Wrapped Forages for Horses

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Abstract

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Wrapped forages, in the form of silage and haylage, have become more common in horse diets during recent years. Silage and haylage is commonly produced in big bales. However, for use in stables with few animals, these bales often contain too much forage to be consumed before onset of aerobic deterioration. Smaller bales are therefore of interest, but knowledge of the chemical composition (including vitamin content), fermentation pattern and changes in those variables during storage of small bales is limited, and was therefore investigated. Small bale forage contained higher pH, higher ethanol and lower lactic acid content, compared to general levels in chopped silo silage, but low levels of ammonia-N and butyric acid. There were no general effects of dry matter or extent of fermentation on α-tocopherol and β-carotene contents in the preserved forages, but linear positive correlations between the vitamins and lactic acid existed. In general, long-term storage (14 months) of small bales influenced fermentation variables, yeasts and pH, but silage was affected by storage to a larger extent than haylage. Although changes occurred during storage, values in two-month old bales correlated well with values obtained after 14 months.

The influence of forage conservation methods on horse preference was also investigated. Hay, haylage and silage were produced from the same grass crops and the forages were offered simultaneously to horses. Silage was the first chosen forage, had the highest rate of consumption and the longest eating time, while hay had the lowest consumption rate and the shortest eating time. Haylage was intermediate between hay and silage in both eating time and rate of consumption.

The influence of forage conservation methods on equine hindgut fermentation was studied using fistulated horses. Hay, haylage and silage were produced from the same grass crop and fed in a changeover study. Horses were sampled after being fed the forage for 21 days, and a kinetic study of colon fermentation was performed in each period. Forage conservation method had no effect on microbial or chemical composition in the right ventral colon or faeces on Day 21. All forages showed similar fermentation kinetics in the right ventral colon before (0h) and at 2, 4, 8 and 12 h after feeding.

Keywords: silage, haylage, hay, horse, preference, hindgut fermentation, storage, bales

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Zusammenfassung

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Folienverpacktes Weidefutter, in Form von Silage und Heusilage, hat in den letzten jahren im zunehmenden Maß das Heu in den Pferderationen ersetzt. Silage und Heusilage wird allgemein in den grossen Ballen produziert. Für den Gebrauch in kleineren Pferdeställen, enthalten diese Ballen jedoch häufig zu viel Futter das ein Verfaulungsprozess startet bevor die Ballen verbraucht sind. Aus diesem Grund wurde die Möglichkeit untersucht Kleiballen zu produzieren. Jedoch sint Kenntnisse über die chemischen Zusammensetsung (einschließlich Vitamininhalt), des Gärmusters und der veränderungen während der Lagerung in solchen Ballen begrenzt, und diese Variablen wurden folglich untersucht. Kleine Ballen enthalten einen höherer pH und höheres Äthanolkonzentration aber weniger Milchsäuregehalt, verglichen mit allgemeinen Niveaus in gehhäckselten silage, aber niedrigen Niveaus des Ammoniak-N und Buttersäure. Es gab keine allgemeinen veränderungen der Trockensubstanz und/oder Gärungumfang auf α-tocopherol- und β-carotininhalt in konserviertem Futter. Im Allgemeinen wurden langfristige Lagerung (14 Monate) der kleine Ballen beeinflußten Gärungvariablen, Hefen und pH aber Silage veränderungen in mehr Variablen als Heusilage unterworfen. Obgleich Veränderungen während der Lagerungen eintraten, stimmten Werte in den 2 Monate alten Ballen gut mit den Werten den 14 Monate alten Ballen überein.

Der Einfluß der Konservierungsmethode des Grünfutters auf die Präferenz von Pferden wurde auch untersucht. Heu, Heusilage und Silage wurde aus dem gleichen Gras produziert und angeboten Pferden. Die Konservierungsmethode wirkte auf die Pferd Präferenz zur Bevorzugung von Silage. Silage war die erste Wahl, hatte den höchsten Verzehr und die längste Freßzeit, während Heu den niedrigsten Verzehr und die kürzeste hatte. Heusilage war zwischen Heu und Silage in der Verzehr und in der Freßzeit zufinden.

Der Einfluß der Konservierungsmethode des Grünfutters auf die Fermentation im Dickdarm (rechter ventraler Kolon) wurde an Fistulierten Pferde untersucht. Heu, Heusilage und Silage wurden aus dem gleichen Gras produziert und in einem change-over Experiment vollzogen. Die Konservierungsmethode hatte keinen Effekt auf die Mikrobielle oder chemische Zusammensetzung im Dickdarm gemessen am 21 Tag jeder Fütterungsperiode. Eine kinetische Studie der Kolon wurde auch durchgeführt. Resultate zeigten, daß alle Futtertypen im gleichen bereich nach 0, 2, 4, 8 und 12 stunden Werte in der selben Größenordnung aufwiesen.

Schlüsselwörter: Silage, Heusilage, Heu, Pferd, Präferenz, Dickdarm, Konservierungsmethode, Fermentation

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”Skulle det någon gång undantagsvis förekomma, att man nödgas lägga för sina hästar ett fördärfvat hö, måste man, sedan man valt ut det bästa, sorgfälligt skaka och lufta det, samt minst 12 timmar före fodringen bespränga det med saltvatten. Sedan höet därefter torkat i friska luften, må det i all världens dar läggas för de stackars krakarna.”

Greve och Hippolog Carl Gustaf Wrangel, 1839-1908.

”Should it ever occur, that one must lay before one’s horses a spoiled hay, one must, after selecting the best, carefully shake and aerate it, and at least 12 hours before feeding, sprinkle it with salted water. Thence, after the hay has dried in fresh air, may it in pity be laid before the poor rips.”

Count and Hippologist Carl Gustaf Wrangel, 1839-1908

(free translation by the author of the thesis).

Till Mamma och Pappa

Contents

Appendix

Papers I-V

The present thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Müller, C.E. 2005. Fermentation patterns of small-bale silage and haylage produced as a feed for horses. *Grass and Forage Science* **60**, 109-118.

II. Müller, C.E., Pauly, T.M. & Udén, P. 2007. Storage of small bale silage and haylage–influence of storage period on fermentation variables and microbiological composition. *Grass and Forage Science 62* (3), xx-xx (*In Press*).

III. Müller, C.E., Möller, J., Krogh Jensen, S. & Udén, P. 2007. Tocopherol and carotenoid levels in baled silage and haylage in relation to horse requirements. *Animal Feed Science and Technology* (*In press)*.

IV. Müller, C.E. & Udén, P. 2007. Preference of horses for grass conserved as hay, haylage or silage. *Animal Feed Science and Technology* **132**, 66-78.

V. Müller, C.E. & Udén, P. 2007. Effect of forage conservation method on microbial and chemical composition in the hindgut of horses fed hay, haylage and silage (*Submitted).*

The experiment in Paper **IV** was approved by the Ethical Committee in Uppsala, Sweden. The experiment in Paper **V** was approved by the Department of Health and Animal Care of the French Veterinary Authority, France.

List of abbreviations and definitions

Ammonia-N	ammonia-nitrogen
ATP	adenosine-triphosphate
a_w	water activity
CFU	colony forming units
Co-EDTA	cobalt(III)ethylenediamine-tetraacetate
CP	crude protein
DM	dry matter
DNA	deoxyribonucleic acid
FM	fresh matter
ha	hectare
Haylage	airtight stored grass containing ≥500 g DM/kg
ip	intraperitoneal
LAB	lactic acid bacteria
LD_{50}	lethal dose 50 %
NADH	nicotinamide adenine dinucleotide
NDF	neutral detergent fibre
r^2	square of the correlation coefficient
RAO	recurrent airway obstruction
SCFOS	short-chain fructo-oligosaccharides
SD	standard deviation
SEM	standard error of mean
Silage	ensiled grass containing less than 500 g DM/kg
SLU	Swedish University of Agricultural Sciences
VDMI	voluntary dry matter intake
VFA	volatile fatty acids
WSC	water-soluble carbohydrates

Introduction

The role of forage in equine nutrition

Horses (*Equus caballus*) have evolved as grass eaters, capable of surviving on plant tissues due to microbial fermentation of the feed in the hindgut (Janis, 1976). Hindgut fermentation was adopted as a digestive strategy early in evolution, and enabled equids to survive on grassland characterized by plants with high fibre contents (Sneddon & Argenzio, 1998). Some 25 million years ago, the ancestor of the modern horse, known from fossil records as *Meryhippus*, was adapted to life as a fast-moving grazer of the plains (Sneddon, 1993).

The modern horse is still a grass eater, designed for hindgut fermentation of fibrous feeds into volatile fatty acids (VFA) (Hintz *et al*., 1971; Argenzio, Southworth & Stevens, 1974). The equine digestive system is adapted to grass, and as in other herbivores, the digestive system works best with the feed it is adapted to (Hummel *et al.*, 2006). Feeding horses small amounts of fibrous feeds is connected with digestive upsets (Clarke, Roberts & Argenzio, 1990; Archer & Proudman, 2006) and development of stereotypic behaviour (McGreevy *et al.,* 1995; Goodwin, Davidson & Harris, 2002). Gastric ulcers have also been reported in ponies fed only concentrates, in contrast to ponies fed hay (Coenen, 1990), and the risk for colic incidents has been found to increase if more than 2.7 kg oats is fed daily (Hudson *et al.,* 2001). Recent studies also show that the mandibular motion is different when horses are eating a pelleted concentrate feed compared to hay, and that diets high in pelleted feed may have an impact on normal dental wear (Bonin *et al.*, 2007). Forage should thus constitute the major proportion in diets to horses, and pasture grass would be the simplest way of ensuing this. However, in northern countries, pasture cannot be provided all year round due to the cold winter climate. The summer grass therefore has to be conserved, and traditionally, haymaking has been the most common way to conserve forage for horses (Thompson, 1983).

The need for conserved forages in a transportable form has a long history in equine management. At the end of the 19th century, the UK cavalry had a large requirement of hay in bales, and at the 1878 Agricultural Exhibition in Paris, the British military bought four of the first stationary hay balers available to bale hay for horses in battle (Thompson, 1983). Thompson (1983) also described a similar requirement for forage in a moveable form by the civil society; at the end of 1800 and beginning of 1900, about 200 000 horses existed in London which had to be provided hay and other feedstuffs from farms outside the city. The present structure of the Swedish horse population is somewhat similar. At least 285 000 horses exist in Sweden (Bratt, 2001), and 75% of these are housed in areas close to cities (Persson, 2005). In recent years, hay in horse diets has been partly replaced by baled silage (<500 g dry matter (DM)/kg) and haylage (≥500 g

DM/kg) (Billysson, 2002; Holmquist & Müller, 2002; Schwarz *et al.,* 2005). The idea of feeding silage to horses is however, not new, as Nourse reported in 1897 that maize silage was a suitable feed for horses and mules if the animals were successively adapted to it.

A common way to produce silage and haylage is in the form of big bales. Today, bales account for approximately 60% of the total amount of silage conserved in Sweden (Wilkinson & Toivonen, 2003). Big bales are, however, not ideal for use in most horse stables, as more than 75% of the Swedish stables house only one to four horses (Persson, 2005). Thus, the big bales contain too much forage, if the bale is to be consumed before onset of aerobic deterioration. Handling of big bales has also been mentioned as a problem at small un-mechanized horse farms (Müller, 2002). Therefore, smaller bales are of interest for horse feeding purposes, but machinery equipment for production of small bale silage and haylage is not readily available at present. Also, scientific knowledge of wrapped forages as substitutes for hay in horse diets is limited and needs further study.

Forage conservation methods

Hay

Hay has been the traditional type of conserved forage used for horses, but if not produced and stored properly (i.e. dry and airy), it is readily subjected to mould growth (Lacey, 1989). The preservative effect of hay-making is due to a low water activity (a_w), and the dried hay must be kept as such in order to retain its nutritive value and hygienic quality, and for minimizing DM losses (Gregory *et al.,* 1963; Sullivan, 1973; Clevström *et al.,* 1981; Clevström and Ljunggren, 1984; Hlödversson, 1985; Lacey, 1989). The dried hay should have a DM level above 840 g/kg to keep mould counts low and to keep the hay from heating (Gregory *et al.,* 1963). Spontaneous heating of damp hay renders it brown and/or black due to the formation of indigestible Maillard products (Sullivan, 1973).

Barn-drying hay at harvest was found to be superior to field-drying and chemical preservation in restricting DM losses and mould growth under Swedish conditions (Clevström *et al.* 1981; Clevström & Ljunggren, 1984; Hlödversson, 1985). Clevström & Ljunggren (1984) investigated the mycological flora in field- and barn-dried hay, harvested from the same crop at different DM levels (600, 700 and 800 g DM/kg), and stored for six months. There was a heavy growth of *Aspergillus, Penicillium* and occasionally *Rhizopus* in the field-dried hay. The barn-dried hay also showed growth of *Aspergillus* and *Penicillium,* but the number of CFU (colony forming units)/g was much less than in the field-dried hay. The lowest mould spore load was found in the barn-dried hay harvested at 600 g DM/kg. Clevström *et al.* (1981) studied chemical preservation of hay (550 and 730 g DM/kg) by addition of various amounts of sodium chloride, propionic acid or formic acid, and found that mould counts were particularly high in hay bales treated with propionic or formic acid. Treatment with formic acid also resulted in an almost pure culture of *A. flavus*, and both aflatoxins B_1 and G_1 were found (635-1000 μg/l) in the hay.

Dry matter losses in hay during storage are primarily caused by fungal growth (Hlödversson, 1985). The extent of mould growth in stored hay can be different at the surface compared to within the stack or the bales, as direct contact with moist air can create conditions favourable to mould growth. In a preliminary report, Sundberg & Lindahl (2006) showed fluctuations in relative moisture, a_w and the presence and quantity of different mould species at the surface, at 25-cm depth and at 50-cm depth in hay bales stored in stacks from August to May. Registrations or samples were taken once every second month, and at three different farms. Results showed that the relative moisture at the surface, but not at 25- or 50-cm depth, followed the relative moisture in the air to a great extent. Water activity was higher at the surface than at 25- or 50-cm depth, and was ≥ 0.70 at the surface from October until May. At 25- and 50-cm depth, a_w generally fluctuated around 0.60 during the same period. Active growth of most filamentous fungi is restricted at a_w 0.80 (Hocking, Miscamble & Pitt, 1994; Adams & Moss, 1995), although xerophilic fungi may be able to grow at a_w 0.61 (Adams & Moss, 1995). The mould growth in the study of Sundberg & Lindahl (2006) was larger (in CFU/g) at the surface than at 25- and 50-cm depth, and a greater diversity of mould species was also found at the surface.

Respirable particles in forage and respiratory disorders

Mould growth in forage for horses should be avoided as moulds produce both spores and mycotoxins. Mould spores, together with actinomycetes such as *Micropolyspora faeni*, play a large role in the aetiology of recurrent airway obstruction (RAO), also known as "heaves" or "broken wind", which is a chronic respiratory disease in horses (Falk-Rønne *et al.,* 1984; Clarke, 1987; Robinson *et al*., 1996; Vandenput *et al.,* 1998). Airborne endotoxins can also contribute to pulmonary dysfunction like RAO (Künzle *et al.*, 2007). Gohlke (1957) argued that the condition was recognized as a manmade disease already by Aristoteles (384–322 BC), and has since been attributed to the use of mouldy feeds and bedding materials. A genetic background in the susceptibility of acquiring the disease is also suspected (Ewart & Robinson, 2007). Respiratory problems were found to be the second largest reason (8 to 9%) for culling of Swedish Warmblood horses born during 1965–1982, and 93% of the respiratory problems were defined as RAO (Wallin *et al*., 2000). In the UK, the estimated true prevalence of RAO in the horse population was 14 % (Hotchkiss, Reid & Christley, 2007). In a recent Swedish study based on insurance company registrations, Penell *et al.* (2005) found that respiratory diseases accounted for 5% of the proportional morbidity in the insured equine population in Sweden. If these studies are comparable, respiratory diseases seems to have decreased slightly as a cause of culling of horses in Sweden. However, as the disease results in laboured breathing (Robinson *et al*., 1996), it is still a serious welfare issue for affected horses. Also, impaired performance, the chronic state of the condition and veterinary costs associated with care of RAO-horses make preventive measures important.

As RAO is associated with respirable particles (0.5–3.0 μm) such as mould spores, forages with less mould growth or fewer counts of respirable particles are

of interest for horse feeding purposes (Clarke, 1987; Raymond *et al.*, 1997; Vandenput *et al.*, 1997). Vandenput *et al.* (1997) showed that even "good quality" (meaning not visibly dusty when shaken) grass hay contained higher levels of respirable particles than haylage (780 g DM/kg) or silage (480–490 g DM/kg), and silage contained less respirable particles and less thermophilic actinomycetes than haylage. Vandenput *et al.* (1998) also reported that to maintain a RAO-horse in clinical remission, it was more important to eliminate hay from the surrounding environment than to change the type of bedding material. Horses with RAO fed grass silage showed no difference in pulmonary function (mechanics of breathing and arterial blood analysis) compared to healthy control horses fed hay, or compared to themselves after being two months on pasture. However, if RAO-horses were fed hay, they developed clinical signs of RAO and had significant changes in pulmonary function parameters within eight days (s.d.± 3 days) (Vandenput *et al.,* 1998). A pasture-like environment can be achieved by using silage and wooden shavings instead of hay and straw, as showed by McGorum, Ellison & Cullen (1998), who measured total and respirable dust and airborne endotoxins in different equine management systems.

One method to reduce the amount of respirable particles in hay is to soak it in water before feeding. Moore-Colyer (1996) found that soaking hay for 30 minutes reduced the amount of respirable particles present in dry hay by about 90%, and Blackman & Moore-Colyer (1998) showed that soaking hay for 10 minutes, or steaming hay for 80 minutes, reduced the amount of respirable particles in dry hay by 93%. However, the procedure of soaking hay is labour-intensive and leads to nutritional losses (in particular P, K, Mg, Na and Cu), even if they are small when the hay is not "oversoaked" (*e.g*. soaked for longer periods than 30 minutes) (Moore-Colyer, 1996; Blackman & Moore-Colyer 1998). The steaming procedure was found to be very expensive in relation to soaking (Blackman & Moore-Colyer, 1998). As it is not known what actually happens with the mould spores during soaking or steaming, the safety of these procedures can be questioned. Soaking of hay also provides possibilities for other microorganisms to start growing, and hay that has been "oversoaked" may be questionable from this perspective as well. Also, if mycotoxins are present in mouldy dry hay, it is not known whether they remain in the hay or in the water after soaking. Mycotoxins produced by *Fusarium spp*. are well known health hazards for horses (Asquith, 1991), but intoxication of horses by *e.g. Aspergillus spp*., *Penicillium spp*. and *Stachyobotrys spp*. toxins has also been reported (Aller, Edds & Asquith, 1981; Asquith, 1991; Vesonder *et al.,* 1991; Ocholi *et al.,* 1992; Barnett *et al.,* 1995; Le Bars & Le Bars, 1996).

Silage and haylage

Finner (1966) classified silages in three categories: direct-cut silage, wilted silage and low-moisture silage, the latter also named haylage. A typical haylage would, according to Finner (1966), contain 400 to 600 g DM/kg. In this thesis, haylage has been defined as containing ≥500 g DM/kg. Silage is preserved by the fermentative activities of lactic acid bacteria (LAB) in anaerobic environments, resulting in a lowered pH. Production of lactic acid decreases with increasing DM

content (Jackson & Forbes, 1970). Haylage is preserved through a combination of drying, airtight storage and, depending on the possibility for LAB to grow, also presence of lactic acid and decreased pH. Wilting of crops for haylage increases the concentration of solubles in the liquid fraction, which decreases a_w of the crop (Greenhill, 1964). The effect of a lowered a_w is inhibitory on overall microbial activity (Adams & Moss, 1995). Greenhill (1964), however, proposed that in low moisture crops, the limited availability of the plant juice was probably the reason for low lactic acid production, and not the lowered a_w in itself. The differences in the conservation methods generally result in higher content of water-soluble carbohydrates (WSC), higher pH and lower concentration of VFA, lactic acid, ammonia-N and alcohols (ethanol and 2,3-butanediol) in haylage compared to silage (Gordon *et al.,* 1961; Greenhill, 1964; Finner, 1966; Nicholson *et al.,* 1991; Pahlow & Weissbach, 1996; Dawson *et al.*, 1999; Driehuis & van Wikselaar, 2000; Han *et al.,* 2006).

LAB are present on the grass crop in the field, and the most frequently found species are heterofermentative *Leuconostoc mesenteroides* and *Lactobacillus fermentum*, as well as homofermentative *Lactobacillus plantarum* and *Pediococcus spp.* Pahlow & Dinter (1987) reported that the relative proportion of *Pediococci spp.* increased with plant maturity, but the ratio of hetero- to homofermentative LAB remained quite stable at 1:1.2. Wilting has been demonstrated to reduce total LAB counts on the fresh crop (Pahlow & Dinter, 1987). Glucose and fructose present in the crop are considered as the primary energy sources of homofermentative LAB during ensiling. LAB metabolism by the Embden-Meyerhof-Parnas glycolytic pathway yields lactate, adenosinetriphosphate (ATP) and water from pyruvate (Gibbs *et al*., 1950). Grass fructans (mainly β-2,6-linked levans, also known as phleins according to Suzuki (1993)) can be indirectly involved as fructose sources during ensiling as shown by Merry *et al.* (1995), who found fructans to be degraded to fructose during ensiling. Fructans were degraded even in the absence of bacteria, but degradation was more rapid if bacteria were present (Merry *et al.,* 1995). However, isolation of 712 strains of LAB from grass forage showed that only 16 strains were able to ferment phlein-type fructans (Müller & Lier, 1994). Müller & Steller (1995) also demonstrated that different strains of LAB had different abilities to enzymatically degrade fructans of β-2,6-linked levan type and β-2,1-linked inulin type.

Streptococci spp., Pediococci spp. and certain *Lactobacilli spp*. are all classified as LAB, but have the ability to produce other fermentation end-products than lactic acid from pyruvate by the glycolytic pathway. Products such as acetate, CO_2, ethanol and 2,3-butanediol, among others, can be detected when glucose availability is low (Thomas, Ellwood & Longyear, 1979). In a heterofermentative process, the end-products formed depend on the type of hexose substrate utilized and on the bacterial species. Lactate, ethanol and acetate are, however, normally predominating (McDonald, Henderson & Heron, 1991).

Badly fermented silage

Badly fermented silage is often characterized by high concentrations of butyric acid and/or ammonia, and can have a pH between 5 and 7 (or higher). Presence of butyric acid and ammonia in silage is normally a result of the activity of clostridia or enterobacteria (McDonald, Henderson & Heron, 1991). The clostridial species found in silages usually belong to one of three phenotypically different groups, described by Pahlow *et al.* (2003) as; 1) proteolytic clostridia producing ammonia, amines, acetic acid and butyric acid from peptides and amino acids. This group includes *Clostridium sporogenes* and *C. bifermentans,* which have a limited ability to ferment carbohydrates; 2) the *C. butyricum*-group, which ferments monosaccharides to mainly butyric acid and acetic acid; and 3) the *C. tyrobutyricum* group, which can use a limited number of carbohydrates, but mainly ferments lactic acid to butyric acid even when pH is low. The latter group is the most studied clostridia in silage due to its economic impact in the dairy industry, causing late blowing of hard cheese. *C. tyrobutyricum* has also been found to be the most common clostridial species in Swedish big bale silage (Jonsson, 1990). The studies of clostridial species isolated from silage have been based on classic microbiology rather than modern DNA-based techniques. Therefore, the species reported should be looked upon as phenotypic groups, rather than strict taxonomic species (Pahlow *et al.,* 2003).

The growth of clostridia in silage has been shown to depend on a_w and DM content (Hengeveld, 1983), the relation between a_w and DM content in different crops, the amount of WSC in relation to DM level, buffering capacity of the crop, degree of laceration, NO_3-concentration of the crop, epiphytic micro-flora, ensiling technology and the use of additives (Wieringa, 1958; Weissbach, Schmidt & Hein, 1974; Spoelstra, 1990; Pauly, 1999). The problem with prediction of clostridial growth in silage is that silage is a heterogenous material. Spoelstra (1990) showed that the magnitude of variation in pH and DM content in a clamp silo was much smaller than the magnitude of variation in counts of clostridial spores in the same samples. Thus, clostridial growth in micro-niches might not have a very large influence on the chemical quality of silage (microbiological niches can exist at distances of about 10 μm), and may not be ver well correlated to the counts of clostridial spores, but the effect of clostridial growth (or of other microbial species), may seriously affect the hygienic quality of the forage (Spoelstra, 1990; Pahlow *et al.,* 2003). Long-stemmed bale silage is even more heterogenous than chopped clamp silage, and niches of clostridial growth with high levels of spores, butyric acid and ammonia have been found in bales where the general DM level would indicate restricted clostridial growth (Pauly, 1999).

The main concern of feeding badly fermented silage to horses is health disturbances. Strictly proteolytic clostridia such as *C. botulinum* are rarely found in silage (Notermans, Kozaki & van Schothorst, 1979; Spoelstra, 1981), but can produce the lethal neurotoxin botulin under certain conditions (Roberts, 1988; Hatheway, 1989). Notermans, Kozaki & van Schothorst (1979) showed that toxin production by *C. botulinum* in grass took place only at $a_w \geq 0.94$ at pH 6.5 and 5.8.

At pH 5.3, toxin production (with grass as a substrate) was demonstrated only at $a_w \geq 0.985$. Case reports of feed-related botulism in horses often fail to show the presence of botulinum neurotoxin in affected animals or in suspected feed or water, due to difficulties with sampling and analytical methods, as very small amounts (LD_{50}/kg body weight 0.5–2.0 ng i.p. in mice and guinea pigs) of the toxin is lethal (Gill, 1982). Also, the clinical signs usually appear three to seven days after ingestion of the toxin, at which time there may be no detectable toxin in the serum or gut content of the horse (Blood *et al.,* 1979) and most often no feed left to sample. A number of published case reports have therefore based the botulism diagnosis on clinical symptoms. A clear connection between feeding silage and incidents of botulism does not seem to be evident in the literature. Rather, inclusion of cadavers or a general poor feed hygiene in feedstuffs of different types seems to be involved. In cases of botulism where silage was fed, the forage was reported as being badly fermented with a high pH (Haagsma *et al.,* 1990), with inclusion of cadavers (Gudmundsson, 1997) or with a high pH and a strong smell of ammonia (Ricketts *et al.,* 1984). In cases were silage was not fed, inclusion of cadavers in oat chaff (Kelly *et al.,* 1984) or alfalfa hay cubes (Kinde *et al.,* 1991), grass clippings subjected to heating (Switzer *et al.,* 1984), feed-through dirt from a rack where only green oat hay had been fed (Heath *et al.,* 1990), feed and water contaminated with carcasses *via* birds as vectors (Schoenbaum *et al.,* 2000) and round bale hay stored outdoors resulting in rotten and mouldy material in the bale centre (Hunter *et al*., 2002), were all reported as sources of the neurotoxin.

The other group of bacteria associated with high levels of ammonia in silage is enterobacteria. Enterobacteria found in silages are Gram-negative bacteria which are facultatively anaerobic and have catalase activity as well as NO_3-reducing ability (Spoelstra, 1987; Heron, Wilkinson & Duffus, 1993). The fermentation products from enterobacteria, using glucose as a substrate in anaerobic environments, are acetic and formic acid as well as ethanol and butanediol, depending on the bacterial species (Pahlow *et al.*, 2003). Ethanol and butanediol are not desirable as they do not contribute to a decrease in pH. The number of enterobacteria can increase during wilting of grass crops (Spoelstra, 1987) and during the first days of ensiling (Heron, Wilkinson & Duffus, 1993), but after four (Östling & Lindgren, 1995) to nine (Heron, Wilkinson & Duffus, 1993) days, they are usually not present in the silage. Also, Byrne *et al.* (2002) did not find any detectable levels of enteric bacteria in grass after 19 days of ensiling, and *Escherichia coli* O157:H7 added at harvest did not survive the ensiling process. Although the species of enterobacteria most frequently found in silages are considered to be non-pathogenic, they can contain endotoxins in the outer cell membrane, which may be associated with health problems in dairy cows (Lindgren, 1991). Also, van Duijkeren, van Aasten & Gaastra (2000) found F17-fimbriae positive *E. coli* genes and haemolytic *E. coli* strains only in faeces from diarrhoeic horses, but not in faeces from healthy horses. However, if F17-positive or haemolytic *E. coli* were responsible for the diarrhoea in the horses was not known.

Tocopherols and carotenes in forage in relation to equine nutrition

Specific factors influencing the content of tocopherols (vitamin E) and carotenes (provitamin A) in wrapped forages have not been investigated to any large extent. Vitamin data in equine feed tables is largely based on vitamin contents in hay and, to a lesser extent, silo silage. As wrapped forages are gaining popularity as feeds for horses (*e.g*. Holmquist & Müller, 2002), knowledge of the influence of forage conservation methods on vitamin content is important. Blakley & Bell (1994) reported that a low vitamin A and E status in horses was associated with winter feeding of harvested feeds. It has also previously been reported that a diet consisting of dried hay and oats does not contain sufficient amounts of vitamin A and E to maintain adequate serum levels of these vitamins in weanlings and pregnant mares during the winter period (Mäenpää, Koskinen & Koskinen, 1988). It is known that airtight storage of moist barley (280 g water/kg) can lower the total amount of vitamin E, compared to airtight storage of grain at a lower water content (200 g water/kg) and compared to heat-dried grain (Hakkarainen, Työppönen & Bengtsson, 1983 a, b). Haylage is also preserved largely due to airtight storage, but it is not known if a reduction in total vitamin E, similar to the reduction in grain, takes place in haylage. Ballet, Robert & Williams (2000) showed in a review that the levels of different vitamins in forages were highly variable, and that the average (and range) of α-tocopherol and β-carotene content was generally lower in hay than in silage. As both α-tocopherol and β-carotene are destroyed by oxidation, ultraviolet radiation and heat (Seshan & Sen, 1942; Sullivan, 1973), different forage conservation methods may influence the content of these vitamins differently. Bauernfeind (1980) suggested that α-tocopherol was more stable in an acid than in an alkali environment, and that is was relatively stable to heat and light in the absence of oxygen.

There are eight forms of vitamin E found naturally in plants, of which α-tocopherol has the widest distribution and the highest vitamin E activity in animal tissue (Aitken & Hankin, 1970). Pagan, Kane & Nash (2005) showed that vitamin E from a natural source was more effective than synthetic vitamin E at elevating plasma α-tocopherol levels in horses. This should be both a physiological and economical incentive to preserve vitamin E levels in conserved forages close to the initial levels of the fresh crops. Saastamoinen & Juusela (1993) found a seasonal variation in serum vitamin E content in horses. Serum levels of vitamin E was highest during grazing, and decreased during a subsequent indoor period when horses were fed timothy hay, oats and a supplement containing 1 mg vitamin E/kg bodyweight. Vitamin E is an important anti-oxidant in the blood (Moffarts *et al.,* 2005), and is vital for the humoral immune response in horses (Baalsrud & Øvernes, 1986). Diseases responsive to vitamin E and selenium supplementation, such as muscular dystrophy (Wilson *et al.*, 1976; Ronéus, 1982) and a form of degenerative disease of the spinal cord and brainstem (equine degenerative myeloencephalopathy) (Mayhew *et al.,* 1987), exist in foals and young horses. In horses 2 years of age or older, a neurodegenerative disorder

of the somatic lower motor neurons (equine motor neuron disease) can be prevented by supplementing the stable diet with vitamin E, or by providing access to green forage (Divers *et al.*, 2006).

β-carotene in green forage is the main source of vitamin A (retinol) to grazing animals, although other carotenes may also be nutritionally important (Britton & Goodwin, 1973). β-carotene is mainly associated with reproductive function in mares (Peltier *et al.,* 1997), function of mucous membranes, cell differentiation at tissue growth and normal function of retinal cells (Lewis, 1996). Saastamoinen & Juusela (1992) found no seasonal variation of serum retinol in horses that were grazing or subsequently kept indoors, where they were fed timothy hay, oats and different amounts of a supplement containing vitamin A. On the other hand, Blakley & Bell (1994) found higher plasma levels of retinol in horses from May to August, compared to other times of the year, and Mäenpää, Koskinen & Koskinen (1988) also found higher serum retinol levels in mares and foals when they were on pasture compared to when kept and fed indoors. Excess intake of carotene from pasture has not been reported to result in toxicity in horses (NRC, 1989), but excess feeding of vitamin A (retinol) supplements have been reported to result in *e.g*. bone fragility, teratogenesis, loss of hair and epidermis, ataxia and depression (Donoghue *et al.*, 1981; NRC, 1989). Naturally occurring β-carotene in forage is therefore a safer (and less expensive) source of vitamin A than supplements, but information on the content of β-carotene in wrapped forages is scarce. The influence of different forage conservation methods on carotene content in grass has been reported to vary from an 80% reduction after slow field-drying of hay (Sullivan, 1973) to less than 30% in well-preserved and rapidly acidified silage (Watson & Nash, 1960; Shahane & Mungikar, 1991).

Factors influencing forage intake and preference in horses

Ensiling has been reported to reduce the voluntary intake of forages in horses (McLean *et al.*, 1995; Moore-Colyer & Longland, 2000), and in sheep and cows (Buchanan-Smith, 1990; Erdman, 1993). However, in comparative studies of voluntary intake in horses, forages conserved as hay, haylage and silage had very different neutral detergent fibre (NDF) contents and different nutritive values (McLean *et al.,* 1995; Moore-Colyer & Longland, 2000), making it difficult to know if the nutrient content (plant maturity and botanical composition) or the conservation method caused the differences in intake. Voluntary dry matter intake (VDMI) of forage is probably partly related to the NDF content of the forage, but other factors are clearly involved, as prediction equations based on NDF content have been reported to have r^2-values of 0.11 (Dulphy *et al.*, 1997) to 0.50 (Lawrence, Lawrence & Coleman, 2001). Other factors influencing voluntary intake of silage may be acetic acid concentration, as foals have been reported to reject drinking water containing more than 0.16 ml acetic acid/100 ml water (Randall, Schurg & Church, 1978). Austbø (1990) also reported that horses rejected clamp silage when it had a noticeable smell of butyric acid.

Voluntary intake and preference do not measure the same thing (Forbes, 1986; Lawrence *et al.,* 1987; LaCasha *et al.,* 1999), and the relation between voluntary intake and preference is unknown. Opinions about horse preferences for different forages exist, but the scientific knowledge in the area is scarce. Reports have mainly dealt with voluntary intake and digestibility of forage (Austbø, 1990; Smolders, Steg & Hindle, 1990; Istasse *et al.,* 1996; Moore-Colyer & Longland, 2000; Bergero, Peiretti & Cola 2002), effects of forage on behaviour (Goodwin, Davidson & Harris, 2002) or effect of plant species on voluntary intake and digestibility (Darlington & Hershberger, 1968; LaCasha *et al.,* 1999). Studies concerning horse preference for forages have, to the authors' knowledge, mainly covered pasture (Archer 1971, 1973, 1978; Naujeck, Hill & Gibb, 2004). The digestibility and nutritive value of a forage are important feed attributes, but only if the forage is not rejected by the horse. When voluntary intake of single forages is studied, the results give no information of the preferences when horses are given multiple choices. One example of this is the experiment of Lawrence *et al.* (1987), who studied intake of dried or acid treated (0.80 propionic acid and 0.20 acetic acid) lucerne (*Medicago sativa*) hay in horses. When horses were given each forage separately, there was no intake difference among treatments, but when given both forages simultaneously, horses consumed more of the dried hay. These "Cafeteria" type tests with more than one feed at a time may therefore be a method to gain better knowledge of active feed choices made by the horse, than studies of one feed at a time.

Factors influencing the ecosystem in the equine hindgut

The equine hindgut is inhabited by a number of different microorganisms using different substrates and producing mainly microbial cells, gases, VFA, ammonia and some lactate (Hintz, Argenzio & Schryver, 1971; Argenzio, Southworth & Stevens, 1974; Udén & van Soest, 1982; de Fombelle *et al.*, 2003). de Fombelle *et al.* (2003) demonstrated the presence of microorganisms all along the digestive tract of horses, but the number of cellulolytic bacteria and VFA production was highest in the hindgut. Changes in the microbial community and biochemistry in the hindgut (including faeces) of horses have been associated with different types of colic (Reeves, Salman & Smith, 1996), laminitis (Garner *et al.*, 1978; Goodson *et al.,* 1988; Rowe, Lees & Pethick, 1994; Milinovich *et al.,* 2006; van Eps & Pollitt, 2006), treatment with antibiotics (Kropp, 1991), equine intestinal clostridiosis (Wierup, 1977) and change of faeces' appearance, bad coat and lowered performance (Ronéus *et al.,* 1993). The microbial and fermentation profiles of both caecum and right ventral colon have been found to be directly related to the composition of the diet in studies examining different ratios of hay to concentrates (Kern *et al.*, 1973; Moore & Dehority, 1992; de Fombelle *et al.*, 2001; Julliand *et al.,* 2001; Medina *et al.,* 2002). The microbial profiles of faeces in horses on early or late summer pasture were also found to differ (Darby *et al*., 1995). Corresponding knowledge of the influence of hay, haylage and silage on the hindgut fermentation in horses is lacking. The changes taking place in the forage during conservation, such as the increase in VFA and lactic acid content, decrease in pH and change in WSC fraction in silage, and sometimes also haylage,

affects not only the forage itself, but may also have an influence on digestion and hindgut fermentation in the horse.

Aims of the thesis

The general aim of the thesis was to investigate production and storage of small bale grass silage and haylage, and to study the influence of forage conservation method on chemical and microbiological variables in small bale silage and haylage, on horse preference and on equine hindgut fermentation. The specific objectives were:

- To investigate the possibility to use high-density hay balers for production of small bale silage and haylage, and to study the resulting fermentation pattern and microbial composition in the small bales
- To investigate stability of chemical and microbiological variables during long-term storage of small bale silage and haylage
- To investigate the influence of extent of fermentation on tocopherol and carotene content in small bale silage and haylage
- To investigate horse preferences for silage, haylage and hay
- To investigate the influence of silage, haylage and hay on hindgut fermentation and microbiology in horses

Materials and methods

This thesis is based on five experiments. Three of the experiments (**I–III**) focused primarily on production and storage of small bale silage and haylage, and influence of DM on fermentation and chemical composition (including tocopherols and carotenes in Paper **III**) of the forage. In Paper **IV**, horse preference for grass conserved as hay, haylage and silage was investigated. In Paper **V,** hindgut fermentation in horses fed hay, haylage and silage was studied. The experiments were mainly conducted at Kungsängen Research Centre, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala. Analyses of tocopherols and carotenes (**III**) were performed at Department of Animal Health, Welfare and Nutrition, Danish Institute of Agricultural Sciences, Research Centre Foulum, Tjele, Denmark. Experiments with horses in Paper **V** were done at Établissement National d'Enseignement Supérieur Agronomique de Dijon, France. Details of experimental, analytical and statistical procedures are given in the respective papers, but a general description is given below.

Grass crops

The grass crops used in the experiments consisted mainly of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*). The crop in Experiment II in Paper **I** also contained 0.05–0.1 of perennial ryegrass (*Lolium perenne*) and 0.05 red clover (*Trifolium pratense*). Couch grass (*Agropyron repens*) was present in the crop in Papers **II–V** in approximate amounts of 0.1. In Paper **V**, dandelions (*Taraxacum vulgare*) compromised about 0.05 of the sward. The crops used in Paper **II–V** were first cuts of a permanent grass ley, which was fertilized with NPK 21-3-10 (430 kg/ha) before the first cut. The same crop was used for the different treatments within experiments in each paper (**I–V**), as the main interest was the influence of different conservation methods, and not plant maturity, botanical composition or other factors related to different crops.

Production of small bale silage and haylage

The production of silage and haylage of different DM levels was done by varying wilting times of the cut crop before baling. In Papers **I–IV**, small square bales were produced directly at harvest using high-density balers intended for hay production. The high-density baler used in Paper **III** and **V** was modified by replacing the original knotters with knotters intended for a large square baler. This made it possible to use a stronger twine for binding the bales. In Paper **III**, a mini-round baler was also used. Wrapping of small square bales was done using a mini-wrapper with a rotating table (**I–V**), and small round bales were wrapped using a conventional round bale wrapper (**III**), also with a rotating table. In Paper **V**, a rebaling method was employed. The forage was harvested in big round bales, ensiled for approximately seven months and then opened and rebaled into small square bales, using the machinery equipment mentioned previously. All wrapped bales in Papers **I–V** were stored in a fenced bale-yard during ensiling, with care taken to protect the bales from birds and rodents. Bales were inspected regularly, and if damages of the stretch film were detected, they were immediately repaired with special plastic tape. Bales with more serious damages in the stretch film were excluded for further use in the studies.

Use of additives (chemical and biological) and number of stretch film layers (6, 8 or 10) were studied in Paper **I**, which consisted of two repeated experiments, with variations in crops and additive inoculants. The same chemical additive was used in Experiments 1 and 2, but the type of bacterial inoculant varied. In paper **V**, a LAB inoculant was added to both silage and haylage.

A method to study tightness in wrapped bales was used in Paper **I** and **III**. The method was based on the gas entry rate of bales. A small negative pressure was created inside the bale, using a one-way valve inserted through the plastic and a hand-operated vacuum pump. An injection needle connected to a manometer was then inserted through the valve membrane, and the gas entry rate was recorded as the time it took for the negative pressure to rise from -20 to -15 mm water column.

Aerobic storage stability

The aerobic storage stability of silage and haylage upon exposure to air was investigated in Experiment 1 (**I),** by measuring increase in CO_2 in forage samples placed in a standardized aerobic environment (constant temperature 24°C). Forage samples (0.187 kg DM) were placed in identical vessels, and about 500 l of CO_2-free air/kg DM was passed through the vessels. CO_2 was measured in the outflow air at 1 h intervals. Production of CO_2 indicated microbial activity in the sample (Honig, 1990), and when the level reached 10 ml CO_2/l, the recording was terminated. The microbial growth during aerobic storage was also studied by quantitative determination of fungi and WSC in bales sampled at Day 6 after opening (Experiment 1, Paper **I**).

Preference study

Wrapped forages used in Paper **IV** were produced as described previously for small bale silage and haylage, and hay was harvested in small square bales and barn-dried. All forages originated from the same crop and harvest. The horses used in the preference study were privately owned, of different breeds and with different previous experiences of eating silage and haylage. The horses were kept at an early autumn pasture during the experiment. The experimental model was most easily described as a Cafeteria model, where each horse had access to equal amounts (on dry matter basis) of all forages during two hours daily. The first choice, forage consumption, eating time and eating behaviour of individual horses were registered daily for five consecutive days in four periods.

Studying the equine hindgut

Haylage and silage in Paper **V** were harvested as described previously, and hay was barn-dried (small square bales). The experimental horses used in Paper **V** were fistulated both in the caecum and the right ventral colon, but only the colon fistula was used for sampling. The experimental model was a crossover design where all horses were subjected to all treatments (Table 1) after a common pre-period. Horses were fed the experimental forage, salt and minerals, and had free access to water. Colon and faecal samples were taken at Day 21 in each period, and analyzed for microbial and chemical composition. Fermentation kinetics of the right ventral colon was followed during two subsequent days in each period, with sampling before (0 h) and 2, 4, 8 and 12 h after the morning meal. Samples from the kinetic study were analyzed for chemical composition, but not for microbial variables.

Table 1. *Experimental design in Paper V*

Horse	Pre-period	Period I	Period II	Period III
Horse G	hay	haylage	hay	silage
Horse K	hay	silage	hay	haylage
Horse P	hay	haylage	hay	silage

Horse D	hay	silage	hay	haylage

Statistical analysis

The statistical methods and models used are described in detail in each paper. Variables were normally distributed except for pH in Paper **IV**. pH was therefore transformed to $[H^+]$ before statistical analysis. Analysis of variance in Papers **I–V** was performed using SAS general linear models procedure (SAS Institute, SAS Inc. Cary, NC, USA). Correlation analysis in Papers **II–III** was performed using SAS correlation procedure, and Pearson correlation coefficients (ρ) were calculated according to Milton (1992) as:

$$\rho = \mathrm{Cov}(X,Y) / \sqrt{(\mathrm{Var}X)(\mathrm{Var}\ Y)}.$$

SAS mixed models procedure was used in Paper **IV**, where the variables "eating time" and "forage consumption" were analysed using a multivariate repeated measurements model. In all experiments, differences were regarded as statistically significant when $P<0.05$.

Results

Production of small bale silage and haylage

Production of small square bale silage and haylage suffered from problems with the twine at baling (**I, II, IV**), but these were minimized with the replacement of the knotters (**III** and **V**). Small quare bales generally had a higher bale density than small round bales, but interactions between bale type and DM content were also present (**III**). Bale weight was found to be important for the possibility to wrap the bales correctly, as bales weighing less than 30–35 kg were pulled off the wrapper table as by the plastic film. Increasing the number of stretch film layers from six to ten resulted in higher levels of CO_2 inside the bale, but gas entry rate or fermentation variables were not affected by the amount of stretch film applied (**I**).

Fermentation pattern and microbial counts in small bale silage and haylage

Use of inoculant additives at DM levels between 350 and 600 g/kg improved lactic acid fermentation and decreased pH compared to controls in haylage in Experiment 1 and in silage in experiment 2 (**I**). Haylage generally showed less signs of lactic acid fermentation and had higher pH than silage, but levels of butyric acid and ammonia were low (**I–V**). Ethanol content was higher than lactic

acid content in control haylage in Experiment 1 (**I**), in both types of haylage in Paper **II–IV** and in haylage in Paper **V** (Table 2). Concentration of ethanol and lactic acid was similar in silage in Paper **III** (Table 2). However, ethanol concentration was higher in silage than in haylage in Paper **II**–**V**, but not in Paper **I** (Experiment 2). Water-soluble carbohydrate contents were generally higher in haylages than in silages (**I–V**). The WSC fraction differed between forages in Paper **V**, where the fresh crop for hay contained half the concentration of fructans found in the fresh crops for silage and haylage. This difference was the opposite in the preserved forages, where hay had the highest fructan content (**V**). Mould counts and enterobacteria were generally higher in hay than in silage or haylage (**IV**, **V**). Clostridial spore counts were higher in silage than in haylage only in Paper **III**.

Aerobic storage stability

Additives prolonged aerobic storage stability in Experiment 1 (**I**), as control forage reached 10 ml CO_2/l in outflow air earlier than forages treated with inoculants or chemicals. Additive-treated bales also expressed lower mould counts than control bales after being open for six days. Bales treated with the chemical additive had the highest WSC content both at Day 0 (opening day) and at Day 6 after opening in Experiment 1 (**I**), and the WSC content at Day 6 did not diverge to any large extent from WSC content at Day 0.

Table 2. Content of dry matter, ethanol, lactic acid and pH in silage and haylage in Papers **I–V**

Paper	Forage type/treatment	Dry matter g/kg	Ethanol g/kg DM	Lactic acid g/kg DM	pH
I[1]	Haylage (control, Exp.1)	544	19.3	4.9	6.07
	Silage (control, Exp.2)	339	4.5	30.4	5.19
	Haylage (control, Exp.2)	496	4.6	12.5	5.46
II[2]	Silage	306	26.9	38.3	4.65
	Haylage (HLL)	565	13.8	2.0	5.55
	Haylage (HLH)	672	8.6	0.4	5.72
III[3]	Silage	285	32.8	32.9	5.16
	Haylage (HL1)	505	21.8	3.1	5.51
	Haylage (HL2)	564	17.3	1.4	5.51
IV[4]	Silage	309	22.8	31.8	4.94
	Haylage (HLL)	577	7.9	2.6	5.63
	Haylage (HLH)	684	4.3	0.3	5.81
V[5]	Silage	343	9.7	43.0	4.44
	Haylage	548	5.9	1.3	5.60

[1] Data from Table 2 (Experiment 1), Table 3 and Table 4 (Experiment 2) in Paper **I**, [2] From Table 2 in Paper **II,** [3] From Table 1 in Paper **III**, [4] From Table 3 in Paper **IV**, [5] From Table 2 in Paper **V**.

Storage of small bale silage and haylage

Long-term storage of small square bales generally resulted in lower pH-value, 2,3-butanediol and WSC concentration, and higher content of lactic and acetic acid, ethanol, ammonia-N and yeast counts, compared to short-term storage (**II**).

Interactions between forage type and storage time were present, as silage responded differently to long-term storage than either of the two haylage types. pH, succinic acid and 2,3-butanediol were lower and lactic and acetic acid higher after long- than after short-term storage in silage. The same variables of both haylage types were unaffected by long-term storage. It was observed that silage bales deviated from a square shape, while haylage bales were rectangular (**II**). A reduction in WSC content during storage was similar (about 20 g WSC/kg DM) for all forage types (**II**).

Tocopherols and carotenes in small bale silage and haylage

The DM level of grass conserved as silage or haylage did not have any general linear effect on the content of α-tocopherol or β-carotene in the preserved forages (**III**). However, haylage with lower DM level (500 g DM/kg) contained less α-tocopherol and β-carotene than silage (300 g DM/kg) and haylage with higher DM level (600 g DM/kg). Interactions between DM treatment and bale type (round or square) were found for several variables, and α-tocopherol and β-carotene content were highest in silage in square bales. Proportions of α-tocopherol and β-carotene in preserved forages in relation to initial content in the fresh crop were highest in silage in square bales and high DM haylage in square and round bales, and were about 0.6 for α-tocopherol and 0.85 for β-carotene. Weak linear correlations were found between fermentation variables and α-tocopherol or β-carotene, of which lactic acid had the highest Pearson correlation coefficients (positive for both α-tocopherol and β-carotene).

Horse preference for hay, haylage and silage

In Paper **IV**, silage, haylages (two different DM levels) and hay from the same crop had different chemical and microbial compositions with the exception of ash and crude protein content and counts of yeast and clostridial spores. Horses preferred silage when given the opportunity to choose simultaneously between hay, haylages and silage (**IV**). Eating time was longest and consumption highest for silage, and silage was never left in favour of any other forage. In 72 out of 84 times, silage was the first chosen forage. Hay had the shortest eating time and lowest consumption, and was never completely consumed. The haylages were intermediate between hay and silage in eating time and amount consumed. At the end of each observation period, horses alternated between the forages more frequently than at the beginning.

Composition of hindgut content in horses fed hay, haylage and silage

Hay, haylage and silage produced from the same crop had different chemical composition except content of acid detergent fibre, *in vitro* digestible organic matter, estimated metabolizable energy and counts of yeasts and clostridial spores (**V**). The different forages did not produce different responses in microbial or

chemical composition in colon or faeces of fistulated horses sampled at Day 21 (**V**). Colon samples were generally lower than faecal samples in DM content, counts of lactobacilli and lactate utilizing bacteria, and higher in pH, acetic, propionic and butyric acids as well as in total organic acids. When haylage was fed, fermentation kinetics in colon was different for acetic acid, valeric acids and total organic acids, which concentrations were lower compared to when hay and silage was fed. Also, feeding hay resulted in higher DM, *i*-butyric and *i*-valeric acid concentration in the kinetic study, but differences were small and the proportions of acetate:propionate:butyrate:valerate to total organic acids in colon were similar among forage treatments. The content of WSC in colon samples from the kinetic study was close to zero. There were general differences, although very small, between sampling times in the kinetic study, as butyric and valeric acids were highest at 0 h, but no interaction between forage type and sampling time, meaning that all forages behaved similarly in the colon at the different sampling times. Small differences among horses were observed in chemical variables in colon both at Day 21 and in the kinetic study, indicating some individual variation in colon fermentation in horses.

Discussion

Production of small bale silage and haylage

The modification of the knotters in the high-density baler was a simple and low-cost way to improve the possibility to use this type of machine for baling crops with a lower DM content than hay (**III, V**). The number of stretch film layers only influenced the amount of CO_2 inside the bales, and a difference existed only between 6 and 10 layers (**I**). As CO_2 in silage is produced during plant respiration and the following fermentation, the higher CO_2-concentration in bales wrapped with 10 layers of stretch film may indicate a tighter seal, as suggested by Paillat & Gaillard (2001). In Finland, Heikkilä *et al.* (2002) found lower yeast and mould counts in bales with six layers of white stretch film compared to four layers, but there was no difference between six and eight layers. O'Kiely, Forristal & Lenehan (2000 a) compared 2, 4 and 6 layers of stretch film on big round bale silage. An improvement in digestibility and less surface rotting and mould development were found when the number of layers was increased from 2 to 4, but only a smaller improvement from 4 to 6 layers. No differences in yeast or mould counts were found between 6, 8 and 10 layers of stretch film in Experiment 1 (**I**). Black stretch film was used by O'Kiely, Forristal & Lenehan (2000 a), and as black stretch film has been shown to have a higher CO_2-permeability than white stretch film, due to different absorption of radiation (Möller, Klaesson & Lingvall, 1999), studies using different film colours may not be directly comparable. Paillat & Gaillard (2001) also stressed the fact that the stretch film could react quite differently in different climates.

The bale density was generally higher in square than in round bales (**III**), but interactions between bale type and DM content were also present. Bosma (1981) found a large impact of crude fibre content in the crop on porosity or density (kg DM/m^3) under laboratory conditions. The higher the crude fibre content, the lower the density. Plant maturity stage, chop length and moisture content have also been found to influence silage density (Bosma, 1981; Honig, 1987). Honig (1987) found that the higher the DM and the coarser the grass, the higher consolidation was needed to keep the gas flow at a constant level. At 200 g DM/kg, a density of 160 kg DM/m^3 and at 400 g DM/kg, a density of 225 kg DM/m^3 was needed to keep the gas flow in the grass at the same level. A high gas flow may facilitate oxygen penetration (Williams, 1994) and subsequent microbial growth. In haylages in Papers **I–V**, the DM level was higher than 400 g/kg and density was seldom larger than 170 kg DM/m^3 in small bales. Still, mould growth was low in all haylages (**I–V**). This supports the findings of O'Kiely, Forristal & Lenehan (2000b), who reported that neither bale density nor degree of wilting were important for avoidance of mould growth when a sufficient number of stretch film layers was used to exclude oxygen-containing air from the forage. The same conclusion about wilting was made by Undersander, Wood & Foster (2005), who showed that a DM range from 370 to 770 g/kg in lucerne or lucerne-grass mixtures was acceptable for preserving the crop as silage or haylage, if sufficiently thick plastic (more than 13µm in total) was used, and bales were wrapped within 24 hours after baling.

Influence of conservation method on forage composition

The fermentation pattern differed between silage and haylage treatments in all experiments in this thesis. The fermentation was more extensive in silage than in haylage, as indicated by a higher concentration of lactic acid and a lower pH in silage (**I–V**). The haylages generally had higher concentration of ethanol than lactic acid (**I–V**) except in Experiment 2 (**I**), and silage in Paper **III** contained equal amounts of ethanol and lactic acid. Ethanol fermentation in silage or haylage is not desirable as it does not contribute to a decrease in pH, and as CO_2 is produced during ethanol formation, DM losses occur (McDonald, Henderson & Heron, 1991). Ethanol in silage can be produced during heterolactic fermentation by LAB (Buyze, Vanden Hamer & De Haan, 1957), by yeasts (Jonsson & Pahlow, 1984), by enterobacteria and by proteolytic clostridia through Stickland reactions (Pahlow *et al.,* 2003). The production of ethanol in high-dry matter grass silage (400–500 g/kg) was discussed by Driehuis & van Wikselaar (1996, 2000), who suspected yeast to be the responsible organism. Driehuis & van Wikselaar (2000) also found a positive correlation between ethanol and WSC content in silages, which indicated that the ethanol producing microorganism was more osmotolerant than epiphytic LAB, and that a substrate other than WSC was used for ethanol formation. O'Brien *et al.* (2007 b) reported the yeast species *Pichia anomala* and mucoraceous moulds to be positively correlated with ethanol content in baled silage, and also that a higher DM level favoured *P. anomala* and mucoraceous moulds. Yeast counts were found to increase in wilted material (up to 500 g DM/kg), but ethanol content did not follow the increase in DM or yeast counts,

but rather tended to change in opposite direction (Jonsson *et al.,* 1990). In Papers **II–III,** yeast counts seemed to be higher in silage and haylage containing more ethanol than lactic acid. However, mixed fermentations with both LAB and yeasts have also been reported to occur, typically when crops rich in WSC are used for ensiling purposes (Ashbell *et al.,* 1987).

Driehuis *et al.* (1997) found that high DM silages (i.e. haylage) contained much lower amounts of ethanol when inoculated with LAB than when not. Adding osmotolerant inoculants to high-DM crops could therefore be a way to enhance the rate of lactic acid production and pH decline, and to avoid ethanol formation. In Paper **I**, where two different inoculants were used, fermentation in haylage in Experiment 1 was similar to the results of Driehuis *et al*. (1997), but in Experiment 2, content of lactic acid, ethanol and pH were not different between control and inoculated haylage.

Production of ethanol in silage and haylage can also occur as a result of plant enzyme activity in the initial stages of anaerobiosis. In absence of oxygen, glycolysis produces ethanol, lactic acid and alanine from pyruvate as a consequence of inhibition of the respiratory chain. Pyruvate decarboxylase is the initial enzyme in the reaction and has an acid pH optimum. If accumulation of organic acids occurs during initial stages, as proposed by McDonald, Henderson & Heron (1991), pH is reduced and pyruvate decarboxylase activated. However, haylages in Papers **I–V** were heavily wilted, and as plant respiration generally decreases and ceases with increasing DM content (Wylam, 1953; Sullivan, 1973), plant enzymes seem less likely as the main cause of ethanol formation.

Ethanol can be rapidly absorbed from the gastrointestinal tract, but the horse appears to have an unusually high concentration of alcohol dehydrogenase in the liver (Chapman & Rudram, 1978). These authors administered 0.57 and 1.14 g ethanol/kg body weight using a nasogastric tube and found the highest ethanol content in the blood 1 h after administration, irrespective of the amount infused into the stomach. The mean rate of ethanol elimination from the blood was 6.3 mg/100 ml/h. Kristensen *et al.* (2007) concluded that alcohol concentrations typical in corn silage (4 to 14 g ethanol and 1 to 6 g propanol per kg DM), did not show any indication of saturation of hepatic alcohol metabolism in dairy cows fed the silages, but that ruminal metabolism and post-ingestion blood levels of alcohols could be affected. Silage in Papers **II** and **III** also contained 2,3-butanediol in amounts close to the ethanol content. Studies on utilization of 2,3-butanediol by horses have, to the author's knowledge, not been published, but Mathison, Fenton & Milligan (1981) concluded that feed intake, growth rate and diet digestibility in sheep were not affected by a 5% inclusion of 2,3-butanediol to a hay diet.

Content of WSC were generally higher in haylages than in silages (**I–V**), which was in agreement with the results of Gordon *et al.* (1961), Finner (1966) and Nicholson *et al.* (1991). The higher WSC content in haylage could be explained by a generally lower microbial activity compared to silage. The differences in the WSC fraction among the preserved forages in Paper **V** could be explained by

respiration and hydrolysis during wilting and ensiling (Wylam, 1953; Sullivan, 1973). Content of sucrose and fructans was lower and glucose and fructose higher or similar in preserved forages compared to their corresponding fresh crops, which indicated a degradation of sucrose and fructans to hexoses during storage. This degradation was larger in silage and haylage than in hay (**V**). Lundén Pettersson & Lindgren (1990) found values of glucose, fructose, sucrose and fructans in wilted fresh grass similar to the values in the fresh crop in Paper **V**, and also that the main changes in WSC, both during wilting and ensiling, was seen in the sucrose and fructan fractions.

The baled forages in Papers **I–V** contained low levels of lactic acid and had higher pH-values than usually found in chopped silages stored in silos, which is in accordance with previous studies (Haigh, 1990; Field & Wilman, 1996; Slottner & Bertilsson, 2006). However, the wrapped forages could not be considered as badly fermented, as levels of butyric acid and ammonia-N were low, or badly preserved, as mould counts were low. pH has previously been reported to differ between bale silage and silo silage. Field & Wilman (1996) used data from 5650 clamp silages and 820 baled silages, and found that pH was generally higher in bales than in clamps at similar DM levels. Also, Haigh (1990) made an on-farm survey of big bale silages, and showed that total lactic and acetic acid concentrations were lower and pH 0.8 units higher than in comparable bunker silages. This indicates that intervals for quality variables used for conventional silo silage cannot be applied directly to baled forages. Also, Wieringa (1958) found the correlations established between pH, butyric acid, ammonia and lactic acid in wet silage not to be applicable to heavily wilted silages. The equation of Weissbach (1968), relating pH to DM for avoidance of clostridial (butyric acid) fermentation, was valid for silages containing 150 to 500 g DM/kg. Thus, these equations can not be directly applied to haylage. This is in accordance with Muck (1988), who stated that fermentation was not as important in silages with higher DM levels (>550 g/kg, i.e. haylage) to prevent general growth of clostridia, as clostridial activity was inhibited at these DM contents (Wieringa, 1958; Leibensperger & Pitt, 1987).

Hengeveld (1983) found a decrease in clostridial spore counts in silage when tedding frequency was increased from once to twice per day during wilting. This is in accordance with the results of Paper **III,** where clostridial spore counts decreased with increased number of mechanical field treatments and increasing dry matter level. In Papers **I**, **II**, **IV** and **V**, there were no differences in clostridial spore counts between DM treatments. Enterobacterial counts were higher in hay than in wrapped forages (**IV**, **V**), but were present also in silages and haylages (**II–V**). The number of enterobacteria usually decreases during the first days of ensiling (Heron, Wilkinson & Duffus, 1993; Östling & Lindgren, 1995), but can increase during wilting of grass crops (Spoelstra, 1987).

Mould counts were generally higher in hay than in silage or haylage (**IV, V**) but no differences in mould counts between silage and haylage were detected (**I–V**). Due to the detrimental effects of mould spores on equine airways (*e.g.* Robinson *et al.*, 1996), and the presence of mycotoxins in mouldy forages (O'Brien *et al.*, 2006), care should be taken to inhibit mould growth in feeds. Roquefortin C,

mycophenolic acid and andrastin A were found in amounts of up to 20 mg/kg, and roquefortines A, B and D, festuclavine, marcfortine A and agroclavine in amounts of 0.1–5 mg/kg, in visibly mouldy bale silage (O'Brien *et al.*, 2006). Although effects of the different mycotoxins have not been not studied to any large extent in animals, detrimental influence on health cannot be ruled out (Scudamore & Livesey, 1998; Wilkinson, 1999). Intoxication of horses by both *Aspergillus spp*. and *Penicillium spp*. has been reported (Aller, Edds & Asquith, 1981; Asquith, 1991; Vesonder *et al.,* 1991; Ocholi *et al.,* 1992; Barnett *et al.,* 1995).

Influence of epiphytic microflora on fermentation

As the majority of horses in Sweden are used for "leisure riding" and have comparably low requirements for energy and protein (NRC, 1989), silage and haylage crops are cut rather late in the season, compared to silage harvested for lactating dairy cows. Cutting the crop at a late maturity stage also results in the standing crop being inhabited by a different epiphytic microflora than at an early cut (Behrendt, Müller & Seyfarth, 1997). This might influence the direction of fermentation and the hygienic quality of the preserved forage. The epiphytic microflora on fresh crops consists mainly of LAB, enterobacteria and fungi (Lacey, 1989; Pahlow, 1991; Behrendt, Müller & Seyfarth, 1997). Pahlow (1991) presented data showing the influence of cutting date on the composition of the epiphytic microflora on grass, and found late cuts to result in increased counts of LAB, enterobacteria, clostridia and moulds but not yeasts. Lacey (1989) also found filamentous fungi to develop during plant growth, especially during senescence and ripening. Behrendt, Müller & Seyfarth (1997) found higher numbers of heterotrophic bacteria and filamentous fungi on a late first cut crop than on a regrowth crop cut at the same time (mid-July). The microbial populations were generally characterized by a low diversity, and the largest numbers of taxa were found on late cut material (mid-July). Enterobacteria were found particularly on overmature grass, did not show up until the end of May and were higher in numbers on the late first cut crop than on the regrowth crop. The dominating species among enterobacteria was *Pantoea agglomerans* (teleomorph *Erwinia herbicola*) (Behrendt, Müller & Seyfarth, 1997). In Papers **I, II, IV** and **V**, microbial counts of LAB on the fresh crop were within normal ranges (Pahlow & Dinter, 1987) or lower, and enterobacteria counts were higher than any other microbial variable in the fresh crop (**II**, **IV**,**V**), probably reflecting the epiphytic flora.

The type of sward used for silage or haylage production may also influence the fermentation outcome. Swards commonly used for forage production at small horse farms in Sweden are extensively managed, old, permanent grass leys also used for grazing. Keating & O'Kiely (2000) found differences in conservation characteristics between *Lolium spp.* swards managed for intense silage production and old, permanent grass swards. Silage from permanent grass swards was higher in pH and had lower ratios of lactic acid to acetic acid + ethanol, compared to silage from *Lolium spp*. swards. The sward used in Papers **II–V** was a permanent grass ley, and this might have contributed to the higher ethanol and lower lactic acid content in the forages.

Wilting and pre-treatment of the crop may also influence the epiphytic microflora. Pahlow & Dinter (1987) found that the number of LAB/g fresh matter of the harvested crop ranged from log 2.85–6.78 for both grass and maize, that chopping increased and wilting (in most cases) reduced the number of LAB (wilted to 330 g/kg within 24 h). Muck (1987) found that the major factors correlated to the number of LAB (on chopped lucerne) were wilting time, average wilting temperature and the rate of drying. A slow drying rate resulted in higher LAB numbers at longer wilting times. The same author also found that the greatest rate of LAB growth occurred during the first day of wilting. Wilting for extended periods during wet weather conditions may also increase the number of enterobacteria (Beck, 1965). In this thesis, only Paper **V** allowed a proper comparison of fresh crops within an experiment, but no effect of wilting on numbers of LAB or enterobacteria was found. However, enterobacteria compete with LAB and can produce the undesired compunds ethanol and butanediol during fermentation (Pahlow *et al.*, 2003). Also, enterobacteria usually die off in silage due to presence of lactic and acetic acid and a lowering of the pH (Heron, Wilkinson & Duffus, 1993; Östling & Lindgren, 1993, 1995). In baled haylage, lactic acid contents were very low and pH rarely below 5.40 (**I**–**V**). The influence of high counts of enterobacteria on the fresh crop on direction of fermentation in baled haylage may therefore be an area requiring further study.

Aerobic storage stability

Haylage may heat very rapidly after opening, due to a high content of residual WSC (Savoie & Jofriet, 2003). Therefore, it may be important to inhibit microbial activity in haylage during aerobic storage, especially if big bales are used in small stables where the number of horses is low. When oxygen diffuses into the silage, lactate-assimilating yeasts grow and a gradient of pH, lactic acid as well as other acids, and oxygen is created from the silage surface, producing heat and niches with micro-environments suitable for different micro-organisms (Jonsson, 1991). Using additives prolonged aerobic storage stability in opened bales (**I)**. The reason for this was probably the presence of sodium benzoate and sodium propionate in the additives. In order for sodium benzoate to be effective against fungi, pH should be lower than 6 (Woolford, 1975), which was the case in Paper **I**. Some LAB inoculants may also have the ability to reduce aerobic deterioration. Ström (2005) used *Lactobacillus plantarum* MiLAB 393 as an inoculant in silage, and found it to inhibit spoilage yeast. The precise mode of inhibition was not found, but synergy between lactic acid and other antimicrobial substances produced by MiLAB 393 probably explained the anti-fungal activity. The antifungal properties of the inoculants used in Papers **I** and **V** were not known.

Compaction of the silage has been regarded as a factor having a major influence on the aerobic stability. Less dense silage has higher oxygen diffusion rates (Rees, Audsley & Neale, 1983), as oxygen penetration depends mainly on diffusion and volumetric flow (McGechan & Williams, 1994). Oxygen entering the forage allows growth of aerobic microorganisms (Jonsson, 1991). However, Hoxey & Billington (1987) found no differences in aerobic deterioration for low dry matter

silage with different compaction levels. For high dry matter silages, the aerobic deterioration was limited to a region close to the silage face, but compaction reduced oxygen penetration and aerobic deterioration (Hoxey & Billington, 1987). Thus, to minimize aerobic deterioration in baled haylage after opening, a high bale density and use of additives at ensiling (**I**) may be helpful.

Storage of small bale silage and haylage

There was a risk of poor bale density (**I, III**) or deviations from a square bale shape (**II**) in small bale silage and haylage. As long as the stretch film layers provide an airtight seal, bale density is less important for storage stability (O'Kiely, Forristal & Lenehan, 2000 b). However, bales with an uneven shape may be difficult to wrap with a proper 50% overlap of the layers which in turn could provide a way for air to enter. Also, bales with a tendency to lose shape, such as low DM and/or low density bales may be more subjected to damage during handling (Randby & Fyhri, 2005). Disruption of the stretch film layers could impair storage stability, and in such cases, bale density may be important for storage stability. In Paper **II**, silage bales deviated from a rectangular form, and silage was the only treatment where mould counts were above detection level. However, mould counts in Paper **II** were on average very low. If the deviating bale shape seriously impaired tightness of the wrapping, mould counts would probably have been much larger, especially in long-term stored bales.

There is little information on losses in baled silage during storage (Savoie & Jofriet, 2003). The model of aerobic fungal growth in silage developed by Muck, Pitt & Leibensperger (1991) and Pitt, Muck & Pickering (1991), and the model of air infiltration losses by McGechan & Williams (1994), were predominantly based on information from silage stored in conventional silos, which differs from baled silage in both fermentation variables (Haigh, 1990; Field & Wilman, 1996; Fychan & Jones, 1996) and density (Ruxton & Gibson, 1995). Bales also have a much higher surface to volume ratio than silos (Forristal & O'Kiely, 2005), resulting in larger volumes of forage being close to the surface in bales. The sensitivity of baled silage during storage was demonstrated by McNamara *et al.* (2002), who investigated bird damages on wrapped silage bales in Ireland. McNamara *et al.* (2002) found that even small stretch film damage could result in quantitative DM losses due to mould growth, especially in high DM silages. The extent of mould growth in baled silage in Ireland was studied by O'Brien *et al.* (2005, 2007 a), who confirmed that unsatisfactory storage conditions and insufficient amounts of stretch film layers caused mould growth in bales. O'Brien *et al.* (2005) found 40% of all bales examined (on 35 farms) to have visible damages in the stretch film, and 90% of the bales had visible mould growth. It was also found that well-managed bales contained lower counts of mould propagules than normal on-farm managed bales. In well-managed bales, *P. roqueforti* spores and visible mould growth was absent, in contrast to on-farm managed bales (O'Brien *et al.*, 2007 b). In Paper **II**, storage of small square bales for 14 months generally resulted in changes in fermentation parameters, WSC content and yeast counts, and silage was more prone to change than haylage. No increase in mould growth was

detected during storage in any of the forages, but yeast counts were generally higher in long-term stored bales. O'Brien *et al.* (2007 b) and Jonsson *et al.* (1990) both found *Candida lambica* (teleomorph *Pichia fermentans*) and *Hansenula anomala* (teleomorph *Pichia anomala*) to be the most common yeast species in baled silage. These genera were also the dominating yeast species in silage where air was allowed to penetrate during fermentation (Jonsson & Pahlow, 1984).

Tocopherols and carotenes in preserved forage

The reduction in tocopherol and carotene contents in silage and haylage in Paper **III** was not clearly associated with the extent of fermentation, but positive linear correlations were strongest between lactic acid and α-tocopherol, and between lactic acid and β-carotene. This may indicate that lactic acid fermentation was more important than the extent of fermentation (or DM level) itself for preservation of α-tocopherol and β-carotene in grass. Thus, the larger reduction in total vitamin E content associated with decreasing DM contents in airtight stored grain (Hakkarainen, Työppönen & Bengtsson (1983 a, b) was not evident for grass silage and haylage (**III**).

The results of Paper **III,** as well as the variation reported in the reviews of Ballet, Robert & Williams (2000) and of Nozière *et al.* (2006), indicated that the conservation method was not the primary determinant of α-tocopherol and β-carotene content in forage. The content of α-tocopherol probably depends to a larger extent on plant maturity, since mature plants have lower leaf:stem ratio than young plants (Miller, 1958), and leaves contain higher levels of α-tocopherol than stems (Brown, 1953; Horvath *et al.,* 2006). Also, the botanical composition of the crop influences the amount of carotenes in forages. Grasses have been shown to have lower losses of β-carotene than lucerne and red clover during wilting, probably due to lower levels of an unidentified aerobic oxygenase enzyme in grasses (Kalač & Kyzlink, 1980), as well as lower leaf losses during harvest. For determination of factors affecting both α-tocopherol and β-carotene levels in forage, the influence of harvest methods and wilting need further attention.

Some, but not all, of the forages in Paper **III** contained sufficient amounts of α-tocopherol to theoretically satisfy the vitamin E requirements of horses at maintenance. Exercise, growth, pregnancy and lactation require additional vitamin E (NRC, 1989), and the content of α-tocopherol in the conserved forages might be too low if forage constitutes the entire ration. All treatments contained sufficient amounts of β-carotene to, in theory, cover the requirement of provitamin A for all categories of healthy horses (NRC, 1989).

Influence of forage conservation method on preference of horses

The horses' preference for silage in Paper **IV** was clear, although the reason for this preference was unknown. One cause may be the lower DM level, making silage resemble pasture grass in its physical appearance. Waring (1974) observed horses that had developed a "hay wetting procedure", in which the horses soaked

mouthfuls of hay in their water supply before chewing. The reason for this behaviour was not explained, but Waring (1974) also mentioned the resemblance of wet hay to fresh grass as a possible reason. The silage in Paper **IV** had a slightly lower NDF content than the other forages and this might have played a role in the preference of the horses. Although preference and VDMI do not measure the same thing, it should be noted that correlations between NDF content and VDMI in horses are poor (Dulphy *et al.,* 1997; Cuddeford, 2005). Also, there was no difference in NDF content between hay and any of the haylages, but eating time and consumption differed between the same forages (**IV**). Silage and hay contained equal levels of *in vitro* digestible organic matter and estimated metabolizable energy, whereas both of the haylages contained approximately 97 % of the same variables in silage and hay. Therefore, NDF concentration, *in vitro* digestible organic matter or metabolizable energy content was not considered to have had any major impact on preference.

Comparisons of studies investigating horse preference are problematic, as the number of published studies is few. The area is also difficult to interpret and understand, partly because preference is dependent on the available alternatives. Studies of feral horses in Wyoming (Crane, Smith & Reynolds, 1997), Camargue (Duncan, 1992) and Alberta (Salter & Hudson, 1979) reported that abundance and succulence (measured as percent moisture) of graminoid vegetation in proximity to preferred habitats seemed to be primary factors influencing habitat selection. Also, the diet composition followed seasonal availability of different plant species, but proportional intake of different species was fairly constant between seasons (Salter & Hudson, 1979; Duncan, 1992; Crane, Smith & Reynolds, 1997) with a few exceptions reported by Duncan (1992). Horses are selective but do not consume only the preferred feed but survey their environment and move back and forth between different feed alternatives both at pasture (Archer, 1971, 1973, 1978; Naujeck, Hill & Gibb, 2004) and when fed different preserved feeds indoors (Goodwin, Davidson & Harris, 2002). This behaviour was also seen in Paper **IV**, as horses did not ignore the less preferred forages, but were observed to alternate between the forages, even though a clear preference for silage existed.

A lower voluntary intake of clamp silage compared to big bale silage, haylage and hay, was reported by Moore-Colyer & Longland (2000), who found ponies to consume only about half the amount of clamp silage compared to the other forages (on a DM basis). However, the forages used in the study had substantial differences in nutritive value (CP content was 154, 111, 70 and 44 g/kg DM in clamp silage, big bale silage, haylage and hay respectively), and were therefore not suitable for comparing the effects of different conservation methods. McLean *et al.* (1995) compared hay and clamp silage fed to horses and also found lower voluntary intakes of the silage, but the nutritive content of the forages used differed greatly, as hay contained 728 and silage 540 g NDF/kg DM. Austbø (1990) used the same crop for production of clamp silage, big bale haylage and hay, and found a lower intake and larger feed refusals of silage, but only when it had a noticeable smell of butyric acid. When the silage had a pleasant smell, intake was not different from hay or haylage. Rejection of butyric acid-containing silage has also been reported in ruminants (Waldo, Keys & Gordon, 1972; Demarquilly

& Dulphy, 1977). The silage in Paper **IV** had very low concentration of butyric acid, and there was no general rejection of silage by the horses. The results of Paper **IV** showed that different forage conservation methods can influence horse preference for forage. This finding can perhaps contribute to future studies of factors that regulate forage intake in horses, which at present is limited (Cuddeford, 2005).

Individual preferences existed as there were interactions between horses and forages in Paper **IV**. Individual variation in selectivity has previously also been reported among horses on pasture (Marinier & Alexander, 1991). This individual variation, together with the influence of previously fed diets, constitutes additional difficulties in studying preference. Confounding effects from previous feeds were reported by LaCasha *et al.* (1999), who measured VDMI and digestibility of three different forages in a changeover study. At the end of the experiment, horses were given simultaneous access to the three forages fed previously, and were found to consume less of the forage they had been fed immediately before. How long this memory of a feed lasts is uncertain, making it difficult to take even a known feeding history of an animal into account.

Influence of forage conservation method on hindgut fermentation in horses

There were no differences in chemical or microbial composition of colon content or faeces in horses fed hay, haylage and silage. This showed that the forage conservation methods employed did not affect hindgut fermentation differently (**V**). Hindgut fermentation values were comparable to a previous study where horses were fed hay with a similar CP content (Hintz, Argenzio & Schryver, 1971). In comparison with horses fed timothy hay (Kern *et al.*, 1974) or pasture grass and hay (Mackie & Wilkins, 1988), acetate was lower and propionate higher in colon samples in Paper **V**. It should however be noted that the crop used in Paper **V** was harvested early in the season and contained twice as much CP compared to the hay used by Kern *et al.* (1974). Crops harvested early contain less indigestible and more readily digestible components, which may explain the somewhat higher propionate percentage in the colon fluid in Paper **V**. Propionate and valerate concentrations were found to increase in caecal fluid of horses when grain was added to forage diets (Hintz, Argenzio & Schryver, 1971; Kern *et al.,* 1973). This indicated that some of the grain reached the caecum and provided a substrate for microbial fermentation (Crawford *et al*., 1968).

Small differences in hindgut fermentation variables between individual horses were noted in Paper **V**. However, these differences were not considered to have had any large influence on the results. The differences were probably a sign of individual variation in hindgut fermentation among horses. Individual variations have previously been reported to be larger in horses compared to ruminants and rabbits, when measuring digesta particle size (Udén, 1982), digestibility (Udén & Van Soest, 1982) and retention time of liquid (CoEDTA) and solid (Cr) digesta markers (Udén *et al*., 1982). It should also be noted that caecal cannulation has

been reported to increase total tract mean retention time of both hay and concentrates compared to before cannulation (Pulse, Baker & Potter, 1973; Austbø & Volden, 2006). The horses in Paper **V** were cannulated in both the caecum and right ventral colon, which have previously been reported to lower the total tract mean retention time (Drogoul, Poncet & Tisserand, 2000). The mean retention time could affect the extent of digestion and fermentation of the feed (Van Weyenberg, Sales & Janssens, 2006), and therefore studies using fistulated horses can produce different results than studies using euthanized animals.

In the kinetic study of the colon in Paper **V**, no evident pattern of cyclic changes in VFA or pH was found, but minor irregular differences in pH, total organic acids and individual acids were present between sampling times. As there were no interactions between forage type and sampling time, all forages had similar fermentation kinetics in the right ventral colon. The amount of total and individual VFA in the colon at a given time is the result of production and absorption of VFA. Argenzio, Southworth & Stevens (1974) studied *in vitro* transport of VFAs in isolated mucosa from the large intestine of horses, and found that although individual acids were present in equal 30 mM concentrations in the lumen, they were transported to the blood side in the order of acetate > propionate > butyrate. Acetate was transported to the blood side at a greater rate than propionate or butyrate, but the absorption ($\mu mol/cm^2$) of acetate from the lumen bath was not different from absorption of propionate or butyrate. Mucosa of caecum and colon absorbed VFA at approximately equivalent rates (Argenzio, Southworth & Stevens, 1974). The net appearance and disappearance of VFA in the equine large intestine has also been correlated with cyclic changes of Na^+ in the same compartment (Argenzio and Stevens, 1975).

Lactate levels in colon and faeces were low in Paper **V**. Alexander, MacPherson & Oxford (1952) and Alexander & Davies (1963) reported that lactic acid was not commonly found in colon liquid. However, the anaerobic Gram-negative cocci *Veillonella gazogenes* was frequently isolated from intestinal sites, and this cocci was found to produce gas and VFA only with lactate as substrate (Alexander, MacPherson & Oxford, 1952). The reason for the low lactate levels in equine colon in Paper **V** could thus have been due to conversion of lactate to propionate, as has been reported in the rumen of sheep (Mackie, Gilchrist & Heath, 1984). However, these authors found only 0.025 of the total VFA to come from lactate on a high-forage diet low in readily fermentable carbohydrates. Also, the propionate concentration and propionate proportion of total organic acids were not exceptionally high in Paper **V**. Alexander & Davies (1963) found the highest amount of lactic acid in the stomach of horses, but could not show that lactic acid was absorbed in the small intestine as the lactate concentration was sometimes higher in the caudal ileum compared to in the cranial ileum. Argenzio, Southworth & Stevens (1974) and de Fombelle *et al.* (2003), on the other hand, found decreasing concentrations of lactic acid from the stomach and along the small intestine in horses fed different diets, and in the large intestine, lactic acid had almost completely disappeared. Both lactate-producing and lactate-utilizing organisms were present in different compartments of the alimentary canal. The dominance of lactic acid in the stomach, and of VFA in the hindgut, may represent

different environments or substrate availability favouring different microorganisms (Alexander & Davies, 1963; de Fombelle *et al*., 2003). In Paper **V**, the number of lactate utilizing bacteria in the colon was close to log 7 CFU/ml, and the number of lactate producing bacteria was about log 6 CFU/ml, which were both considered as normal levels (Julliand, 2005). Chamberlain, Thomas & Anderson (1981) found that protozoa played a central role in metabolism of lactic acid in the rumen of sheep fed grass silage diets. The role of protozoa in the equine hindgut is, however, not very well understood (Ike *et al.*, 1983; Moore & Dehority, 1992).

Cellulolytic bacteria were a minor component of total anaerobic bacteria in colon (**V)**, which was in accordance with Kern *et al.* (1973) and Julliand (2005). *Ruminococcus flavefaciens* has been identified as the predominating cellulolytic species in pony and donkey ceacum (Julliand *et al.*, 1999), together with *R. albus* and *Fibrobacter succinogenes* (Julliand, 2005). Major end-products from cellobiose fermentation by isolated strains of *R. flavefaciens* from equines were comparable (with some deviations) to corresponding rumen strains, except for ethanol production, which was larger in the equine strain, and malate and fumarate, which were not detected from isolated equine species of *R. flavefaciens* (Julliand *et al.*, 1999). *In vitro* fermentation end-products of the equine fungi *Piromyces citronii,* found in caecum of ponies and donkeys, were formate, acetate and ethanol from fermentation of glucose and cellobiose, in contrast to rumen strains of the fungi, which also produced lactate (Julliand *et al*., 1998).

Using faecal samples as indicators of the status in the colon of forage-fed horses were applicable for lactic acid, *i*-butyric acid, valeric acids, total anaerobic bacteria, cellulolytic bacteria and streptococci (**V**). However, as concluded by da Veiga, Chaucheyras-Durand & Julliand (2005), more information of the influence of different diets on caecal, colonic and faecal ecosystems is needed before faeces can be regarded as a substitute for caecal and colon samples in horses. However, if variables in faeces diverge strongly from normal, they may serve as indicators of upsets in the alimentary canal. Milinovich *et al.* (2006) and van Eps & Pollitt (2006) induced laminitis in horses using an overload of fructo-oligosaccharides administered via nasogastric tubing, which resulted in a faecal pH of 4 to 5 in conjunction with watery diarrhoea. In these cases, faecal pH and consistency can clearly be regarded as a sign of a disturbed gut function.

Digestion of water-soluble carbohydrates in forages

Although there were differences in the water-soluble carbohydrate fraction between hay, haylage and silage, these differences were not manifested in colon samples (**V**). The very low concentrations of glucose, fructose and sucrose in colon (**V**), can be explained by the presence of brush border disaccharidases in the small intestine and the transport of glucose and fructose across the small intestinal membrane (Roberts, 1975; Dyer *et al.,* 2002). Varloud *et al.* (2004) also reported that "sugar and starch" in different diets were digested to about 0.90 in samples taken from the caecum of horses. Fructan content was also very low in colon samples in Paper **V**. The fructans were probably degraded prior to right ventral

colon, as the glycosidic linkage between C2 and C6 (β-2,6-linkage) in the furanosyl units is regarded as very unstable in acid environments (Smith, 1973), such as in the pyloric part of the equine stomach (Frape, 2004). Temperate grasses such as Timothy and Meadow fescue contain fructans mainly of the levan type, which consists of β-2,6-linked fructose units and a sucrose residue (Chatterton *et al.,* 1990; Suzuki, 1993; Cairns *et al.,* 1999). Very small amounts of cereal fructans (β-2,1-linked) have been found to be absorbed in the small intestine of rats (Nilsson *et al*., 1988), but in fistulated pigs fed β-2,1-linked fructan (inulin), more than half of the oral dose had disappeared prior to the caecum (Böhmer, Branner & Roth-Maier, 2005). In horses, Coenen, Mößeler & Vervuert (2006) argued evidence for microbial digestion of inulin (from Jerusalem artichoke) pre-caecally through measurement of fermentative gases in the horses' breath. Also, Respondek *et al*. (2005) were unable to recover any short-chain fructo-oligosaccharides (SCFOS, β-2,1-linked) from the digesta in the stomach and small intestine of horses fed a diet of straw, concentrates and added SCFOS when horses were sampled two hours after feeding. Pre-caecal microbial digestion of SCFOS and pre-colonic digestion of grass fructans in horses thus seems likely to exist, and could be supported by the findings of de Fombelle *et al.* (2003), who demonstrated the presence and activity of microorganisms along the entire gastrointestinal tract of horses, and of Yuki *et al.* (2000) who reported the presence of *Lactobacillus spp*. in the non-secreting area of the horse stomach.

It has recently been reported that equine laminitis can be experimentally induced by an orally administered overload of β-2,1-linked inulin from chicory roots (van Eps & Pollitt, 2006; Milinovich *et al.,* 2006). Berg *et al.* (2005) demonstrated lower pH and higher lactate and VFA content in faeces from horses receiving increasing amounts of a β-2,1-linked SCFOS in the feed. However, a connection between an overload of β-2,1-linked SCFOS and presence of β-2,6-linked levans in grass in their abilities to induce laminitis or disturbances in the gastrointestinal tract has not yet been established. Bailey, Marr & Elliot (2004) also argued that the induction of laminitis by different models may not precisely mirror naturally occurring laminitis. If a connection between the fructan types were to be proven, the reduced fructan levels in silage or haylage (**V**) would render these forages more suitable than hay to horses prone to develop laminitis. On the other hand, no difference in fructan content in the colon was found between horses fed hay, haylage or silage (**V**). It should also be noted, that the lowest dose of fructans used to induce laminitis was 7.5 g/kg body weight (van Eps & Pollitt, 2006). The highest fructan conctent found in Paper **V** was 34 g/kg DM in the fresh crop used for silage. To reach the dose 7.5 g fructans/kg body weight by eating this forage, a horse weighing 500 kg would have to consume 110 kg DM. Thus, the levels of fructans present in the fresh crops or preserved forages in Paper **V** do not seem to be sufficient to be able to induce laminitis in horses.

Main conclusions

- Small bale silage and haylage production is possible using high-density hay balers, but modification of the knotting mechanism may be necessary. Ethanol can be a common fermentation product in small bale silage and haylage, and can exceed the content of lactic acid.
- Long-term storage (14 months) of small silage bales resulted in changes in fermentation variables compared to short-term storage (2 months), but values at 14 months were well correlated with values at 2 months. Small haylage bales were not affected by the same storage time.
- Content of α-tocopherol and β-carotene in small bale silage and haylage was not clearly associated to the extent of fermentation, but correlated to lactic acid content.
- Different forage conservation methods had an impact on horse preference in favour of silage. Silage had the longest eating times and highest consumption, followed by haylage and hay in descending order.
- Conservation of forage as hay, haylage or silage did not produce different responses in microbial or chemical composition in the hindgut of fistulated horses fed the forages.

Populärvetenskaplig sammanfattning

Det har länge varit tradition att utfodra hästar med vallfoder i form av hö. På senare år har dock ensilage och hösilage (≥50 % torrsubstanshalt (ts)) i inplastade balar i viss utsträckning ersatt hö i hästfoderstaterna. Det kan finnas flera anledningar till detta, men en orsak är sannolikt svårigheten att producera och lagra hö torrt. Torr lagring av hö är en förutsättning för att undvika tillväxt av mögel. Förekomst av mögelsporer i foder och strömedel är förknippat med sjukdomen Recurrent Airway Obstruction (RAO), eller ”kvickdrag” som den också kallas. RAO är en kronisk luftvägssjukdom som innebär att hästens lungor inte har samma kapacitet som lungorna hos en helt frisk häst, vilket påverkar hästens prestationsförmåga negativt. Ensilage och hösilage innehåller i allmänhet färre mögelsporer än hö, och både skörd och lagring är mindre väderberoende än skörd och lagring av hö.

Inplastat vallfoder produceras vanligen i stora runda eller fyrkantiga balar (ca 400-700 kg). Problemet med stora balar i hästsammanhang är att de flesta hästar finns på gårdar eller i stall med ett fåtal djur. För ett sådant stall innehåller stora ensilage- och hösilagebalar i allmänhet för mycket foder för att de skall hinna utfodras innan fodret blivit skämt, särskilt under de varmare årstiderna. Små hästgårdar utan mekaniserad balhantering kan också ha problem med att hantera

storbalar. Ensilage och hösilage i mindre balar kan därför vara ett intressant alternativ, men det finns i dagsläget inga kommersiellt tillgängliga balpressar som är anpassade för en sådan småbalsproduktion. Kunskapen om hösilage och ensilage med avseende på fodrets användbarhet och inverkan på digestionskanalen hos hästar är också begränsad. Syftet med de studier som avhandlingen grundar sig på var därför att närmare studera både produktion av småbalar med tillgängliga metoder, och användning av inplastat vallfoder i hästfoderstater.

I den första studien (**I**) undersöktes om det var möjligt att använda en konventionell höpress (glidkolvspress) för att producera ensilage (35 % ts) och hösilage (50-60 % ts) i småbalar. I försöket studerades också om det var nödvändigt att använda ensileringsmedel (biologiska och kemiska) för att få en bra hygienisk kvalitet på fodret, om balarnas hygieniska kvalitet var stabil efter öppning och hur många lager plast (6, 8 eller 10) som behövdes för att balarna skulle vara täta. Resultaten visade att ensileringsmedel inte var nödvändigt för att få en acceptabel hygienisk kvalitet, men att det förlängde hållbarheten på fodret efter öppning av balarna. Antalet plastlager hade främst effekt på mängden koldioxid inne i oöppnade balar, vilken var högst med 10 lager plast. Det fanns en del praktiska problem med att få tillräckligt hårt pressade balar utan att äventyra pressens knytmekanism, och utan att riskera att balbanden gick sönder direkt vid pressning. I studie **III** och **V** löstes detta problem genom att byta ut pressens originalknytare till sådana knytare som normalt sitter i stora fyrkantsbalpressar. På så sätt gick det att använda starkare och grövre pressgarn, och balarna kunde pressas hårdare och få högre baldensitet (kg ts/m^3) utan att balbanden gick sönder.

För att undersöka om småbalarna var hållbara över en längre lagringsperiod, och om den kemiska och mikrobiologiska sammansättningen i fodret förändrades över tiden, gjordes en lagringsstudie med provtagning av balarna 2 och 14 månader efter inplastning (**II**). Denna studie gjordes på ensilage (35 % ts), hösilage med låg ts-halt (55 %) och hösilage med hög ts-halt (70 %), som producerats från samma vall och samma skörd. Resultaten visade att 14 månaders lagring av balarna påverkade alla kemiska fermentationsvariabler utom smörsyra- och etanolhalt i ensilaget, men de båda hösilagen uppvisade inte några förändringar. Av de mikrobiologiska variablerna var det bara jästantalet som ökade med lagringstiden. Även om förändringar i de kemiska fermentationsvariablerna ägde rum under lagringen överensstämde analysresultaten efter 2 och 14 månaders lagring mycket väl med varandra. Det innebar att prov som togs 2 månader efter inplastning var godtagbara för att åskådliggöra sammansättningen i fodret även efter 14 månaders lagring. Detta gäller under förutsättning att plasten bibehållits intakt, att balarna inte skadats på något sätt och att ingen smörsyrajäsning skett.

I samband med att framför allt hösilage börjat användas i större utsträckning till hästar, har också funderingar kring vitamininnehållet i inplastat vallfoder uppkommit. Det är sedan tidigare känt att E-vitamininnehållet i lufttätt lagrat korn kan minska kraftigt under lagringen, särskilt då ts-halten är runt 72 %. Eftersom hösilage också till stor del konserveras genom lufttät lagring, uppstod frågan om innehållet av vitamin E i gräs påverkades av lagring på samma sätt som korn. Eftersom naturligt vitamin E tas upp bättre i hästens kropp än syntetiskt framställt

vitamin E, är det en god idé att försöka bevara så mycket som möjligt av det naturliga E-vitaminet i fodret. Likaså uppkom frågeställningar kring hur betakaroten, ett förstadie till vitamin A, påverkades av lufttät lagring eftersom tidigare studier visat att mängden betakaroten kan minska med upp till 80 % vid långsam torkning av gräs till hö, men att förlusterna i allmänhet är mindre än 30 % i välfermenterat ensilage. En studie av innehållet av vitamin E och betakaroten i ensilage (30 % ts), hösilage 1 (50 % ts) och hösilage 2 (60 % ts) genomfördes därför (**III**). Gräs ensilerades i små fyrkantiga och små runda balar som lagrades i 11 månader innan de öppnades och provtogs. Resultaten visade att ts-haltens inverkan var svår att tolka. Ensilage i fyrkantsbalar och hösilage 2 i rund- och fyrkantsbalar innehöll 60 % av den initiala mängden E-vitamin i grönmassan, och motsvarande siffra för betakaroten var 86 %. Ensilage i rundbalar och hösilage 1 i rund- och fyrkantsbalar innehöll 39 % av den initiala E-vitaminhalten, motsvarande siffra för betakaroten var 33 %. Det fanns alltså ingen tydlig generell effekt av ts-halt och fermentationsgrad (eller balform) på vitamininnehållet. Det fanns dock ett samband mellan innehållet av mjölksyra och båda vitaminerna, vilket indikerade att en bra konserveringsprocess (mjölksyrajäsning) kan vara fördelaktig för att bevara innehållet av vitamin E och betakaroten i inplastat vallfoder. Andra faktorer än konserveringsmetoden verkar också spela större roll för innehållet av vitamin E och betakaroten i vallfoder, som t ex växternas botaniska utvecklingsstadium vid skörd, den botaniska sammansättningen av vallen och förtorkningen vid skörd.

Att använda ensilage och hösilage som hästfoder har ibland diskuterats, eftersom enstaka studier påvisat en nedgång i hästarnas konsumtion när de utfodrats med ensilage jämfört med hö. Att hästen äter sitt vallfoder är naturligtvis viktigt, eftersom fodervägran för just grovfoder kan ge mycket stora problem med störningar i mag-tarmkanalen (som tex kolik). För att undersöka om hästar hade någon preferens för vallfoder konserverade på olika sätt, genomfördes därför en studie där försökshästar fick välja fritt vilket vallfoder de ville äta (**IV**). För att kunna göra en sådan jämförelse var det viktigt att de foder som ingick i studien inte hade olika näringsinnehåll eller olika botanisk sammansättning. I de tidigare studierna användes foder från olika vallar och skördar, vilket gjorde det svårt att tolka resultaten. Ensilage (30 % ts), hösilage med låg ts (55 %), hösilage med hög ts (70 %) och hö (88 % ts) producerades därför från samma gräsvall och samma skörd i små fyrkantsbalar. Hästarna erbjöds sedan 1 kg ts av varje foder samtidigt, under 2 timmar per dag i 20 dagar. Hästarnas förstahandsval, hur länge och hur mycket de åt av varje foder registrerades. Hästarna åt mest av ensilaget, därefter kom hösilaget med låg ts, sedan hösilaget med hög ts och sist höet. Samma ordning gällde för ättiden och för hästarnas förstahandsval. Höet åts aldrig upp helt och hållet, och hästarna lämnade aldrig ensilaget för att äta av ett annat foder efter att ha luktat och/eller smakat på det förstnämnda. Studien påvisade alltså att konserveringsmetoden kunde inverka på hästars preferens för vallfoder, och att det var till ensilagets fördel, även om orsaken till denna preferens inte kunde förklaras. Detta resultat gäller för välfermenterade ensilage. Feljästa ensilage med höga halter av smörsyra och ammoniak kan inte förväntas ge samma resultat.

Torrsubstanshalten i gräs som pressas och plastas in spelar en avgörande roll för hur fodret kommer att konserveras. Grönmassa som har en låg ts-halt kommer att ensileras i större utsträckning än grönmassa med en hög ts-halt. Det beror på att mjölksyrabakterierna, som sköter om själva ensileringen, behöver vatten för att kunna bilda mjölksyra från socker i gräset. Det innebär också att ensilage innehåller mer mjölksyra och andra organiska syror, samt mindre socker och har ett lägre pH-värde jämfört med hösilage och hö. Skillnaden mellan hö och hösilage kan variera beroende på hösilagets ts-halt, men generellt sett innehåller hö mer socker och har ett något högre pH-värde än hö. Mängden mjölksyra och andra organiska syror i hösilage är vanligtvis mycket låg och skiljer sig inte nämnvärt från hö, men kan variera beroende på ts-halten och mjölksyrabakteriernas aktivitet. Hur dessa olikheter i den kemiska sammansättningen i hö, hösilage och ensilage påverkar hästens grovtarmsmiljö har inte undersökts tidigare, och därför genomfördes ett utfodringsförsök (**V**). Hö, hösilage (55 % ts) och ensilage (35 % ts) producerades från samma vall och samma skörd. För att kunna studera aktiviteten i hästens tarm vid utfodring med de olika vallfodren användes fistulerade försökshästar. Fisteln var placerad i högra ventralkolon och möjliggjorde provtagning av hästens grovtarmsinnehåll. Provtagning av tarminnehållet och av träck gjordes efter 21 dagars utfodring med respektive vallfoder. Dessutom utfördes en kinetikstudie, som innebar att tarminnehållet provtogs före utfodring, och sedan efter 2, 4, 8 och 12 h, för att följa fermentationsförloppet i kolon. Utfodring med de olika vallfodren gav inte upphov till några skillnader i mikrobiologiska eller kemiska variabler i varken tarminnehåll eller träck. Det var alltså inte möjligt att från proverna på tarminnehåll eller träck avgöra om hästen hade ätit hö, hösilage eller ensilage. Kinetikstudien påvisade också att de olika fodren uppförde sig likadant i kolon vid de olika provtagningstidpunkterna.

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Meine Oma, Du bist mein Idol.

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Min mamma Kerstin och min pappa Heribert – jag har så oerhört mycket att tacka er för, men mest av allt vill jag säga tack för er outsinliga uppmuntran för alla mina påhitt, och för all hjälp jag alltid fått när något har varit problematiskt eller svårt. Ni har alltid gett mig alla möjligheter, och jag har lärt mig mycket mer av er än vad ni nog egentligen själva tror.