indicated that the coefficients obtained using the ADL method were different from the others for DM, OM, CP, CF, NDF, ADF and GE at a 0.01 or a lower P level.

The predicted vs. observed values of DM, OM, GE and CP digestibility coefficients are shown in Fig. 1. The corresponding r^2 values of the linear regressions are 0.268, 0.245, 0.174 and 0.300, respectively; the relevant SEE values are 5.75, 5.16, 5.48 and 5.65, respectively.

Discussion and Conclusions

In earlier studies [8] we compared the AIA and ADL markers with the total collection of faeces method, for horse digestibility studies, using rations composed of hay and different concentrates. In those studies we found no significant difference between the total collection and AIA methods and a lack of recovery with a consequent great underestimation of the digestibility rate using the ADL method. In the present study, the same trend was not confirmed, because the lack of recovery is less evident: we can therefore conclude that, when studying the digestibility of hay alone, the high level of fibrous fractions and ADL in particular led to better results than those obtained with the addition of concentrates.

Peiretti et al.[27] compared the ingesta/egesta and AIA methods in three *in vivo* digestibility trials with feedstuffs that differed according to the forage/concentrate rate. The differences between the apparent digestibility coefficients were found to be influenced by - and to increase with – the percentage of concentrate in the diet, therefore confirming the previous data. In the same paper, a series of problems concerning the calculations of the digestibility coefficients related to the concentrate alone were pointed out. When digestibility coefficients of concentrates are drawn from those obtained using the forage alone and the whole ration, negative values are in fact very often observed, in particular for the fibre fractions. This is, in our opinion, the main problem of all the methods that are used, but this does not interfere with the estimation of the digestibility of hays, that is comparable in the different works found in international literature. Other internal markers, that differ according to the different feedstuffs used in horse rations, such as N-alkanes, could be the best choice in this kind of study.

In particular, we tested the previously proposed equation, and obtained low correlation coefficients and high SEE (in all cases, more than 5%). This indicates that, at present, there is no real possibility of deriving digestibility coefficients obtained with total collection using other, easier, internal marker methods and AIA in particular.

The characteristics of the Mediterranean forages that are used in Italy, as underlined in previous works [28-30], could determine some differences between their energy values and the data of other similar forages listed in the INRA table, mainly due to the different botanical composition, the climatic conditions of harvesting and the soil structure in which they are grown. For this reason, a wider survey on the forages used for horse feeding in Mediterranean countries could be of the utmost importance for their correct evaluation and for a proper rationing.

The work is attributable in equal part to the authors.

Table 1. Chemical composition (% on DM basis) of the hay in the three trials.

	Trial 1	Trial 2	Trial 3
DM	88.2	90.4	89.6
OM	94.4	92.9	92.2
Ash	5.6	7.1	7.8
CP	8.1	8.9	8.0
CF	37.5	34.3	32.1
NDF	61.7	64.0	63.7
ADF	38.3	39.3	39.1
ADL	5.1	6.4	5.3
NFE + EE	48.8	49.7	52.2
GE (MJ/kg)	18.9	18.1	19.1
AIA	1.42	1.75	2.61

DM dry matter (% as fed), OM organic matter, CP crude protein, CF crude fiber, NDF neutral detergent fiber, ADF acid detergent fiber, ADL acid detergent lignin, NFE +EE nitrogen free extract + ether extract, GE gross energy, AIA acid insoluble ash.

Table 2. Percent apparent digestibility coefficients (mean±SE) of the three hays, obtained using the total collection method.

	Trial 1	Trial 2	Trial 3
DM	56.7±4.3	52.1±1.5	48.1± 2.3
OM	56.4±3.8	53.4±1.3	49.5±2.3
CP	57.5±3.0	59.0±3.3	54.7±3.0
CF	43.0±4.2	43.9±2.2	36.7±4.5
NDF	50.7±6.5	47.3±1.4	42.4±3.3
ADF	43.2±8.0	39.5±1.7	35.6±4.0
GE (MJ/kg)	54.5±4.3	49.5±1.5	48.1±1.9

DM dry matter (% as fed), OM organic matter, CP crude protein, CF crude fiber, NDF neutral detergent fiber, ADF acid detergent fiber, ADL acid detergent lignin, GE gross energy.

Table 3. Percent apparent digestibility coefficients (mean ± SE) of the three hays, obtained using AIA (acid insoluble ash) as the internal marker.

	Trial 1	Trial 2	Trial 3
DM	60.1±3.4	55.0±2.5	48.2±1.9
OM	61.4±3.6	56.1±2.7	49.5±1.9
CP	57.3±2.8	61.2±4.1	55.0±1.5
CF	47.3±3.1	47.2±3.6	37.1±2.7
NDF	54.6±5.2	50.3±3.4	42.5±2.2
ADF	47.7±6.4	43.1±3.5	35.8±2.6
GE (MJ/kg)	58.0±3.3	52.6±2.8	48.0±2.4

DM dry matter (% as fed), OM organic matter, CP crude protein, CF crude fiber, NDF neutral detergent fiber, ADF acid detergent fiber, ADL acid detergent lignin, GE gross energy.

Table 4. Percent apparent digestibility coefficients (mean ± SE) of the three hays, obtained using acid detergent lignin (ADL) as the internal marker.

	Trial 1	Trial 2	Trial 3
DM	64.9±1.9	56.5±1.8	65.1±2.4
OM	66.0±1.9	57.6±2.0	66.0±2.4
CP	62.7±9.0	62.8±3.1	69.7±2.1
CF	52.7±3.1	49.1±2.4	57.8±2.4
NDF	59.8±2.7	52.0±2.6	61.4±2.5
ADF	53.5±3.5	45.1±2.3	56.9±2.7
GE (MJ/kg)	63.2±1.8	54.2±2.0	64.9±3.1

DM dry matter (% as fed), OM organic matter, CP crude protein, CF crude fiber, NDF neutral detergent fiber, ADF acid detergent fiber, ADL acid detergent lignin, GE gross energy.

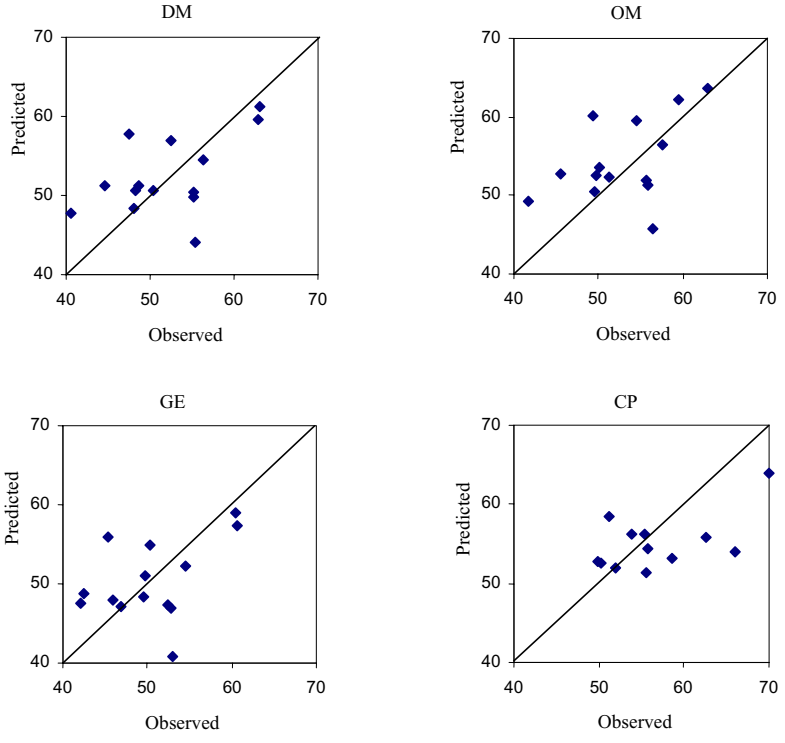

Figure 1. Predicted vs. observed values of the DM, OM, GE and CP digestibility coefficients.

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