Content of nutrients and energy in pasture vegetation and their apparent digestibility in Old Kladruber horses

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Received April 23, 2012
Accepted October 23, 2012

Abstract

This study focuses on the determination of apparent digestibility of pasture vegetation nutrients in horses of the Old Kladruber breed through a balance indicator method (determination of acid insoluble ash). When evaluating the nitrogenous substances, their determination was based on the content of pure protein (sum of amino acids) which is more accurate than crude protein. There were differences in the chemical composition of dry matter from pasture vegetation and horse excrements. Regarding crude fat, crude fibre, acid detergent fibre, neutral detergent fibre, acid detergent lignin, ash, P and Mg, the average values in dry matter of horse excrements were significantly ($P \leq 0.01$) higher; on the contrary, we found significantly ($P \leq 0.01$) lower mean values of nitrogen-free extract, organic matter and Ca in the dry mass of pasture grass. The highest coefficient of apparent digestibility for organic nutrients was determined in the nitrogen-free extract (73%) followed with organic matter (71%), pure protein (68%), crude protein (65%) and crude fat (61%). In case of fibre and its fractions, the apparent digestibility coefficients decreased as follows: crude fibre (66%), neutral detergent fibre (66%), acid detergent fibre (63%) and acid detergent lignin (42%). Significantly lower values were determined for coefficients of apparent digestibility for minerals P (16%) and Mg (50%), which highlights the need for their regular supplementation in horses on pasture. This study extends the current state of knowledge regarding the apparent digestibility of pasture vegetation which is a prerequisite for optimal nutrition of horses.

Equine nutrition, grazing, forage, chemical composition, excrements, indicator method

One of the important internal factors contributing to the welfare of horses is genetics, as reported by Vostrý et al. (2012). One of the important external factors is nutrition, especially the nutritional value of feed and digestibility of basic nutrients. Currently, there are several methods for determining the apparent digestibility of feed and feeding rations in horses. Generally, most of them are *in vivo* methods based on the use of external or internal markers. Indigestible detergent fibre, acid-detergent lignin and n-alkanes can be used as internal markers (Miraglia et al. 1999). A proven and most widely used method for determining the apparent digestibility can be considered the method of determining the digestibility using the marker in the form of acid insoluble ash as presented by Sales and Janssens (2003) or Zeyner et al. (2003). Forage digestibility is influenced by many factors such as forage botanical composition (Pearson et al. 2006), forage mechanical processing (Drogoul et al. 2000), forage preserving (Miyaji et al. 2008) and forage quality (Miyaji et al. 2011), fibre content and especially energy content in a feeding ration (Homolka et al. 1995). Varloud et al. (2004) proposed that the energy content significantly influences the digestibility of structural carbohydrates. The balance result is also affected by the method used for assessing the total digestibility, especially by the selected marker.

The aim of this study was to determine the apparent digestibility of nutrients and energy with regard to the feeding ration in horses of the Old Kladruber breed based on the total daily intake of pasture vegetation.

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Our experiment was based on the scientific hypothesis that the digestibility of monitored nutrients contained in pasture vegetation would be higher compared to the digestibility of nutrients contained in processed feeds, especially after their conservation or heat processing during which individual nutrients, mainly proteins, are denatured, which results in their lower digestibility. The results achieved will significantly contribute to balancing nutrients and energy in feed rations for horses.

Materials and Methods

During the first week of June 2011, sampling of pasture vegetation and faeces was carried out within one pasture area of 6 ha where 33 two-year-old Old Kladruber horses were grazed. Pasture vegetation samples were taken from 10 sites within the pasture area, each sample from the area of approximately 1 m^2 at the amount of about 1 kg of fresh matter, without the individual identification of horses. Similarly, 10 samples of fresh faeces in roughly the same amount were collected from the same pasture area to which the animals had been adapted.

From the botanical point of view, the pasture vegetation quality can be assessed as very high with the prevalence of grasses such as cock's foot (*Dactylis glomerata*), meadow foxtail (*Alopecurus pratensis*), red fescue (*Festuca rubra*), meadow fescue (*Festuca pratensis*), commonmeadow grass (*Poa pratensis*). Tall oatgrass (*Arrhenatherum elatius*), couch grass (*Agropyrum repens*), perennial ryegrass (*Lolium perenne*) and creeping bentgrass (*Agrostis stolonifera*) were less frequent. As the pasture stands were regularly treated with herbicides, the incidence of dicotyledonous plant species like shepherd's purse (*Capsella bursa pastoris*), sticky willy (*Galium aparine*), white clover (*Trifolium repens*), dandelion (*Taraxacum officinale*) or lawndaisy (*Bellis perennis*) was very low. Sporadically, we identified representatives of the buttercup (*Ranunculus*) and sorrel (*Rumex*) genus.

In the pasture vegetation and faeces, we determined the content of dry matter, nitrogen (N), amino acids (AA), fat, crude fibre, neutral-detergent fibre (NDF), acid-detergent fibre (ADF), acid-detergent lignin (ADL) and gross energy (GE).

Dry matter was determined by weight upon drying the sample at 105 °C under prescribed conditions. The content of nitrogenous substances (crude protein) was calculated from the nitrogen content, determined by Kjeldahl method using the Buchi analyzer (company Centec automatika, Czech Republic), multiplied by the coefficient of 6.25. The amino acid content was determined after acid hydrolysis by 6 N HCl at 110 °C for 24 h using the automatic amino acid analyzer AAA 400 (company INGOS, Czech Republic) on the basis of colour reaction between amino acids and an oxidizing agent (ninhydrin). The analysis of amino acids served for determining the pure protein which covers Σ AA (Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, His, Lys and Arg). Fat was determined by the device ANKOM^{XT10} Fat Analyzer (company O.K. SERVIS BioPro, Czech Republic). Gross fibre, acid-detergent fibre, acid-detergent lignin and neutral-detergent fibre were determined by the device ANKOM^{220} fibre Analyzer (company O.K. SERVIS BioPro, Czech Republic).

Ash was determined by weight after the sample incineration at 550 °C under prescribed conditions. Calcium, phosphorus and magnesium were determined through incinerating and leaching the sample by extraction and subsequent titration.

Then we calculated the nitrogen-free extract and organic matter content. Regarding minerals, we determined calcium, phosphorus and magnesium. The apparent digestibility of nutrients and energy was determined using the indicator method (acid-insoluble ash) from the pasture vegetation nutrients and faeces as recalculated to 100% dry matter.

The results achieved were processed by mathematical-statistical methods involving the statistical program Unistat 5.6. We evaluated average values and their differences through a multiple comparison using the test Tukey-HSD at significance levels of $P \leq 0.01$ and $P \leq 0.05$. Each indicator was presented by its average value (x) and standard deviation (± SD).

Results

In this work, we present the results of determining the apparent digestibility of pasture vegetation nutrients and energy in Old Kladruber horses. Analyses of pasture vegetation samples and freshly collected faeces were conducted prior to the actual determination of digestibility coefficients. The average dry matter was 425.5 g/kg and 217.8 g/kg for the pasture vegetation and excrements, respectively. Results of dry matter chemical analyses concerning pasture vegetation and excrements are shown in Table 1.

Regarding nitrogenous compounds such as crude protein (N × 6.25), we also stated pure protein due to a very low informative value of crude protein with respect to the nutrition of monogastric animals although this indicator is still used. For the pure protein we considered the sum of all the amino acids (Σ AA g/kg) in the feed which represent the real feed protein in

Table 1. Average content of single nutrients in the dry matter of pasture vegetation and excrements in horses.

Component (g/kg)	Pasture vegetation (x ± SD)	Excrement (x ± SD)
Crude protein	68.67 ± 15.376	75.75 ± 14.895
Pure protein	51.48 ± 13.312	50.60 ± 10.438
Crude fat	20.79^{A} ± 3.422	25.80^{B} ± 3.191^{B}
Crude fibre	348.94^{A} ± 19.287	387.94^{B} ± 17.260
ADF	432.49^{A} ± 15.821	530.28^{B} ± 25.395
NDF	672.47^{A} ± 25.531	756.55^{B} ± 24.095
ADL	80.408^{A} ± 6.225	152.390^{B} ± 9.031
Nitrogen-free extract	501.61^{A*} ± 29.699	438.77^{B*} ± 15.510
Organic matter	940.01^{A} ± 6.829	905.15^{B} ± 8.449
Ash	59.99^{A} ± 6.829	94.85^{B} ± 8.449
Ca	5.25^{A} ± 0.893	3.85^{B} ± 0.635
P	1.44^{A} ± 0.283	3.94^{B} ± 0.701
Mg	1.51 ± 0.551	2.18 ± 0.464
Gross energy (MJ/kg)	18.20^{A} ± 0.228	18.59^{B} ± 0.298

^{AB} $P \leq 0.01$, ADF – acid detergent fibre, NDF – neutral detergent fibre, ADL – acid detergent lignin, SD – standard deviation

Table 2. Apparent digestibility coefficients of pasture vegetation nutrients and energy in horses.

Component	Digestibility coefficient (% ± SD)
Crude protein	64.62 ± 13.575
Pure protein	67.72 ± 14.050
Crude fat	61.04 ± 11.072
Crude fibre	66.17 ± 5.186
ADF	62.67 ± 6.180
NDF	65.70 ± 5.912
ADL	42.24 ± 9.441
Nitrogen-free extract	73.09 ± 6.020
Organic matter	70.60 ± 5.290
Ash	52.70 ± 5.912
Ca	77.64 ± 3.829
P	16.47 ± 13.013
Mg	49.91 ± 22.678
Gross energy	68.82 ± 5.522

ADF – acid detergent fibre, NDF – neutral detergent fibre, ADL – acid detergent lignin, SD – standard deviation

qualitative as well as quantitative terms. The term "pure protein" in Table 1 means average values Σ AA (Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, His, Lys and Arg). As documented in Table 1, a great disproportion considering the values of crude protein (pure protein) is also highlighted by their values of 68.67 g/kg (51.48 g/kg) and 75.75 g/kg (50.60 g/kg) in the pasture vegetation and excrements, respectively. From this perspective, the value of crude protein significantly underestimates the actual protein content in feeds.

With regard to the majority of monitored indicators, i.e. crude protein, crude fat, crude fibre, acid-detergent fibre (ADF), neutral-detergent fibre (NDF), acid-detergent lignin (ADL), ash, P and Mg, their mean values in the excrement dry matter were higher than in the dry matter of pasture vegetation. Average values of the tested indicators were highly significantly different ($P \leq 0.01$) except for crude protein.

As regards pure protein, nitrogen-free extract, organic matter and Ca, the average values in pasture forage dry matter were significantly ($P \leq 0.01$) higher compared to excrements, except for pure protein (Table 1). Results of analysis of the pasture vegetation and excrement samples using the balance indicator method (acid-insoluble ash) were taken to calculate the digestibility of single pasture vegetation nutrients and energy (Table 2). It is evident from the results that organic nutrients of pasture vegetation show higher digestibility compared to mineral substances. The highest digestibility coefficient was determined for the nitrogen-free extract; digestibility coefficients further decreased in the following order: organic matter, pure protein, crude protein and crude fat. Regarding fibre and its fractions, apparent

digestibility coefficients of single nutrients decreased in this order: crude fibre, NDF, ADF and ADL.

Relatively high digestibility was found also with regard to GE of pasture vegetation from which the digestible energy value of 12.525 MJ/kg of dry matter (5.668 MJ/kg of fresh green forage) was determined. Determined coefficients for apparent digestibility of mineral substances including total ash were significantly lower. Regarding minerals, relatively high digestibility was observed for calcium; in contrast, very low digestibility was found for phosphorus. These results are presented in Table 2.

Discussion

It follows from the obtained results that pasture vegetation is nutritionally suitable food for horses in terms of its botanical and nutrient composition. Balance digestibility in horses was determined by Homolka et al. (1995). For the feeding ration, the composition of which is closest to the nutrient content in pasture stands that we examined, they stated the apparent digestibility of dry matter (57%), organic matter (60%), gross energy (58%), crude protein (57%), fat (49%), fibre (49%) and nitrogen-free extractives (NFE, 67%). Miraglia et al. (1999) pointed out the differences in the apparent digestibility in horses, using a range of internal markers. When using acid-insoluble ash as a marker, they came to the following coefficients of apparent digestibility: organic matter (63–66%), gross energy (61–65%), crude fibre (40–42%), crude protein (66–70%), acid-detergent fibre (ADF, 42–49%), neutral-detergent fibre (NDF, 48–54%) and nitrogen-free extract (73–76%). For a high-quality diet, Miyaji at al. (2011) determined the apparent digestibility coefficients: organic matter (0.574–0.587), crude protein (0.514–0.543), NDF (0.549–0.557), and ADF (0.511–0.519). As regards diets based on 85% share of meadow hay in light, heavy, pregnant and lactating mares, Martin-Rosset et al. (1990) determined the apparent digestibility coefficients for *ad libitum* feeding: organic matter (52–57%), crude protein (54–60%), crude fibre (39–43%) and gross energy (49–53%). Peiretti et al. (2006) studied the apparent digestibility of hay in horses and determined apparent digestibility coefficients in a range of values (56–70%), gross energy (55–67%) and crude protein (58–63%).

In terms of digestibility, we determined a higher coefficient of apparent digestibility for pure protein (68%) compared to crude protein (65%).

Markedly lower apparent digestibility coefficients of crude protein were indicated by Homolka et al. (1995) and Miyaji et al. (2011) whereas Peiretti et al. (2006) reported slightly lower digestibility coefficients for crude protein. Our results of determination of the apparent digestibility coefficients for crude protein correspond to the results published by Miraglia et al. (1999). Lower values for crude protein digestibility reported by cited authors may be due to the composition of feed rations based on hay or otherwise thermally treated feed where their lower digestibility is also caused by the influence of thermal protein denaturation. As stated by the authors cited, a relatively high digestibility relates to organic matter. Our results roughly correspond to the apparent digestibility coefficients in horses as reported by Miraglia et al. (1999) or Peiretti et al. (2006). The high value of apparent digestibility that we determined for the pasture vegetation is closely associated with the high digestibility of other organic nutrients. The highest value of the apparent digestibility coefficient was determined for the nitrogen-free extract which corresponds also to the results reported in horses by Homolka et al. (1999) and confirms to the digestibility coefficient mentioned for the nitrogen-free extract by Miraglia et al. (1999). Relatively large differences in digestibility in horses are reported for the crude fibre and individual fibre fractions. Most authors such as Miraglia et al. (1999), Miyaji et al. (2011) and Martin-Rosset et al. (1990) presented significantly lower values of the apparent digestibility coefficient for crude fibre, NDF, ADF and ADL than we found in

the pasture vegetation. We believe that this is due to good quality and appropriate nutrient composition as well as energy content of the pasture vegetation and that it closely relates to the digestibility of organic matter. Compared to organic nutrients, except for Ca, the pasture vegetation provided relatively low apparent digestibility coefficients including the total ash. In the available literature, we found no results regarding the apparent digestibility of mineral substances for pasture vegetation.

The achieved results have confirmed our hypothesis that compared to the authors who studied nutrient digestibility in feeds processed by heat, the digestibility of individual nutrients contained in pasture vegetation, especially the digestibility of protein, was higher than stated by the cited authors.

With respect to the quality nourishment in horses, the low digestibility of pasture vegetation minerals demonstrates the need for their regular supplementation in the form of mineral feed during the pasture period.

Acknowledgements

This work was financially supported by the project NAZV QH 92265 "Optimization of breeding and veterinary care system for the critically endangered population of Old Kladruber horses".

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