Effect of plant maturity at harvest of haylage on digestibility and faecal particle size in horses fed forage-dominated diets

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Plantmognadens inverkan på smältbarhet och partikelstorlek i träck hos hästar utfodrade med hösilage-dominerade foderstater

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SAMMANFATTNING

Tiden för vallskörd påverkar hösilagets näringsvärde och smältbarhet, då vallgrödorna befinner sig i olika botaniska utvecklingsstadium. Målsättningen med den här studien var att undersöka plantmognadens inverkan på smältbarhet samt på partikelstorlek i träck hos hästar utfodrade med hösilage skördat i juni, juli och augusti.

Analys av skattad smältbarhet av torrsubstans samt partikelstorlek i träck genomfördes på foder och träckprov sparade från ett utfodringsförsök genomfört under hösten 2009. Tolv hästar användes i en change-overstudie och delades in i tre grupper och utfodrades under tre perioder med tidigt (juni), mellan (juli) eller sent (augusti) skördat hösilage. Under varje period togs träckprov från hästarna en gång per dag, vilka sedan poolades till ett prov per häst och period. Totalt analyserades 36 träckprover. Smältbarheten av ts för de olika hösilage foderstaterna beräknades efter analys av den inre markören saltsyraolöslig aska, i foder och träckprover. Smältbarheten av ts som skiljde sig mellan de tre hösilagen, var högst för junihösilaget och lägst för augustihösilaget. Mellan perioder eller hästar var det ingen skillnad i smältbarhet. Efter att häst 8 och 9, som hade höga smältbarhetsvärden för foderstaten med augustihösilaget, uteslutits från den statistiska analysen blev det dock skillnad i smältbarhet även mellan hästar.

Partikelstorlek i träck bestämdes genom våtsiktning med såll av olika storlekar. Fördelningen av partikelstorlek i träck skiljde sig mellan foder men inte mellan perioder eller hästar. Foderstaten med junihösilage skiljde sig från foderstaterna med juli- och augustihösilage i alla fraktioner som var mindre än 2,0 mm. Juli- samt augustihösilaget hade en större andel träckpartiklar i de mellersta fraktionerna (0,1 - < 2,0 mm) i jämförelse med juni hösilaget. Foderstaten med junihösilage hade störst andel partiklar i den minsta fraktionen (<0,1 mm). I fraktionen 0,2 mm var det skillnad mellan alla hösilage foderstater, där den största andelen var representerad av augusti hösilaget, följt av juli- och junihösilaget. I fraktionen med störst partikelstorlek (>2,0 mm) var det ingen skillnad mellan foder. Över 70 % av träcken hamnade på sållet med den största maskstorleken (2,0 mm).

Enligt den här studien leder en ökad plantmognad hos vallen till ett mindre smältbart vallfoder för djurslaget häst. Ett hösilage med högre smältbarhet av ts, gav fler partiklar som var mindre än 0,1 mm. Ett mindre smältbart, och mer lignifierat, hösilage verkar dock resultera i fler träckpartiklar mellan 0,1 och 2,0 mm.

ABSTRACT

Time of harvest affects nutritional value and digestibility of haylage since plants are in different developmental stages. The aim of this study was to examine the effect of plant maturity of haylage cut in June, July and August on digestibility and faecal particle size in horses.

Forage and faecal samples used for the analysis of apparent dry matter digestibility (DMd) and particle size distribution in faeces were from a feed-study performed in the autumn of 2009. Twelve horses were used in the study, divided into three groups in a change-over experiment. Each group was fed haylage harvested in June, July or August during three periods. Faecal grab samples were taken from each horse and pooled so that one sample represented one horse during one period. In total 36 faecal samples were analysed. Apparent DMd of the different haylage diets was calculated by using the amount of acid-insoluble ash in feed and faeces as an internal marker. The apparent DMd was different in the three haylage diets. June haylage had the highest apparent DMd and August the lowest. There was no difference in apparent DMd among the periods or horses. However, a difference among horses occurred when two horses with outlier values were excluded from the statistical evaluation.

Particle size distribution in faeces was measured using stainless steel sieves of different mesh sizes. Particle size distribution in faeces did not differ between horses or periods. Particle size distribution in faeces from horses fed the diet containing haylage harvested in June differed from horses fed July/August haylage in all fractions lesser than 2.0 mm. Faecal particles from diets containing haylage harvested in July and August dominated the middle fractions (0.1 – 2.0 mm). The diet containing June haylage had the largest proportion of faecal particles in the smallest fraction size (<0.1 mm). Fraction size 0.2 mm was the only fraction with a difference between all diets. August haylage dominated followed by July and June haylage. More than 0.7 of the faecal particles were found in the largest fraction size (>2.0 mm).

This study confirms that a more mature herbage produces a less digestible forage for horses. A haylage of high DMd gave a higher proportion of faecal particles less than 0.1 mm. A more lignified haylage with lower digestibility results in a higher proportion of faecal particles between 0.1 and 2.0 mm.

INTRODUCTION

Horses are hind-gut fermenters and adapted to consume forage with high fibre content (Duncan *et al.*, 1990; Edouard *et al.*, 2008). Chemical composition and nutritional value of grasses and legumes varies with plant maturity and makes the time of harvest important when producing forage with a specific nutritional value or digestibility. Horses have different nutrient requirements depending on physical activity or stage of life (Frape, 2004; Ellis and Hill, 2006). Lactating mares, growing colts and fillies and horses in tough physical training need forages of high nutritional value and digestibility. However, hobby horses need a forage with lower nutritional value and less digestibility in order to prevent health disorders caused by an excessive intake of nutrients.

The digestibility of forage depends on forage composition with regard to plant structure, chemical composition and level of lignification (McDonald *et al.*, 2002). Plants in an early developmental stage contain a higher proportion of cell content compared to cell wall, and therefore also a higher energy and protein content for animals. During maturity, plant cell walls become lignified which influence digestibility in several ways. Lignin is considered to be indigestible and cross linkage to other nutrients like structural carbohydrates in the plant cell wall decreases the overall digestibility even further. The developmental stage of the plant at time of harvest is therefore a very important factor determining the digestibility (Moore and Jung, 2001).

Since the chemical composition changes during plant maturation, particle size in faeces may be affected as well. A haylage with high digestibility may contain a larger proportion of smaller feed particles, thus a larger area available for the fermenting microorganisms, compared to a less digestible haylage. A more lignified forage should be harder for the animal to masticate, which may lead to an ingesta with a higher proportion of larger particles also in the faeces. Several studies have concluded that horses fed a high fibre forage increases their feed intake and the passage rate of the ingesta to compensate for the reduced digestibility (Janis, 1976; Edouard *et al.*, 2008). An increased feed intake may influence particle size of the ingested feed by less careful mastication, whereas an increased passage rate may decrease mechanical disruption and microbial fermentation.

The aim of this thesis was to evaluate the effect of plant maturity at harvest of haylage on digestibility and faecal particle size distribution in horses.

LITERATURE REVIEW

Plant maturity, nutritional value and digestibility

Stage of growth is the strongest factor influencing the nutritional value of herbage (Bélanger *et al*., 2001; Fogelfors, 2001; McDonald *et al*., 2002; Bélanger *et al*., 2008). The development of grasses is divided into four stages; vegetative, elongation, reproductive and seed ripening. Maturity influences the moisture content in the standing crop as well. Young plant material contains 750-850 g water/kg, whereas mature herbage contains about 650 g water/kg. However, moisture content of forage is also affected by weather conditions. The increase in concentration of dry matter (DM) in grasses is slow in the beginning of development but accelerates as the stem grows and ears emerge. Subsequently, when the ears begin to ripen, the increase in DM concentration is slower (McDonald *et al*., 2002).

Digestibility of a feed declines with increased fibre content (Darlington and Hershberger, 1968; Beever *et al*., 2000; Edouard *et al*., 2008). The proportion of fibre in the feed normally depends on the stage of development of the plant at harvest and older plants are more fibrous in order to maintain the plant structure. The proportion of cell walls to cell contents is larger in more mature plants, and the mass of structural carbohydrates is therefore larger compared to the soluble components (Janis, 1976). The digestibility of cell content is almost complete while the digestibility of cell walls is more variable due to the degree of lignification. Lignification of cell wall components, such as structural carbohydrates, diminishes the possibility of fermentation by the microorganisms (McDonald *et al*., 2002). The concentration of lignin in grasses increases with more than 50% during the development from vegetative stage to reproductive stage, whereas the increase in legumes is less (Bidlack and Buxton, 1992).

The structure of plant tissues influences the cell wall digestibility. Chesson (1993) discussed the proportions of different cell types as a factor influencing the DM digestibility (DMd). The different layers, like cuticle, vascular bundle sheets and warty layers, could have an influence on digestibility by restricting the microbial access to the cell walls. The relationship between digestibility, in ruminants, and the proportion of leaves has been discussed by Bélanger *et al*. (2001). In very young grasses, the stem is more digestible than the leaves. The digestibility of the stem fraction decreases rapidly during maturation, whereas digestibility of leaves does not decrease as fast (Bélanger *et al*., 2001). Developmental stage and increased forage yield results in a decrease in the proportion of leaves while the proportion of stems increases. The change in proportion between leaf and stem leads to a reduction in digestibility due to the higher content of lignified cell walls in the stem.

The digestibility of legumes decreases more slowly during plant growth, compared to grasses (Kaldmäe *et al*., 2003). Legumes have a higher concentration of lignin than grass when expressed as proportion of fibre. Even though the lignified legume cells are virtually indigestible, the concentration of fibre is lower in legumes compared to grasses. This means a higher concentration of digestible cell contents in legumes compared to grasses (Moore and Jung, 2001). The concentration of pectic substances is higher in legumes, but the proportions of hemicellulose in cell walls are lower, compared to grasses (Beever *et al*., 2000).

Plant species, plant variety, environment and previous management of the sward are factors that influence the nutritive value of herbage (Beever *et al*., 2000). For example, the concentration of the sugars (glucose, fructose, sucrose, raffinose and stachyose) and fructans is influenced by environmental conditions such as light and temperature (McDonald *et al*.,

2002). High temperature and cloudy weather results in a rapid reduction of energy content in the herbage. This is mainly due to a decreased sugar content since the photosynthesis, which converts carbon dioxide and water into simple sugars, is favoured by sunny weather (Parsons and Chapman, 2000). Mineral content in herbage is very variable since it is dependent on soil type, plant species, stage of growth, and management factors like cultivation and fertilizer application. Clover species generally contain more minerals compared to grasses, in particular calcium, phosphorus, magnesium, copper and cobalt (Fogelfors, 2001).

A major factor influencing digestibility is the chemical composition of a feed and similar feeds may have different digestibility due to different nutrient content. The chemical composition of a feed may influence the action of digestive enzymes, while addition of protein or soluble carbohydrates may increase the digestibility of a feed (Khan *et al*. 2003). The maturation process, including the increased stem to leaf ratio and the decreased amount of cell content, usually results in a decreased protein concentration in grass over the season. In some cases, an increased concentration of non-structural carbohydrates may also occur. The latter is mainly due to an increase of fructans in the stem, stem base and inflorescence. In the plant, the cell content (i.e. cell nucleus and cytoplasm) contain most of the proteins, peptides, nucleic acids, lipids, sugars and starches (Beever *et al*., 2000). The crude protein content in herbage may vary between 30 and 300 g/kg DM, where the higher value represents young, heavily fertilised grass. Compared to grasses, legumes contain a higher proportion of crude protein (McDonald *et al*., 2002). Legumes have the ability to fix nitrogen, leading to a high level of nitrogen in the leaves, and therefore high crude protein content in the plant. The decrease of crude protein content with maturity is equal in grasses and legumes. However, since legumes have higher nitrogen content, due to the nitrogen fixation, the crude protein level at harvest is usually higher than in grasses (Jönsson, 1981).

Finally, the digestibility of a feed may also be affected by feed processing i.e. chemical or biological treatment of silage consumed by cattle (Khan *et al*. 2003).

Cell wall

The cell wall surrounds the protoplasm and protects and provides rigidity to the plant tissue (Fogelfors, 2001). In addition to mechanical support, the cell wall in plants is also important for water balance, ion exchange, cell recognition, and protection from biotic stresses. The cell wall is composed of water and structural carbohydrates such as celluloses, hemicelluloses and pectins (Moore and Jung 2001).

The primary cell wall of the young plant cell is generated from the cell membrane. The main constituents of the primary cell wall are pectin, xylan and cellulose. The cellulose micro fibrils, i.e. compact aggregates joined by hydrogen bindings, of the primary cell wall are randomly arranged, making the cell elastic and able to expand (Figure 1). Older cells develop a secondary cell wall where the cellulose micro fibrils are arranged in the same direction in each layer, creating a less elastic cell (Fogelfors, 2001). The secondary cell wall is located outside the cell membrane, but within the primary cell wall (Figure 1). In older grasses, the secondary cell wall consist of a considerable amount of lignin (around 15% of DM) (Janis, 1976), nearly 45% cellulose, hemicelluloses and hence a lesser amount of pectin (Fogelfors, 2001). In grasses, all main cell types undergo secondary cell wall thickening and lignification, which is not the case for legumes (Wilson and Hatfield, 1997).

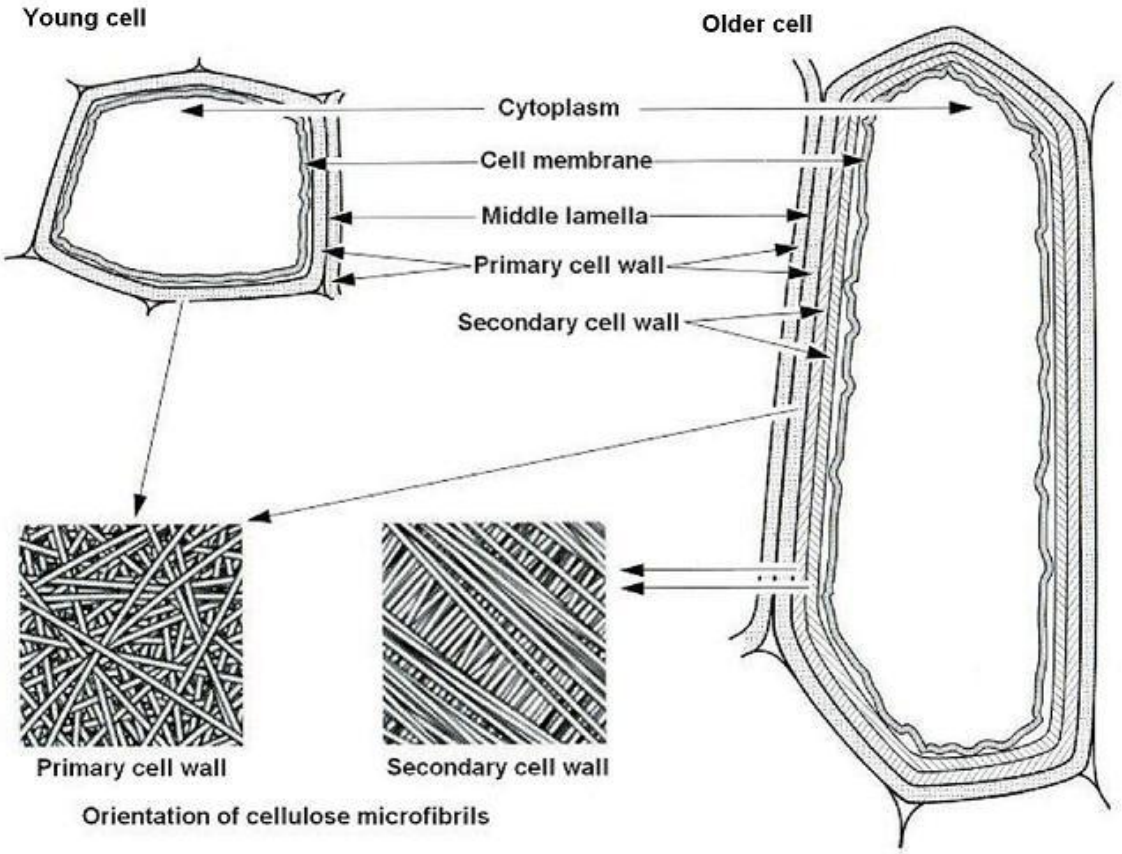

Figure 1. The cellulose micro fibrils in the primary cell wall of the young plant cell are randomly arranged, making the young cell elastic and able to expand. The cellulose micro fibrils in the secondary cell wall are arranged in the same direction in each layer, making the older plant cell less elastic (Illustration by Fredrik Stendahl in Fogelfors, 2001).

Legume plant cells are divided into cells with high digestibility; cortex and pith cells, and cells that appear to be next to indigestible; xylem cells. The xylem cells have highly lignified secondary walls and histological studies indicate that all lignin in mature legume stems appears to be concentrated in the xylem ring (Wilson & Hatfield, 1997). Xylem is important for the water and ion transport in the plant. Together with phloem which is involved in the transport of organic solutes in the plant, xylem forms a vascular system throughout the plant. Some phloem fibre cells may also contain lignin (Alberts *et al*., 2002). However, the cortex and pith cells do not undergo lignification, although secondary walls or thickened primary walls may develop (Wilson & Hatfield, 1997).

Structural carbohydrates

It is difficult to provide an accurate definition of different carbohydrates since structure and function varies considerably and role and function overlap between different groups (McDonald *et al.*, 2002). In this thesis, carbohydrates degraded by enzymes secreted by the horse are referred to as non-structural carbohydrates, while structural carbohydrates like cellulose, hemicelluloses and pectin, are carbohydrates that are degraded by microorganisms in the digestive tract of the horse.

Cellulose

Cellulose is the most abundant carbohydrate in the plant kingdom, and is fundamental in the structure of plant cell walls. In grasses, cellulose content varies from 200 to 300 g/kg DM, with the higher value in mature herbage (McDonald *et al*., 2002).

The cellulose molecule, synthesized at the plasma membrane, is a straight chain β-1,4 linked polymer of glucose that may contain over 10 000 glucose monomers. Molecules of cellulose form chains which produce micro fibrils. The micro fibrils are important for the cell wall structure and function. The degradation of cellulose, forming glucose units, requires cellulases that are produced by microorganisms in the digestive tract of the horse. Cellulose is closely associated with other components in the cell wall, like hemicelluloses and lignin, which can have an impact on the degradation process during digestion (McDonald *et al*., 2002).

Hemicelluloses

Hemicelluloses are important for the structure of cell walls, and the content in grasses ranges from 100 to 300 g/kg. Hemicelluloses are, like cellulose, polysaccharides composed of glucose but also of other sugar components like D-galactose, D-mannose, D-xylose and L-arabinose units. Unlike cellulose molecules, hemicellulose molecules are shorter and branched. The components of hemicelluloses can be joined together in different combinations and by various glycosidic linkages. The main chain of hemicelluloses from grasses is generally composed of β-1,4 linked D-xylose units with glucuronic acid and glucose, galactose and arabinose. Hemicelluloses can be linked to lignin, which inhibit the action of hemicellulases and the digestion of the polysaccharide (McDonald *et al*., 2002).

Pectic substances

The pectic substances are often referred to as ’intracellular cement’ since they are important in the action of bringing primary cell walls, of two adjacent cells, together (Fahey and Jung, 1983). Pectic substances are important constituents in the primary cell wall and intracellular regions, like the middle lamella (Figure 1). Pectic substances are a heterogeneous group of polysaccharides and consist mainly of galacturonic acid residues. Pectin, the most common member of the pectic substances, is composed of a linear chain of D-galacturonic acid units, interrupted by L-rhamnose residues and D-galactose, L-arabinose and D-xylose as side chains. Pectinases, produced by the microorganisms in the digestive tract of the horse, are needed for the digestion of pectic substances (McDonald *et al*., 2002).

Lignin

The most important function of lignin is to give strength and rigidity to the cell wall. Other functions of importance are to limit water loss by reducing permeability of the cell wall, and to protect the plant from harmful organisms (McDonald *et al*., 2002).

Lignin is a complex polymer originating from the monolignols; p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Monolignols are members of the phenylpropanoids and cross-links between many units form lignin (McDonald *et al.*, 2002). The complex molecule of lignin has a high resistance to chemical degradation and decreases digestibility of forage through interactions with structural carbohydrates in the plant cell wall. Lignin hampers the microbial fermentation by acting as a physical barrier between the microbial enzymes and the target polysaccharide or protein (Moore and Jung, 2001).

The concentration of lignin in the plant increases with maturity due to morphological changes such as an increased proportion of cell walls. Temperature, soil moisture, light and soil fertility also have direct or indirect effects on lignification of the plant. The indirect effects are mainly due to changes in development and morphology of the plant. The increase of lignified plant tissue in more mature plants results in a negative relationship between digestion and plant maturity (Moore and Jung, 2001). Structural tissues, like epidermis, xylem and sclerenchyma in stems are commonly exposed to lignification. The lignification process differs in different species and even between genotypes within species, hence the different concentrations and composition of lignin in plant varieties (Akin *et al*., 1990).

The digestive system of the horse

Horses are hindgut fermenters with the ability to consume diets containing high levels of plant fibre. Unlike ruminants, horses digest most of the fibrous fractions in the caecum and colon which gives them the ability to consume more of less digestible forage. This is possible since the passage of digesta in horses is not dependent on the reduction of feed particles in the same manner as ruminants (Duncan *et al*., 1990; Edouard *et al*., 2008). Cattle have to reduce the feed particles to a size less than 3-4 mm that enables passage from rumen further through the gastrointestinal (GI) tract (McDonald *et al*., 2002). The GI tract of the horse is illustrated in Figure 2.

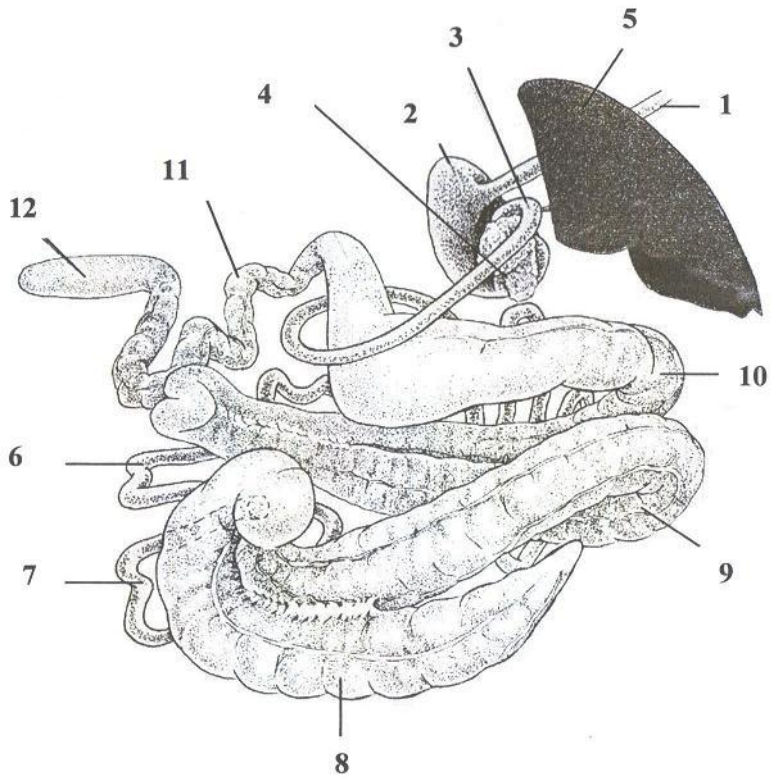

Figure 2. The gastrointestinal tract of the horse. 1) Oesophagus, 2) Stomach, 3) Duodenum, 4) Pancreas, 5) Liver, 6) Jejunum, 7) Ileum, 8) Caecum, 9) Ventral colon, 10) Dorsal colon, 11) Small colon, 12) Rectum (Illustration by Bo Furugren from Attrell et al., 1999).

Oral cavity

Horses use their lips and teeth for ingestion of feed. Unlike ruminants, horses have incisor teeth in both premaxilla and mandible which are used during grazing (Ellis and Hill, 2006). The tongue is important for the manipulation of feed in the oral cavity and it moves the feed to the cheek teeth for grinding (Frape, 2004). The mandible of the horse is approximately one third narrower than the maxillary jaw, resulting in a lateral movement while chewing. This leads to sharp prominent ridges of the molars which efficiently masticate the feed (Frape, 2004; Ellis and Hill, 2006).

Eating stimulates the secretion of saliva which facilitates the swallowing of feed. Each day, a horse produces 10-12 litres of saliva, composed mostly of water but also mucus and bicarbonate, sodium, potassium and chloride ions. There is a lack of knowledge concerning the production and composition of saliva in horses and therefore the knowledge about the pre-gastric digestion of feed in horses is also poor (Frape, 2004). There is no amylase activity in the saliva of the horse, but the microflora present in the mouth can to some extent degrade starch. Bicarbonate from the saliva buffers the digesta in the proximal region of the stomach. This is important for the microbial environment in the stomach and to prevent gastric ulcers (Ellis and Hill, 2006).

The secretion of saliva is greater when horses consume roughage compared to concentrates. Horses consuming 1 kg of long-stemmed hay, make between 3000 and 3500 chewing movements whereas 1 kg of concentrates requires between 800 and 1200 chewing movements. Since saliva is stimulated by mastication of feed, horses secrete less saliva when consuming concentrates compared to roughage (Frape, 2004). As a consequence, horses consuming excessive levels of concentrates may increase the acidity of the stomach and the risk for gastric ulcers (de Fombelle *et al.*, 2003).

The chewed feed is moved, aided by the tongue, towards the back of the mouth where it is swallowed and transported from the pharynx, through the oesophagus to the stomach (Frape, 2004).

The stomach

The stomach of the horse represents approximately 10% of the volume of the total GI tract. The small volume is suited for frequent consumption of small quantities of food. Fresh ingesta enter the stomach through the cardiac sphincter which is a powerful muscular valve preventing digesta to go back through the oesophagus. The stomach is rarely empty since the expulsion of digesta through the pyloric sphincter to the duodenum declines as ingestion of feed ends (Frape, 2004).

The horse stomach is divided into glandular and non-glandular regions (Frape, 2004). Microbial activity occurs primarily in the non-glandular region *saccus caecus*, but also in cardiac- and fundic gland regions. Since the retention time of digestion in the stomach is short, 2-6 hours, the microbial fermentation is limited (Hintz *et al*., 1971; de Fombelle *et al*., 2003; Frape, 2004). The fermentation which yields moderate concentrations of volatile fatty acids, amino acids and lactic acid, diminish near the glandular regions of the stomach. The ceased microbial activity is due to a reduction of pH due to secretion of hydrochloric acid (HCl) in the glandular area (Frape, 2004).

The glandular area is divided into three different regions; the cardiac-, the fundic- and the pyloric region. The division depends on the type of glands present in the specific area (Ellis and Hill, 2006). The cardiac glands, situated between the non-glandular area, *saccus caecus*, and the fundic gland region, secrete mucus and bicarbonate by exchange of chloride ions (Singer, 1998). Pepsinogen, HCl and gastric juices are secreted in the fundic region where pH is between 5 and 5.5 (de Fombelle *et al*., 2003). The pH of the digesta falls to 2.6 or lower in the pyloric region, due to the secretion of HCl. The lower pH enables the proteolytic activity of pepsin which is 15 to 20 times higher in the pyloric compared to the fundic region. However, the degradation of protein in the stomach of the horse is limited due to the short retention time (Frape, 2004). The pyloric glands secrete mucus and the polypeptide hormone gastrin, which is activated by the distension of the stomach wall. Gastrin stimulates the secretion of HCl and gastric juices in the fundic region. Together with the buffering properties of bicarbonate, mucus is important as a protective barrier to prevent gastric ulceration due to HCl and pepsinogen (Singer, 1998).

The small intestine

The small intestine, representing about 30% of the volume of the GI tract and 75% of its length, is divided into duodenum, jejunum and ileum (Frape, 2004). Digesta entering from the pylorus, with a pH of 2.5 to 3.5, is neutralized to a pH between 7.0 and 7.5, by the action of bile buffers and secretion of bicarbonate by duodenal glands (de Fombelle *et al*., 2003). The pH of the digesta increases even further in the proximal jejunum and ileum to 7.8 to 8.2 (Ellis and Hill, 2006). The horse has no gall bladder but the presence of HCl in the duodenum stimulates the secretion of bile from the liver. Gastric HCl also stimulates the secretion of pancreatic juice by release of the polypeptide hormone secretin. The secretion of pancreatic juice which provides sodium, potassium, chloride and bicarbonate ions, and some active trypsin, is also stimulated by signals from vagal nerve fibres due to presence of food in the stomach (Frape, 2004).

Enzymes like α-amylase, sucrase and α-glucosidases (glucoamylase, maltase and isomaltase) are important for the digestion of soluble carbohydrates. Oligosaccharides and starch are broken down by enzymes into monosaccharides that can be absorbed as glucose and fructose through the intestinal mucosa. Structural carbohydrates like cellulose and hemicelluloses are not degraded in the small intestine but have an important role for the viscosity and water holding capacity of the digesta which influence the passage rate. A decrease in passage rate promotes uptake of nutrients. Soluble carbohydrates that are not degraded in the small intestine are rapidly fermented by the microbes in the large intestine (Ellis and Hill, 2006).

The amount of protein digested in the small intestine is about three times higher than in the stomach. Inactive pancreatic proteases secreted into the small intestine converts into active endopeptidases like trypsin, chymotrypsin and elastase which hydrolyse peptides into amino acids. The amino acids are absorbed across the intestinal wall and undegraded protein will be degraded by the hind-gut microflora (Frape, 2004).

The small intestine is also the main site for digestion and absorption of dietary fat and long-chain fatty acids. Bile salts emulsify fat, enabling a more efficient hydrolyse by lipase into fatty acids, mono-glycerides and glycerol. These are absorbed through the intestinal wall and re-esterified to triglycerides (Frape, 2004).

The large intestine

The large intestine of the horse is the major site of microbial fermentation and it is divided into the caecum, the large colon, the small colon and the rectum. Different compartments in the large intestine are defined due to the position of flexures and degree of sacculation (de Fombelle *et al*., 2003).

Microbial fermentation takes place in the caecum and the large colon. The caecum of the horse is approximately one meter in length and holds 25-35 litres, which is about 16% of the total volume of the GI tract. The large colon is divided into the right ventral colon, the left ventral colon, the left dorsal colon and the right dorsal colon which in total is approximately three to four meters in length and with a capacity of 50-60 litres. Passage of digesta through the large intestine depends on gut mobility and a rapid passage rate can be a limiting factor in degradation of nutrients. Muscular contractions, which increase during feeding, also enable mixing of digesta, which is important for the microbial digestion. Retrograde of digesta through the large intestine is obstructed by valves forming barriers between ileum and caecum, caecum and ventral colon, ventral and dorsal colon and also between dorsal colon and small colon (Frape, 2004).

The digestive tract contains no enzymes that can digest complex molecules such as cellulose, hemicellulose, pectin, fructo- and galacto-oligosaccharides. Microbial fermentation is therefore essential. Lignin on the other hand cannot be digested by the microbes and reduces the fermentation of the plant cell walls. Besides structural carbohydrates, lactic acid, undigested protein and soluble carbohydrates are also fermented in the large intestine. The fermentation process is slow in comparison to the enzymatic digestion of starch and protein (Frape, 2004). In order to enable digestion of the structural carbohydrates, the passage rate of the digesta has to be slow and the retention time in the caecum and colon is on average 35 hours (Van Weyenberg *et al.,* 2006). Dietary proteins and urea, as a source of nitrogen, is important for the microbial growth. Urea from the liver is secreted into the ileum and transported to the large intestine where it is degraded by microbes to ammonia. The degradation of urea is possible due to the enzyme urease produced by the intestinal bacteria. Some of the ammonia produced diffuses into the blood while most of it is reutilized by the microbes (Frape, 2004).

Acetate, butyrate, propionate and lactate are short chain fatty acids (SCFA), produced during the microbial fermentation in the hindgut (Glinsky *et al*., 1976; Frape, 2004). Acetate and butyrate originate mainly from the digestion of structural carbohydrates, whereas propionate and lactate increase by the digestion of starch. The SCFA are absorbed across the epithelium of the large intestine and are later utilized as a source of energy. The microbes also produce essential amino acids for the horse, vitamins of the B group and vitamin K2. Carbon dioxide, methane and hydrogen, are also produced during fermentation. The gases are absorbed, ejected from the rectum or participate in further metabolism (Frape, 2004).

The small colon, following the large colon, with a length of approximately 3.5 meters, is one of the main sites of water and mineral absorption. Rectum, 30 cm in length, store and expulse gases and faeces and is the last part of the GI-tract (Ellis and Hill, 2006).

Factors causing variation in digestibility between horses

Digestibility may vary among horses, *e.g.* Udén and Van Soest (1981) noticed a large individual variation in digestibility between horses in their study. The results showed a lower value (0.297) for cell wall digestibility of the largest horse, weighing 500 kg, compared to the smallest horse (0.437), weighing 90 kg. Besides forage quality and composition, feed intake, breed effect, dental health and physical condition are some factors that have been discussed, causing variation in digestibility between horses (Janis, 1976; Pond *et al*., 1984; Pagan *et al*., 1998; Edouard *et al*., 2008).

Some studies have concluded that horses that are fed forage of low digestibility respond with an increased feed intake (Janis, 1976; Edouard *et al*., 2008). The increased intake makes it possible to still fulfil the nutritional needs. Unlike ruminants, horses are not limited by rumen capacity, and the reduction of feed particles to the small size required passing through the GI tract. The reduction in particle size and the slower passage rate in ruminants results in a higher digestibility of fibre compared to horses. A limited feed intake is, however, a drawback for the ruminant in maintaining the nutritional needs when fed a low quality diet. It should be mentioned that, in the study performed by Edouard *et al*. (2008) all horses did not respond in the same way when fed different forages. Some horses compensated for the low nutritional value of the forages by an increased intake, whereas a few horses decreased forage intake, but not enough to cause any nutrient deficit. However, for grass forages with a decline in digestibility, most individuals compensated by an increased voluntary intake (Edouard *et al.*, 2008).

The amount of feed may also influence digestibility of forage, since an increased passage rate results in lower DMd, due to incomplete digestion and absorption of feed particles. According to Cuddeford *et al*. (1995), digestibility tends to be more efficient on lower DM intakes. This is in accordance with Ragnarsson (2009), who found that apparent DMd was reduced as feeding level increased.

Chewing and disruption of forage is important since it makes the feed particles smaller and increases the area available for the fermenting microorganisms. The crushing action of the mastication disrupts barrier tissues and exposes more digestible tissue (Ellis *et al.*, 1979). Important factors in the mastication of feeds, that may cause individual variation between horses in the digestion of a feed, are the surface morphology, the shape of crown of tooth, and contact of occlusal surfaces (Murphy and Kennedy, 1993). Sharp edges, broken or missing teeth may reduce the grinding capacity (Lewis, 1995) or cause damage to the inside of the mouth leading to a reduction in chews. However, small points and hooks does not seem to affect digestibility and the effect of severe abnormalities has been proven in a controlled study (Ralston *et al.*, 2001). A greater degree of lignification will influence the mastication of forage to smaller particles since the fracture properties of the plant changes. Lignin increases the rigidity of the cell wall and makes it more likely to break or shatter than bend. Therefore, smaller particles do not have to be more digestible if the constituents are indigestible, like lignin (Pond *et al*., 1984).

Exercise has been shown to reduce the apparent DMd and also the passage rate of digesta (Pagan *et al*. 1998). An increased intake of water may contribute to the increased mean retention rate. The question is how the exercise influences the digestibility and how other factors, *e.g.* frequency of feeding and amount of water contribute to the differences in digestibility among horses (Khan *et al*., 2003).

Measurements of digestibility

Digestibility is a measure of how much of a particular nutrient or DM that is absorbed by the animal during the passage of digesta through the GI tract (Frape, 2004). Digested feed may be defined as the proportion of digesta which is not excreted in the faeces. In a digestibility trial, animals are fed with a particular feedstuff during three to four weeks and faeces are collected during the last one or two weeks. The proportion of absorbed nutrients can be underestimated due to excretion of enzymes and other substances in the GI tract, which are included in the faeces. For that reason, digestibility is expressed as apparent digestibility since true digestibility can be difficult to determine. A common equation to calculate the apparent digestibility as a coefficient is:

$$\text{Apparent digestibility} = \frac{\text{(Nutrient consumed – Nutrient in faeces)}}{\text{Nutrient consumed}}$$

(McDonald *et al*., 2002).

Quantitative collection technique

The quantitative or total collection of faeces technique is the method considered to be most accurate in the assessment of nutrient digestibility. The method has been tested on a large number of feeds and a significant number of horses. During the quantitative collection technique, total feed intake is registered and a total collection of faeces is performed. A sub-sample of the feedstuff is retained for analysis and approximately 10% of the daily faecal output is pooled together to represent faecal output (Bergero *et al*., 2009). To collect all faeces and, if necessary all urine, the horses are either equipped with a collection harness or placed in metabolism stalls for five to six days (Goachet *et al*., 2009). Even if the total collection method is the most accurate measure of apparent digestibility, it is time consuming, costly and difficult to perform with athletic horses in training and competition. The necessity to change the environment of the horse due to the collection equipment or use of metabolism stalls can also be a drawback of the quantitative collection technique (McDonald *et al*., 2002).

Indirect measurement with an indicator

An alternative method to total collection of faeces and urine in digestibility studies could be the use of an indigestible marker, i.e. an indicator (McDonald *et al*., 2002). This method allows faecal grab samples, instead of total collection of faeces, which is less time consuming and enables minimal changes of daily routines (Van Keulen and Young, 1977; Miraglia *et al*., 1999; Goachet *et al*., 2009). Apart from being indigestible, the indicator should be evenly excreted in the faeces and easy to analyse. The indicator can be a natural constituent of the feed, an internal marker, or an added chemical, an external marker (Palmgren Karlsson *et al*., 2001). Examples of internal markers are acid-insoluble ash (AIA), acid-detergent fibre, lignin and some naturally occurring n-alkanes (C25-C35). Chromic oxide (Cr_2O_3), ferric oxide, silver sulphide and polyethylene glycol are examples of external markers (Miraglia *et al*., 1999; McDonald *et al*, 2002).

The concentration of the indicator in the feed and also in faecal grab samples from each horse participating in the trial needs to be determined to give an estimation of the digestibility. However, the use of markers and estimation of apparent digestibility by faecal grab samples should be interpreted with caution. An important indicator of the reliability of a marker is the

recovery, which is the quantity collected from the total collection of faeces expressed as a proportion of that consumed (Jagger *et al*., 1992).

Acid-insoluble ash (AIA) as an internal marker

Acid insoluble ash (AIA), which may be composed predominantly of silicate (Van Keulen and Young, 1977), can be used as an indicator in digestibility trials since it is an easy and cheap method (Bergero *et al*., 2009). Satisfactory results by the use of AIA as an internal marker in digestibility trials with horses have been reported by Sutton *et al*. (1977), Van Keulen and Young (1977) and Bergero *et al*. (2004). However, other reports have observed overestimated digestibility values with the AIA-method (Miraglia *et al*., 1999; Goachet *et al*., 2009). Even if the digestibility values were overestimated, there were no statistical differences in digestibility coefficients between the AIA method and total collection of faeces (Miraglia *et al*., 1999). The overestimation of apparent digestibility coefficients with the AIA-method may be due to an underestimation of the concentration of AIA in the feed or an overestimation of AIA in the faeces. The content of AIA in both feed and faeces may be influenced by environmental contamination, such as soil particles (Goachet *et al*., 2009).

Bergero *et al*. (2009) compared the use of 2N HCl with 4N HCl for analysis of AIA in feed and faeces, and came to the conclusion that 2N HCl was easier and cheaper, since no differences were found between 2N and 4N HCl regarding averages and accuracy. This conclusion corresponds with results from Van Keulen and Young (1977), who compared concentrated HCl, 2N HCl and 4N HCl, and concluded that 2N HCl was the most convenient and the least time consuming of the three AIA procedures evaluated.

Factors affecting faecal particle size

Forage particles are minced in the oral cavity of the horse and sound teeth reduce forage particles to less than 1.6 mm in length (Meyer *et al*., 1975; Frape, 2004). Two thirds of the hay particles in the stomach of the horse are less than 1 mm across (Meyer *et al.*, 1975), but according to Meyer *et al*. (1985), more than 30% of the particles were above 1.5 mm and up to 12 mm in length.

Ellis (2003) compared faecal particle size distribution between hay and silage, where silage seemed to fragment more easily than hay. A reason for this may be the lower physical resilience to mastication of silage (Vincent, 1990). Another suggestion was a slower rate of ingestion of the ensiled feed and therefore a more careful mastication resulting in a greater proportion of smaller particles in faeces by horses fed silage (Ellis, 2003). In a study by Müller (2009), faecal particle size distribution of long-stemmed and cut haylage was analysed. The results showed no difference between the forage types. However, differences among individual horses occurred in particle size fraction 4.0 mm, 1.0 mm and 0.25 mm.

Fragmentation of forages by ingestive mastication by cattle was examined by Pond *et al*. (1984). Their study showed that the smallest fraction sizes were composed of rigid tissues and vascular bundles. Pond *et al*. (1987) examined fragmentation in the digestive tract of cattle, and their study showed that fracture occurs between the vascular bundles and the severing of vascular bundle ends. Particles between 0.1 mm and 0.25 mm contained more lignified tissue and less leaf material compared to larger particles, collected using the 0.25 mm sieve. Based on particle anatomy and histochemistry, particles less than 0.1 mm was composed of more indigestible tissue compared to most of the larger particles. This is in accordance with the

fracture properties of lignin, which increases the rigidity of the cell wall and makes it more likely to break than bend (Pond *et al*., 1984).

Carmalt *et al*. (2005) examined if the occlusal angle of the molars had an effect on faecal particle size in adult horses, but according to the results this was not the case. In another study, Carmalt and Allen (2008) came to the conclusion that faecal particle size was not associated with oral pathology score, since there were no differences in faecal particle size distribution between horses with high and low scores. The oral pathology score was determined through the sum of all dental lesions found (Carmalt *et al.*, 2004). Stomach content and faecal particle size distribution did not differ between horses that were given three different feeds with different particle sizes. Since there were no differences between the particle size of the stomach content and faecal particle size, results indicate that there were no reduction in particle size as the digesta passed through the GI tract. This suggests that most of the reduction in feed particle size is due to mastication in the oral cavity (Carmalt and Allen, 2008).

MATERIAL AND METHODS

The aim of the experiment was to estimate the apparent DMd of haylages harvested from primary growths of the same sward in June, July and August, with AIA as an internal marker. The effect of plant maturity at harvest of haylage on particle size distribution in faeces was also evaluated using wet sieving of faecal samples.

Experimental design and faecal samples

The forage and faecal samples were collected during a change-over experiment performed at Jällaskolan, Uppsala, Sweden, during the autumn 2009. The experiment was carried out during three periods. Each period consisted of three weeks. Twelve horses (Warmblood type) were included in the trial. These were divided into three groups (A, B and C) depending on age, size and gender (Table 1). All horses were clinically examined and measured for live weight estimation, body condition scored (Carroll and Huntington, 1988) and checked for oral cavity status (dental floating was performed if necessary). During the experimental period, the horses were used in the ordinary school activities (riding lessons) and kept in the same stable under the same conditions. Each horse was kept in a single box with wood shavings and peat as litter. During daytime they were kept single or in pairs in paddocks without edible material. The horses were fed four times a day; morning and lunch meals were fed outside in the paddocks, whereas afternoon and evening meals were fed inside the stable.

Table 1. Information about the horses taking part in the trial. Gender are expressed as mare (M) or gelding (G). Weight and body condition score are mean values of data assembled from 5th of October to 4th of December 2009

	Group	Gender	Age	Weight (kg)	Body Condition Score
Horse 1	A	M	14	645	3.2
Horse 1b	A	M	15	579	3.1
Horse 2	A	G	12	561	2.9
Horse 3	A	G	19	617	3.0
Horse 4	A	G	9	563	3.4
Horse 5	B	M	7	563	3.1
Horse 6	B	G	17	556	3.2
Horse 7	B	G	13	581	3.0
Horse 8	B	G	16	601	3.1
Horse 9	C	M	13	585	3.4
Horse 10	C	G	17	611	3.0
Horse 11	C	M	7	630	3.5
Horse 12	C	G	14	627	3.3

Three different haylages, harvested from the primary growth of the same sward but at different plant maturities (June, July and August), were used in the experiment. During each period, all horses in the same group were given the same haylage harvest (Figure 3). Feed changes between periods were done during two days. The two first weeks in each period were adaptation weeks, and faecal samples were collected during the third week. Fresh faeces from each horse was collected once daily during five days. The samples were stored in plastic bags in a temperature of -18 ºC. Since each horse participated during three periods, there was a total amount of 15 faecal samples per horse. One horse, nr 1, was put away during the experiment (for other reasons), but was replaced during the last period with a new horse, 1b.

	June haylage	**July haylage**	**August haylage**
Period I	Group A average BW 602 kg (sd 42.6 kg)	Group B average BW 613 kg (sd 17.4 kg)	Group C average BW 570 kg (sd 16.7 kg)
Period II	Group B average BW 575 kg (sd 17.4 kg)	Group A average BW 591 kg (sd 42.8 kg)	Group C average BW 609 kg (sd 20.9 kg)
Period III	Group C average BW 609 kg (sd 23.1 kg)	Group B average BW 572 kg (sd 20.9 kg)	Group A average BW 572 kg (sd 28.5 kg)

Figure 3. Experimental design. Group averages for horse live weight during each period and the standard deviations (sd) are given within in the figure.

Forage

The primary growth was harvested from the same ley during three different occasions; 8th June, 2nd July and 5th August 2009 at Kungsängen, SLU, Uppsala. After cutting with a mower conditioner with flails (Kverneland Taarup 4028, Kverneland, Nyköping, Sweden), the herbage was wilted, tedded with a conventional hay tedder (Claas WaS 730, CLAAS, KgaAmbH, Harsewinkel, Germany), raked using a windrower (Krone KS 3.80 - 4.20 Vario, Bernard Krone Holding GmbH & Co., Spelle, Germany) and baled with a combined baler/wrapper (Taarup Bale-in-One, Kverneland Taarup, Nyköping, Sweden). The bales, with a width of 1.22 m and a diameter of 1.25 m, were wrapped with ten layers of white stretch film (Silotite, BPI Agri, London, UK), marked individually and stored at Kungsängen, Uppsala until transport to Jällaskolan.

The haylage, with DM content between 500-600 g/kg, contained timothy, meadow fescue, red clover and a small amount of perennial ryegrass. For chemical analysis, a minimum of six samples, from each haylage bale, were pooled together to produce one sample per bale. Samples were collected at opening of haylage bales for feed-out and then stored at –18ºC before analysis. Chemical composition and the proportion of clover in the haylage was given before the experiment (Müller, C. pers. comm., 2010) and depended on harvest date (Table 2).

Table 2. Chemical composition of haylage and proportion of red clover in the haylages used in the experiment (data from other report (Müller, C. pers. comm., 2010))

Variable	June harvest	July harvest	August harvest	Std err	P
Dry matter, g/kg	549	573	583	10.8	0.09
Estimated metabolizable energy for horses, MJ/kg DM	12.4[a]	9.1[b]	7.7[c]	0.10	<0.0001
Crude protein, g/kg DM	130[a]	93[b]	80[c]	3.6	<0.0001
Estimated digestible crude protein, g/kg DM	91[a]	56[b]	44[c]	3.4	<0.0001
Neutral detergent fibre, g/kg DM	522[a]	610[b]	637[c]	9.0	<0.0001
Lignin, g/kg DM	56[a]	85[b]	100[c]	2.4	<0.0001
Total water soluble carbohydrates (calculated), g/kg DM	101[a]	73[b]	49[c]	3.2	<0.0001
Ash, g/kg DM	75	71	70	1.5	0.08
Calcium, g/kg DM	7.1[a]	7.3[a]	8.7[b]	0.41	0.0165
Phosphorous, g/kg DM	1.7[a]	1.3[b]	1.0[c]	0.05	<0.0001
Magnesium, g/kg DM	1.5	1.5	1.7	0.11	0.3730
Proportion of red clover	0.003[a]	0.036[a]	0.262[b]	0.03851	0.0001

[a,b,c] *Different letters within rows indicate difference at the given P-value.*

In addition to haylage, all horses were fed pelleted commercial mineral feeds (Krafft Miner Blå pellets and Krafft Miner Vit pellets, Krafft, Falkenberg, Sweden) and molassed sugar-beet pulp (Betfor®, Nordic sugar, Copenhagen, Denmark), depending on their nutrient requirement. The chemical composition of these feeds given by the commercial companies is reported in Table 3. All horses also had access to salt lick stones and water *ad libitum*. Average feeding level was 1.7 kg DM/100 kg body weight (sd 0.22). The proportion of haylage fed was on average 0.94 (sd 0.011), whereas the proportion of molassed sugar-beet pulp fed was 0.05 (sd 0.009). For more details about the feed rations see Appendix 1.

Table 3. Chemical composition of mineral feeds (Krafft Miner Blå and Krafft Miner Vit) and molassed sugar-beet pulp (Betfor) according to the information available from the manufacturers, and dry matter content analysed in this experiment

Variable	Krafft Miner Blå	Krafft Miner Vit	Betfor
Dry matter, g/kg	947 [a]	937 [a]	900
Energy MJ/kg			11.2
Digestible crude protein g/kg			57
Water soluble carbohydrates g/kg			200
Calcium g/kg	120	55	7
Phosphorous g/kg	30	65	1.1
Magnesium g/kg	60	60	1.1
Sodium chloride g/kg	125	125	12
Copper mg/kg	900	*900*	
Selenium mg/kg	15	15	

[a] *Analysed in this thesis*

Digestibility

In order to estimate the apparent DMd of the different haylage diets, acid insoluble ash (AIA) was used as an internal marker. Equal amounts of five faecal samples from each horse during each period were pooled together to one sample weighing 250 g, representing one horse during one period/haylage. The mixed samples were dried at 60 ºC during 24 h. After air equilibration, the samples were weighed and ground in a hammer mill (KAMAS 200 B) fitted with a 1 mm screen.

The forage was prepared in a manner similar to the faecal samples; after drying at 60 ºC during 24 h the samples were ground in a hammer mill with a 1 mm sieve (KAMAS 200B). Haylage samples for AIA analysis were taken from specific bales that were used during the collection of faecal samples. Grab samples taken daily from one bale were pooled to produce one sample. If more than one bale from the same harvest were used during the faecal collection week, samples from all used bales were pooled to one sample. There was a total amount of nine haylage samples for the AIA analyses; one sample per haylage harvest (June, July and August) and period (three periods). Molassed sugar-beet pulp, mineral feeds and salt were also analysed for AIA.

Faecal and feed samples were also analysed for total DM and ash content. From each ground sample, 2 g were dried for 16 h at 103ºC. After cooling in an desiccator, the samples were weighed and DM content calculated. Ash content was determined by incineration of the DM samples in 550 ºC for 3 h.

For analysis of AIA-content, 6-7 g of the ground faecal samples was used and 10-15 g of the different feedstuffs. A control sample and an empty crucible were also included. The empty crucible was used to determine the weight of an ashed quantitative filter paper used for the filtration process described below. The weight of the ashed filter paper was deducted from the weight of the final samples of AIA (ashed during one hour in 600ºC), since the samples were ashed together with the filter paper. To begin with, the samples were ashed for 16 h at 450 ºC and then moved to 250 ml test tubes (Kjeldahl). Then 100 ml of 2M HCl was added and the

mixture was boiled in a 2020 Digestor (Foss Tecator) for five minutes. At last, the samples were filtered through 150 mm quantitative filter paper (00R, Munktell Filter AB). Boiled distilled water was used to wash both the sample and filter in order to get rid of the acid. The sample and filter were then transferred back into the crucible and dried for 16 h at 103ºC and after that ashed during one hour in 600ºC. After cooling to room temperature in a desiccator, the crucible was weighed with ash (Wf) and then re-weighed without ash (We). The percentage of AIA could then be calculated from the equation:

$$(Wf - We) / Ws * 100$$

where, Wf is the weight of the crucible and ash, We is the weight of the empty crucible and Ws is the weight of sample dry matter.

The DMd was calculated according to Van Keulen and Young (1977).

$$DMd (\%) = (1-A/B) * 100$$

where, A and B are the AIA concentrations in feed and faeces, respectively.

Particle size distribution in faeces

To determine the particle size, wet sieving with stainless steel sieves of mesh size 2.0, 1.0, 0.63, 0.315, 0.2 and 0.1 mm were used. Equal amounts of the five faecal samples from each horse during each period were pooled together to one sample weighing 250 g per horse and period. The frozen faecal samples were thawed and 100 g of each sample was mixed with tap water with a temperature of approximately 37ºC. The dissolved sample was poured through the sieve rack followed by 8 l of water to distribute the particles to the right sieve size. No sieve shaker was used. Gentle rinsing with water transferred all particles from the sieve to a cloth filter. The particles from the different fractions and the cloth filter were dried in 55ºC for 20-24 h, and then weighed immediately after drying. The proportion of the different fractions (F_X) of particle sizes was calculated as:

$$WF_X / Ws * 100$$

where, WF_X was the weight of a particular fraction and Ws was the total sample weight.

Statistical evaluation

For statistical evaluation SAS 9.2 for Windows was used (SAS, 2001). For differences between horses and haylage (harvested in June, July or August) in apparent DMd and particle size distribution in faeces the SAS Mixed Models Procedure was used with the model:

$$Y_{ijk} = \mu + (horse)_i + (period)_j + (haylage)_k + (error)_{ijk}$$

and with statements: "DDFM = kenwardroger" and "repeated/sub horse*period type = un" to take into account that individual horses were sampled repeatedly within forage type and period. Differences among means where P-values <0.05 were regarded as statistically different.

RESULTS

Digestibility

The content of DM, ash and AIA in the different haylages is reported in Table 4. Content of DM, ash and AIA in minerals, molassed sugar-beet pulp and salt are reported in Table 5.

Table 4. Mean values of dry matter content (DM), total ash content, and content of acid-insoluble ash (AIA) in the different haylages. Values within parenthesis represent standard deviation

Variable	June haylage	July haylage	August haylage	P
Dry matter, g/kg	536 (3.8)	554 (35.3)	554 (35.0)	0.6946
Ash, g/kg DM	81 (0.5)	74 (6.1)	78 (7.8)	0.4076
AIA, g/kg DM	14 (0.7)	21 (5.9)	24 (7.7)	0.2316

[a,b,c]*Different letters within rows indicate significant difference at the P level listed.*

Table 5. Dry matter content (DM) (n=1), ash content (n=1) and content of acid-insoluble ash (AIA) (n=2) in mineral feed (Krafft Miner Blå and Krafft Miner Vit), molassed sugar-beet pulp (Betfor) and salt. Values within parenthesis represent standard deviation

Variable	Krafft Miner Blå	Krafft Miner Vit	Betfor	Salt
Dry matter, g/kg	947	937	900	997
Ash, g/kg DM	623	529	75	990
AIA, g/kg DM	45 (0.8)	27 (0.6)	8 (0.3)	0 (0.1)

The apparent DMd differed between the three diets (Figure 4). The diet containing haylage harvested in June had the highest digestibility (P=0.0022), and the diet containing haylage harvested in August had the lowest digestibility (P=0.0005).

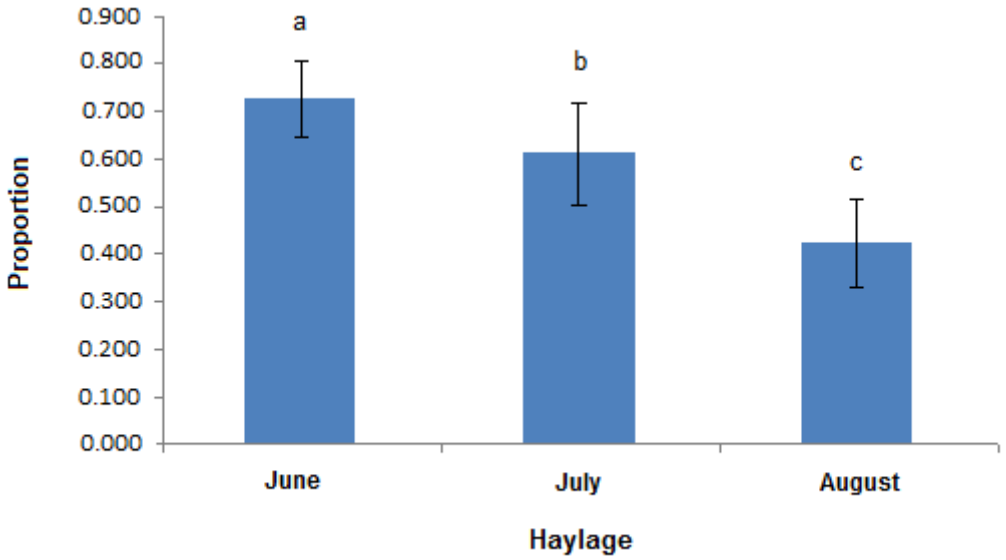

Figure 4. Digestibility of the three haylage diets. Different letters indicate difference at P<0.0022. Bars show standard deviation.

There was no general difference in digestibility between horses or periods, but digestibility values for haylage diets differed. Most of the horses showed highest digestibility values when fed the diet containing haylage harvest in June, followed by lower values for diets containing haylage harvested in July and August (Table 6). The exceptions were horse number 6, 8 and 9. Horse number 6 had a similar digestibility between the diets containing June and July haylage (0.66), whereas horse number 8 had higher digestibility for the diet containing haylage cut in August (0.54) than the diet containing haylage cut in July (0.45). Horse number 9 differed from all other horses since the digestibility increased with maturity of haylage (0.61<0.64<0.68). Horse number 9 had the highest value for apparent DMd, when fed the diet with August haylage, of all horses in the trial. A statistical run excluding horses number 8 and 9, resulted in a general effect of horse in in DMd (P=0.0101).

Table 6. Acid-insoluble ash (AIA) in feed rations and faeces, and apparent dry matter digestibility (DMd) of the different diets containing haylage cut in June, July and August

	AIA feed, g/kg DM	AIA faeces, g/kg DM	DMd
June haylage diet			
Horse 1	13.2	75.4	0.82
Horse 2	13.2	49.2	0.73
Horse 3	13.2	59.6	0.78
Horse 4	13.2	78.9	0.83
Horse 5	14.5	61.9	0.79
Horse 6	14.5	42.7	0.66
Horse 7	14.7	43.8	0.67
Horse 8	14.6	48.3	0.70
Horse 9	14.2	61.2	0.61
Horse 10	14.2	57.9	0.59
Horse 11	14.3	55.3	0.74
Horse 12	14.3	67.4	0.79
July haylage diet			
Horse 1	13.0	60.3	0.79
Horse 2	13.0	28.1	0.54
Horse 3	13.0	38.9	0.67
Horse 4	13.0	50.0	0.74
Horse 5	23.6	54.1	0.69
Horse 6	23.6	50.1	0.66
Horse 7	23.7	43.4	0.46
Horse 8	23.7	43.2	0.45
Horse 9	24.1	66.0	0.64
Horse 10	24.0	58.0	0.59
Horse 11	24.3	58.3	0.58
Horse 12	24.3	51.5	0.53
August haylage diet			
Horse 1			
Horse 2	16.9	25.3	0.34
Horse 3	16.8	31.7	0.47
Horse 4	16.8	30.0	0.44
Horse 5	32.2	60.0	0.46
Horse 6	32.2	57.9	0.44
Horse 7	32.6	52.0	0.37
Horse 8	32.4	71.0	0.54
Horse 9	20.2	45.6	0.68
Horse 10	20.2	31.9	0.37
Horse 11	20.2	40.1	0.50
Horse 12	20.3	37.0	0.45

Particle size distribution in faeces

There was no difference in faecal particle size distribution between horses or periods, but between diets differences were present. In all particle size fractions, except the fraction with the largest particle size (>2.0 mm), there was a difference between the diet containing June haylage compared to both July and August haylage diets (Figure 5). The proportion of faecal particles from horses fed June haylage compared to horses fed July and August haylages, was less from fraction size 0.1 to 2.0 mm, but higher in fraction size <0.1 mm. In particle size fraction 0.2 mm there was also a difference between July and August haylage diets. The largest proportion of particles occurred in fraction size >2.0 mm followed by fraction size <0.1 mm. Minimum, median and maximum of the particle size distribution in faeces among horses and the different haylage diets are displayed in Table 7.

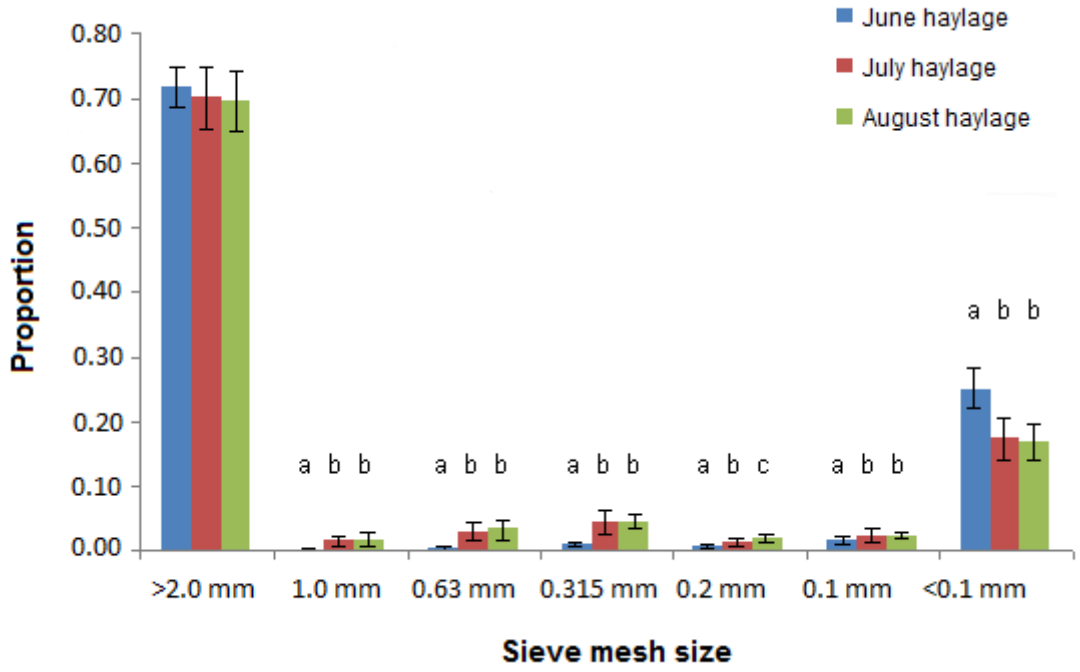

Figure 5. Particle size distribution in faeces from horses fed diets containing haylage harvested in June, July and August. Different letters (a, b, c) indicate difference at P<0.0346. Bars show standard deviation.

When all faecal particles less than 2.0 mm were pooled together and compared with particles larger than 2.0 mm, there was no difference in particle size distribution (smaller or larger than 2.0 mm) in faeces from horses fed haylages harvested in June, July or August.

Table 7. Proportions of minimum, median and maximum values of particle size distribution in feaces between horses fed diets containing haylage harvested in June, July and August

	>2.0 mm	**1.0 mm**	**0.63 mm**	**0.315 mm**	**0.2 mm**	**0.1 mm**	**<0.1 mm**
June haylage							
Min	67.4	0.0	0.0	0.4	0.0	0.8	19.8
Median	70.8	0.0	0.0	0.9	0.6	1.3	25.6
Max	78.9	0.9	0.9	1.6	1.4	2.7	29.6
July haylage							
Min	61.9	0.5	1.5	2.4	0.0	0.8	9.4
Median	70.4	1.6	2.9	3.7	1.5	2.2	17.5
Max	75.6	2.9	5.0	8.8	2.3	4.4	21.6
August haylage							
Min	62.2	0.0	0.9	1.8	0.9	1.3	13.5
Median	67.9	2.1	3.3	4.6	1.8	2.6	16.8
Max	77.4	3.2	4.7	5.9	3.5	3.1	23.6

DISCUSSION

Digestibility

The apparent DMd decreased with increased plant maturity. This is in accordance with the literature (Darlington and Hershberger, 1968; Fogelfors, 2001; McDonald *et al*., 2002; Edouard *et al*., 2008). The haylage harvested in June probably had a lower proportion of cell wall compared to the haylage harvested later in the season (Janis, 1976). An increased stem/leaf ratio and a higher proportion of lignified plant cell walls are certainly main reasons for decreased digestibility of the more mature August haylage. Feeding level may have influenced the results as well, since feed rations differed between diets.

The difference in apparent DMd between June and July haylage compared with August haylage would probably be even more evident with a pure grass haylage, since the August haylage had a red clover proportion of 0.25. The increase in lignin concentration during growth is less dramatic among legumes compared to grasses. This, together with a higher concentration of cell contents, make legumes more digestible compared to grasses in a later developmental stage of growth (Moore and Jung, 2001). Unharvested grass goes through a reproductive phase and seed ripening, producing new tillers. Younger plants are more digestible than older lignified plants. However it may not affect the digestibility of the forage. When plants grow close together the competition of important resources such as light, water and nutrients, limits the growth and appearance of new tillers (Fogelfors, 2001). For example, red clover is growing tall, reducing the grasses possibilities to catch sunlight. The growth rate in the established sward is also affected by environmental factors such as temperature and light intensity. The development of leaves is more effective in the beginning of the season since the photosynthesis is more intense in spring than later in the season. This contributes to the slow development of new plants in the sward (Parsons and Chapman, 2000). The haylage harvested in June probably had a larger proportion of leaves and less lignified cell walls due to the developmental stage of the plants, less competition of important resources between plants in the sward and an effective leaf growth due to a more intense photosynthesis.

There was no general effect of horse or period concerning apparent DMd when all horses were included in the statistical evaluation. Most of the horses followed the expectations with the highest DMd value for the early harvested forage, followed by a decrease in DMd with increasing plant maturity. Since the feed rations were calculated to maintain the same energy content irrespective of which haylage they were fed, the amount of feed or feeding level also increased with haylage maturity. This is due to a decrease in energy content with plant maturity. The higher feed intake may increase passage rate and contribute to a lower digestibility of the later harvested haylages (Ragnarsson, 2009). However, some values of apparent DMd for individual horses were exceptions, i.e. horse number 6, 8 and 9. Horse number 9 had increasing apparent DMd values with plant maturity, which is the opposite to most of the horses in the trial. When fed the diet containing the haylage harvested in August, the apparent DMd value of horse number 9 was highest among all horses.

There may be several reasons for an increased digestibility with increased plant maturity for horse number 8 and 9. Registered and calculated data concerning horse number 9 showed a higher proportion of ash in faeces when fed the diets containing July and August haylage, compared to the other horses in the same group. The higher proportion of ash in faeces may be due to a better utilization of fibrous feed compared to the other horses in the group. The high apparent DMd of horse number 9, when fed the diet with August haylage, indicates a better utilization of fibrous feed compared to the other horses in the trial. Other reasons for

high apparent DMd, when fed August haylage, may be an underestimation of AIA in feed or an overestimation of AIA in faeces (Goachet *et al*., 2009). The underestimation of AIA in feed is possible due to unexpected intake. This may be due to ingestion of sand which increases the total intake of ash content. Environmental contamination of grab samples may cause an overestimation of AIA in faeces. It is also important not to forget about the human factor. Giving a horse the wrong feed ration, making mistakes in the laboratory, register wrong values or making errors while calculating are factors that influence the results.

The difference between horses in apparent DMd, when horses number 8 and 9 were excluded from the statistical evaluation, was probably due to removal of the outlier values. The decreased distribution of apparent DMd values contributed to a more gathered data set and a significant difference between horses. Since a lower DM intake may lead to a more efficient digestibility (Cuddeford *et al.*, 2005; Ragnarsson, 2009), the individually calculated feed rations may be a reason for differences in apparent DMd between horses. Chewing and disruption of forage are other factors that may contribute to differences in apparent DMd between horses (Murphy and Kennedy, 1993; Ralston *et al*., 2001).

AIA as an internal marker for digestibility measurements

The use of AIA as an internal marker made it possible to estimate the apparent DMd since quantitative collection techniques was not feasible for the horses participating in the trial. To take grab samples once a day does not influence the daily routines with the school horses like the quantitative collection of faeces would do. The equipment used for total collection of faeces would prevent horses from participating in the riding-lessons. A total collection of faeces is more time consuming, needs larger room for storage and is in total more expensive. The use of an internal marker and grab samples also made it possible to do measurements on several horses at the same time since no special equipment was needed (Van Keulen and Young, 1977).

Using a natural constituent of the feed as an indicator also facilitates the performance of the trial. An external marker, i.e. chromic oxide, needs to be added to the feed ration specifically or added orally. However, chromic oxide is not recommended due to its irregular faecal excretion (Palmgren-Karlsson, 2001). According to several studies (Cuddeford and Hughes, 1990; Miraglia *et al*., 1999; Palmgren-Karlsson, 2001), AIA is more reliable as an indicator in digestibility trials, compared to other internal markers, i.e. lignin. The conclusions of earlier studies facilitated the choice of AIA as an internal marker of apparent DMd in this trial.

It is impossible to know if the values for apparent DMd in this trial were satisfactory since no total collection of faeces was made to compare the results with. When comparing mean values of apparent DMd from the different haylages in this study with other studies (Darlington and Hershberger, 1968; Miraglia *et al*., 1999; Bergero *et al*., 2002; Ragnarsson, 2009), the apparent DMd of the diet with June haylage was quite high, whereas the DMd of the diet with August haylage was low. As mentioned in the discussion about digestibility, there was a difference between horses in DMd. Looking at the individual values for DMd of June haylage diet, some horses (number 1, 3, 4, 5 and 12) had DMd close to or above 0.80. The same individuals also had the highest amount of AIA in faeces (g/kg DM) for the June haylage diet. This indicates a very good ability to digest the June haylage or an overestimation of DMd. Looking at the individual values for DMd of the diet containing August haylage, horse number 2, 7 and 10 had values below 0.40. Horse number 2 and horse number 10 also had the

lowest amount of AIA in faeces (g/kg DM), indicating a poor digestibility of August haylage or an underestimation of DMd.

The values of the AIA control samples seemed to be in the range of what was normal and that is an indicator of a successful laboratory work. The estimation of digestibility may be influenced by other constituents in faeces, i.e. enzymes, minerals and other substances secreted into the gut (McDonald *et al*., 2002). The excretion of other substances into the faeces leads to an underestimation of digestibility. Underestimation of digestibility may also be due to overestimation of AIA in feed, which is possible if the horse refuses to consume the forage. An increased feed intake may also lower the digestibility because of an increased passage rate, which may have influenced the DMd of the horses consuming the August haylage (Ragnarsson, 2009). The large variation of AIA in feed rations (Table 6) is probably due to low values of AIA in the June and August haylage used for group A (horse number 1 to 4) compared to the haylages used for group B and C. It is important to remember that the estimation by AIA is *apparent digestibility* and not *true digestibility*. As mentioned earlier in the discussion about the digestibility, contamination of faecal grab samples can contribute to an overestimation of AIA in faeces. All faecal samples used in the analysis of digestibility were carefully cleaned from litter, but since the grab samples were picked up from the littered stall floor the risk of environmental contamination should not be excluded. Ingestion of sand in the paddock during day time may also contribute to an overestimation of AIA in faeces and this may be a reason for the high DMd for some horses when consuming June haylage. However, the horses were fed in the same way during the entire experiment and an overestimation of AIA in faeces is therefore possible for the July and August haylage as well.

Particle size distribution in faeces

The difference in particle size distribution between the diet containing June haylage and the diets with later harvested haylage, in six out of seven particle size fractions, was probably due to different chemical composition in forages harvested early or late. A greater content of lignified tissue in the more mature forage may influence the fracture properties of the plant (Pond *et al*., 1984). When fed July and August haylage, horses had a higher proportion of faecal particles in fraction sizes 1.0 mm, 0.63 mm, 0.315 mm, 0.2 mm and 0.1 mm compared to when fed June haylage. This was probably due to the fracture properties of lignin (Pond *et al*., 1984). The increased rigidity of the cell wall due to lignification makes the plant fraction more likely to break than bend, and this result in smaller particles of the late harvested forage. Chewing data was not included in this thesis, but more fibrous forage may increase the number of chewings. The fracture properties of the more lignified forage together with more chewings will result in a higher proportion of smaller faecal particles when fed a more fibrous haylage. In the smallest fraction size (<0.1 mm) horses that consumed June haylage had the highest proportion of particles. Since this fraction was calculated using the amount left from the total sample weight after all fractions had been weight, the actual content of this fraction is unknown. Hopefully the weight is represented by particles less than 0.1 mm, where microbes may be included as well.

There was no difference between the haylage diets in faecal particle size distribution in the largest fraction size (>2.0 mm). Approximately 70% of the faecal particles from the total sample ended up in the largest fraction. This means that most of the faecal particles, independent of the haylage ingested, were above 2.0 mm. However, the distribution in faecal particles above 2.0 mm is unknown. If sieves of a larger mesh size were used, more information about the particle size distribution in the largest fraction would have been known.

The results could have shown differences in particle size distribution between early and late harvested haylages, e.g. if early harvested haylage gives a larger proportion of smaller particles within the fraction size above 2.0 mm. For example, the statistical evaluation with all fractions under 2.0 mm pooled together showed no differences between haylages, however during comparison of all fractions there was a difference between haylages in all fraction sizes under 2.0 mm. However, using sieves with larger mesh size was not possible for this study, since the equipment was not available.

The proportion of faecal particles over 2.0 mm was much higher in this study compared to other studies (Ellis, 2003; Müller 2009). To compare different studies may be difficult and in some cases impossible, due to different physical and chemical composition of the feed and also different analyses. The idea in this discussion is to draw attention to the remarkable differences that seems to occur between this study and the two other mentioned studies. According to Ellis (2003), around 50% or less of faecal particles was larger than 2.0 mm, when grass silage and hay were fed to horses. The faecal particle size distribution of horses fed cut or long-stemmed haylage presented by Müller (2009) showed less than 15% of the total faecal sample in fractions larger than 2.0 mm. This is a great difference in particle size distribution compared to the results of this study with approximately 70% of the total faecal sample in fraction size >2.0 mm. According to Frape (2004), horses masticate feed particles to less than 1.6 mm in length. This is not in accordance to the results of this study, where most of the particles were above 2.0 mm in length. The cause of these differences in faecal particle size distribution between trials is difficult to explain without knowledge concerning the forage composition, feed ration and trial performance. Dental health may be a reason for differences between horses but the large difference between the studies mentioned is probably due to other reasons.

CONCLUSIONS

The haylage diets had different apparent DMd, which decreased with plant maturity, i.e. June haylage diet had the highest digestibility followed by the diet containing July haylage, whereas the diet with haylage harvested in August was least digestible. After the exclusion of two horses, both with a high DMd for the diet containing August haylage, a general difference in DMd between horses appeared. There was no difference in DMd between periods.

The distribution of particle sizes in faeces differed among haylages but was not different between horses or periods. Between the June and July/August haylages, six out of seven fractions exhibited a difference. June haylage produced the highest proportion of all three haylages in the smallest fraction size (<0.1 mm), whereas July and August had the highest proportion in fraction sizes between 0.1 mm and 2.0 mm.

This study confirms that apparent DMd of haylage decreases with increased plant maturity. Faecal particle size distribution may vary between an early and late cut haylage, but 70% of all faecal particles were in the largest fraction. It is therefore difficult to draw conclusions about particle size distribution in faeces when horses were fed haylages harvested in different stages of maturity. However, forage harvested early and with high digestibility seems to have a larger proportion of particles smaller than 0.1 mm, compared to forage harvested later in the season and with lower digestibility. A less digestible, thus more lignified, forage have a larger proportion of particles between 2.0 and 0.1 mm.

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APPENDIX 1

Daily feed rations (kg DM) for the horses during the different periods

	Period	Bale nr	June haylage	July haylage	August haylage	Betfor	Krafft Miner vit	Krafft Miner blå	Salt
Horse 1	1	103	8.54			0.45	0.06	0.06	0.01
Horse 2	1	103	8.81			0.45	0.06	0.06	0.01
Horse 3	1	103	8.01			0.45	0.06	0.06	0.01
Horse 4	1	103	6.41			0.45	0.05	0.05	0.01
Horse 5	1	121			8.30	0.45	0.07	0.00	0.01
Horse 6	1	121			8.86	0.45	0.07	0.00	0.01
Horse 7	1	121			12.46	0.45	0.07	0.00	0.01
Horse 8	1	121			9.97	0.45	0.07	0.00	0.01
Horse 9	1	112		8.87		0.45	0.07	0.00	0.01
Horse 10	1	112		8.32		0.45	0.07	0.00	0.01
Horse 11	1	112		11.64		0.45	0.07	0.00	0.01
Horse 12	1	112		11.64		0.45	0.07	0.00	0.01
Horse 1	2	114		8.82		0.45	0.07	0.00	0.01
Horse 2	2	114		10.90		0.45	0.07	0.00	0.01
Horse 3	2	114		9.34		0.45	0.07	0.00	0.01
Horse 4	2	114		7.78		0.45	0.07	0.00	0.01
Horse 5	2	106	6.41			0.45	0.05	0.05	0.01
Horse 6	2	106	6.41			0.45	0.05	0.05	0.01
Horse 7	2	106	10.15			0.45	0.07	0.07	0.01
Horse 8	2	106	9.08			0.45	0.06	0.06	0.01
Horse 9	2	123			10.61	0.45	0.07	0.00	0.01
Horse 10	2	123			10.61	0.45	0.07	0.00	0.01
Horse 11	2	123			11.79	0.45	0.07	0.00	0.01
Horse 12	2	123			12.37	0.45	0.07	0.00	0.01
Horse 1	3	126&127			9.35	0.45	0.07	0.00	0.01
Horse 2	3	126&127			11.42	0.45	0.07	0.00	0.01
Horse 3	3	126&127			10.91	0.45	0.07	0.00	0.01
Horse 4	3	126&127			8.83	0.45	0.07	0.00	0.01
Horse 5	3	118		9.43		0.45	0.07	0.00	0.01
Horse 6	3	118		9.43		0.45	0.07	0.00	0.01
Horse 7	3	118		12.97		0.45	0.07	0.00	0.01
Horse 8	3	118		11.79		0.45	0.07	0.00	0.01
Horse 9	3	108	7.03			0.45	0.05	0.05	0.01
Horse 10	3	108	7.03			0.45	0.05	0.05	0.01
Horse 11	3	108	8.65			0.45	0.06	0.06	0.01
Horse 12	3	108	8.65			0.45	0.06	0.06	0.01

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