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SIGNIFICANCE OF NUTRIENT DIGESTIBILITY IN HORSE NUTRITION – A REVIEW

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Abstract

The aim of the review is to present mechanisms of digestion in horses through a functional description of the digestive system's structure with emphasis on nutrient digestibility. In the era of an increasingly intensive and specialised sport usage of horses, also the significance of balancing their dietary nutrient value in accordance with individual requirements of these animals is gaining in importance. At the same time, it is not possible to meet nutritional requirements of a sport horse without knowledge about feed utilisation. In many farm animal species, digestibility is measured *postmortem* or via complicated and expensive cannulation of the digestive system. In horses, these methods are not applied due to ethical approaches or other limitations (e.g. sport horses); therefore, the importance of marker-based techniques is growing, although not much data is published in the available literature (Sales, 2012). Moreover, in contrast to other non-ruminants, horses are naturally adapted to a constant intake of large quantities of roughages. However, during intensive sport training, they are primarily fed concentrated diets with high amounts of easily digestible, non-structural carbohydrates and reduced amounts of dietary fibre fractions. Therefore, the risk of metabolic diseases and behavioural disorders in the horse increases.

Key words: horses, nutrients, digestion

Horse gastrointestinal tract – its anatomy and digestive functions

The horse belongs to the group of monogastric (non-ruminant) animals, which possess a single-chamber stomach (*ventriculus*) (Nomina Anatomica Veterinaria, 2002). Nevertheless, its gastrointestinal system is described by features that are unique among monogastric animals; therefore, it requires exceptional attention in intensively schooled and managed saddle horses. In non-ruminant animals, the prevailing digestion mechanisms are based on endogenous enzymes, which take place, primarily, in the stomach and small intestine, and the utilisation of dietary fibre (DF) in these animals is significantly restricted (Choct and Kocher, 2000). The significance of this group of dietary constituents is confined mainly to their mechanical assistance of bowel functions, and their excess (in particular, of the so called fraction of soluble fibre) disturbs absorption processes of nutrients (Schneeman, 1998).

In horse nutrition, although these animals belong to non-ruminants, the activity of the endogenous microbiota of the digestive system plays a very important role. As in the case of other monogastric animals, enzymatic degradation of proteins, fats and carbohydrates takes place in the stomach and the small intestine. The chyme is then transferred into an expanded caecum where it is subjected to microbiological fermentation (Figure 1). This activity involves, primarily, the breakdown of the dietary fibre fraction, as well as other diet constituents, which were not absorbed in the small intestine. For the above reasons, maintaining microbiological equilibrium in this part of the digestive system of horses is essential, not only from the point of view of their health but, equally importantly, also for ensuring appropriate energetic balance (Pagan, 2008).

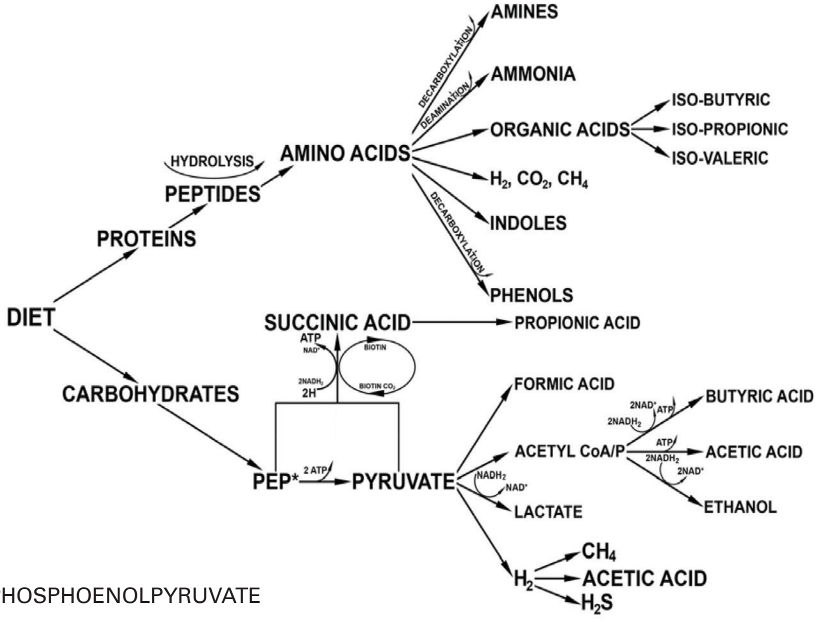

Figure 1. Fermentation pathways in the horse gastrointestinal tract, modified after Cummings and Macfarlane (1991)

Digestive processes in horses begin in the oral cavity (*cavum oris*). Saliva is secreted by the sublingual, mandibular and parotid salivary glands (*glandulae: sublingualis, submandibularis et parotidea*) in amounts ranging from 35 to 40 l/daily in adults (Moeller et al., 2008). Horse saliva contains only small amounts of amylase, but the presence of chlorine and acid sodium carbonate gives horse saliva properties that buffer the content of the stomach blind ventricular sac (*saccus caecus ventriculi*)

(Alexander and Hickson, 1970). The feed goes from the oral cavity (*cavum oris*) to the throat equipped in an expanded lymphoid ring and then to the oesophagus, which is 1 to 1.5 m long and lined with stratified squamous epithelium. In comparison to the entire gastrointestinal tract, the stomach volume is small (approximately 8%, i.e. 8–15 litres). This causes the horse to be adapted to the intake of small quantities of feed at short time intervals. Horses reared in an indoor system, which have permanent access to roughages such as hay and straw, and which also obtain concentrates several times a day in small doses, are not exposed to excessive, single stomach loads. On the other hand, the necessity to apply highly concentrated diets in the case of sport horses exposes their low-capacity stomachs to overloads. This is an easy road to excessive fermentation in this organ, which contributes to a further tightening of the already strong pyloric sphincter *(musculus sphincter pylori*) of the stomach inflow, making gas eructation impossible. Their excess is a frequent cause of various kinds of ailments of the digestive system (colic pains, laminitis, insulin resistance), including even the rupture of the stomach (Kutzner-Mulligan et al., 2013). Furthermore, in the stomach pylorus area (Figure 2), there are glands that secrete the gastrin hormone into the blood, which is responsible for controlling hydrochloric acid secretion through the lining cells of the stomach's main body. Other glands situated in the mucous membrane of the stomach main body secrete pepsinogen which – under the influence of acid environment (pH 2–4) – is activated into pepsin. Swallowed feed usually remains in the stomach only for about 15 minutes and then it is transferred into the duodenum. Some nutrients, particularly structural carbohydrates, can stay in the stomach for up to 6 hours; in fact, this organ is never completely empty (Jackson, 1997; Frape, 2004). The horse's stomach microbiota is not a very rich microecosystem, primarily due to the chyme acid reaction. The dominant bacteria in this segment of the gastrointestinal tract include *Lactobacillus equigenerosi* and *Streptococcus criceti* (St. Pierre et al., 2012). The small intestine of the horse constitutes 30% of the entire gastrointestinal tract and is about 10 m shorter than in cattle, measuring from 19 to 30 m (Dyce et al., 2010; Moore et al., 2001).

Pancreatic juice is secreted continually in the amount of 10–12 l per 100 kg body mass and it increases the pH of the chyme transported from the stomach to the duodenum from about 2 to 7. The digestive enzymes contained in the pancreatic juice such as: α-amylase, lipase, elastase, trypsin and chymotrypsin are characterized by lower activity in comparison with other farm animals (Frape, 2004). In the region of the brush border (*limbus absorbens*) of the small intestine mucosa, digestion of carbohydrates containing α-1,4-glycosidic bonds, which can be hydrolysed by the animal's own enzymes, takes place. In draught horses, the main source of starch is provided by cereals, which contain approximately 70% of this sugar in dry matter. They are also described by a high glycemic index making them a good source of rapidly released energy, very important for horses subjected to great physical effort (Rodiek and Stull, 2007). Also in the small intestine, fat digestion takes place. Lipases, produced by the pancreas, split fats into glycerol and free fatty acids. However, as compared to other non-ruminants (Józefiak et al., 2014; O'Neill et al., 2014) apparent digestibility coefficients of fat in the horse's small intestine are relatively low within the range of 55–70% (Swinney et al., 1995). The mucous membrane of the horse intestines does not secrete fibrolytic enzymes and, therefore, dietary fibre – until it reaches the caecum – fulfils a mechanical function by stimulating peristalsis of the gastrointestinal system (Hintz and Cymbaluk, 1994; Kalck, 2009; Lawrence, 2008).

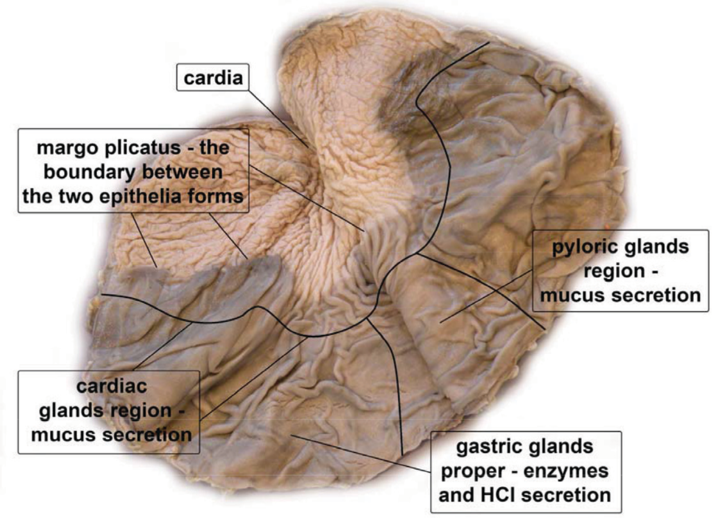

Figure 2. Horse's stomach (M. Komosa)

Chyme passage through the large intestine takes from 36 to 72 hours. The blind gut (*caecum*) is situated in the central part of the abdominal cavity with its apex pointing cranially, and reaches the xiphoid region. It is the caecum that makes the horse's entire gastrointestinal system unique as it comprises up to 15% of its total volume. This expanded organ is inhabited by a multitude of bacteria and protozoa, similarly to the forestomachs of ruminants in a way reminiscent of ruminant forestomachs. The intestinal microbiome is able to hydrolyze proteins which were not digested in the intestine, but in contrast to ruminants, the microbial protein does not become a source of amino acids for the horse, i.e. they are not absorbed in significant quantities by the caecum walls; they move on with the chyme to further parts of the large intestine. Also, horses cannot utilise microbiologically transformed plant protein of lower biological value into more valuable animal protein (cells of protozoa). Nevertheless, the animals derive considerable benefits from the microbiological dietary fibre breakup in the caecum. The processes taking place there are identical to those occurring in the forestomachs of ruminant animals (Sneddon and Argenzio, 1998; Reece, 2009). Microbiota in the caecum is capable of hydrolyzing β-1,4-glycosidic bonds, hence, cellulose, hemicelluloses, lignocelluloses, fructans or galactans are broken down into simple sugars. The remaining products undergo fermentation (Figure 1) into short-chain fatty acids (primarily acetic, propionic and butyric acids), which become a source of energy for the horse (about 30% of the entire digestible energy). The possibility of deriving significant quantities of energy from dietary fibre makes it possible for mildly utilised horses to be successfully maintained, even exclusively on good quality roughages. Diets based only on forages significantly increased levels of acetate in blood plasma, pre- and post-training, showing an important influence of caecal fermentation and energy balance (Jansson and Lindberg, 2012; Clauss et al., 2013). In the case of relatively low starch supplies to the caecum, the dominant short-chain fatty acid that develops there is acetic, primarily as a result of fibre fermentation carried out by anaerobic bacteria like: *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Propionibacterium*, *Selenomonas* and *Streptococcus* (Mackie and Wilkins, 1988; Costa et al., 2012). On the other hand, high starch supplies to the large intestine can be a cause of diseases associated with the rapid course of lactic fermentation. Therefore, it is very important to utilise simple sugars in the small intestine in the best way possible, and to avoid an excessive supply of concentrates in diets for horses (Potter et al., 1992; Kienzle, 1994). During one feeding, the consumption of non-structural carbohydrates corresponding to over 0.4% of the horse's body weight (±2–4 g/kg BW) constitutes a real threat to its health (Cuddeford, 2000). Excessive concentrations of organic acids in the caecum result in a decline of the chyme pH from the optimal value of 6.5 for cellulolytic microbiota. Reduced chyme pH in the caecum destroys its mucosa and leads to the reorganisation of the microbiota in this organ (predominance of lactic fermentation bacteria, e.g. *Lactobacillus sp*., increase in *Clostridium sp.* populations) or even to its total elimination. Unfavourable changes in the microbiota of the horse's large intestine may influence not only the occurrence of disturbances in the function of the gastrointestinal system (colic, diarrhoea, endotoxemia), but they can also cause illnesses such as laminitis or other diseases of the locomotor system (Milinovich et al., 2007; Costa et al., 2012). It should be remembered that even relatively small changes in the pH value can result in the occurrence of the so-called subclinical acidosis. In a healthy equine large intestine, protozoa – together with bacteria – create a harmoniously functioning microecosystem which is, nonetheless, capable of rapid reactions to dietary changes. The dominant protozoa here include: *Buetschila*, *Cycloposthium*, *Blepharocorys* and *Paraisotricha sp*. The defaunation of this organ leads to the decreased digestibility of the feed's dry matter, although it does not exert any impact on the decreased fibre digestibility (Moore and Dehority, 1993). Simultaneously, the horse's caecum is inhabited by fungi, e.g. *Caecomyces equi* which participate in the breakdown of lignocellulosic complexes (Nagpal et al., 2009). It is also well documented that the activity of properly functioning microbiota supplies the horse, apart from short-chain fatty acids, with some of the vitamins (Mackenthun et al., 2013; Faubladier et al., 2013). The colon is colonised by similar microorganisms to that of the caecum, but their density here is lower; hence, processes of nutrient microbial degradation also take place below the blind gut (Mackie and Wilkins, 1988). In addition, mucus is secreted here profusely by goblet cells which facilitates, together with rhythmical and arhythmical contractions of the muscular coat, the movement of the chyme towards the anus. The large colon has a shape of a hoofed, two-level loop with the convexity directed towards the diaphragm (Figure 3). The average retention time of the markers in the solid state of the feed in horses lasts approximately 42 hours (Frape, 2004; Lopes and Pfeiffer, 2000; Sneddon and Argenzio, 1998).

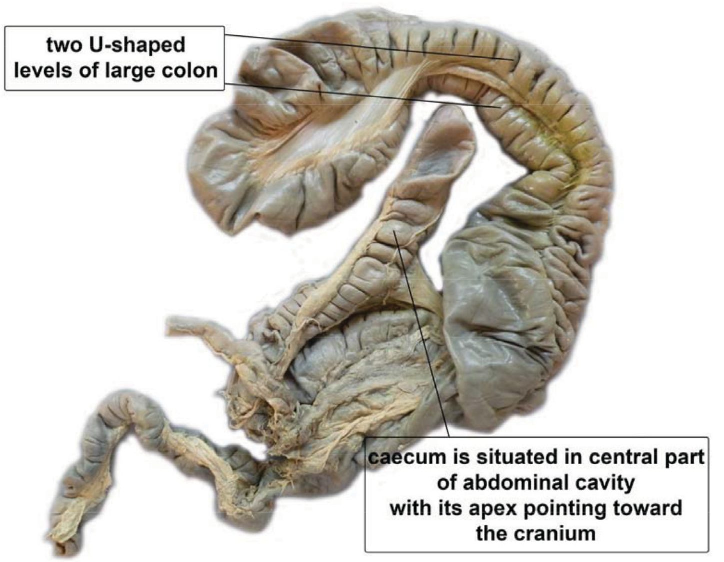

Figure 3. The anatomy of the horse's large intestine (M. Komosa)

Nutrient digestibility evaluation in horses

Due to the high proportions of structural carbohydrates found in feeds most frequently employed in the nutrition of horses, the value of fibre digestibility is very important as it exerts a significant impact on energy balance. Considering individual structural carbohydrate fractions, it can be said that hemicelluloses are characterized by higher digestibility coefficients than celluloses, whereas lignin passes through the gastrointestinal system almost intact (Figures 4 and 5). It should also be emphasised that Polish Horse Feeding Standards (1997) contain information only about the requirements of these animals for crude fibre, without breaking it up into individual fractions. On the other hand, American NRC standards from 1989 fail to provide any recommendations concerning fibre levels required in horse nutrition; instead, they suggest that the horse consumes, daily, high quality roughages in amounts corresponding to at least 1% of its body weight (Pagan, 1997). Recent studies recommend 20 g of roughages intake per kg BW per day (Coenen et al., 2011). However, available literature provides limited data on the proportions of carbohydrate fractions. This data is more important than the content of crude fibre itself in the ration, as it has been proved in ruminants and many non-ruminants. Dry matter digestibility coefficients (Table 1) in the case of diets consisting only of roughages, are lower in comparison to mixtures of roughages and concentrates most popular among draught horses, and this is due to the fact that concentrates contain high quantities of easily available structural carbohydrates which are sources of rapidly available energy. In the case of applying the most popular feed composition in diets for horses, i.e. hay plus cereal grains, the choice of cereal does not remain neutral with respect to dry matter digestibility of the entire dietary ration. When comparing dry matter digestibility of diets based on meadow hay supplemented with oat, maize or barley grains (in different physical forms), the highest coefficients were obtained using pressed maize grains followed by flaked oats. The lowest digestibility coefficients were found in diets containing flaked maize and then whole oats, while intermediate values were determined when diets were supplemented with flaked barley (Bergero et al., 2009). In the case of dietary fibre, the one derived from roughages is digested in a better way (Pagan et al., 1998). Dried lucerne and hay are more easily available for horses than hay from meadow grasses. Earlier developmental stages during cutting and low lignifications associated with it result in higher digestibility coefficients of nutrients (Barriere et al., 2007). It should be added that the digestibility of timothy readily consumed by horses, in comparison with lucerne and cock's foot, falls less rapidly together with later developmental stages (Darlington and Hershberger, 1968; Cuddeford and Hughes, 1992). Moreover, it is believed that high lignin concentrations in feeds reduce hemicellulose and cellulose digestibility as a result of creating a physical barrier for fibrolytic enzymes of microbiological origin (Barriere et al., 2007).

It can be assumed, on the basis of available literature data, that in the case of horse feeding, starch derived from cereal grains is a controversial compound (Cuddeford, 2000). It is a very important source of energy – especially in sport horse nutrition – but its excess contributes to disorders in the functioning of the gastrointestinal system. The optimal intake of starch in horses is 2 g per kg BW per day (Geor, 2010). However, it should be emphasised that the coefficient value of starch digestibility in horses is very high, exceeding 90% (Meyer et al., 1993) (Figure 6). It was found that starch derived from oat and sorghum grains is most readily available for horses, while starch from maize and barley is not so easily digested. This is primarily attributed to the fact that starch from oats assumes the form of very small granules (Lindeboom et al., 2004). Apart from granule sizes, it is believed that other factors affecting the digestibility level of this compound include: applied feed technological process (e.g. pelleting, cooking, micronisation, roasting), association with elements of the plant cell wall, time of passage through the small intestine, as well as amylase and amylopectinase availability and concentration. On the other hand, crushing and grinding cereal grains fails to have a significant impact on the improvement of starch availability in horse feeding (Cuddeford, 2000). The digestibility of thermally processed grains is higher than that of mechanically processed ones (Nielsen et al., 2010). Bearing in mind the role of a building material which is played by protein, it is also important to determine its digestibility, especially in the case of horses used in competitive sports and reproduction. The highest protein digestibility of cereal grains was determined in hulless oat, while the lowest – in maize (Sarkijarvi et al., 2000). Dry matter digestibility coefficients of roughages used in horse nutrition differ significantly, depending on the species, part of plants (generative or vegetative) and cultivation processes (Table 1). The large variety of feed compounds used in horse rations, in combination with differences in breeds and their activity, affects digestion processes (Jensen et al., 2010; Ragnarsson and Jansson, 2011). Furthermore, gastrointestinal disorders have become very important issues in this group of animals. Therefore, digestibility measurements are essential not only in terms of the feed evaluation, but also in the aspect of gut health and performance.

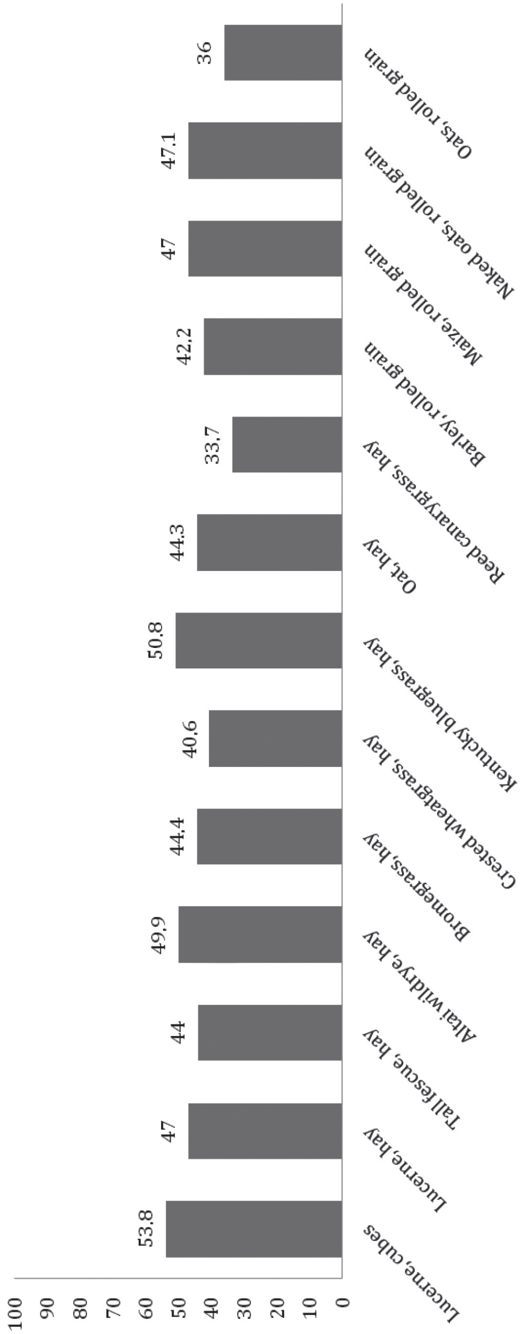

Figure 4. Neutral detergent fibre total-tract apparent digestibility coefficients (%) of selected feeds used in horse nutrition (modified after Hainze et al., 2003; Crozier et al., 1997; Cymbaluk, 1990; Hussein et al., 2004)

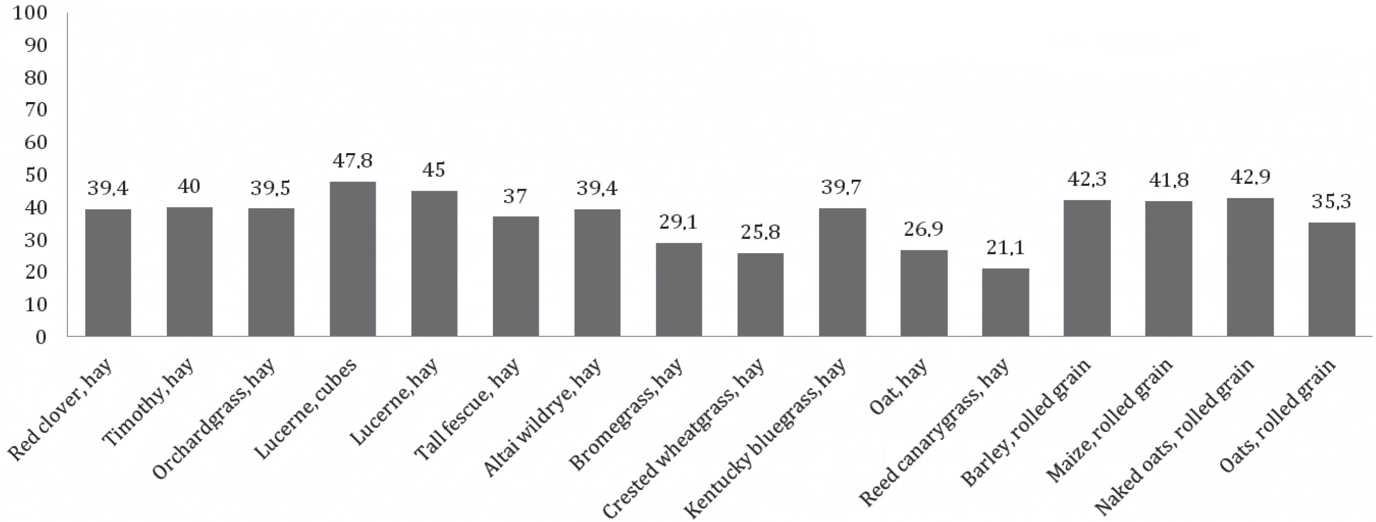

Figure 5. Acid detergent fibre total-tract apparent digestibility coefficients (%) of selected feeds used in horse nutrition (modified after Fonnesbeck, 1968; Haizne et al., 2003; Crozier et al., 1997; Cymbaluk, 1990; Hussein et al., 2004)

Table 1. Dry matter digestibility coefficients of selected feeds used in horse nutrition

Feed constituent	DM digestibility coefficient (%)	Source
Tall fescue, hay	48.0	Crozier et al. (1997)
Maize, flaked grain	74.7±17.8	Peiretti et al. (2011)
Maize, ground grain	83.1±10.5	Peiretti et al. (2011)
Lucerne, hay	58; 58.4±4.9	Crozier et al. (1997), Edouard et al. (2008)
Lucerne, cubes	62.0	Hainze et al. (2003)
Oats, whole grain	38.0±10.9; 72.7	Peiretti et al. (2011), Hainze et al. (2003)
Oats, flaked grain	88.9±2.8	Peiretti et al. (2011)
Oats, grain cleaned three times	64.5-70.5	Ott and Barnett (1984)
Grass hay	50.8±6.2	Edouard et al. (2008)
Meadow forage	58.1±4.2	Edouard et al. (2008)
Perennial ryegrass, haylage	57.7±1.0	Bergero et al. (2002)
Red clover, hay	59.2	Fonnesbeck (1968)
Timothy, hay	49.9	Fonnesbeck (1968)
Reed canarygrass, hay	49.4	Fonnesbeck (1968)
Orchardgrass, hay	44.5	Fonnesbeck (1968)

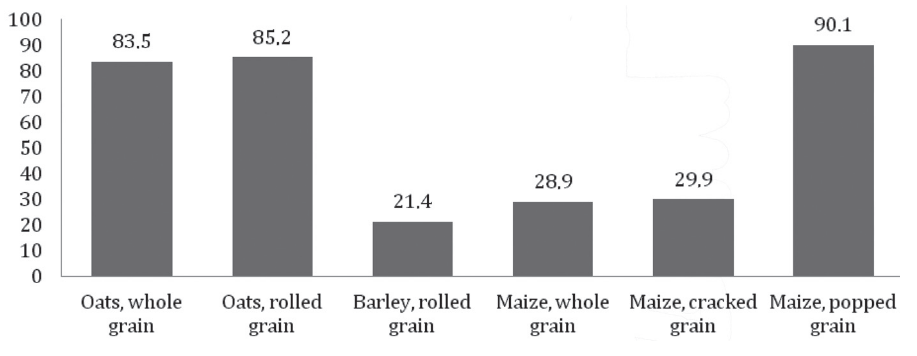

Figure 6. Starch preileal apparent digestibility coefficients (%) of selected grains used in horse nutrition (modified after Meyer et al., 1993)

Digestive processes taking place in the gastrointestinal tract of horses can be illustrated by employing a relatively simple method – so-called faeces sieves (Nørgaard et al., 2004). They consist of 3 segments of different screen meshes (top 4.76 mm, middle 2.4 mm, and bottom 1.6 mm). Excreta are placed on the highest segment with the largest mesh and then water under pressure is poured from the top until the water flowing at the bottom is clean (instruction of faeces sieves producer). The higher the dry matter digestibility coefficient of the diet, the higher the quantities of the finest faeces particles finding their way to the bottom sieve together with water. In our observations (unpublished), there is definitely a smaller fraction of particles with a diameter below 1.6 mm in horse faeces than in ruminants. Data from our own experiments (unpublished) conducted on sport horses in a Stallion Stud in Gniezno (Poland) indicates that, in the case of horses fed a diet based on meadow hay, oat grain and wheat straw (40:40:20), the distribution was not far from mean values: 18, 42 and 40% (Figure 7). The coarsest fraction was rich in hay and straw, as well as oat grain particles, which passed through the horses' digestive systems unaffected by digestive enzymes. Moreover, this easy-to-use in field conditions determination of DM digestibility seems to be highly correlated with the values estimated by marker retention (TiO_2); however, more research is needed to confirm this finding.

Figure 7. Horse's faeces distribution on three segments of faeces sieves (S. Klessa)

Methods of *in vitro* and *in vivo* digestibility determination

In vitro methods are most important in studies on ruminants and on the fermentation processes taking place in their forestomachs. They are carried out on a wide scale because of the possibility of cannulating polygastric animals and the ease of collecting rumen fluid. In order to accelerate, facilitate and reduce costs of digestibility trials on horses, researchers began to use the above methods also in horses. At the very beginning of *in vitro* digestibility determination in horses, scientists employed systems used for cows (Daisy^{II} Alkom, USA) which did not to give fully reliable results (Wilman and Adesogan, 2000). Experiments demonstrated that the application of horse faeces instead of the caecum chyme as inoculum in trials with the use of the Daisy^{II} Alkom system made it possible to obtain results consistent with the results of classical *in vivo* tests (Earing et al., 2010). Horse faeces turned out to be a good source of inoculum also when a modified method for the determination of *in vitro* digestibility developed for ruminants by Tilley and Terry in 1963 was used (Treverton and Friend, 2000). Initially, the Tilley and Terry method consisted in the incubation of the examined feed first in the sampled rumen fluid, and then in pepsin and hydrochloric acid solution (Tilley and Terry, 1963) for 24 hours, mixing it frequently. Following this, after centrifuging, the sample was exposed to a cellulase mixture for another 24 hours. Carrying out parallel *in vitro* experiments for diets based on different components – by employing the balance method and n-alkanes as an indicator – a regression equation was developed to calculate the feed digestibility coefficient (O'Keeffe et al., 1998). Despite the fact that in *in vitro* conditions, feed digestibility can be determined much faster, the above methods have not found wider application. Their basic disadvantage is that it is impossible to identify individual differences in metabolic conditions of animals. In addition, each *in vitro* method requires systematic validation involving *in vivo* experiments (Coles et al., 2005).

In order to study digestive processes in horses taking place before the caecum, it is possible to carry out experiments using nylon bags, a method known as *in sacco*. However, it is a very invasive method which requires the use of cannulas, hence, it cannot be applied in animals used in sports. Cannulation allows the placement of even several sacs in the horse's digestive system filled with any feed, making it possible to test a number of diets simultaneously. Thanks to the *in sacco* method, scientists were able to study, among others, how horses digest starch derived from different concentrates. Once digestion in the small intestine comes to an end, nylon bags are recovered from the caecum with the assistance of a magnet. The digested feed contained in them provides good material for various kinds of analyses. This method is applied very rarely, because it requires surgery prior to the experiment (De Fombelle et al., 2004).

In vivo methods are more time and labour consuming than those conducted in laboratory conditions. In all *in vivo* methods, one – at least a week-long – introductory period is necessary during which experimental animals are fed the test diet. The period is essential for the gastrointestinal microbiota to adapt to the new element of the diet. Among *in vivo* methods, the classical (balance) method requires the complete collection of the faeces excreted. This experimental procedure is very controversial in the case of horses used in sports as the animals must be kept in closed litter-free boxes or stalls for the entire period of the experiment. The collected faeces are mixed and comminuted, pooled, and then a representative sample for appropriate chemical analyses is taken. Identical procedures are performed with respect to the feed fed to animals. Next, on the basis of differences in the content of individual components in the feed and faeces, digestibility coefficients are calculated (Schneider and Flatt, 1977). Numerous attempts were made to shorten the period of the entire faeces collection to a number of days, which would still allow the proper evaluation of digestibility coefficients. According to Goachet et al. (2009), shortening the collection period from 5–6 days to 3–4 days can be considered as optimal. However, even a 3-day confinement in a box/stall of the horse thus far used in sport with no possibility of movement is a serious interference in its welfare, and may exert a negative influence on its behaviour, and therefore upset digestive processes. That is why attempts are continued to find other research methods that would give similarly reliable results.

In the 1960s, a unique harness incorporating a special sac was elaborated, which enabled total faeces collection to determine digestibility using the balance method (Friend and Nicholson, 1965). The application of this harness reduces the time-consuming nature of experiments and allows grazing or even moderate use of horses. Nevertheless, the use of harnesses and bags for faeces collection will always restrict, to a certain extent, the horse's movements, which influences its behaviour and, consequently, affects the harmonious course of digestive processes (Sales, 2012).

Indicator methods are much simpler in carrying out digestion experiments on horses, since they do not require complete faeces collection, but only representative samples, either from the ground or *per rectum*. Therefore, horses can be maintained on their usual management regime, which allows the performance of experiments even on sport horses. This, in turn, makes it possible to obtain the most reliable information for formulating feeds intended for these horses. In the indicator method, the digestibility coefficient is calculated on the basis of ratio comparison of the nutrient content and the indicator in representative feed and faeces samples.

Indicators naturally occurring in feeds include, among others: lignin (ADL), chromogen dyes, acid insoluble ash (AIA, primarily silica), indigestible acid-detergent fibre (IADF), indigestible neutral detergent fibre (INDF), indigestible cellulose, chain odd alkanes and cutins. The indicators incorporated into diets include: chromium oxide (Cr_2O_3), titanium oxide (TiO_2), labelled fibre (Cr-EDTA and Co-EDTA), polyethylene glycol, rare earths elements (ytterbium, lanthanum, cerium, dysprosium) and even alkanes in the chain. Markers naturally occurring in feeds make them more practicable in field conditions as they make it unnecessary to add indicators to diets (Miraglia et al., 1999). The application of a given marker depends on the percentage proportion with which the digestibility coefficient determined by the method coincides with the coefficient calculated on the basis of the classical method (complete faeces collection).

Lignin was the first indicator used in history. Digestibility studies using them were carried out as early as the 19th century (Kotb and Luckey, 1972). With the passage of time, results obtained with their assistance turned out to be unreliable. This can be attributed to the fact that lignin was partially degraded by fungi settling in the large intestine of the horse (Nagpal et al., 2009). Digestibility coefficients determined in competitively trained Arab horses, employing naturally occurring markers such as lignin and AIA, turned out to be overestimated in comparison with the classical method (Goachet et al., 2009). In another experiment, the application of AIA yielded several percent more highly reliable results as compared to lignin (Martin et al., 1989). In still other experiments employing AIA and ADL, again lignin turned out to be unreliable and this time, it was justified by its poor recovery in the faeces. In the above trial, AIA gave results that coincided with the balance method (Miraglia et al., 1999). Following the trials in which digestibility of Mediterranean grasses for horses was determined, it was concluded that AIA can only be used for the rapid evaluation of digestibility coefficients. For these reasons, some researchers recommend that in experiments which are used to provide reliable data serving as models in widely available tables of nutritional values and feeding standards, the classical balance method should be applied (Bergero et al., 2004). Nevertheless, there are numerous reports in available literature of considerable practical implications which are based completely on AIA evaluations with no parallel verification with the balance method. All in all, AIA is a very popular marker used in digestibility studies on horses conducted so far. AIA can be identified in faeces and feed samples in two ways, namely using 2- or 4-normal hydrochloric acid. Experiments showed that both methods are equally reliable, although the one employing 2-normal acid is cheaper and simpler to apply, making it more practical (Bergero et al., 2009).

Alkanes with the odd carbon number constitute part of the waxy plant cuticle. Alkanes most common in fodder plants are C31 and C29 (Ordakowski et al., 2001). By simultaneously using natural and synthetic (having an even carbon number) alkanes added into diets in experiments, it is possible to determine feed intake (Mayes et al., 1986). For this experiment model, also other combinations of natural and added markers can be applied. This method of determination of feed intake is particularly suitable when horses graze on pastures, where it is impossible to calculate feed intake by the difference of the supplied forage and that left. Another method of estimating forage intake from pastures, in which the basis for calculation are data from the National Research Council (NRC) standards regarding the intake of digestible energy and body weight and horse physiological status turned out to be so inaccurate that errors frequently exceeded 100% (Kronfeld, 1998). Therefore, the alternative method to the one above based on two indicators makes it possible to conduct a wide spectrum of investigations where reliable data concerning feed intake are essential, e.g. when determining the horses' preferences for specific constituents (free choice method).

So far, chromium oxide appears to be the most popular marker among those employed as a feed supplement in digestibility experiments on horses (Sales, 2012). In a comparative experiment with the most commonly applied natural markers (as well as ytterbium (Yb), a chemical element derived from rare earths and applied as an additive to feeds), this compound turned out to be the most effective (Martin et al., 1989). However, other studies reported an underestimation of the result in the case of applying Cr_2O_3 and higher reliability of the results obtained on the basis of the AIA natural marker (Orton et al., 1985). Cr_2O_3 is described by 24-hour secretion variability (Haenlein et al., 1966; Cuddeford and Hughes, 1990), which may affect the consistency of the results obtained. In addition, there are reports about the carcinogenic nature of this compound (Myers et al., 2004). As in the case of AIA, there are investigations based exclusively on assessments resulting from the content of Cr_2O_3 in the feed and faeces. In Japan, attempts were made to determine the digestibility of individual nutrients in a wide range of different feed constituents used in horse nutrition (Tagaki et al., 2003).

Titanium oxide has not enjoyed significant popularity in scientific investigations so far. However, certain characteristics of this marker appear to give it some advantages, making it more attractive in comparison with Cr_2O_3. Titanium oxide is a compound that is simple to administer to animals and to determine in samples thanks to spectrophotometric methods (Myers et al., 2004; van Bussel et al., 2010; Józefiak et al., 2011, 2014) and its recovery from faeces is by more than 20% higher than the recovery of chromium oxide (75% vs. 98%) (Jagger et al., 1992). The history of the use of this indicator in experiments on horses is very short. Recent trials showed the lack of statistically significant differences between digestibility coefficients in horses (Visser, 2010), determined using the indicator method with titanium oxide and the balance method. In trials carried out on ponies, it was demonstrated that titanium oxide yielded good results in comparison with natural markers (ADL and AIA) used in this experiment. Digestibility coefficients calculated with the assistance of titanium oxide turned out to be most consistent with the results obtained using the complete faeces collection (Schaafstra et al., 2012).

Conclusions

There are many possibilities of using markers to facilitate digestibility experiments on horses. Non-invasive indicator methods appear to be a better solution when compared to both the *in sacco* and classical balance methods. Nevertheless, it is still necessary to keep working on and improving methods of applying individual markers and to verify the reliability of the results obtained using them. Together with the continuously increasing requirements with regard to the training of sport horses, the significance of digestibility investigations will also increase. There are numerous studies in available scientific literature dealing with attempts to improve feed utilisation by horses without negative effects on the health of the gastrointestinal tract. It was demonstrated that horses subjected to demanding training digest feeds better than less extensively trained horses. This refers not only to the comparison of horse groups subjected to training at different levels of intensity (Orton et al., 1985). This hypothesis is also true with respect to long-term training. Digestibility coefficients of dry matter, organic matter and NDF in Arab horses trained for a period of two years for long-distance races turned out to be lower before the beginning of training (Goachet et al., 2010). In other trials, it was shown that digestibility coefficients of fibre, dry matter and organic matter declined together with the increase in the intensity of training horses (Bergero et al., 2002). Recent studies performed on Standardbred horses indicate that regular training increases nutrient digestibility and short-chain fatty acids concentration in the hindgut (Goachet et al., 2014). There may be many causes of the above differences in the results obtained including, among others: differences in maintenance conditions and diets, different kinds of training and physical condition of animals, as well as individual variability. The newest studies indicate that digestibility coefficients also depend on horse breeds (Jensen et al., 2010; Ragnarsson and Jansson, 2011), while age seems to be less important (Earing et al., 2013). Therefore, it can be assumed that investigations on digestibility in horse nutrition can exert a significant impact on expanding our knowledge in the field of sport use of this group of animals. On the other hand, improvement in feed utilisation can contribute to the improvement of health and the occurrence of many metabolic diseases, frequently difficult to diagnose due to their subclinical nature.

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