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Original Research

The Content of Amino Acids in Pasture Vegetation and Their Apparent Digestibility in 2-year-old Horses

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ABSTRACT

Ten samples of plant vegetation and 10 samples of fresh excrement were taken from the same pasture area. The excrement were collected from 10 2-year-old Old Kladruber horses that received the pasture vegetation daily. The apparent digestibility of nitrogen and amino acids in pasture vegetation was determined by using the acid-insoluble ash marker method. In comparison with excrement, the pasture vegetation contained higher levels of Ser, Ala, Leu, and His and higher levels of Pro ($P \leq .01$), Met ($P \leq .01$), and Arg ($P \leq .05$). The mean level of Ile in pasture vegetation was lower than in excrement ($P \leq .05$). The apparent digestibility of amino acids from pasture vegetation was high for Pro and Met (86.75 and 89.39%), moderate for Ser, Ala, Leu, His, and Arg (68.61%-76%), and low for Asp, Thr, Glu, Gly, Val, Leu, Tyr, Phe, and Lys (56.15%-66.03%). The digestibility of lysine and Ile was relatively low (56.39% and 56.15%, respectively). The total content of nitrogen per dry matter was 10.98 ± 2.46 g/kg for pasture vegetation and 12.12 ± 2.38 g/kg for excrement, whereas the content of protein nitrogen was 7.20 ± 0.25 g/kg and 6.89 ± 0.21 g/kg of dry matter in pasture vegetation and excrement, respectively. This means that only 65.55% and 56.90% of N is bound in proteins in pasture vegetation and excrement, respectively. Non-protein nitrogen accounts for 34.45% in pasture vegetation and for 43.10% in excrement.

1. Introduction

The digestibility of forage in farm animals is affected by a number of factors such as botanical composition [1], mechanical treatment of forage [2], methods of forage preservation [3], forage quality [4], and particularly the content of fiber and energy per feed dose [5]. Varloud et al [6] concluded that the content of energy has a profound effect on the digestibility of structural saccharides.

While the apparent digestibility of basic nutrients (organic matter, crude protein, crude fiber, acid detergent fiber, neutral detergent fiber and nitrogen-free extractives) and gross energy in horses has been reported by many authors [4,7-10], data on the apparent digestibility of amino acids are rare in the available literature [11] and remarks about their need are also scarce.

The distribution of amino acids in a diet is very important particularly in growing horses and in pregnant and lactating mares. Adult horses are less sensitive to protein quality than growing horses [12]. The level of lysine is particularly important in this respect as it is the first limiting amino acid in horses [13]. Threonine is the second limiting amino acid [14].

As in other monogastric animals, protein digestibility in horses increases with a decreasing content of dietary fiber [15,16] and with an increasing content of proteins contained in a diet [11,17]. Most proteins contained in forage are digested in the horse's large intestine, however, the importance of small intestine increases when high quality

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hay is fed [17]. Microbial degradation is required to digest a large portion of proteins in fiber-rich feed.

The results of nutrient balance assessment are strongly affected by the method used to determine the apparent digestibility, particularly by the selected marker [8]. There are a number of methods to determine the apparent digestibility of nutrients in feed and feed doses. *In vivo* methods based on external or internal markers are mostly used. Indigestible detergent fiber, acid detergent lignin, and n-alkanes can be used as internal markers [8]. The acid insoluble ash marker method as a well established method is frequently used [18,19].

The apparent digestibility of basic nutrients and energy in pasture vegetation in Old Kladruber horses has been investigated by Straková and Suchý [10]. The main aim of this pilot study was to determine the distribution and apparent digestibility of amino acids in pasture vegetation and excrement from two-year-old Old Kladruber horses.

2. Materials and Methods

The samples of vegetation (approximately 1 kg of fresh matter) were collected from an area of approximately 1 m^2 at 10 different sites of the respective pasture land (approximately 6 ha). The samples of vegetation taken were of high quality in terms of the botanical spectrum, and consisted mainly of orchard grass (*Dactylis glomerata*), meadow foxtail (*Alopecurus pratensis*), red fescue (*Festuca rubra*), meadow fescue (*Festuca pratensis*), and common meadow grass (*Poa pratensis*). The incidence rate of these grass species was approximately 80%. The following species of grass also occurred but at a low incidence rate (approximately 20%): false oat-grass (*Arrhenatherum elatius*), couch grass (*Agropyrum repens*), perennial ryegrass (*Lolium perenne*) and creeping bentgrass (*Agrostis stolonifera*). Since the pasture vegetation had been treated with herbicides before the grazing began (Refine® herbicide product, 20 g/ha), the occurrence of dicotyledonous plant species in vegetation was negligible.

Along with the samples of pasture vegetation, 10 samples of fresh excrement (each containing 1 kg of excrement) were taken from the same pasture area. Excrements were obtained individually from two-year-old Old Kladruber horses that received the pasture vegetation daily for a long period of time (starting in April) and were adapted to receiving it. The body weight of horses was 550 ± 50 kg. The samples of pasture vegetation and excrement were collected in June.

The dry matter content of samples was determined gravimetrically as residue after drying at 105°C at the prescribed conditions. The resultant dry matter of pasture vegetation and excrement was analyzed for the content of nitrogen (N) and amino acids (AAs). The apparent digestibility of AAs was determined using the acid-insoluble ash marker method. The content of nitrogen was determined by using the Kjeldahl method and the Buchi analyzer (Centec automatika, Prague, Czech Republic). The content of nitrogen-containing substances (crude protein) was calculated by multiplying the nitrogen values obtained by the Kjeldahl method by a factor of 6.25. The content of AAs was determined after acid hydrolysis in 6 N HCl at 110°C for 24 hours using the automatic amino acid analyzer AAA 400 (Ingos , Prague, Czech Republic). The AAA 400 works on the principle of ion-exchange chromatography with ninhydrin derivatization and photometric detection.

The results of amino acid analysis were used to calculate values of protein nitrogen bound in amino acids in both pasture vegetation and excrement. The nitrogen content in respective amino acids (14.008-56.027 g N) was multiplied by 100, divided by molecular weight of the respective amino acid to calculate the percentage of protein nitrogen. The value determined by amino acid analysis for the respective amino acid (AA/g) in pasture vegetation and in excrement, multiplied by the percentage of nitrogen content (N AA%) and divided by 100, gives the value of protein nitrogen (N AA g/kg) bound in the respective amino acid.

The results obtained were processed using mathematical and statistical methods implemented in Unistat version 5.6 software. The mean values and their differences were evaluated by the Tukey-HSD multiple comparison test, at the level of significance being $P \leq .01$ and $P \leq .05$. Each parameter is represented by the mean value calculated from ten analyses (x) and by the respective standard deviation (±SD).

3. Results

The amino acid analysis of samples of pasture vegetation and excrement was performed prior to the determination of the apparent digestibility coefficients. The results of amino acid analysis of pasture vegetation and excrement are provided in Table 1.

Unlike excrement, pasture vegetation contained higher levels of Ser, Ala, Leu, and His, higher mean levels of Pro ($P \leq .01$), Met ($P \leq .01$), and Arg ($P \leq .05$). The mean values of AAs such as Asp, Thr, Glu, Gly, Val, Ile, Tyr, Phe, and Lys in pasture vegetation were lower than those in excrement.

Table 1
g/kg levels of amino acids per dry matter of pasture vegetation and excrement

Amino Acid	Pasture Vegetation (n = 10)	Bermudagrass Hay*	Excrement (n = 10)
Asp	5.27 ± 1.540	7.85	6.04 ± 1.254
Thr	2.61 ± 0.691	3.08	3.00 ± 0.595
Ser	2.51 ± 0.694	3.60	2.40 ± 0.474
Glu	5.84 ± 1.483	8.83	6.49 ± 1.291
Pro	5.74^{A} ± 1.236	-	2.45^{B} ± 0.555
Gly	3.07 ± 0.822	3.38	3.28 ± 0.721
Ala	4.14 ± 1.102	4.55	3.92 ± 0.854
Val	3.18 ± 0.884	4.00	3.37 ± 0.853
Met	0.50^{A} ± 0.087	0.93	0.18^{B} ± 0.240
Ile	2.59^{b} ± 0.742	3.20	3.41^{a} ± 0.567
Leu	4.41 ± 1.278	5.63	4.06 ± 1.021
Tyr	1.43 ± 0.706	1.48	1.65 ± 0.395
Phe	2.43 ± 1.210	3.70	2.86 ± 0.701
His	1.50 ± 0.675	2.25	1.38 ± 0.339
Lys	2.93 ± 0.916	4.73	3.69 ± 0.781
Arg	3.33^{a} ± 0.997	4.90	2.40^{b} ± 0.640

Tryptophan and cysteine are destroyed in the acid hydrolysis.
^{AB}Means with a different superscript letter differ ($P \leq 0.01$).
^{ab}Means with a different superscript letter differ ($P \leq 0.05$).
* Antilley et al [13].