Metabolism and Hindgut Ecosystem in Forage Fed Sedentary and Athletic Horses

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Abstract

The objective of this thesis was to study the effects of forage crude protein content and forage conservation method on metabolism and hindgut ecosystem, abrupt feed changes and exercise response.

In total 22 horses, both sedentary and athletic, were used. The sedentary horses were fistulated in right ventral colon and the athletic horses were in Standardbred race training. In the sedentary horses bacterial counts, volatile fatty acid (VFA), pH and dry matter (DM) in colon content and faeces and in the athletic horses nitrogen intake and excretion, fluid and acid-base balance, digestibility and exercise response were measured. Forages used in the studies were 1) grass silage with 13% (RP) and 17% (HP) crude protein, 2) grass hay, haylage and silage (same cut): 81%, 55% and 36% DM, 3) grass hay and silage (same cut): 82% and 45% DM and 4) grass haylage and silage (same cut): 68% and 41% DM.

Colon VFA concentrations were higher and pH was lower on the HP diet than the RP diet. No other major alterations in composition and activities of the colon ecosystem were found. In the athletic horses urine and faecal nitrogen excretion and water intake increased and faecal DM decreased on the HP diet compared to the RP diet already within 48 h. Colon lactobacilli increased on the silage diet and streptococci decreased on the haylage diet compared to the hay diet, but there were no changes in VFA concentrations or pH. Apparent digestibility was higher on the silage diet compared to the hay diet. Estimated evaporative fluid loss was higher and tended to be higher on the silage diet than the hay diet and on the HP diet than the RP diet, respectively. Changes were observed already within 48 h. Heart rate, blood pH, plasma lactate and total proteins did not differ between the RP diet and the HP diet or between the haylage diet and silage diet during exercise and recovery. However, the increased urine and evaporative fluid losses might be a disadvantage during more prolonged exercise when fluid loss is a limiting factor.

Keywords: Colon microflora, Digestibility, Equine, Exercise response, Feed change, Fluid balance, Haylage, Nitrogen, Protein, Silage.

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List of Publications

This thesis is based on the work contained in the following Papers, referred to by Roman numerals in the text:

I Muhonen, S., Connysson, M., Lindberg, J E., Julliand, V., Bertilsson, J. and Jansson, A. (2008). Effects of crude protein intake from grass silage-only diets on the equine colon ecosystem after an abrupt feed change. *J Anim Sci.* 2008 doi:10.2527/jas.2007-0374.

II Connysson, M., Muhonen, S., Lindberg, J E., Essén-Gustavsson, B., Nyman, G., Nostell, K. and Jansson, A. (2006). Effects on exercise response, fluid and acid-base balance of protein intake from forage-only diets in Standardbred horses. *Equine vet J Suppl.* Vol 36, 648-653.

III Muhonen, S., Julliand, V., Lindberg, J E., Bertilsson, J. and Jansson, A. (2008). Effects on the equine colon ecosystem of silage and haylage diets after an abrupt change from hay (submitted).

IV Muhonen, S., Lindberg, J E., Bertilsson, J. and Jansson, A. (2008). Effects on fluid balance, digestion and exercise response in Standardbred horses fed silage, haylage and hay (submitted).

Papers I-II are reproduced with the permission of Journal of Animal Science and Equine Veterinary Journal.

Abbreviations

BW	Body weight
CP	Crude protein
DM	Dry matter
Exp	Experiment
HP	High protein
LSM	Least square means
N	Nitrogen
ME	Metabolisable energy
MJ	Megajoule
MSB	Molassed sugar beetpulp
RP	Recommended protein
TPP	Total plasma protein
VFA	Volatile fatty acids
WSC	Water-soluble carbohydrates

Thesis at a Glance

Year	Horses	Feeds	Feeding level	Measurements	Main results	Paper
2005	4 sedentary (crossbreed) colon fistulated horses, BW: 412-452 kg.	Two grass silages: RP: 13% CP, HP: 17% CP, ~ 50% DM, 11.0-11.5 MJ ME/kg DM.	Maintenance: ~ 11 g DM/kg BW	Bacterial counts, VFA, pH, DM in colon content and faeces.	Higher VFA and lower pH on HP than RP diet but no other major alterations in composition and activities of colon ecosystem or faecal DM.	I
2005	6 athletic (Standardbred) trotters, BW: 443-548 kg.	Two grass silages: RP: 13% CP, HP: 17% CP, ~ 50% DM, 11.0-11.5 MJ ME/kg DM.	Maintenance × 2: ~ 19 g DM/kg BW	N intake and excretion, fluid and acid-base balance, exercise response.	Increased urine, faecal N excretion and water intake, lower faecal DM and pH on HP than RP diet at rest. Blood pH, plasma lactate did not differ during intensive exercise.	II
2006	4 sedentary (crossbreed) colon fistulated horses, BW: 431-515 kg.	Hay, haylage, silage (same cut): 81, 55, 36% DM, 11.5-11.7 MJ ME/kg DM.	Maintenance: ~ 11 g DM/kg BW	Bacterial counts, VFA, pH, DM in colon content and faeces.	Slight decrease in colon and faecal DM on the haylage and silage diets. Increase in lactobacilli on the silage diet and decrease in streptococci on the haylage diet. No changes in VFA or pH.	III
2007	5 athletic (Standardbred) trotters, BW: 493-527 kg.	Hay, silage (same cut): 82, 45% DM, 11.6 MJ ME/kg DM.	Maintenance × 2: ~ 19 g DM/kg BW	Fluid balance and digestibility.	Faecal DM and plasma lactate did not differ at rest. Apparent digestibility and estimated evaporative fluid loss was higher on the silage than the hay diet.	IV
2007	6 athletic (Standardbred) trotters, BW: 453-588 kg.	Haylage, silage (same cut): 68, 41% DM, 11.2-11.3 MJ ME/kg DM + MSB (18% of total DM intake).	Maintenance × 2: ~ 20 g DM/kg BW	Water intake, exercise response.	Higher water intake on the haylage than the silage diet. No difference in heart rate, respiratory rate, plasma lactate, blood pH and TPP.	IV

Background

The use of haylage and silage in horse feeding is becoming more and more frequent in Sweden. Surveys have shown that 32% of Swedish riding horses were fed silage and 14% hay and silage (Henricson, 2007) and 56% of trainers of trotters in the North of Sweden fed silage or haylage, usually in combination with hay, straw or lucerne (Westerlund, 2007). Haylage or silage as feed for horses is also seemingly increasingly popular in other parts of Europe (Moore-Colyer and Longland, 2000; Peiretti and Bergero, 2004; Ursin and Johannessen, 2004; Ragnarsson and Lindberg, 2008). Silage production depends less on weather conditions than hay production, and therefore a feed with high nutritional and hygienic quality can easily be produced. Feeding silage instead of hay has also been recommended because it can reduce the amount of respirable particles and allergens to which the stabled horse is exposed and maintain clinical remission in horses with chronic obstructive pulmonary disease (Vandenput et al., 1997; Vandenput et al., 1998). In Sweden, diseases of the respiratory system have been the third and the sixth most common cause of death in horses (Wallin et al., 2000; Egenvall et al., 2006). In other European countries, diseases of the respiratory system have also been a common cause of loss or ailment (Clausen et al., 1990; Wilsher et al., 2006).

The silage qualities, popularly, called "cow silage" has a bad reputation among horse owners and trainers. These silages are often characterized by a low DM and high lactic acid content with a high energy and CP content. There have been reports of increased faecal water content (slight diarrhoea) (Holmquist and Müller, 2002; Müller, 2002) and anecdotal reports of reduced exercise performance when feeding or when introducing silage. However, few studies are published on grass silage diets for horses and very few for athletic horses.

Due to different conservation methods the chemical composition of hay and silage differ. Silage usually contains lactic and acetic acid, has a lower pH and lower NDF and WSC content compared to hay. Differences in digestibility of hay and silage in equines have also been suggested (Moore-Colyer and Longland, 2000). Feeding silage high in CP concentration may lead to an excessive CP intake. An increase of N compounds in the large intestine could possibly alter the composition of the microbial flora, increase the ammonia production, and affect the pH and osmolality (Meyer, 1983). A high CP intake is associated with a higher heat production, higher water intake and a more acidic urine (Blaxter, 1989; Houpt, 1993a; Meyer, 1987) and has been suggested to be a limiting factor for the athletic horse (Glade, 1983).

Horses water intake by drinking decreases as the DM concentration of forage decreases, which is logical because more water is provided via the feed (Austbø, 1990). However, Austbø (1990) showed that the total water intake (water by drinking + water in feed) was higher as the DM concentration of the forage decreased suggesting that the total fluid balance might be altered. An abrupt introduction of new roughage might also be a reason for disturbances in the ecosystem of the gastrointestinal tract. However, there is a lack of studies on the effects of abrupt changes and no studies of abrupt changes between different forage diets or diets with different CP content.

In order to study the effects of forage conservation method and forage CP content on metabolism, the hindgut ecosystem and exercise it could be of interest to feed forage-only diets. To meet the energy requirements of athletic horses the forages have to be harvested early to have high energy content, corresponding to the energy content in conventional race horse diets consisting of low energy forage and energy dense concentrate. The experimental diets in this thesis were only forages supplemented with minerals and salt to both sedentary and athletic horses, except for the second experiment in Paper IV where the diets had to be supplemented with MSB. Moreover, abrupt feed changes were performed to investigate both the acute effects of abrupt feed changes and the adaptation.

Today there is no clear definition separating haylage and silage. Generally most would agree that haylage is drier than silage and therefore contains less lactic acid and has a higher pH. In the Papers included in this thesis forages with DM concentrations of about 50% and lower have been called silage but for example Ragnarsson and Lindberg (2008) uses the term haylage for forages of 43-50% DM. When referring to the literature, the same term has been used as by the authors in the referred papers.

Introduction

Forage and Silage Qualities

The crude fibre content increases as forage matures and the CP content decreases. Therefore the digestibility and nutritive value of forage decrease with maturity. This has been shown with alfalfa, timothy and orchard grass diets where apparent DM and crude fibre digestibilities decreased with increasing maturity, and the digestibility of CP was directly proportional to the CP content of the forage (Darlington and Hershberger, 1972; Ragnarsson and Lindberg, 2008). Different species of grasses and legumes also differ in nutritive value (Fonnesbeck et al., 1967).

During silage making the rate of fermentation is affected by the amount of moisture present in the ensiled crop. Wilting delays the bacterial multiplication and leads to higher pH and WSC, and lower levels of lactic, acetic and butyric acids. Wilting has no major inhibitory effect on proteolysis, but seems to inhibit the deamination of amino acids probably due to mainly decreased clostridial activity (McDonald, 1981). Lower N concentrations and higher NDF concentrations in hay compared to silage simultaneously harvested have been reported (Jaakkola and Huhtanen, 1993) and can be explained by higher leaf losses during handling in the field because hay is drier and more brittle (Honig, 1980).

In ruminants, effects of ensiling on digestibility are generally considered to be small (Demarquilly and Jarrige, 1970). However, higher utilisation (kg milk/kg DM silage) of silage compared to hay from the same cut has been reported for dairy cows and was partly explained by differences in energy and protein content of the forages (Bertilsson and Burstedt, 1984). The impact of the conservation method on the rumen fermentation seems to depend mainly on the extent of silage fermentation (Jaakkola and Huhtanen, 1993).

Moore-Colyer and Longland (2000) showed different apparent DM digestibilities, 39%, 57%, 61% and 67% for hay, haylage, big bale silage and clamp silage respectively. However, the forages were not from the same swath and varied considerably in fibre and CP content. Istasse et al. (1996) compared two first-cut and one second-cut silage with DM contents of 43%, 66% and 79%, and with DM intakes of 8.4 kg/d, 8.6 kg/d and 7.0 kg/d. The apparent DM and CP digestibilities averaged 64% and 62%, 68% and 76%, and 59% and 80% respectively. Särkijärvi et al. (2002) reported apparent DM digestibilities of 57%, 69% and 52% for silages with DM and NDF contents of 37% and 62%, 24% and 49%, and 56% and 49% respectively. The above reported apparent digestibility values are contradictory, although different techniques for measuring digestibility have been used. However, forage maturity and botanical composition has a large impact on digestibility and to determine the effect of conservation method the forages used must be from the same swath and harvested simultaneously.

Fate of Lactate

For herbivores lactate intake via the feed is generally low, but lactate-utilising bacteria colonies the stomach. Lactate concentrations are highest in the stomach and the lowest values are found in the jejuno-ileum, caecum and ventral colon (de Fombelle et al., 2003). Varloud et al. (2007) measured lactate-utilising bacteria in gastric contents of horses ranging from 3.18 to 5.38 $\log_{10}$ cfu/ml. The stomach and the large intestine are the major sites of VFA production and the large intestine has a high capacity of VFA absorption but the stomach appears unable to transport organic acids to the blood side (Argenzio et al., 1974). The gastric and pyloric mucosa absorbs VFA but transports small amounts to the blood side (Argenzio et al., 1974). The decrease in gastric lactic acid concentration has been shown to accompany the disappearance of food from the stomach and it would be reasonable to assume that lactate is absorbed from the small intestine (Alexander and Davies, 1963).

Gill et al. (1986) showed that 90% of total lactate flux was found to be metabolized in the rumen. It has been suggested that silage-fed ruminants are more adapted to use lactate. An in vitro study showed a more rapid metabolism of lactate when animals consumed silage instead of hay (Newbold et al., 1987). The rate of disappearance of lactate in protozoal incubations were up to 15 times higher than in bacterial incubations, showing that the protozoa play a major role in lactate uptake (Newbold et al., 1987). Jaakola and Huhtanen (1992) calculated the metabolic fate of infused lactate into the rumen on a molar basis and 21% of lactate was converted to acetate, 52% to propionate, and 27% to butyrate when bulls were kept on grass silage-based diet.

Microbiology of the Equine Gastrointestinal Tract

The microbial flora inhabits the entire equine gastrointestinal tract (de Fombelle et al., 2003; Julliand et al., 2008; Kern et al., 1973; Mackie and Wilkins, 1988). High counts of total anaerobic bacteria has been found throughout the digestive tract, with higher counts in the stomach than the small intestine. Cellulolytic bacterial concentrations are high in the hindgut and low in the stomach and small intestine suggesting a low forage degradation in the precaecal section of the digestive tract (de Fombelle et al., 2003). Proportionally, cellulolytic bacteria appear to be higher in caecum than in the lower parts of the hindgut, and the lowest concentrations of lactobacilli, streptococci and lactate-utilising bacteria can be found in the caeum. This suggests the caecum as the most favourable for cellulolysis and less exposed to rapidly fermentable carbohydrates that seem to flow quickly through the caecum and have a higher impact on the colon flora (de Fombelle et al., 2001, 2003). The microbial profile of the caecum and right ventral colon is related to the diet composition (Julliand et al., 2001). The amount of forage and concentrate fed also affects the volatile fatty acid composition in the hindgut, with higher acetate concentrations on a roughage diet and higher propionate concentrations on a high starch diet (Hintz et al., 1971; Julliand et al., 2001). In caecum and colon biochemical fermentation parameters, VFA and ammonia concentrations and pH depend on the sampling time after feeding (Medina et al., 2002). Diet composition also affects the DM concentration of the digesta. The DM concentration of digesta in the caecum was higher on a mixed feed compared to a hay diet, 8.7% vs. 4.5% respectively (Schwabenbauer et al., 1982). Varloud et al. (2004) reported DM concentrations of 9.9-8.6% in caecum and 15.6-15.9% in right ventral colon when feeding pelleted fibre and starch diets.

The proteolytic bacteria form a high proportion of the total culturable bacteria (Baruc et al., 1983; Kern et al., 1973; Mackie and Wilkins, 1988; Maczulak et al., 1985). Mackie and Wilkins (1988) showed a higher number of colony counts of proteolytic bacteria in the caecum than in the colon and a close relationship between total culturable and proteolytic bacteria in gut segments of the small intestine. Kern et al. (1974) showed that proteolytic activity per gram ingesta was 30-fold or more higher in the ileum than in the caecum or colon of ponies.

An in vitro study has shown that lactic acid bacteria from silage can survive in rumen fluid (Weinberg et al., 2004). *Lactobacillus rhamnosus* has been shown to survive transit through the gastrointestinal tract of horses; however, the extent of colonization was low (Weese et al., 2003). Drouault et al. (1999) showed, using *Lactococcus lactis* in rats, that the way bacteria were administered had a dramatic impact. If transiting with the diet it was resistant to gastric acidity (90-98% survival and 10-30% survival in the duodenum).

Abrupt Feed Changes

There is a lack of studies on the effects of abrupt feed changes. de Fombelle et al. (2001) investigated the effects of an abrupt change from a hay diet to a hay and barley diet. This study showed that concentrations of total anaerobes tend to be lower 5 h after the abrupt introduction of barley, and that there was a sharp increase in the concentrations of lactobacilli, streptococci and lactate at 5 and 29 h post prandial. Goodson et al. (1988) studied the effects of an abrupt change from an alfafa hay diet to a concentrate diet (ground corn and soybean meal) and then back again to alfalfa hay using one Shetland pony. Caecal starch-utilising bacteria rose to 92.2% of the total bacterial numbers 24 h after the abrupt change to concentrate and the lowest caecal pH was 5.8. The shift back to hay did not cause any rapid changes in the caecum ecosystem. Goodson et al. (1988) reported that after the change to concentrate the animal appeared dull and depressed standing with its head down, but changing the diet to hay caused no observable disorders. As far as I know there are no published studies on abrupt changes between forage diets or diets with different CP content.

Nitrogen Metabolism

A positive N balance is when an animal retains more N than is excreted as during growth and pregnancy, and a negative N balance is an excess of N excretion over intake (Beitz, 1993). As mentioned above, CP digestibility is proportional to the CP content of the forage (Darlington and Hershberger, 1972; Ragnarsson and Lindberg, 2008). An increased N intake causes an increase in plasma urea concentration and an increased N excretion via both urine and faeces (Freeman et al., 1988; Prior et al., 1974; Slade et al., 1970). Miller and Lawrence (1988) found higher blood urea-N levels on a high protein diet (1807 vs. 1256 g CP/d) but plasma ammonia concentration was not affected.

Caecum N content has been found to be of a similar order of magnitude to that in the feed ration (Schwabenbauer et al., 1982). The pre-caecal N digestion seems to decrease with increasing fibre content of the forage (Gibbs et al., 1988). Nitrogen balance studies have shown that urea is recycled in the equine gastrointestinal tract (Houpt and Houpt, 1971; Prior et al., 1974; Slade et al., 1970). When urea is recycled to the large intestine it is degraded to ammonium ions by urease-catalyzed hydrolysis (Wootton and Argenzio, 1975). Hintz et al. (1970) measured urease activity in caecal fluid of 17 to 25% of that reported in bovine rumen fluid. Maczulak et al. (1985) found that few caecal bacterial isolates had the ability to use ammonia or urea as N sources for growth. This may reflect the medium used to isolate these organisms or urease activity in the equine caecum may be related to a distinct population associated with the caecal wall not comprised by the sampling procedure, or both (Maczulak et al. 1985).

Slade et al. (1971) and McMeniman et al. (1987) demonstrated that labeled amino acids were absorbed from the caecum and colon. However, the quantities were small and microbial amino acids synthesized in the large intestine probably do not contribute significantly to the amino acid supply of the horse (McMeniman et al., 1987).

Acid-Base Balance

Grasses contain anions of malic, aconitic and citric acids in the form of potassium, calcium and magnesium salts or complexes. As the anions are oxidised, hydrogen ions are removed and bicarbonate ions accumulate. Therefore, horses, like other herbivores, have an excess of base and excrete an alkaline urine with a high bicarbonate concentration (Houpt, 1993a).

When amino acids are metabolized in the liver the primary nitrogenous end product is ammonia which reacts with carbonic acid yielding ammonium and bicarbonate. Most of the ammonium ions are used in the hepatic synthesis of urea when also hydrogen ions are formed. The hydrogen ions react with the bicarbonate and overall there is no effect on acid-base balance. However, a high protein diet can cause an excess of acid leading to a lowered urine pH (Houpt, 1993a).

Heat Increment of Feeding

The increased heat production after feeding has multiple causes. For example the actual act of eating, fermentation heat, hydrolyses in the lumen of the gut, absorption processes, transport of absorbed nutrients, provision of oxygen at the cell level, disposal of carbon dioxide and other products of oxidation (Blaxter, 1989). The dominant causal factor seems to be the heat associated with the generation of ATP. The efficiency of utilization of ME varies with the chemical nature of the nutrient absorbed (Blaxter, 1989). Protein is particularly associated with a low efficiency of utilization of ME and consequently a high increment of heat. The yield of ATP from oxidation of protein is low, largely due to the ATP required for synthesis of urea (Blaxter, 1989). Pagan (1985) found that the protein oxidation accounted for approximately 1/4 of the total heat production in horses.

Fluid Balance

An increased CP intake causes an increased heat production (Blaxter, 1989) and excessive heat is evaporated from the body (Andersson and Jónasson, 1993). Therefore, increased CP intake causes increased water consumption (Meyer, 1987). However, water intake was not consistent with CP intake and water intake per kg of DM was not correlated to the CP content of the diets when several all-hay and hay-grain diets with CP contents ranging from 8.3 to 16.0% were compared (Fonnesbeck, 1968a). In the same experiment total, faecal and urinary water excreted and faecal DM concentration was not significantly correlated to CP content of the feeds.

Horses water intake by drinking decreases as the DM concentration of forage decreases, which is logical because more water is provided via the feed (Austbø, 1990; Dahlqvist, 1999). However, Austbø (1990) also showed that the total water intake (water by drinking + water in feed) was higher as the DM concentration of the forage decreased when comparing bunker silage (23% DM), big bale silage (53% DM) and hay (82% DM) from the same swath. In the same study the faecal DM concentration tended to differ between the hay, big bale silage and bunker silage diets (20, 18 and 17% respectively) but the differences were not statistically significant. For all treatments the faeces had normal consistency, but it was observed that with abrupt feed changes loose faeces were occasionally seen.

The NDF concentration of hay is usually higher than of silage simultaneously harvested (Jaakkola and Huhtanen, 1993), probably due to higher leaf losses during handling in the field since hay is drier and more brittle (Honig, 1980). If the fibre fractions of hay and silage differ, the water-holding capacity of the ingesta might differ (Eastwood, 1973). The type of diet influences the water and electrolyte content of the large intestine, which affects the total fluid balance (Meyer and Coenen, 1989).

Feeding Level and Exercise

The feeding level affects the utilisation of ME (Blaxter, 1989). Feeding restricted amounts compared to ad libitum of alfalfa and oat straw in ponies lead to longer mean retention times, but this did not result in any significant difference in apparent digestibilities (Pearson et al., 2001). Pearson et al. (2006) found no consistent effect of feeding level on apparent digestibilities for different forage diets in ponies. The same study showed longer mean retention times of fibre when diets were fed restrictedly compared to ad libitum but results were not significant.

Most feeding trials have been performed on horses at maintenance and the impact of exercise on digestion is contradictive. At the same level of feeding, intense exercise lead to lower apparent DM and ADF digestibility, a decreased mean retention time and higher water intake (34.4 vs. 29.4 l/d) (Pagan et al., 1998). Bergero et al. (2002) found, at the same level of feeding, an increased workload to decrease DM and crude fibre apparent digestibility. Worth et al. (1987) found that exercise increased apparent DM, ADF and CP digestibilities in two groups of horses when feed intake was adjusted to maintain BW. Exercise increased voluntary feed intake and apparent digestibility and the mean retention time decreased for particulate marker but increased for fluid marker in yearling horses (Orton et al., 1985). Nitrogen retention has been shown to increase when horses were subjected to an increased programme of exercise (Freeman et al., 1988). Increased N intake has also been shown to increase N retention in growing non-exercised horses (Prior et al., 1974) and adult non-exercised horses (Slade et al., 1970). Athletic horses fed at twice the maintenance level of energy requirements might have a different digestive response and fluid regulation than sedentary horses fed at maintenance level of energy requirements.

Exercise Response

An excess protein intake has been suggested to decrease performance in horses causing; longer time to finish races (Glade, 1983), higher heart and respiratory rates during and after endurance rides (Ralston et al., 1995). It has also been suggested that a restriction of dietary protein moderates the acidogenic effect of exercise (Graham-Thiers et al., 1999; 2001). However, lower heart rates, lower lactate accumulation and lower muscle glycogen utilization with high protein diets during stepwise and high speed exercise have been reported (Pagan et al., 1987). In the same study also lower muscle glycogen content on the high protein diet during long slow exercise was shown. Other studies have found no evidence for a negative or positive effect of excess dietary protein during exercise (Frank et al., 1987; Hintz et al., 1980; Miller and Lawrence, 1988; Miller-Graber et al., 1991). Slade et al. (1975) measured higher heart and respiratory rates and observed more profused sweating when horses were fed a high protein diet compared to a high starch or fat diet, but sweating was not measured and the data were not statistically treated.

There have been anecdotal reports of reduced exercise performance when feeding silage but Austbø (1990) found no differences in heart rate during a treadmill exercise test when horses were fed hay compared to bunker silage. As far as I know, there are no other extensive experiments or published studies on exercise response comparing conservation method; silage with hay, or silage with any other feedstuff.

Aims of the Thesis

The general aim of this thesis was to investigate how the colon ecosystem, fluid balance and exercise performance of horses are affected by different silage qualities. More specifically:

- To study the effect of a high CP intake from silage-only diets and the abrupt change between silage high in CP and silage providing a recommended CP intake on the composition and activities of the colon microflora in sedentary horses (**Paper I**).
- To study the effect of a high CP intake from silage-only diets and the abrupt change between silage high in CP and silage providing a recommended CP intake on N metabolism, fluid and acid-base balance and exercise response in athletic horses (**Paper II**).
- To study the effect of an abrupt change, and the following adaptation, from hay to haylage or silage on the composition and activities of the colon microflora in sedentary horses (**Paper III**).
- To study the effect of an abrupt change, and the following adaptation, between hay and silage on the fluid and acid-base balance and digestion in athletic horses (Exp 1, **Paper IV**).
- To study the effect of haylage or silage on the exercise response in athletic horses (Exp 2, **Paper IV**).

Hypotheses:

- A CP intake exceeding 40% of the recommendation (NRC, 1989) changes the composition and increases the activities of the colon microflora. Changes can be observed within 24 h (**Paper I**).

- A high CP intake alters fluid balance and enhances the exercise induced acidosis during intensive exercise. Changes in fluid balance and N excretion can be observed within 48 h (**Paper II**).
- A fermented forage (> 3% lactic acid) changes the composition and activities of the colon microflora. Changes can be observed within 28 h (**Paper III**).
- A fermented forage (~ 45% DM) diet changes the digestive response and fluid balance and might affect the plasma lactate concentration during rest and exercise. Changes in digestive pattern, fluid balance and plasma lactate can be observed within 48 h (**Paper IV**).

Materials and Methods

The experiments in this thesis were performed at Etablissement National d'Enseignement Supérieur Agronomique de Dijon (ENESAD), France, The National Trotting School, Wången, Nälden, Sweden and at the SLU research facility outside Uppsala, Sweden. The methods used in this thesis are presented or referred to in the accompanying publications. An overview of the forages used in this thesis is presented in Table 1.

Sedentary and Fistulated Horses

In total seven fistulated geldings were used in Paper I and III, one horse was used in both experiments. The experiment for Paper I was performed in spring 2005 and the experiment for Paper III in spring 2006. The horses were born between 1994 and 1999 and were fistulated in caecum and right ventral colon before 2003. The surgical technique used to fistulate the horses is described in Drogoul et al. (2000). The horses were six French crossbreeds and one French trotter. The whither heights were between 145 to 160 cm and during the experiments their BW ranged between 412 to 515 kg.

Athletic Horses

In total 15 Standardbred geldings were used in Paper II and IV, 2 horses were used in two of the three experiments. The experiment in Paper II was performed in spring 2005 and the two experiments in Paper IV in spring and autumn 2007. The horses were born between 1996 and 2002 and during the experiments BW ranged between 443 and 588 kg. All horses were in race training.

Table 1. *An overview of ME and chemical and microbial composition of the forages (mainly timothy and meadow fescue) in Paper I-IV (g/kg DM if not otherwise stated), more detailed analyses are shown in the accompanying publications*

	Paper I		Paper II		Paper III			Paper IV, Exp 1		Paper IV, Exp 2	
	RP	HP	RP	HP	Hay	Haylage	Silage	Hay	Silage	Haylage	Silage
ME, MJ/kg DM[1]	11.0	11.4	11.5	11.7	11.7	11.5	11.5	11.6	11.6	11.2	11.3
DM, %	49	52	45	50	81	55	36	82	45	68	41
CP, %	131	168	125	166	170	152	174	155	167	145	131
NDF	497	492	492	488	483	469	429	479	430	477	456
ADF	306	289	-	-	273	280	277	263	273	281	283
WSC[2]	100	93	52	81	117	122	80	157	140	132	106
Lactic acid	9.9	0.8	45.4	4.3	0.5	2.0	34.7	-	6.7	1.7	29.9
Acetic acid	2.8	0.9	0.7	0.3	0.1	0.8	4.0	-	1.3	0.3	2.9
pH	5.2	5.4	4.4	5.4	6.0	5.6	4.5	-	5.3	5.8	4.8
LAB[3]	6.2	4.6	5.7	5.3	0	4.3	6.6	0.4	5.1	-	-
Mould[3]	0	0	1.5	0	2.6	0.8	1.7	1.1	0	2.2	< 2.0

[1]The estimated energy values were calculated from the in vitro digestible organic matter values according to Lindgren (1979).

[2]Free glucose, free fructose, sucrose and fructans.

[3]$\log_{10}$ cfu/g fresh matter.

Feeding and Sampling of Horses

Paper I: The horses were fed approximately 20% of the daily feed allowance at 08:00, 10:00 and 15:00 and 40% at 17:30. Colon and faecal samples were taken before the feed change (0 h), then 4, 12, and 24 h after the abrupt feed change (at 07:30, 12:00, 20:00 and 08:00 respectively) and 7, 14 and 22 days after the feed change (at 08:00).

Paper II: The horses were fed at 06:00 (~11% of daily feed allowance), 12:00 (~23%), 17:00 (~22%) and 21:00 (~44%). The first day of each experimental period started with an abrupt feed change at 06:00 and a 48-h total collection of urine and faeces in collection harnesses. During day 20-22 a 72-h total collection of urine and faeces was performed.

Paper III: The horses were fed approximately 40% of the daily feed allowance at 08:00 and 60% at 17:30. Colon and faecal samples were taken before the feed change (-20 h), then 4 and 28 h after the abrupt feed change (all samples at 12:00), and 8, 15 and 21 days after the feed change (at 12:00).

Paper IV: The horses were fed at 06:00 (~11% of daily feed allowance), 12:00 (~21%), 17:00 (~21%) and 21:00 (~47%). The first day of each experimental period started with an abrupt feed change at 07:00 and a 48-h total collection of urine and faeces in collection harnesses. During day 18-20 a 72-h total collection of urine and faeces was performed.

Exercise Tests

In Paper II a standardised exercise test and a field test was performed. The standardised exercise test was performed on a treadmill on day 19 in both periods. The test started with a warm up with 5-min walk (1.8 m/s), 3-min trot (9 m/s), 45-s fast trot (11 m/s), and 4-min walk (1.8 m/s) designed to correspond to pre-race occurrences. After the warm up, the horses trotted for 3 min 15 s at 10 m/s at 5% incline and then at 9.5 m/s for 1 min with no incline.

The field test was performed on an oval racetrack and started with 4000-m slow trot (6.3-6.7 m/s), 2080-m trot (10.7-10.9 m/s) and 10-min walk. After the warm up the horses trotted 1600 m at 11.3-11.5 m/s and a 480-m finish as fast as they could (13.7-14.1 m/s). The field test was performed in pairs including one horse on each diet. The same driver drove the same horse at both test occasions but did not know which diet the horse was fed. After the field tests the drivers ranked the condition of the horses according to the following options; very dull, dull, alert, very alert or pulling.

In Paper IV an incremental interval exercise test was performed on an oval racetrack (approximately 0.6% incline). The horses were tested in pairs, one horse on each diet, after 17 days of adaptation to the diets. The test started with a 3000-m warm up at slow trot (9 m/s), followed by 5-min walk and then slow trot to the start point (250 m). The horses completed four intervals of 1000 m with 5-min walk in between and then trotted to the start. After the last interval the horses walked to the stable, approximately 5 min. The first interval was run at a speed of 9 m/s, the second interval at 11 m/s, the third interval at 11.6 m/s and the fourth interval at 12 m/s. The same driver drove the same horse at both test occasions. The exercise temperament was assessed post exercise by the drivers in a blind test, using an 11.5-cm linear index (exercise temperament: lazy = 0, very hard pulling = 11.5; calm = 0, very nervous = 11.5).

Statistical Analyses

The additional statistical analyses in this thesis have been performed according to the descriptions in the accompanying publications if not otherwise stated. Differences were considered statistically significant at $P < 0.05$. Table 5 and Figure 8 in the results section only show the values for 'at rest', 'peak value' and '15 min post exercise' but the statistical analysis include all values and are the same as in the Papers.

Simple correlations (r) were calculated using PROC CORR of SAS/STAT (Version 9.1, SAS Inst., Inc., Cary, NC) and *P*-values were calculated for $r = 0$. In these calculations individual values from two or four of the experiments in this thesis were used (Figure 1 - 3). Graphs with the regression equation and r^2 were done using Microsoft Office Excel 2003. Which experiments are included and how samples were obtained is described in the figures.

Results

Effects of Forage Diets After Adaptation

N Balance

In Paper II, N intake and faecal and urinary N excretion were higher on the HP diet than the RP diet, but N balance did not differ between the diets (Table 2). Urine pH was lower on the HP diet than the RP diet.

Table 2. *N intake and output (g/d) during 72 h total collection on the RP diet and the HP diet*[1] *after 20 to 22 days of adaptation (Paper II)*

	RP	HP	SEM	*P*-value
N intake	167	244	5	< 0.001
Faecal N	52	64	2	0.009
Urinary N	117	171	9	0.002
Total N ouput	169	234	7	< 0.001
N balance	-2	9	7	0.268

[1]Two silage diets, differing in CP concentration, were fed restrictedly to result in similar DM intakes, but a CP intake close to recommended (NRC, 1989) daily CP intake (diet RP, 1044 g CP/d) or higher than recommended daily CP intake (diet HP, 1525 g CP/d).

CP intake was correlated ($P = 0.006$) with blood/plasma urea across Paper I and II (Figure 1).

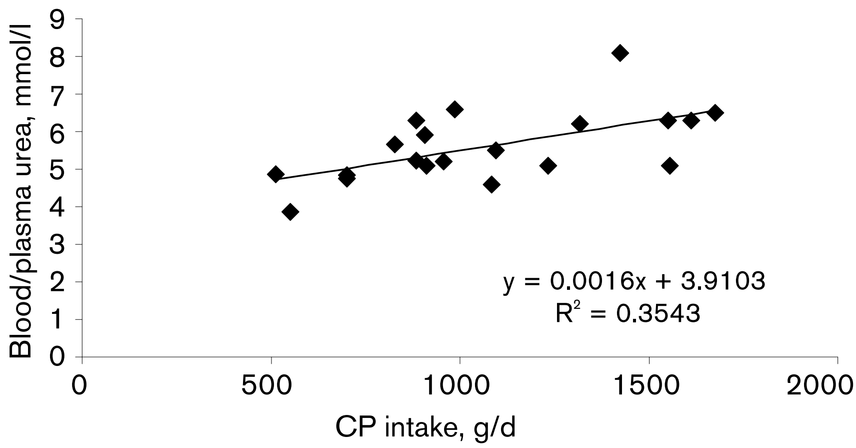

Figure 1. Blood/plasma urea vs. CP intake, values are from horses in Paper I and II. Plasma urea measured day 22, blood samples drawn before the morning feeding, sedentary horses (Paper I). Blood urea measured day 23, blood samples drawn after the morning feeding, athletic horses (Paper II).

Colon and Faecal Biochemical Pattern

Colon VFA concentrations were higher and pH was lower on the HP diet compared to the RP diet in Paper I (Table 3). Colon propionate concentrations (Paper I) were higher on the HP than the RP diet, but the VFA ratios (acetate + butyrate)/propionate) from day 7-22 were not significantly different between the diets (3.7 vs. 3.9 respectively, SEM = 0.3, P = 0.608). Iso-butyrate and iso-valerate concentrations did not differ; they were 0.62 vs. 0.65 and 0.69 vs. 0.81 mmol/l on the RP diet and the HP diet respectively. Colon lactate concentrations were below the detection limit (< 0.2 mmol/l). Faecal VFA concentrations were almost twice as high in the athletic horses than the sedentary horses (Table 3). Faecal DM and pH did not differ between diets for the sedentary horses in Paper I but was significantly lower on the HP diet than the RP diet for the athletic horses in Paper II (Table 3). Plasma urea tended to be higher on the HP diet than the RP diet for the sedentary horses in Paper I and was significantly higher for the athletic horses in Paper II (Table 3).

Table 3. *Colon and faecal total VFA*[1] *and pH, faecal DM, water intake and plasma urea LSM of four sedentary (maintenance-fed) horses (Paper I) and faecal total VFA, pH and DM, blood urea and water intake LSM of six athletic (twice maintenance-fed) horses (Paper II) fed the RP and the HP diet*[2]

	Sedentary horses[3] (Paper I)				Athletic horses[4] (Paper II)			
	RP	HP	SEM	*P*-values	RP	HP	SEM	*P*-values
Colon total VFA, mmol/l	45.1	51.8	5.8	0.034	-	-	-	-
Colon pH	7.2	6.9	0.1	0.035	-	-	-	-
Faecal DM, %	21.3	21.8	0.7	0.571	20.9	19.5	0.2	0.004
Faecal total VFA, mmol/l	35.4	34.8	5.2	0.797	53.5	59.6	3.5	0.258
Faecal pH	6.6	6.4	0.1	0.223	6.3	6.1	0.02	< 0.001
Water intake, kg/d	11.4	12.4	0.4	0.088	16.4	20.8	0.4	< 0.001
Plasma/blood urea, mmol/l	4.6	5.8	0.3	0.086	5.5	6.4	0.1	0.008

[1]Acetate, propionate, butyrate, isobutyrate, isovalerate, n-valerate, lactate.

[2]Two silage diets, differing in CP concentration, were fed restrictedly to result in similar DM intakes, but a CP intake close to recommended (NRC, 1989) daily CP intake (diet RP, 615 and 1044 g CP/d for Paper I and II respectively) or higher than recommended daily CP intake (diet HP, 873 and 1525 g CP/d for Paper I and II respectively).

[3]Colon VFA and pH, faecal DM and pH: LSM over time for samples taken after 7, 14 and 22 days, faecal VFA and plasma urea: samples taken after 22 days of adaptation. Colon, faecal and blood samples were taken before the morning feeding, water intake measured daily.

[4]Faecal DM and pH: 72 h total collection after 20-22 days, faecal VFA: 24 h total collection after 22 days, blood urea: LSM over time for samples taken after 19 and 23 days of adaptation, water intake measured daily.

Colon VFA concentrations did not differ between the haylage and silage diet in Paper III and the VFA ratios (acetate + butyrate)/propionate) did not differ between the diets (4.1 vs. 3.9 respectively, SEM = 0.1, P = 0.396). Colon lactate concentrations were almost exclusively under the detection limit (< 0.2 mmol/l).

Faecal pH was correlated with faecal total VFA (P < 0.001) across Paper I to IV, and faecal and colon pH (P = 0.003) across Paper I and III (Figure 2).

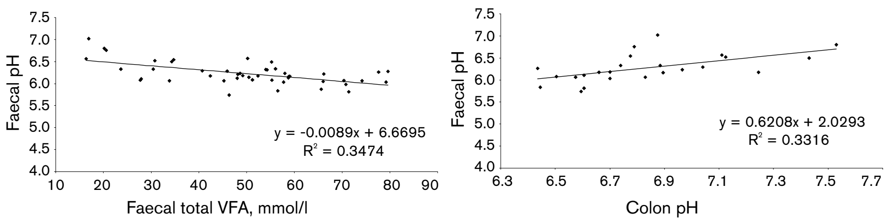

Figure 2. Faecal pH vs. faecal total VFA, data from Paper I to IV (Exp 1), and faecal pH vs. colon pH, data from Paper I and III, after three weeks of adaptation to the diets. Paper I and III are grab faecal samples and Paper II and IV are 24 h collections.

No diarrhoea was observed in any of the experiments. Figure 3 shows correlations between faecal DM concentration and DM concentration of the forage (P = 0.035), total water intake (water by drinking + water in feed) (P < 0.001), lactate (P = 0.221), CP (P = 0.018), NDF (P = 0.026) and ADF (P = 0.053) intake across Paper I to IV.

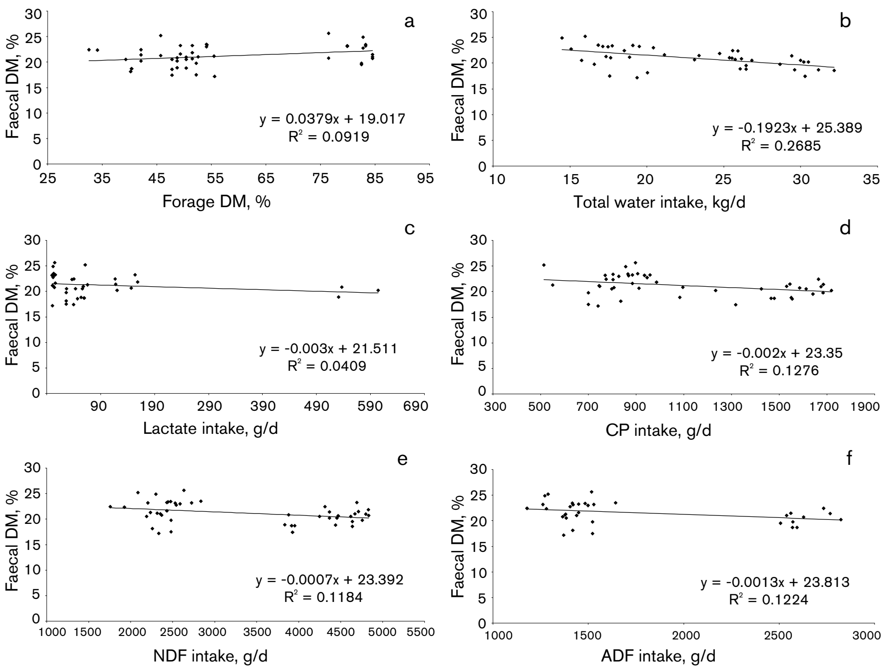

Figure 3. Faecal DM vs. forage DM, total water, lactate, CP, NDF and ADF intake after three weeks of adaptation to the diets. Data from Paper I to IV (Exp 1) are included in the figures. Paper I and III are grab faecal samples and total water intake from the same day (b), Paper II and IV are 72 h collections, total water intake from the same collection periods (b). c) lactate was not analysed on the hay in Paper IV. f) ADF was not analysed in Paper II.

Fluid Balance

In Paper II horses drank more on the HP diet than the RP diet, and the total water intake was also higher on the HP diet (Table 4). Total water output via faeces and urine did not differ between the diets but the difference in total water intake minus output tended to differ. Which indicate that more water was lost via evaporation on the HP diet (Table 4). TPP did not differ between the RP diet and the HP diet.

Table 4. *Water intake by drinking and in feed and water output via faeces and urine (kg/d) during 72 h total collection on the RP diet and the HP diet[1] after 20 to 22 days of adaptation (Paper II)*

	RP	HP	SEM	*P*-value
Water intake	16.7	19.1	0.7	0.045
Water in feed	8.3	9.5	0.2	0.007
Total water intake[2]	25.0	28.6	0.8	0.014
Water in faeces	10.8	12.2	0.6	0.107
Water in urine[3]	9.6	10.5	0.7	0.382
Total water output	20.4	22.7	0.8	0.069
Difference intake-output	4.6	5.9	0.5	0.095

[1]Two silage diets, differing in CP concentration, were fed restrictedly to result in similar DM intakes, but a CP intake close to recommended (NRC, 1989) daily CP intake (diet RP, 1044 g CP/d) or greater than recommended daily CP intake (diet HP, 1525 g CP/d).

[2]Water intake by drinking + water in feed.

[3]With an estimated urine DM of 9.5%.

The oxidation of one gram of protein yields about 0.4 ml of water (Houpt, 1993b). Assuming all urine N (Paper II) came from the oxidation of protein to urea the amount of metabolic water produced from protein oxidation would be 294 and 427 ml/d for the RP diet and the HP diet respectively (for calculations see Appendix 1a). Hence the production of metabolic water from protein oxidation is larger on the HP diet than the RP diet. Adding this to the total water intake and subtracting the total water output, the estimated evaporation would differ by even more. Although 200-400 ml of water is within the margin of error for measuring water intake.

The trends in water intake by drinking and total water intake were similar for Paper III and IV (Table 5). The drier the feed the higher water intake by drinking but this did not reflect the total water intake (drinking + water in feed) which showed an opposite pattern. In Paper IV total water output (water in faeces + water in urine) did not differ between the hay and the silage diet. The difference in total water intake minus output differed indicating that more water was lost via evaporation on the silage diet than the hay diet (values for water intake and output are presented more in detail in the paper).

Table 5. *Water intake by drinking and total water intake (drinking + water in feed) (kg/d) in sedentary horses (Paper III) and athletic horses (Paper IV)*

Paper and feeding level	Feed	Water intake by drinking	Total water intake
III[1]	Hay	15[a]	16[a]
Maintenance fed	Haylage	15[a]	19[b]
	Silage	12[b]	20[c]
	SEM	0.9	0.9
	P-value	< 0.001	< 0.001
IV Exp 1[2]	Hay	24	26
2 × maintenance fed	Silage	17	28
	SEM	0.4	0.4
	P-value	0.001	0.032
IV Exp 2[3]	Haylage	25	35
2 × maintenance fed	Silage	18	36
	SEM	1	1
	P-value	< 0.001	0.186

[abc]Within a column and exp without common superscript letter differ ($P < 0.01$).

[1]Measured daily for 21-day periods, period excluded from the statistical model.

[2]Measured daily for 21-day periods.

[3]Measured day 6-17 for 17-day periods, between 16:00 and 8:00 h. Total water intake: drinking + water in forage + 6-7 l of water for soaking MSB.

In Exp 1 (Paper IV) TPP was higher on the hay than the silage diet (65 vs. 63 g/l, SEM = 2, $P = 0.011$). Assuming horses on the hay diet had a total plasma volume of 31.6 l, the difference in TPP between the hay and the silage diet would correspond to 1 l plasma (31.6 l × 63 g/l ÷ 65 g/l = 30.6 l). (The average plasma volume of a "hot-blooded" horse is 61.9 ml/kg BW (Julian et al., 1956) and for a 510-kg horse the total plasma volume would be 31.6 l.) In Exp 2 (Paper IV) resting values of TPP did not differ between the silage and the haylage diet.

Digestibility, Evaporation and Heat Production

The apparent DM, OM, ADF and CP digestibility was higher on the silage than the hay diet (Exp 1, Paper IV). The estimated evaporation on the silage diet in Paper IV was 2.8 kg/d higher compared to the hay diet after 18-20 days of adaptation. It takes about 600 calories to evaporate 1 g of water from the body (Andersson and Jónasson, 1993), hence 2.8 kg/d corresponds to a heat loss of ~ 7 MJ (for calculations see Appendix 1b). The estimated evaporation on the HP diet was 1.3 kg/d higher than on the RP diet (Paper II) corresponding to a heat loss of ~ 3 MJ (for calculations see Appendix 1c).

One gram of urinary N derived from protein is associated with the production of 26.5 kilocalories of heat (Brody, 1945). Assuming that all the urine N (Paper II) came from the oxidation of protein to urea the heat production from protein metabolism would be higher on the HP diet than the RP diet (19 vs. 13 MJ/d, SEM = 0.9, $P = 0.002$) (for calculations see Appendix 1d). The daily estimated energy (Lindgren, 1979) intake was about 15 MJ ME higher on the HP diet than the RP diet, but the BW of the horses did not differ.

Plasma lactate concentrations measured at day 21 in Paper III did not differ between the haylage and the silage diet. In Paper IV plasma lactate concentrations did not differ between the hay and the silage diet in Exp 1 or between the haylage and silage diet in Exp 2.

Effects of Abrupt Changes Between Forage Diets

Short-Term Observations

The faecal DM concentration during the first 48 h after an abrupt feed change showed a similar pattern in the athletic horses in both Paper II and IV (Figure 4). Faecal DM increased from 7-9 h to 46-48 h after the abrupt feed change on the RP diet and during hours 10-21 and 40-45 on the HP diet, which corresponds to 15:00-03:00 and 21:00-03:00. On the hay diet faecal DM increased during 10-24 and 34-48 h corresponding to 16:00-07:00 and 16:00-07:00. On the silage diet faecal DM increased during 10-18, 34-36 and 40-45 h corresponding to 16:00-01:00, 16:00-19:00 and 22:00-04:00. It seemed that faecal DM concentration increased during nighttime. During 25-30 h, faecal DM concentration dropped on the silage diet corresponding to 07:00-13:00.

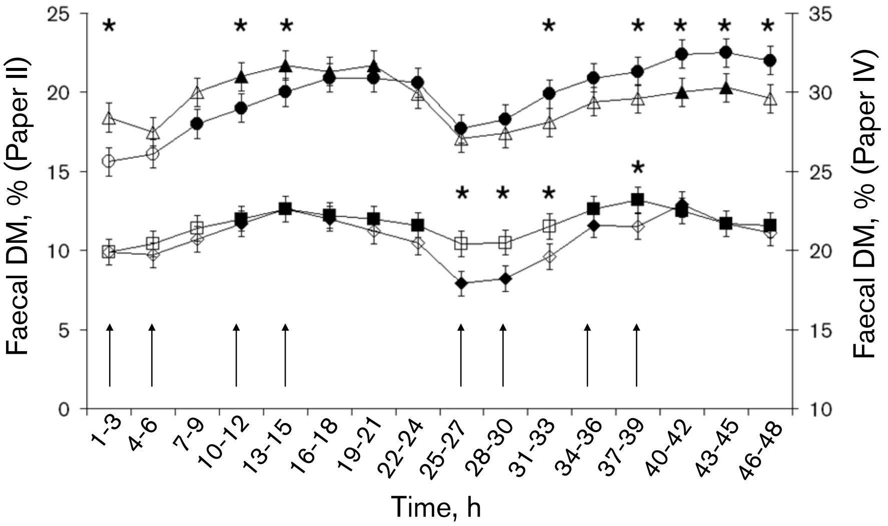

Figure 4. Faecal DM concentration the first 48 h after an abrupt feed change between silage RP ○ and silage HP Δ (Paper II) and hay □ and silage ◊ (Paper IV). In Paper II the feed change started at 06:00 and in Paper IV at 07:00. Arrows indicate when horses were fed. A total faecal collection of 6 and 5 athletic horses, respectively, was done and the figure shows LSM of every third hour. The SAS Mixed Procedure was used for both experiments. RP vs. HP: effect of time $P < 0.001$, time × treatment interaction $P = 0.002$. Hay vs. silage effect of treatment $P = 0.019$, effect of time $P < 0.001$. Pairwise t-test: ⋆differ between diets ($P < 0.05$), filled markers differ from 1-3 h ($P < 0.05$).

Within 36-48 h after the abrupt feed change between the HP and the RP diet (Paper II) faecal DM concentration was lower and N concentration was higher on the HP diet than the RP diet. Faecal pH was unchanged during the first 48 h post feed change. At 15-18 h post feed change urine pH was lower on the HP diet than the RP diet. On day 3, water intake decreased on the RP diet and was significantly lower than on the HP diet.

During the first 24 h after the abrupt feed change between the RP and the HP diet in the sedentary horses (Paper I) concentrations of colon total anaerobic bacteria were higher on the HP diet than the RP diet. Concentrations of proteolytic and lactate-utilising bacteria and streptococci decreased on both diets. Lactobacilli were higher on the HP diet than the RP diet. Colon VFA concentration increased on both diets after the abrupt feed change. Bacterial counts and VFA concentrations are shown in Appendix 2 (Table 7). Colon and faecal pH (Figure 5) and DM concentrations were unchanged throughout the 24 h post feed change.

During the first 28 h after the abrupt feed change from hay to haylage and silage (Paper III) colon bacterial counts were unchanged. VFA concentrations were also unchanged (Appendix 2, Table 8). Colon and faecal pH were unchanged throughout the first 28 h post feed change (Figure 6). Colon DM concentrations were unchanged but faecal DM tended to decrease at 28 h on both diets.

In Paper IV, the apparent digestibility for DM, OM, ADF and CP, calculated for the first 48 h after the abrupt change between diets, were higher on the silage compared to the hay diet. There was also a rapid response in water intake that was higher and total water intake that was lower on the hay diet compared to the silage diet within 48 h.

Long-Term Observations

Colon pH in Paper I seemed to start changing during the first 24 h after the abrupt feed change between the RP and the HP diet and lead to a lower pH on the HP diet than the RP diet days 7-22 (Figure 5). The faecal pH in Paper I did not differ between diets, but during days 7-22 the pH was numerically lower on the HP diet than the RP diet (Figure 5). Concentrations of colon VFA did not differ over time (7-22 d). Colon and faecal DM concentrations were unchanged throughout the weekly observations.

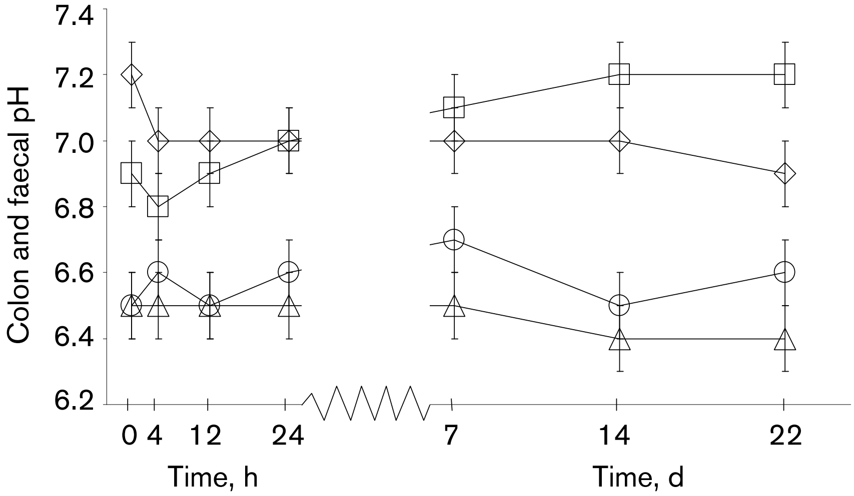

Figure 5. Colon (right ventral) pH (□ RP, ◊ HP) and faecal pH (○ RP, Δ HP), LSM of 4 sedentary horses sampled before (0 h) and after (1 h, 4 h, 24 h, 7 d, 14 d and 22 d) an abrupt feed change (Paper I). Two silage diets, differing in CP concentration, were fed restrictedly to result in similar DM intakes, but a CP intake close to recommended (NRC, 1989) daily CP intake (diet RP, 615 g CP/d) or higher than recommended daily CP intake (diet HP, 873 g CP/d). The statistics are run separately for the first 24 h and 7 - 22 d. Colon pH first 24 h: effect of treatment $P = 0.073$, 7 to 22 d: effect of treatment $P = 0.035$. Faecal pH 7 - 22 d: effect of time $P = 0.063$.

After the abrupt change from hay to haylage and silage (Paper III), colon and faecal pH did not differ between diets (Figure 6). Colon VFA concentrations were unchanged throughout the weekly observations. Colon and faecal DM concentrations decreased on both the haylage and silage diet.

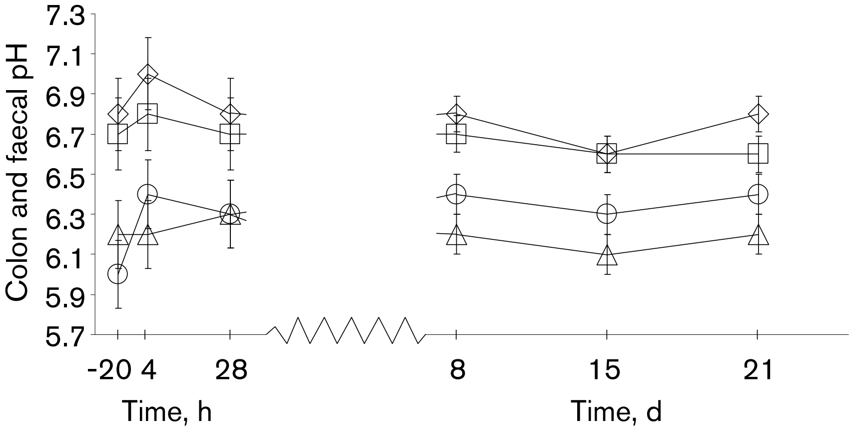

Figure 6. Colon (right ventral) pH (□ haylage, ◊ silage) and faecal pH (○ haylage, Δ silage), LSM of 4 sedentary horses sampled before (-20 h) and after (4 h, 28 h, 8 d, 15 d and 21 d) an abrupt feed change from hay to haylage and silage (Paper III). The statistics are run separately for the first 28 h and weekly (-20 h, 8 – 21 d). Colon pH first 28 h: effect of time P = 0.088. Faecal pH weekly observations: effect of time P = 0.095.

In Paper IV faecal acetate, propionate, butyrate, isobutyrate and total VFA concentrations were increased from days 1-2 to days 18-20 on the hay diet and decreased from days 1-2 to days 18-20 on the silage diet. Faecal pH tended to decrease on the hay diet and tended to increase on the silage diet (P = 0.053).

In Paper I the weekly observations of colon bacterial counts did not differ between diets, but total anaerobic bacteria and streptococci increased over time on both the HP and the RP diet (Figure 7). In Paper III there was a shift in lactobacilli and streptococci. At 21 d, lactobacilli concentrations were higher on the silage diet than the haylage diet, and day 15 and 21 streptococci concentrations decreased and were lower on the haylage diet than the silage diet (Figure 7).

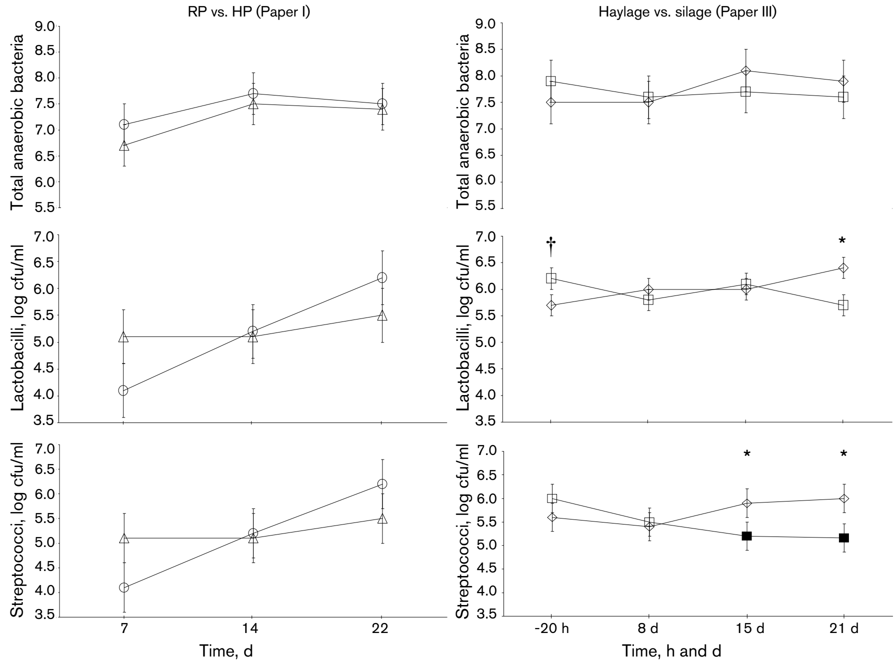

Figure 7. Weekly observations of colon (right ventral) bacterial counts after an abrupt feed change between silage RP ○ and silage HP Δ (Paper I) and after an abrupt feed change from hay to haylage □ and silage ◊ (Paper III). In Paper I, 4 sedentary horses were sampled at 08:00 before the morning feeding and in Paper III, 4 sedentary horses were sampled at 12:00 and fed at 08:00. RP vs. HP: total anaerobic bacteria: effect of time $P = 0.015$, streptococci: effect of time $P = 0.010$, time × treatment interaction $P = 0.096$. Haylage vs. silage: lactobacilli: time × treatment interaction $P = 0.023$, streptococci: time × treatment interaction $P = 0.046$. Pairwise t-test: differ between diets ⋆ $P < 0.05$, † $P < 0.10$, filled markers differ from -20 h $P < 0.05$.

Effects of Forage Diets on Exercise Response

Three exercise tests were performed (Paper II and IV) and in none of them did diets differ in the post exercise respiratory rate, rectal temperature, heart rate, TPP and 24 h post water intake (Table 6). After both exercise tests in Paper II horses had lost BW, but BW loss did not differ between diets.

Table 6. *Respiratory rate, rectal temperature, heart rate, total plasma protein and 24 h post water intake for athletic horses fed the RP and HP diet*[1] *(Paper II) and haylage and silage (Paper IV) during and after exercise tests*[2]*, there were no significant differences between diets*

	Standardised exercise test (Paper II)		Field test (Paper II)		Incremental exercise test (Paper IV)	
	RP	HP	RP	HP	Haylage	Silage
Respiratory rate, breaths/min						
At rest	13	11	22	19	12	14
15 min post	82	93	100	86	62	67
SEM	6	6	9	9	6	6
Rectal temperature, °C						
At rest	37.6	37.6	37.4	37.4	37.7	37.8
15 min post	40.1	40.1	39.1	39.5	39.3	39.4
SEM	0.1	0.1	0.3	0.3	0.1	0.1
Heart rate, beats/min						
At rest	34	38	35	39	33	34
Peak value	213	216	222	215	213±17[3]	217±8[3]
15 min post	70	72	80	78	81	80
SEM	3	3	7	7	3	3
Total plasma protein, g/l						
At rest	65	63	65	66	62	64
Peak value	77	77	82	82	74	74
15 min post	68	65	74	74	68	69
SEM	1	1	1	1	2	2
24 h post water intake, l						
	16.8	20.7	26.8	24.3	17.8[4]	14.5[4]
SEM	1.7	1.7	2.9	2.9	4.0	0.7

[1]Two silage diets, differing in CP concentration, were fed restrictedly to result in similar DM intakes, but a CP intake close to recommended (NRC, 1989) daily CP intake (diet RP, 1044 g CP/d) or higher than recommended daily CP intake (diet HP, 1525 g CP/d).

[2]Standardised exercise test: race-like exercise test on treadmill, Field test: race-like exercise test on an oval racetrack, Incremental exercise test: interval exercise test on an oval racetrack.

[3]Due to technical problems with heart rate recorders these are mean values with the standard error based on 3 observations on the haylage diet and 4 observations on the silage diet.

[4]Values are only from period 1, 3 observations on the haylage and 2 observations on the silage diet.

In the three exercise tests (Paper II and IV) plasma lactate and venous blood pH did not differ between diets (Figure 8). At 15-min post exercise, in the standardised exercise test on a treadmill, the venous blood pH did no longer differ from resting values on both the RP and HP diet (Paper II).

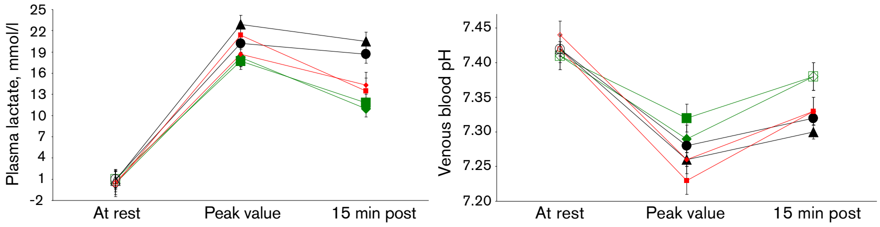

Figure 8. Plasma lactate and venous blood pH values at rest, the peak value and 15 min post exercise during three different exercise tests, green: RP diet □ and HP diet ◊ during a standardized exercise test (LSM of 5 horses) on a treadmill, black: RP diet ○ and HP diet Δ during a field test (simulated race, LSM of 4 horses) on an oval racetrack (Paper II) and red: haylage □ and silage ◊ during an incremental interval exercise test (LSM of 5 horses) on an oval racetrack (Paper IV). Pairwise t-test: filled markers differ from 'at rest' ($P < 0.05$).

Drivers Ranking

The drivers ranking of the horses in the field test (Paper II) did not differ between diets, two horses were ranked as 'dull' and one horse as 'alert' at both test occasions. One horse was 'very alert' on diet RP and 'alert' on diet HP. The drivers ranking of the horses in the incremental interval test (Paper IV) did not differ between diets (Lazy – very hard pulling: haylage 5.5 and silage 5.4, SEM = 0.7, $P = 0.215$; Calm – very nervous: haylage 3.9 and silage 5.4, SEM = 1.7, P = 0.362).

General Discussion

Effects of Forage Diets After Adaptation

The RP and HP Diets

The higher colon VFA concentrations and lower colon pH on the HP compared to the RP diet in the sedentary horses suggests that a CP intake of 140% of the recommendation (NRC, 1989) increases the activities of the colon microflora. However, bacterial counts did not differ. Colon propionate concentrations were also higher on the HP than the RP diet, but the VFA ratios (acetate + butyrate/propionate) did not differ suggesting no alteration in the fermentation pattern of the microflora. Higher colon propionate concentration might suggest that more lactate was available (Jaakola and Huhtanen, 1992), but this seems unlikely because the lactate concentration was lower on the HP than the RP diet. Iso-butyrate and iso-valerate are mostly derived from proteins (France and Dijkstra, 2005). Although not significant, iso-butyrate and iso-valerate concentrations were numerically higher on the HP diet than the RP diet. Although the RP and HP silages had similar chemical composition except for CP content, the silages were not from the same swath and other differences than CP content between the feeds might also have had an impact on the activity of the microflora. Although a lack of difference in bacterial counts between the diets, there could be other microbes than those cultivated in the current study that may have been affected. The tendency for an increased plasma urea concentration and water consumption in the sedentary horses, suggests that there was an increased N absorption and metabolism on the HP diet.

In the athletic horses, water intake was higher, total water output and the difference water intake minus output tended to be higher on the HP diet compared to the RP diet. This suggests an alteration in fluid balance on the HP diet due to an increased heat production and urea synthesis supported by increased urinary N excretion and lower urine pH. Water intake only tended to be higher on the HP diet in the sedentary horses but the difference was significant in the athletic horses. The athletic horses consumed a larger daily amount of CP, but exercise also leads to a higher daily water intake (Pagan et al., 1998) which might have enhanced the possibility to observe differences between the RP and the HP diet.

Horses were never in negative N balance. The numerically higher faecal VFA concentration, significantly lower faecal pH and higher blood urea in the athletic horses altogether indicates an increased N absorption and metabolism on the HP diet than the RP diet. In addition faecal DM concentration was lower on the HP diet compared to the RP diet in the athletic horses. It can be concluded that the HP diet provided an excess N intake. Variations in CP and water content in the silages cause variations in the daily CP intake. During the 72-h collection period the CP intake for the HP diet was 140% of CP intake for the RP diet. Calculating the average CP intake during the whole experimental periods the CP intake for the HP diet was 160% of CP intake for the RP diet. It is difficult to adjust for these variations in this type of experiments.

For the sedentary and athletic horses fed the RP and HP diets, blood/plasma urea was correlated to CP intake, which has been shown previously (Prior et al., 1974; Slade et al., 1970). The sedentary horses were sampled before and the athletic horses after the morning feeding, but plasma urea concentration does not seem to vary in time after feed intake (Jansson et al. 2006).

Hay, Haylage and Silage

Colon lactobacilli and streptococci bacterial counts in the sedentary horses, were altered in two to three weeks after a feed change from hay to haylage and silage. At 21 d lactobacilli concentrations were higher on the silage diet and day 15 and 21 streptococci concentrations were decreased on the haylage diet. This suggests that a fermented feed changes the composition of the colon microflora. The increase in total water intake, hay < haylage < silage, also supports an altered metabolism. However, the results are difficult to interpret when the changes in colon bacterial counts were not accompanied by any changes in colon VFA concentrations or pH. It is noteworthy that the faecal pH was numerically lower at day 8, 15 and 21 on the silage diet compared to the haylage diet. Colon and faecal DM decreased on both the haylage and silage diet compared to the hay diet.

In the athletic horses, differences in apparent digestibilities and faecal VFA concentrations on hay and silage diets indicate changes in the digestive response. The apparent digestibilities of hay and silage in the athletic horses were almost as high as the values reported by Ragnarsson and Lindberg (2008) for a first cut grass haylage (66-68% vs. 72%). This is not surprising since the forages used were early cuts high in estimated ME, CP and low in NDF and ADF concentrations. The total collection of faeces was only for 72 h, but digestibility coefficients have been shown to be similar comparing 3, 4 and 5 days of collection (Goachet et al., 2005) and 2 and 3 days of collection (Muhonen et al., unpublished). The higher apparent digestibility of the silage compared to the hay might be explained by differences in the fibre fractions. The major factor for differences in digestible energy and digestible organic matter among forages is the carbohydrate composition (Fonnesbeck, 1968b). Although simultaneously harvested the hay had a higher NDF content than the silage, which was probably due to higher leaf losses during handling in the field. However, also chemical changes during the ensiling process (McDonald, 1981) might induce changes in the carbohydrate and fibre fractions and contribute to higher digestibility. The NDF and ADF analyses only roughly estimates the fibre fractions and a more specific analysis of non-starch polysaccharides might give a more comprehensive understanding (Longland et al., 1997; Moore-Colyer et al., 2002).

The silages and haylages in paper III and IV were treated with a combination of bacterial inoculant and enzymes. Bacterial inoculant-treated silages have lead to higher DM, higher crude fibre and higher N digestibility compared to untreated silages in cattle (Keady et al., 1994; Keady and Steen, 1995). In these experiments DM concentrations ranged between 16.4-19.3% and the higher digestibilities were suggested to be related to the effects of the bacterial inoculant on plant structural carbohydrates during the ensilage process. Rooke and Kafilzadeh (1994) found no differences in digestibility between bacterial inoculated silage and untreated silage, fed to sheep. Interestingly, when a bacterial inoculant was applied to the silage immediately before feeding there was no effect on total tract digestibility, or degradability or fermentation in the rumen of cattle (Keady and Steen, 1996).

A combination of bacterial inoculant and enzymes usually improves silage fermentation leading to lower pH, higher lactic acid concentration and reduced crude fibre content (Selmer-Olsen, 1994). However, wilting to a higher DM content (> 45%) causes the effect of enzyme addition on fibre degradation and lactic acid production to diminish (Beuvink and Spoelstra, 1994).

In the athletic horses fed hay and silage, during days 18-20 the difference in CP intake was small (15 g/d more on the silage diet); however the apparent CP digestibility was still 3% higher on the silage diet than the hay diet. Because of hydrolysis of proteins to amino acids during ensiling much of the silage N can be in a highly degradable form and most of the non-protein-N in silage is in the form of amino acids (McDonald P, 1981). Maybe there was a higher availability of soluble protein in the silage than the hay.

The higher total water intake, estimated evaporative fluid loss and lower TPP on the silage compared to the hay diet in the athletic horses altogether suggest there was a change in fluid balance. The higher apparent digestibility of the silage also suggests a higher production of metabolic water; hence the production of metabolic water depends on the feed's digestibility (Frape, 1998). However, in the athletic horses in Exp 2 (Paper IV), the total water intake and TPP did not differ between the haylage diet and the silage diet. The difference in DM content between the feeds was larger in Exp 1 and the difference in lactic acid content between the feeds was larger in Exp 2. The DM content of the silage in Exp 2 was 4% lower and the lactic acid content 23 g/kg DM higher than the silage used in Exp 1. This suggests that the lactic acid fermentation in the silage did not affect the fluid balance; instead it was more likely the larger difference in DM content. However, in Exp 2 the haylage and silage diets were complemented with MSB, which might have decreased the possibility in observing the response in water intake and TPP from the haylage and silage intakes.

Resting values of plasma lactate after three weeks of adaptation, in both sedentary and athletic horses, did not differ between haylage and silage despite large differences in lactate ingested via the forages. In the sedentary horses ~ 170 vs. 10 g/d on the silage and haylage diet respectively; in the athletic horses ~ 257 vs. 15 g/d on the silage and haylage diet respectively. The lack of difference in plasma lactate concentrations could be because the ingested lactate was converted to acetate, propionate or butyrate (Jaakola and Huhtanen, 1992; Varloud et al., 2007). Measuring lactate concentration in the portal blood might have given a different result to sampling the jugular vein as in the present experiments (Alexander and Chowdhury, 1958).

Sedentary and Athletic Horses

Among horses, large differences in the utilization of forage nutrients have been reported (Fonnesbeck et al., 1967). A low number of animals require large differences to become significant when the parameter has a large variation. A higher number of animals were used in the experiments on athletic horses than in the experiments on sedentary horses. Comparing the same measurements between the experiments, such as water intake, faecal pH and DM, plasma/blood urea, which was not significant or only showed tendencies in the sedentary horses was often significant in the athletic horses. However, number of animals is not the only difference between these experiments. The fact that the sedentary horses were fed at the maintenance level of energy requirements and the athletic horses were fed at twice the maintenance level might have influenced the response. For example the feeding level for the HP diet in the athletic horses gave ~650 g more CP/d than the feeding level for the HP diet in the sedentary horses.

VFA levels in the gastrointestinal tract can vary markedly with time of sampling after feeding (Argenzio et al., 1974; Medina et al., 2002). Comparing colon VFA concentrations between the sedentary horses fed the RP and HP diets and the hay, haylage and silage diets, colon VFA was lower in the RP and HP diets than in the hay, haylage and silage diets, 45.1-51.8 vs. 54.2-70.5 mmol/l. This could be because the horses fed the RP and HP diets were sampled before the morning feeding and the horses fed the hay, haylage and silage diets were sampled 4 h after the morning feeding.

Faecal VFA concentrations were considerably higher for the athletic horses compared to the sedentary horses when fed the RP and HP diets. However, the sedentary horses were sampled before the morning feeding and for the athletic horses values represent a 24-h period. Faecal VFA concentrations for the sedentary horses fed the hay, haylage and silage diets were lower than the faecal VFA concentrations for the athletic horses fed hay and silage diets (34.7-47.9 vs. 53.2-64.8 mmol/l). Although these sedentary horses were sampled 4 h after feeding it was still grab samples and the faecal VFA concentrations in the athletic horses represented a 72-h period. This aggravates the interpretation of the results, but the athletic horses overall had higher faecal VFA concentrations. Probably due to a higher VFA production because of the higher feed intake.

Overall Correlations in Digestive Pattern

Overall, across experiments and diets faecal pH was negatively correlated to faecal total VFA concentrations. This agrees with Argenzio et al. (1974) who showed that colonic digesta pH correlates inversely with VFA concentration. Faecal pH was also positively correlated to colon pH in the sedentary horses. This suggests faecal biochemical parameters could give an indication of the status in the colon.

Diarrhoea was not observed in any of the experiments but there were differences in faecal DM concentrations between the RP and HP diets in the athletic horses and individual values varied between 17.4 and 25.2% across experiments after three weeks of adaptation. Comparing how faecal DM concentration correlates to forage DM concentration, total water, lactate, CP, NDF and ADF intake, the strongest correlation was with total water intake. That faecal DM concentration correlates to total water intake might be because the diets with the highest total water intake also had higher digestibilities, higher N excretion and were associated with higher heat production. Several factors might have lead to an indirect correlation between faecal DM and total water intake. The correlation between faecal DM concentration and CP intake supports the findings of a lower faecal DM concentration on the HP diet compared to the RP diet for the athletic horses. Fewer observations in the comparison between faecal DM concentration and ADF intake might be the reason why it was not significant despite a higher r^2 value than for NDF intake. However, the water-holding capacity of fibre suggests a higher faecal water content with higher fibre intake (McRorie et al., 2000; Zeyner et al., 2004).

Effects of Abrupt Changes Between Forage Diets

There is a lack of studies on abrupt feed changes between forage diets. de Fombelle et al. (2001) showed a sharp increase in the concentrations of lactobacilli, streptococci and lactate 5 and 29 h after an abrupt introduction of barley in a hay diet. After an abrupt change from the RP diet to the HP diet, in the sedentary horses, colon lactobacilli concentrations were higher during the first 24 h and seemed to peak at 24 h, but lactate levels were consistently under the detection limit. The 24 h post prandial pH was still neutral (7.0), whereas in the study by de Fombelle et al. (2001) it was 6.4 (29 h). In general, an abrupt introduction of barley in the diet carried out in the way described by these authors seems to result in more acidic conditions than introducing a high CP silage. However, within the first 24 h, some long-term alterations of the activity of the colon ecosystem seemed to be initiated leading to higher total VFA concentrations and lower pH after 7-22 days on the HP diet.

Already at 15-18 h after the abrupt change to the HP diet in the athletic horses, urine pH was lower and after 36-48 h faecal N concentration was higher than on the RP diet. This suggests a rapid onset of excretion of excess N. After the abrupt change to the RP diet in the athletic horses, water intake was decreased first on day 3, which suggests a two-day "washout" of the effects of a high CP forage.

The abrupt change from hay to haylage or silage in sedentary horses did not result in any differences in the microbial concentrations or activities during the first 28 h. This indicates that no rapid alterations in the colon ecosystem could be expected if sedentary horses, fed at maintenance level, are subjected to a feed change from hay to silage or haylage from the same crop. However, in the athletic horses, the differences in apparent digestibilities between the hay diet and the silage diet calculated for the first 48 h after the abrupt feed change were similar to those after three weeks. There was also a rapid response in water intake by drinking and total water intake. This suggests there will be a different digestive response with an abrupt change from hay to silage than with an abrupt change from silage to hay. However, there was a delay in the response of faecal VFA concentration that has yet to be explained.

Resting values of plasma lactate 24 and 28 h after the abrupt feed changes, in both sedentary and athletic horses, did not differ between hay, haylage and silage despite large differences in lactate ingested via the forages. As discussed earlier the ingested lactate may have been converted to acetate, propionate or butyrate (Jaakola and Huhtanen, 1992; Varloud et al., 2007) and sampling the portal blood instead of the jugular vein might have given a different result (Alexander and Chowdhury, 1958).

In the athletic horses fed the RP and HP diet and the hay and silage diet, faecal DM concentrations during the first 48 h after the abrupt feed changes seemed to increase during nighttime. A release of aldosterone in a diurnal rhythm with elevated plasma aldosterone concentrations between 21:30 and 04:30 has been reported (Jansson and Dahlborn, 1999). This would imply a sodium and water uptake from the large intestine (Argenzio and Clarke, 1989) during nighttime which would agree with an increased faecal DM concentration. The diurnal changes in faecal DM concentration might be something to consider when taking grab samples for measuring faecal composition.

Effects of Forage Diets on Exercise Response

The two exercise tests performed on the RP and HP diets, lead to maximum heart rates of 213-222 beats/min, peak plasma lactate levels of 17.6-22.9 mmol/l, decreased blood pH with the lowest values of 7.26-7.28 and BW losses of 6-11 kg. However, the intensive anaerobic exercise tests did not lead to any differences between the RP diet and the HP diet. At rest, the increased (P = 0.095) estimated evaporation suggest a higher heat production and the lower urine pH suggest a higher production of hydrogen ions on the HP diet compared to the RP diet. However, the high CP intake did not enhance the exercise-induced acidosis during intensive exercise typically performed by Standardbred horses.

Both tests on the RP and HP diets were designed to resemble racing conditions of Standardbred trotters. To reduce the risk of injuries in the field test, a constant speed of 11.3-11.5 m/s was held the first 1600 m and then horses finished at maximal speed (480 m). If the field test would have been run at maximal speed the full length, the results might have been different. However, the horses showed a more pronounced metabolic acidosis after the field test with higher plasma lactate and lower blood pH values than in the treadmill test. This could be because the field test included the finish at maximal speed (Nostell et al., 2006).

The incremental exercise test performed on the haylage and silage diets lead to maximum heart rates of 213-217 beats/min, peak plasma lactate levels of 18.7-21.4 mmol/l and decreased blood pH with the lowest values of 7.23-7.26. Due to technical problems BW losses could not be obtained in period 2. In period 1, BW losses ranged from 1 to 15 kg. This intensive anaerobic exercise test did not result in any differences between the haylage diet and the silage diet. The objective of performing an incremental exercise test was to establish the lactate threshold; VLa4 (velocity at plasma lactate 4 mmol/l), which can be used as an indicator of aerobic fitness (Persson, 1983). However, the track conditions were heavier than expected and almost every horse exceeded the lactate threshold during the first interval at both test occasions.

No exercise test was performed comparing the hay and silage diet, but resting values for TPP was higher on the hay diet than the silage diet, calculated to correspond to ~ 1 l larger plasma volume on the silage diet. A higher plasma volume suggests an advantage for the performance horse. However, in the same experiment the estimated evaporation on the silage diet was 2.8 kg/d higher on the silage diet than the hay diet. This estimated higher heat production might level out the advantage of the higher plasma volume.

In both exercise tests on an oval racetrack, the field test on the RP and HP diets and the incremental exercise test on the haylage and silage diets, the dietary treatments caused no difference in the ranking of the drivers. The lack of difference in drivers ranking might be explained by the fact that all the dietary treatments were roughage, which might influence the response to exercise less than concentrate diets (Jansson and Lindberg, 2008). The lack of difference in heart rates between the haylage diet and the silage diet might be because BW did not differ. Ellis et al. (2002) found that water intake was higher on high forage diets leading to higher BW and therefore also higher heart rates during exercise and recovery.

In the three different exercise tests the horses were subjected to high intense anaerobic work typically performed by Standardbred horses. These theoretical disadvantages did not lead to any differences between dietary treatments. It would be of interest to examine how the increased urine output and estimated higher evaporative fluid losses and heat production would affect horses during more prolonged exercise or during hot and humid conditions when fluid losses are a limiting factor.

A Horse Perspective on the Definition of Hay, Haylage and Silage

As mentioned earlier there is no clear definition separating haylage and silage. Today the most common definition is that haylage is drier than silage and therefore contains less lactic acid and has a higher pH, and some haylage is so dry it is hay wrapped in plastic. However, the forage conservation method used has an impact on the horse. Clear indications that conservation method has an impact on the horse are the higher apparent digestibilities on the silage diet (45% DM) compared to the hay diet (81% DM). The fact that the ensiling process might lead to more fermentable fibre fractions (McDonald, 1981) is also supported by the higher estimated heat production and higher water intake. The lower TPP on the silage diet than the hay diet also indicates an alteration in the total fluid balance. Warren et al. (1999) suggests there is a higher volume of fluid available in the gastrointestinal tract when more soluble fibre is included in the diet, which may be due to the higher fermentability of soluble fibre and possibly a higher availability of water as the fibre is being fermented in the hindgut.

As discussed earlier the DM content of the forage seems to have a larger impact than the lactic acid content. Digestibility and total fluid balance was not measured in the sedentary horses, but the increase in total water intake; hay diet (81% DM) < haylage diet (55% DM) < silage diet (36% DM) indicates an altered fluid balance. From the studies comprising this thesis there is not enough results to establish contingent clear definitions between hay, haylage and silage from the “horse perspective”.

Conclusions

- An abrupt feed change between silages with different CP content altered the colon bacterial counts within the first 24 h in the sedentary horses, and in the athletic horses faecal water content and N excretion increased within 36-48 h on the high CP silage. Fluid and acid-base balance was also altered at rest.
- The following three weeks after the change to high CP silage a small decrease in pH and increase in VFA concentration was observed in the sedentary horses, but no other major alterations in the composition and activities of the colon ecosystem or faecal water content. In the athletic horses, the changes observed within 48 h persisted after three weeks of adaptation.
- An abrupt feed change from hay to haylage or silage did not have any major short-term effects on the colon ecosystem in the sedentary horses, indicating that forage with similar chemical and botanical composition, but preserved by different methods, could safely be rapidly introduced. For the athletic horses, there was a higher apparent digestibility and estimated evaporative fluid loss on the silage diet compared to the hay diet observed already at day 1-2 after an abrupt feed change between the diets. This indicates rapid and different responses when silage and hay are introduced.
- During the subsequent three weeks, colon and faecal water content increased and there were alterations in the lactobacilli and streptococci bacterial counts in the sedentary horses. These bacterial changes need further investigation. For the athletic horses, differences in apparent digestibility and fluid balance persisted, but feeding silage did not affect faecal water content or plasma lactic acid concentrations.
- Feeding a high CP silage compared to silage providing a recommended CP intake or haylage compared to silage did not adversely affect the

response to intensive exercise, typically performed by Standardbred trotters. However, the increased urine output and estimated higher evaporative fluid losses and heat production might be an unnecessary disadvantage during more prolonged exercise when fluid losses might be a limiting factor.

To summarize this thesis: the CP content and conservation method of forages affects the metabolism and hindgut ecosystem of both sedentary and athletic horses and some effects seem to be more pronounced in the athletic horses. No "disturbances" were observed on either of the diets but theoretically the higher estimated heat production and evaporative fluid losses would be a disadvantage during exercise.

Populärvetenskaplig Sammanfattning

Syfte och Slutsatser

Hösilage och ensilage används alltmer som foder till hästar i Sverige och de verkar också bli mer och mer populära i andra delar av Europa. Vid ensilageproduktion är man mindre beroende av bra väderföhållanden än vid höskörd, vilket underlättar för produktion av ett foder med högt näringsinnehåll och bra hygienisk kvalitet. Lös träck och nedsatt prestation vid utfodring av ensilage har rapporterats av hästägare och tränare, men det finns få ensilagestudier gjorda på häst och väldigt få på högpresterande hästar. De olika sätten att konservera grovfoder gör att den kemiska sammansättningen mellan hö, hösilage och ensilage skiljer sig. Skillnaden mellan hösilage och ensilage är att hösilage är torrare (högre torrsubstanshalt) och att det därför skett mindre eller ingen mjölksyrajäsning. Ensilage däremot innehåller mer vatten och mjölksyra och mindre socker eftersom de mjölksyraproducerande bakterierna ”äter” sockret. Då ensilaget innehåller mera mjölksyra sjunker pH-värdet. Några klara gränsvärden mellan hösilage och ensilage finns inte idag. Så kallat ”ko-ensilage” har ett särskilt dåligt rykte bland hästägare och tränare. Detta ensilage karaktäriseras av låg torrsubstanshalt, välfermenterat (mycket mjölksyra) och med högt innehåll av energi och råprotein.

Syftet med denna avhandling var att undersöka effekterna av grovfoder med olika råproteininnehåll och konserveringsmetod på ämnesomsättning och ekosystemet i grovtarmen, i samband med foderbyten och efter tre veckors anpassning samt i samband med arbete. Alla studierna var upplagda med en cross-over design, det vill säga alla hästar testades på alla foder i olika ordningsföljd.

Sammanfattningsvis, har studierna i den här avhandlingen visat att grovfoder med högt råproteininnehåll ger en ökad aktivitet i ekosystemet i tjocktarmen, en ökad utsöndring av kväve via urin och träck och ett ökat vattenintag. Ett byte från hö till hösilage och ensilage förändrade sammansättningen av bakteriefloran i tjocktarmen. Fodersmältbarheten var högre för ensilage än för hö och ensilagefoderstaten gav ett ökat vattenintag. De olika foderstaterna, olika råproteininnehåll och hösilage jämfört med ensilage, innebar inga skillnader i samband med arbete. Men en ökad urinutsöndring och en beräknad ökad avdunstning av vatten från huden (evaporation) tyder på att ett högt råproteinintag och ensilage kan vara till en nackdel för hästar som gör mycket stora vätskeförluster i samband med arbete, till exempel distansritthästar.

Utförande och Resultat

I den första studien (**I**) var syftet att undersöka effekten av råproteinintag från två gräsensilage med olika råproteinkoncentration, fodrade i lika stor mängd, på ekosystemet i hästens tjocktarm. Det ena ensilaget resulterade i ett överskott av råprotein (rp) i förhållande till behovet (HP, 873 g rp/dag) och det andra resulterade i ett intag av råprotein som motsvarade det rekommenderade behovet (RP, 615 g rp/dag). Under de första 24 timmarna efter foderbytet var totala koncentrationen av anaeroba bakterier och laktobaciller något högre på HP-foderstaten än på RP-foderstaten. Det var ingen skillnad i koncentrationen av kortkedjiga fettsyror mellan foderstaterna under det första dygnet. Dag 7 till 22 var koncentrationen av kortkedjiga fettsyror högre och pH i tjocktarmen lägre på HP än på RP-foderstaten. Vattenhalten i tjocktarm och träck och pH i träck var oförändrade på båda foderstaterna genom hela försöket. Resultaten visar att ett abrupt foderbyte mellan ensilage med olika råproteininnehåll, för hästar i vila på underhållsfoderstat, gav en mindre förändring i antalet bakterier i tjocktarmen det första dygnet. Under de följande tre veckorna ökade koncentrationen av kortkedjiga fettsyror och pH minskade på HP-foderstaten, men inga andra större förändringar i sammansättning och aktivitet i ekosystemet i tjocktarmen eller vattenhalt i träcken observerades.

Hur olika råproteinintag från grovfoder påverkar kväve- och vätskebalansen, och svar på arbete hos högpresterande hästar undersöktes i studie två (**II**). Två grovfoderdieter (kompetterade med mineraler och salt) baserade på gräsensilage med högt energiinnehåll fodrades till 6 varmblodiga travhästar i träning. De två grovfodren bestod till största delen av gräsensilage och fodrades till lika stor mängd torrsubstans, men det ena hade högre råproteinkoncentration och gav ett högt råproteinintag (HP, 1525 g rp/dag) och det andra gav ett lägre men tillräckligt råproteinintag (RP, 1044 g rp/dag) för arbetande hästar. Efter ett abrupt byte till HP-foderstaten minskade pH i urinen medan utsöndringen av kväve, urea-koncentrationen i blodet, vattenintaget, urinvolymen och vattenhalten i träcken ökade inom två dygn. Dessa förändringar kvarstod efter tre veckor. Det totala vattenintaget tenderade att vara högre än den ökade vattenutsöndringen via urin och träck vilket tyder på ökade vätskeförluster via avdunstning från huden (evaporation) på HP-foderstaten. Under och efter arbetstesten var det inga skillnader mellan foderstaterna i andnings- och hjärtfrekvens, total plasmavolym, mjölksyra i plasma och pH i blod. Inga förändringar i kroppsvikt observerades. Resultaten pekar på att, för arbetande hästar på 2 × underhållsutfodring, ett råproteinintag motsvarande 160 % av behovet gav en ökad kväveutsöndring inom två dygn efter foderbyte och förändringar i vätskebalansen i vila. Den ökade urinutsöndringen och även tendensen till ökad evaporation tyder på att ett högt råproteinintag kan vara till en nackdel för hästar som gör mycket stora vätskeförluster i samband med arbete, till exempel distansritthästar.

Syftet med den tredje studien (**III**) var att undersöka effekten av ett abrupt foderbyte från hö (81 % torrsubstans, ts) till ensilage (36 % ts) och hösilage (55 % ts) på ekosystemet i hästens tjocktarm och att jämföra anpassningen till ensilage- och hösilagefoderstaten. Fyra vuxna valacker användes och foderstaterna bestående av hö, hösilage och ensilage kompletterades med mineraler och salt. Antal bakteriekolonier och koncentrationen av kortkedjiga fettsyror i tjocktarmen, pH och vattenhalt i tjocktarm och träck var oförändrade under det första dygnet efter det abrupta foderbytet från hö till ensilage och hösilage. Koncentrationen av laktobaciller var högre dag 21 på ensilage- än på hösilagefoderstaten och koncentrationen av streptokocker minskade på hösilagefoderstaten och var lägre dag 15 och 21 än på ensilagefoderstaten. Den totala koncentrationen av kortkedjiga fettsyror och pH i tjocktarmen och träcken var oförändrade de följande tre veckorna efter foderbytet. Vattenhalten i tjocktarmen och träcken ökade på både hösilage- och ensilagefoderstaten jämfört med höfoderstaten. Resultaten tyder på att, för hästar i vila på underhållsfoderstat, ett abrupt foderbyte från hö till hösilage eller ensilage inte innebar några större förändringar i ekosystemet i tjocktarmen det första dygnet. Under de följande tre veckorna ökade vattenhalten i tjocktarm och träck något och det skedde förändringar i antalet laktobaciller och streptokocker i tjocktarmen. De bakteriella förändringarna blir svårtolkade när inga förändringar i kortkedjiga fettsyror och pH observerades och detta behöver därför undersökas ytterligare.

I den sista och fjärde studien (**IV**) var syftet att undersöka effekter på vätskebalans, fodersmältning och svar på arbete hos varmblodiga travhästar i träning när de fodras med ensilage, hösilage eller hö. I försök 1 användes fem valacker som fodrades med hö (82 % ts) och ensilage (45 % ts) (högt energiinnehåll, kompletterade med mineraler och salt). I försök 2 användes sex valacker som fodrades med hösilage (68 % ts) och ensilage (41 % ts) (högt energiinnehåll, kompletterade med betfor, mineraler och salt). Smältbarheten var högre på ensilagefoderstaten jämfört med höfoderstaten. Hästarna drack mer när de åt hö än när de åt ensilage men det totala vattenintaget (druckit + vatten i fodret) var högre på ensilagefoderstaten. Den totala utsöndringen av vatten skilde inte mellan foderstaterna och därför var den beräknade avdunstningen av vatten från huden (evaporationen) större på ensilage- än på höfoderstaten. Även den totala plasmavolymen var större i vila på ensilage- än på höfoderstaten (försök 1), men det var ingen skillnad mellan hösilage- och ensilagefoderstaten (försök 2). Inga förändringar i kroppsvikt observerades. Andnings- och hjärtfrekvens, total plasmavolym, mjölksyra i plasma och pH i blod skilde inte mellan hösilage- och ensilagefoderstaten under och efter arbetstesten. Resultaten visar att vattenhalten i träcken inte påverkades av utfodring med ensilage men smältbarheten och evaporationen var högre på ensilage- jämfört med höfoderstaten. Ensilagefoderstaten hade ingen negativ påverkan på arbetssvar jämfört med hösilagefoderstaten, men en högre evaporation kan vara till en nackdel för hästar som gör mycket stora vätskeförluster i samband med arbete.

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Appendix 1

Calculations

a) The oxidation of each g of protein yields about 0.4 ml of water, per g metabolised (Houpt, 1993b). Assuming all urine N (Paper II) came from the oxidation of protein to urea the amount of metabolic water produced would be:

RP: 117.4 g N × 6.25 × 0.4 ml = 293.5 ml metabolic water per day

HP: 170.8 g N × 6.25 × 0.4 ml = 427.0 ml metabolic water per day

b) It takes about 600 calories to evaporate 1 g of water from the body (Andersson and Jónasson, 1993). Energy loss from evaporation (Paper IV):

2.8 kg water evaporated/d × 600 calories = 1680 kcal → ~ 7 MJ

c) Calculation of energy loss from evaporation (Paper II):

1.3 kg water evaporated/d × 600 calories = 780 kcal → ~ 3 MJ

d) Assuming that all the urine N (Paper II) came from the oxidation of protein to urea the heat production from protein metabolism would be higher on the HP than the RP diet:

RP: 117.4 g N × (26.5 Kcal × 4.184 J ÷ 1000) = 13 MJ

HP: 170.8 g N × (26.5 Kcal × 4.184 J ÷ 1000) = 19 MJ

Appendix 2

Table 7. *Colon (right ventral) microbial counts (log_{10} cfu/ml) and VFA concentrations (mmol/l) before (0 h) and at 4, 12, and 24 h after an abrupt feed change between grass silage diets with a recommended and a high CP concentration*[1] *(n = 4) (Paper I)*

Item	Diet	0 h	Diet	4 h	12 h	24 h	SEM	*P*-value		
								Effect of treatment	Effect of time	Interaction time × treatment
Microbial counts										
Total anaerobic	HP	6.8	RP	6.4	7.0	6.4	0.3	0.021	0.013	0.153
bacteria	RP	7.6	HP	6.8	6.9	7.1	0.3			
Cellulolytic	HP	4.6	RP	5.0	5.6	4.8	0.3	0.992	0.106	0.525
bacteria	RP	4.9	HP	5.1	5.1	4.9	0.3			
Lactate-utilising	HP	6.6	RP	5.6	6.3	5.8	0.3	0.237	0.001	0.363
bacteria	RP	6.9	HP	5.8	6.2	6.6	0.3			
Proteolytic	HP	7.2	RP	6.4	6.8	6.2	0.2	0.430	0.002	0.147
bacteria	RP	7.1	HP	6.5	6.6	6.8	0.2			
Lactobacilli	HP	5.6	RP	5.2	5.9	5.3	0.4	0.021	0.103	0.180
	RP	6.2	HP	5.4	5.9	6.6	0.4			
Streptococci	HP	5.2[3]	RP	5.0	6.0	5.9	0.5	0.079	0.024	0.314
	RP	6.3	HP	5.2	5.8	6.6	0.4			

VFA concentrations										
Acetate	HP	33.5	RP	39.3	48.4	29.3	4.2	0.422	< 0.001	0.636
	RP	29.2	HP	36.4	45.5	32.4	4.2			
Propionate	HP	11.3	RP	9.9	13.3	8.8	1.3	0.154	0.003	0.336
	RP	8.7	HP	9.2	12.1	9.4	1.3			
Butyrate	HP	3.5	RP	3.9	4.7	2.7	0.6	0.702	0.002	0.505
	RP	3.1	HP	3.9	4.8	3.6	0.6			
Iso-butyrate	HP	0.60	RP	0.60	0.53	0.55	0.24	0.609	0.138	0.422
	RP	0.60	HP	0.60	0.33	0.63	0.24			
Iso-valerate	HP	0.75	RP	0.70	0.70	0.63	0.27	1.000	0.272	0.299
	RP	0.75	HP	0.58	0.73	0.73	0.27			
Total VFA[2]	HP	50.6	RP	55.2	68.8	42.8	6.0	0.473	< 0.001	0.417
	RP	43.2	HP	51.5	64.3	49.4	6.0			
(acetate+butyrate)	HP	3.3	RP	4.4	4.0	3.7	0.3	0.541	0.002	0.598
/propionate	RP	3.8	HP	4.5	4.2	3.9	0.3			

[1]The grass silage diets were fed restrictedly to result in similar DM intakes, but a CP intake close to recommended (NRC, 1989) daily CP intake (diet RP, 615 g CP/d) or higher than recommended daily CP intake (diet HP, 873 g CP/d).

[2]Acetate, propionate, butyrate, isobutyrate, isovalerate, n-valerate, lactate.

[3]Missing values due to technical problems, therefore only two observations at this sampling occasion.

Table 8. *Colon (right ventral) microbial counts (log_{10} cfu/ml) and VFA concentrations (mmol/l) before (- 20 h) and at 4 and 28 h after an abrupt feed change from hay to haylage or silage (n = 4) (Paper III)*

Item	- 20 h Hay	Diet[1]	4 h	28 h	SEM	*P*-value: Effect of treatment	*P*-value: Effect of time	*P*-value: Interaction time × treatment
Microbial counts								
Total anaerobic	7.9	H	7.1	6.7	0.3	0.661	0.119	0.172
bacteria	7.5	S	7.1[3]	7.5	0.4			
Cellulolytic	5.0	H	5.2	5.0	0.4	0.887	0.810	0.554
bacteria	5.0	S	4.8	5.5	0.4			
Lactate-utilising	7.1	H	6.7	6.4	0.3	0.860	0.152	0.564
bacteria	7.0	S	6.5	6.8	0.3			
Lactobacilli	6.2	H	5.8	6.1	0.2	0.213	0.550	0.506
	5.7	S	5.8	6.0	0.2			
Streptococci	6.0	H	5.5	5.5	0.4	0.880	0.360	0.354
	5.6	S	5.6	5.5	0.4			

VFA concentrations								
Acetate	45.8	H	39.3	44.5	8.3	0.990	0.503	0.884
	50.4	S	38.2	41.2	8.3			
Propionate	11.7	H	11.1	11.8	1.5	0.823	0.376	0.544
	13.2	S	9.8	10.7	1.5			
Butyrate	3.9	H	3.7	4.1	0.7	0.992	0.780	0.802
	4.4	S	3.5	3.8	0.7			
Total VFA[2]	63.3	H	56.2	62.3	11.3	0.987	0.554	0.859
	70.5	S	53.9	57.9	11.3			
(acetate+butyrate)/	4.2	H	3.8	4.1	0.4	0.737	0.725	0.660
propionate	4.2	S	4.2	4.2	0.4			

[1]Diets: H = haylage, S = silage.

[2]Acetate, propionate, butyrate, isobutyrate, isovalerate, n-valerate, lactate.

[3]Missing values due to technical problems, therefore only three observations at this sampling occasion.

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