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Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and
Animal Sciences

Free faecal liquid in horses – associations with nutritional factors

Fri fekal vätska hos häst – associationer med nutritionella faktorer

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1. Abstract

The occurrence of free faecal liquid (FFL) in horses is a problem as it leads to contamination of the tail and inside of hindlegs, which may result in skin lesions. Distinct from diarrhoea, horses with this condition often defecate normal faeces, but also void faecal liquid before, during or after defecation of solid faeces. Anecdotally, feed related factors such as feeding of haylage, silage or increased amounts of lucerne has been suggested to cause FFL. The aim of this study was therefore to compare feeding, feed rations and feeding routines between horses affected by FFL (case group) and horses not affected by FFL (control group). Data on feeding and forage samples was collected from 50 stables in a matched case-control study, where each stable housed one pair consisting of one case and one control horse. Horses in each pair were fed the same haylage and were housed in the same stable. The horse owners were requested to complete a survey with questions regarding feeding and management of the horses. The forage used in each stable was sampled at three occasions during the same winter and analysed for its chemical and microbial composition. The results didn't show any differences in amount of kg DM per 100 kg BW/day of forage or concentrates between case and control horses. The daily intake of digestible crude protein (dCP), metabolisable energy (MJ ME_h), and g neutral detergent fibre (NDF) per 100 kg BW did not differ between case and control horses. The results from this study showed that case and control horses were fed similarly, but does not rule out other nutritional causes of FFL. Further studies on causes of FFL are of interest.

2. Sammanfattning

Uppkomsten av fri fekal vätska (FFV) hos hästar är ett problem där kontaminering av svansen och insidan av benen kan leda till skador på hästarnas hud. Till skillnad från hästar med diarré innebär detta tillstånd ofta en normal träckavföring varpå hästarna innan, under eller efter defekering också avger en fri fekal vätska. Tidigare har utfodringsrelaterade orsaker som intaget av hösilage, ensilage eller ett ökat intag av lucern sammankopplats med uppkomsten av FFV. Syftet med denna studie var därför att undersöka utfodring, foderstat, och utfodringsrutiner mellan hästar drabbade av FFV (fall) och hästar icke drabbade av FFV (kontroll) för att om möjligt kunna identifiera utfodringsrelaterade faktorer. Data gällande utfodring samt vallfoderprover samlades in från 50 stall i en matchad fall-kontroll studie, där varje par inkluderade en fall- och en kontroll-häst. Hästarna i varje par utfodrades med samma sorts hösilage och var uppstallade i samma stall. Ägarna till hästarna ombads delta i en enkätundersökning där frågor om utfodring och övrig hantering utav hästarna behandlades. Foderprover från hösilaget som hästarna i varje stall utfodrades med samlades in under tre olika tillfällen under samma vinter och analyserades med avseende på kemisk och mikrobiell sammansättning. Resultaten visade inte några skillnader mellan fall och kontrollhästar i det dagliga intaget av kg torrsbstans grov- eller kraftfoder per 100 kg kroppsvikt. Det dagliga intaget av smältbart råprotein, omsättbar energi och neutral detergent fiber per 100 kg kroppsvikt var också lika mellan fall och kontrollhästar. Resultaten från den här studien visade att fall- och kontrollhästar inte utfodrades på olika sätt, men utesluter inte heller att det kan finnas andra utfodringsrelaterade orsaker till FFV. Ytterligare studier av orsaker till FFV är av intresse.

3. Introduction

Free faecal liquid (FFL), also referred to as free faecal water (Kienzle *et al.*, 2016), is a condition in horses where normal faecal balls but also free faecal liquid is voided before, during, after or independently from defecation of faecal balls. The general health of horses displaying FFL does not appear to be seriously impaired by the condition, as no apparent symptoms as fever or weight loss has been reported (Valle *et al.*, 2013; Kienzle *et al.*, 2016). However, the released faecal liquid has been reported to contaminate tail and hindlegs and in some cases it has been reported to cause skin lesions (Ertelt and Gehlen, 2015; Kienzle *et al.*, 2016). The occurrence of FFL has also been demonstrated in one horse where it was interspersed by outbreaks of diarrhoea (Valle *et al.*, 2013). Anecdotally, both nutritional and non-nutritional factors have been suggested causes for FFL. In Sweden, the condition has previously been referred to as “haylage intolerance” among horse owners, as the feeding of wrapped forages instead of hay has been anecdotally associated to FFL. However, today it is known that FFL also exist in horses eating hay or fresh grass (Kienzle *et al.*, 2016). Other proposed nutritional causes of FFL are feeding of lucerne hay, silage, or drinking very cold water (Kienzle *et al.*, 2016). Examples of non-nutritional factors suggested to cause FFL are poor dental care or inadequate parasite control (Kienzle *et al.*, 2016), whereas previous studies have suggested stress related factors associated to FFL (Valle *et al.*, 2013; Kienzle *et al.*, 2016). The gender and coat colour of horses was reported to be of importance in a German study (Kienzle *et al.*, 2016), as being a gelding and paint was associated to presence of FFL in the investigated group of horses. The occurrence of FFL among horses is unexplored and scientific studies on the subject is scarce. The gastro intestinal tract (GIT) of the horse is complex, and the hindgut microbiota is not well defined. However, both feed source and nutritional composition of the feed plays a key role to a well-functioning hindgut fermentation. For instance, feeding horses with non-structural carbohydrates in amounts larger than the amylolytic capacity of the small intestine increases the risk of undegraded starch ending up in the hindgut. This may lead to lactic acid fermentation in the hindgut, predisposing for osmotic diarrhoea (Garner *et al.*, 1978; Van Soest, 1994). Also, feeding protein in excess (over theoretical requirements) could cause disturbances in the GIT of the horse due to a build-up of nitrogen end-products followed by diarrhoea (Mair and Jones, 1995; Desrochers *et al.*, 2003; Frape, 2010). There are no previously identified nutritional factors associated to FFL. The aim of this study was therefore to investigate nutritional factors as possible risk factors for the occurrence of FFL in horses. The study was performed by comparing feeds and feeding routines between case (FFL) and control (no FFL) horses matched in pairs. The hypothesis was that one or several differences in the nutritional intake between case and control horses existed and could be one factor involved in presence of FFL.

4. Description of free faecal liquid in horses

In horses with FFL, faecal characteristics deviates from normal equine faeces. Faecal balls may be normal but are accompanied by a liquid phase. It may also manifest as a liquid and a solid phase where faecal balls are looser than normal, noticed by falling apart very easily when hitting the ground or floor. Clinical examinations of horses with FFL has not shown any apparent symptoms of fever or weight loss (Valle *et al.*, 2013; Kienzle *et al.*, 2016), indicating that horses are probably not suffering any infectious illness. Nevertheless, GIT of the horse is probably affected as shown by the deviating faecal characteristics. In some cases, both diarrhoea and FFL are present simultaneously. This was demonstrated in a horse with FFL, which proceeded into chronic diarrhoea (Valle *et al.*, 2009). In this case the horse always manifested the problem in a cyclic way starting with an increase of free fecal liquid which was followed by an increase in

number of defecations (5-6 times in an hour), and after a few days watery faeces or diarrhoea was observed. Apart from this, the horse seemed healthy without any other noticeable physiological disturbances and with no decrease in body weight. However, during the voiding of FFL, the horse presented repeated tail swishing and alternate rhythmic movement of the hind limbs. There was no history of GIT related problems as colic, but the horse owner reported that the horse sometimes seemed to be a little apathetic and had some difficulties to be collected during flat work. Examination of the digestive system of the horse didn't show any abnormalities regarding appetite, oral cavity, teeth or function of mastication. However, during auscultation of the abdomen, an increased motility in the left upper abdomen was detected in comparison to the right abdomen (Valle *et al.*, 2013). Based on these findings part of the forage was replaced with ground and pelleted hay, which in previous studies has been reported to help reduce mechanical stress on the colon (Van Weyenberg and Sales, 2006) and to enable healing of a possible mucosal damage (Galvin *et al.*, 2004). Further, the horse responded well to a treatment with a substance (sulfasalazine) commonly used for treatment of colitis both in humans, dogs and cats, and also for typhlocolitis in horses. Faecal examination of the horse didn't show any signs of bacterial infection (Valle *et al.*, 2009).

4.1. Intestinal motility

Horses with colitis may subsequently show FFL (Ertelt and Gehlen, 2015). Colitis in horses often lead to hypermotility of the gut contents, and hypermotility of the gut contents during an active fluid secretion into the bowel lumen during colitis increases fluid and faecal output (Blikslager *et al.*, 2017). Further on, FFL has also been compared to a syndrome in humans called functional gastrointestinal disorder (FGID), as they have common characteristics (Hunter, 2009). This is a syndrome that includes several chronic or recurrent gastrointestinal (GI) symptoms, where the physiological processes behind these disorders are multifactorial and not completely understood. Usually it is an issue of an ongoing inflammation, which may damage the enteric nervous system, causing motility disturbances in the GI tract (Drossman, 2006; Hunter, 2009). In human patients suffering from the form of FGID called irritable bowel syndrome (IBS) group C4, no problems with physical pain in addition to the watery diarrhoea is present (Drossman, 2006). Another aspect on gut motility is the nutritional composition of the feed and its effect on the passage rate of digesta. Feed with a smaller particle size or with a high water-holding capacity will move slower through the gut, whereas factors as increased fibre length, increased feeding level, or an increased forage/concentrate ratio will increase digesta passage rate (Van Weyenberg and Sales, 2006).

5. Nutrition and management factors associated with free faecal liquid

5.1. Nutritional factors

In addition to anecdotal proposals for nutritional causes of FFL, feed related factors have also been identified as possible causes in previous studies (Zehnder *et al.*, 2009; Valle *et al.*, 2013). In the case investigated by Valle *et al.* (2013) a rebalancing of the diet consistent with the theoretical nutritional requirements of the horse, together with changes in the composition of the diet, resulted in diminished or absence of FFL. The horse was estimated to have a body condition score (BCS) of 7 on a nine-point scale which was interpreted as a result of overfeeding the horse. The initial feed ration of the horse consisted of 9 kg DM of first-cut meadow hay (6.5% crude protein (CP), 0.1% crude fat (CF), 33% crude fiber (CFb), 1.5 kg of

cereal-based concentrate (12.4% CP, 3.5% CF, 9.1% CFb), 1.5 kg of lucerne and oat straw chaff (12.8% CP, 32% CFb), and 120 g of soy bean oil. The changes made in the feeding regimen of the horse consisted of avoiding excess of soluble carbohydrates from both forage and concentrate, and to replace some of the provided forage with ground and pelleted meadow hay. The new feed ration consisted of 5 kg DM of first-cut meadow hay (long stem; 7.5% CP, 0.1% CF, 29% CFb) provided in a hay net, 5 kg DM of ground and pelleted meadow hay (8.5% CP, 25.5% CFb, 9.5% CF), 1 kg of cereal-based concentrate, 120 g of soybean oil, 20 g of linseed oil and a vitamin/mineral balancer. The ration was divided into six meals per day and fed every 3 to 4 hours. Within a few days after these changes, signs of free faecal liquid was absent in the horse (Valle *et al.*, 2013). In the previously mentioned German study (Kienzle *et al.*, 2016) no apparent nutritional causes were found to be associated to the presence of FFL among horses in the study. The performed investigation included 42 horses displaying FFL (case horses) where all were fed grass hay all year round, whereas only 7% (n=3) were given silage or haylage in addition to the hay, and only during the winter. The majority of the 42 case horses (n=36, 86%) also received small amounts of various concentrate feeds (mean 1.5 kg/d; range, 0–4 kg), mineral supplements (n= 26, 62%), and were turned out at pasture at least during the summer period. In another linked study (Zehnder *et al.*, 2009) an association between the occurrence of FFL and hours spent per day on a winter pasture was found. The time spent on a field containing grass during winter were for FFL horses on average 15.3 hours per day in comparison to a healthy stable mate control group which spent on average 6.7 hours per day (p=0.003). During grazing in the summer, FFL horses spent longer (p = 0.01) time at pasture with an average of 16.2 hours, while the control horses spent on average 12.1 hours per day on pasture (Zehnder *et al.*, 2009).

5.2. Management factors

Stress-induced changes in peristaltic bowel movements connected to social hierarchy were suggested to cause FFL in the previously mentioned German study (Kienzle *et al.*, 2016). In the gut there are two different kinds of peristaltic movements. One of those are called haustral where the effect is more of a mixing than a propulsive movement of the digesta. The other is called phasic and has a stronger propulsive activity. Haustral contractions are less likely to press liquid out of the digesta compared to phasic contractions. If the digesta receives too much pressure it is possible that the solid and fluid phase is separated irreversibly (Lentle and Janssen, 2010). This type of change in the peristaltic movements in the gut of the horses were proposed to occur in connection to social stress in horses with FFL (Kienzle *et al.*, 2016). Stress has also been seen to induce an increased activity in the intestines followed by an increased faecal amount in studies on rats (Xiaojing *et al.*, 2015) which further confirms the effect stress might induce on the gut peristalsis and changes in faecal departure in animals. Several of the FFL horses were identified to be last or second last in the social hierarchy in the study by Kienzle *et al.* (2016). Hence, in order to further investigate social hierarchy as a contributing factor to FFL, owners of another 37 healthy horses (group housed, or group turned out) were interviewed on their horses' behaviour (behaviour control (BC) group). The results showed that 40 percent (n=15) of the case horses were considered to be last or second to last in the social hierarchy in comparison to 4 percent (n=2) in the BC group ($P < .001$). Further, 62 percent (n=23) of case horses compared to 27 percent, (n=3) in the BC group did not defend their feed against other horses ($P = .002$).

Another stress factor suggested to be associated to FFL were changes in management routines (Valle *et al.*, 2009). In the previously mentioned study by Valle *et al.* (2009) the occurrence of

FFL was seen to appear during stressful events such as changes in the stable management. Further, the owner of the horse also reported an increase of faecal liquid when the horse was subjected to abrupt feed changes. The emergence of FFL was reported to occur mainly when the current forage consisting of a first-cut meadow hay (6.5% CP, 0.1% CF, 33% CFb) was replaced with a first-cut hay mainly composed of ryegrass (7.7% CP, 0.2% CF, 30% CFb). Changes in management factors that were included in the recovery of the horse was to enable an adequate feed consumption time and meal size. This included providing forage in a hay net and dividing the feed into six meals per day every 3 to 4 hours. The horse was also allowed access to an overgrazed paddock (Valle *et al.*, 2009).

6. Nutritional factors associated with diarrhoea

Horses with diarrhoea, unlike horses with FFL, defecate faeces with a soft and watery consistency and the production of faeces is greater than usual (Mair & Divers, 2002). Diarrhoea is induced when there is an increased fluid content in the lumen of the intestines of the horse, and this occurs when the secretion of electrolytes controlling absorption of fluid in the gut is disrupted (Cohen and Divers, 1998). There are several factors classified as either infectious or non-infectious suggested to induce acute respectively chronic diarrhoea in horses (Chapman, 2009). These in turn often causes disruptions of the colonic microbial flora of the horse which could lead to overgrowth of potential pathogens, disruption of gut motility, and electrolyte and acid-base imbalances (Magdesian and Smith, 2002).

6.1 Regulation of water in the GIT

The composition of body fluids is affected by the ingestion of nutrients and water. When osmolality of one compartment increases, water movement through osmosis will occur to equalize the osmolality between the adjacent compartments (Johnson, 1998). The gut barrier transfers water in and out of the lumen, mainly by osmosis following secretory and absorptive transports of solutes. The hindgut absorbs water along with sodium ions (Na^+) exchanged for potassium (K^+) or hydrogen (H^+) ions, chloride ions (Cl^-) exchanged for bicarbonate (HCO_3^-), and short chain fatty acids (Kronfeld, 2001a). When large amounts of highly digestible feeds such as grain is digested and fermented macromolecules are cleaved, producing large numbers of smaller molecules within the gastrointestinal lumen. If the fermentation results in a production of macromolecules higher than the absorption capacity, this will result in hyperosmolality of the GIT contents. This was shown in a study conducted by Argenzio *et al.* (1974a), where ponies fed a hay-grain diet resulted in hyper-osmolality of the large intestinal contents, whereas feeding of a high-fibre low-protein diet resulted in hypo-osmolality. Slowly fermentable fibres (mature hay or straw) sustain a steady rate of production and a moderate concentration of VFA, hence a continuous absorption of VFA, Na, and water occurs from the large bowel (Clarke *et al.*, 1990; Stevens, 1995). Thus, net water absorption is favoured by a moderately low VFA concentration. It is likely to be achieved with frequent small intakes of forages or a feed containing multiple fibres that are fermented at different rates (Kronfeld, 2001).

6.2. Chronic diarrhoea

For diarrhoea to be considered as chronic, it should have been present for at least 7 to 14 days (Mair & Divers, 2002). Sometimes the diarrhoea will persist for weeks or months, with recurrent attacks of changed faecal appearance from “cowpat” consistency to watery diarrhoea, separated by periods of relatively normal faecal voiding (Mair & Divers, 2002).

In chronic diarrhoea, the onset often originates from the large intestine as a result from an upset in the intestinal microflora. This in turn is often connected to colonic dysfunction due to an altered intestinal permeability of nutrients and/or toxins (Barbut and Petit, 2001) or a high osmotic load of the colon (Argenzio *et al.*, 1974; Argenzio, 1990; Field, 2003). Nutritional factors that may lead to disruption in the GIT causing diarrhoea are a soluble carbohydrate or protein overload and a rapid transit of digesta (Garner *et al.*, 1978; Van Soest, 1994; Frape, 2010). When the transit time of digesta through the large intestine is rapid, the fiber digestion will be impaired with a depressed efficiency in reabsorbing water, Na, and K ions (Frape, 2010). Further, when large amounts of carbohydrates such as starch are consumed, the enzymatic capacity of the small intestine to degrade starch becomes overloaded. The undegraded starch will continue to and be rapidly fermented in the hindgut (Hoffman *et al.*, 2001) where accumulation of lactic acid may overpower the buffering capacity of the hindgut and lower the pH-value. A pH < 6 favours a further production of lactic acid and has been shown to be associated with clinical conditions such as osmotic diarrhoea (Garner *et al.*, 1978; Van Soest, 1994) and overgrowth of undesired bacterial populations such as *Salmonella* spp. and *Clostridium* spp. (Sprouse *et al.*, 1987; Bailey *et al.*, 2002). An osmotic diarrhoea may also arise when a buildup of cleaved molecules from highly digestible feed withdraws water into the lumen of colon (Blikslager *et al.*, 2017). Feeding horses with protein in excess of the foregut protein degrading capacity may cause disruptions in the GIT of the horse. Digestion and absorption of amino acids primary occurs in the small intestine, but if protein ingestion overwhelms the digestive capacity in the small intestine, more protein is entering the large intestine where it will be microbially degraded to NH₃ (Frape, 2010). This could cause a buildup of nitrogen end-products (ammonia and urea) that can contribute to health problems such as diarrhoeic states (Mair and Jones, 1995; Desrochers *et al.*, 2003; Frape, 2010). The most potent NH₃ producers are gram-negative aerobic bacilli such as *Escherichia coli*, *Klebsiella*, *Proteus*, and *Pseudomonas* spp. whose presence and activity could lead to accumulation of excessive ammonia (Mair and Jones, 1995).

6.3 Acute diarrhoea

When acute diarrhoea occurs in adult horses, it is usually a clinical sign of a large intestinal disease (Oliver *et al.*, 2006) where severe diarrhoea is frequently caused by *Salmonella* infection precipitated by stress during transport or strongyle worm infection (Frape, 2010). Another important aspect is the hygienic quality of the feed. For instance, forage crops might be subjected to mould growth already in the field with species with the potential to produce different mycotoxins. Mycotoxins are harmful to horses and other animals (Scudamore and Livesey, 1998) and could cause disruptions in the GIT of the horse with following diarrhoea (Kamphues, 2013). Further, forage can also be contaminated by soil or manure where strains of *Clostridium* spp. predisposing for GI disorders, toxicosis and diarrhoea may occur (Wilkinson, 1999; Weese *et al.*, 2001).

7. Digestion of various feeds or feed components and its effect on faecal characteristics

Historically, horses were steppe-living animals adapted to eat a fibre-rich diet through a continuous ingestion of grass. However, today it is very common to feed both performance horses (Richards *et al.*, 2006) and pleasure horses (Murray *et al.*, 2015) with cereal based concentrates as an energy supply. Intake of concentrates have been reported to cause hyperosmolality of GIT contents (Argenzio *et al.*, 1974a) whereas intake of fibre rich feed as forage enabled a steadier rate of production and absorption of nutrients and water in the bowel (Argenzio *et al.*, 1974a; Clarke *et al.*, 1990; Stevens, 1995). Previous studies have also shown that faeces from horses fed forage and grains in comparison to forage only became fetid and less formed (Robinson *et al.*, 1976; Lopes *et al.*, 2004). To understand how different feeds affect the GIT and the faecal characteristics in horses, a deeper understanding of their structure and digestion is needed.

7.1 Carbohydrates

Carbohydrates can be divided into structural and non-structural, which may be hydrolysed or fermented in the GIT of horses depending on the linkage of their sugar molecules (Hoffman, 2009). Neutral detergent fibres (NDF) includes cellulose, hemicellulose, and lignin which are examples of structural carbohydrates. The non-structural carbohydrates, also called soluble carbohydrates, includes e.g. sugar, starch and fructans. These are digested in different ways in the GIT, generating different bacterial populations and metabolites (Hoffman, 2009). Grains contain starch which are subjected to enzymatic hydrolysis of α -1,4 linked molecules in the small intestine. This yields monosaccharides, mainly glucose, which is effectively absorbed in the small intestine (Dyer *et al.*, 2002). Forages contains carbohydrates with β -1,4 linked molecules, which cannot be enzymatically degraded in the small intestine but are fermented by microorganisms in the hindgut (Hoffman, 2009). Fermentation of carbohydrates yields volatile fatty acids (VFA), where fermentation of structural carbohydrates mainly yields acetate, propionate, and butyrate. If the non-structural carbohydrates escape enzymatic hydrolysis in the small intestine, they will be fermented by the microorganisms, mainly yielding propionate and lactate (de Fombelle *et al.*, 2001; Hoffman *et al.*, 2001). Hence, the relative proportions of VFA produced are dependent on the type and amount of substrate (i.e. the proportions of forage and concentrate) (de Fombelle *et al.*, 2001; Hoffman *et al.*, 2001).

7.1.1. Grains

Cereals are energy dense feeds containing a considerable amount of starch. The starch concentration in some grains commonly fed to horses are presented in Table 1. Lactate producing microorganisms such as *Lactobacilli* and *Streptococci* favours starch as a substrate for fermentation, why they proliferate in a starch rich environment and produce excess amounts of lactic acid (Miwa *et al.*, 1997). Species that readily ferment starch, in preference to structural carbohydrates, will not only produce excess amounts of lactate, but also large amounts of CO₂, which can cause gut distension and pain. This excess gas production can lead to different forms of colic (McGavin *et al.*, 2002). Lactic acid is poorly absorbed in the large intestine and do not serve as a major nutrient for the horse (Argenzio *et al.*, 1974; Argenzio, 1990). The lactate levels in the hindgut are normally low because specific bacterial groups convert lactate to other short chain fatty acids (Biddle *et al.*, 2013). If, however, large amounts of lactic acid are produced in the large intestine, this would lead to intraluminal acidosis and an increase in intraluminal osmolality (Argenzio *et al.*, 1974; Argenzio, 1990) where diarrhoea can occur (Rowe *et al.*, 1994). Lactate is known to irritate the gut lining which has been demonstrated in

rats (Saunders *et al.*, 1982). Induction of lactate in the rat intestine was shown to cause an impaired activity of the absorptive cells with a decreased water absorption (Saunders *et al.*, 1982). Further, when horses were fed a starch-based diet resulting in the production of high lactate levels in the hindgut, the voided faeces became soft and unformed (Rowe *et al.*, 1994). Feeding horses 4.55kg of grains twice daily has been reported to reduce water content of colon ingesta but not in faeces, in comparison to a hay-only diet (Lopes *et al.*, 2004). Grain ingestion resulted in colon contents being more homogenous, dehydrated, foamy, and dense in comparison to a hay only diet, whereas the faeces became less compact and smelled more in comparison to a hay only diet (Lopes *et al.*, 2004).

Table 1. Starch content and starch digestibility in feeds commonly used for horses. Modified from Richards *et al.* (2006)

Grain	Starch content in feed (%)		Small intestinal starch digestibility (%)	
	Mean	Range	Mean	Range
Oats (<i>Avena sativa</i>)	41.3	36.4–46.8	60.5	52.0–66.3
Barley (<i>Hordeum vulgare</i>)	60.0	58.5–61.5	42.3	41.0–43.5
Corn (<i>Zea mays</i>)	70.6	66.2–76.8	35.6	29.1–41.5
Commercial concentrates	32.6	6.4–52.2	58.7	32.2–92.1

7.1.2 Forages

The digestive strategy of equines is characterised by a high chewing efficiency and a relatively short digesta retention time (Clauss *et al.*, 2009; Fritz *et al.*, 2009). Further, the intake of fibre rich feed encourages the performance of a more natural feeding behaviour (Thorne *et al.*, 2005) and prolongs eating time (Ellis *et al.*, 2005). Further, the greater bulkiness of forage stimulates peristaltic contractions and leaves less space in the intestines for accumulation of gas bubbles (Frape, 2010). Intake and chewing of fibrous feeds as forage increases saliva production which counteracts a decreasing pH in the intestines of the horse in comparison to concentrates (Willard *et al.*, 1977). Low roughage diets could result in digestive disturbances (hindgut acidosis, colic, gastric ulcers) and behavioural problems, and in the Swedish national feed recommendations for horses a DM intake of 1.5-2 kg per 100 kg BW/day is recommended (Jansson *et al.*, 2013). Harvested forages and pasture grass may differ greatly in nutritional content and structure depending on several factors (*e.g.* botanical origin and time of harvest) (Hoffman, 2009), thus influencing the gut of the horse in different ways. For instance, during ingestion of coarse forage with high NDF concentration, the passage rate of digesta increases in comparison to ingestion of feeds with smaller particles (Van Weyenberg *et al.*, 2006) which could result in an impaired efficiency in reabsorbing water and nutrients. Further, grazing of pasture grass may risk overwhelming the capacity of the large intestines, as cool season grasses may contain a substantial amount of fructans (Longland *et al.*, 1999; Cuddeford, 2001). Fructans has been shown to induce an even greater rapid fall in caecal pH (Van Eps and Pollitt, 2006) than an equal amount of corn starch (Bailey *et al.*, 2002). Fibre-degrading bacteria such as *Fibrobacter* spp. are predominantly acid-intolerant bacteria whose growth is greatly suppressed at acidic pH (Miwa *et al.*, 1997). Hence, different feeds may change the microbiota and the milieu of the bowel in different directions.

7.2 Effects on the microbiota during feed changes

The hindgut is mainly inhabited by fibrolytic bacteria (e.g. *Clostridiaceae*, *Fibrobacter*, *Spirochaetaceae*) that ferment fibre to short-chain fatty acids primarily consisting of acetate, propionate, and butyrate (Hintz *et al.*, 1971; Daly *et al.*, 2001). To a lesser extent, there is also a bacterial population of saccharolytic species in the hindgut (e.g. *Bacillus*, *Lactobacillus*, *Streptococcus*), fermenting soluble carbohydrates that has escaped small intestinal digestion to propionate and lactate (Hintz *et al.*, 1971; Hoffman *et al.*, 2001; Daly *et al.*, 2006). In an experiment where cereals were included abruptly in a hay-based diet, changes in the microbial profiles were seen together with increased lactate levels and a subsequent decrease in pH in the hindgut of horses (De Fombelle *et al.*, 2001). This was shown by feeding three ponies meadow hay with or without an abrupt inclusion of different proportions of rolled barley (*Hordeum vulgare*) (100% hay; 70% hay and 30% barley, or 50% hay and 50% barley). Differences were present in the microbial profiles, VFA concentrations and lactate levels 29 hours after the diet was changed from 100% hay to hay and barley. When 30% barley was included in the diet, propionate and lactate levels increased (propionate (molar %) from 19 to 24; and Lactate (mg/L) from 35 to 305), together with increased counts of anaerobic bacteria (*Streptococci*). When 50 % barley was included in the diet, the total VFA concentration increased ((mmol/L) from 74 to 98) together with an increased propionate concentration (from 19 to 28) and a decreased acetate concentration (from 73 to 64). The counts of *Lactobacilli* and *Streptococci* sharply increased in the colon with the incorporation of grain in the diet, while cellulolytic bacterial species remained unchanged. However, the fibrolytic activity was assumed to be affected as the VFA profile was modified by an increased propionate level (De Fombelle *et al.*, 2001). Alterations in both the microbial community and their metabolites result in a reduction in the fermentation of structural carbohydrates, and apart from the importance of VFA as an energy source for the horse, butyrate seems to have an essential role in maintaining gut health by regulating the expression of genes and controlling colonic tissue homeostasis. Disturbances in the microbiota of the large intestines could also cause increased risk of colonic acidosis and/or colic (Cuff *et al.*, 2005; Daly and Shirazi-Beechey, 2006).

7.3 Protein

Nitrogen availability is crucial for microbial growth, hence for the breakdown of dietary fibre in the hindgut. Nitrogen is achieved from the degradation of dietary protein and through secretion of urea into the hindgut lumen from the blood (Frape, 2010). Protein is primarily absorbed as amino acids in the small intestine, whereas protein escaping digestion pre-caecally is degraded by bacteria in the large intestine and absorbed as NH₃ (McMeniman *et al.*, 1987; Hintz and Cymbaluk, 1994). At intake of protein with low digestibility, more nitrogen will end up in the large intestine where it will be degraded to NH₃ (Frape, 2010). The metabolism of protein in excess of requirements may cause a build-up of nitrogen end-products (ammonia and urea) in the hindgut that can contribute to health problems such as diarrhoeic states (Mair and Jones, 1995; Desrochers *et al.*, 2003; Frape, 2010).

An excessive CP intake with an increased availability of nitrogen compounds in the large intestine will possibly increase the ammonia production and ingesta osmolality (Meyer, 1984). An increase of both nitrogen and VFA concentration in the hindgut induces an osmotic drive resulting in an increase in the ingesta water content (Brownlow and Hutchins, 1982). Race horses fed a high amount of dCP (323±12 g dCP/100 kg BW) in comparison to recommended

amount of dCP (216 ± 8 g dCP/100 kg BW) had a lower DM content in faeces (19.5 ± 0.6 vs. 20.9 ± 0.6 %) (Connysson *et al.*, 2006). However, an abrupt feed change between either a recommended CP intake or a high CP intake using two silage diets didn't show any differences in faecal DM (Muhonen *et al.*, 2008a).

7.3.1. Hyperammonemia

Animals with intestinal disease may produce excessive amounts of NH_3 because of bacterial overgrowth or may absorb increased amounts of NH_3 because of inadequate intestinal barriers (Desrochers *et al.*, 2003). Hyperammonemia (HA) is a state that might develop through an increased production or absorption, or a decreased clearance, of the substance. This condition has been found in conjunction with an abnormal faecal output in horses and has been suggested to be caused by intake of high amounts of protein. However, in several cases of equine HA including various diarrhoeic state, no clear association with protein level in the feed ration was found (Peek *et al.*, 1997; Desrochers *et al.*, 2003; Sharkey *et al.*, 2006; Stickle *et al.*, 2006). In two different cases, horses were diagnosed with HA in conjunction with episodes of watery diarrhoea. No other horse in the same stables as case horses and with access to the same diet was affected. Faecal samples from both case horses showed heavy bacterial growth of *Clostridium sordelli* (Desrochers *et al.*, 2003) and *Clostridium perfringens*, respectively, where the latter was found to have severe colitis (Stickle *et al.*, 2006). In four other horses diagnosed with HA and signs of dysfunction of the GIT (colic with or without diarrhoea), other horses on the same pasture were unaffected, and no overgrowth of specific bacteria in the intestinal tract was identified (Peek *et al.*, 1997).

8. Aim and objective

Since previous studies have shown that different feed components may affect the environment and/or function of the colon of the horse, it is of interest to investigate whether such factors may be involved in the occurrence of FFL. The aim of this study is therefore to compare feeds and feed rations for horses with and without signs of FFL by using survey data and feed samples from horses in a case-control study. The goal was to find out if differences in feeds and feed rations existed between case and control horses, with special emphasis on daily intake of metabolisable energy (MJ ME_h), g digestible crude protein (dCP), and g neutral detergent fibre (NDF) per 100 kg BW and day.

9. Materials and methods

9.1. Experimental design

A matched case-control study of horses with and without presence of FFL was performed, with sampling of forages used for the horses as well as data collection from all owners by use of an online questionnaire. Questionnaire data and forage samples was obtained from 50 case-control horse pairs from Sweden and Norway. One of the matched pairs of case and control could not complete their participation in the study and was excluded from further analyses. The final number of participating pairs of case and control was therefore 49. Each horse pair was housed in the same stable, were fed the same forage and were managed under the same husbandry

practices. The forage in each stable was subjected to three samplings, while data collection was performed once (in conjunction to the first forage sampling occasion).

9.2. Design of questionnaire

The questionnaire consisted of 53 questions including general information about the horse and overall management factors. One part of the questionnaire comprised feeds and feeding practices, and this part was selected for further analysis in this thesis. The full questionnaire is reported in Appendix 1. Nutritional factors included in the statistical analysis were type and amount of feed, daily intakes of MJ ME_h; g NDF; g dCP; kg DM (all per 100 kg body weight (BW)) and proportion of concentrate in the total feed ration (%).

9.3. Preparation and analysis of forage samples

The forage samples were taken by the horse-owners and sent to the Feed Laboratory at the Department of Animal Nutrition and Management, SLU. Samples were stored in a freezer until analysis of chemical composition. Each sample was prepared for analysis by weighing, drying and milling. From each sample, 75 g was weighed and dried in a forced air cabinet for 18h at 55 °degrees C. After air equilibration, samples were weighed and ground in a hammer mill to pass a 1.0-mm sieve. Ground samples were used for analysis of *in vitro* digestibility of organic matter (IVDOM) for estimation of content of ME_h, and concentrations of CP, NDF and DM. Concentration of ME_h per kg DM forage was estimated using the following formula: ME_h = 1.12x – 1.1 where x = MJ ME_r/kg DM where ME_r is metabolisable energy for ruminants (Jansson *et al.*, 2011). The following formula was used for estimation of ME_r from IVDOM (for forages containing <50% legumes) ME_r = (0.160x-1.91) where x = IVDOM (Spörndly, 2003). To estimate the concentration of digestible CP in forages the following formula was used: g dCP = dCP x CP/100 where dCP = (93.9-313/y), CP= g CP/kg DM and y = percent CP of DM (Jansson *et al.*, 2011).

9.4. Transformation of data

The responses from the survey were transferred to a Microsoft Excel worksheet for processing and quality control of data and preparation for statistical analysis. Some questions had the response alternative “other” if none of the pre-given options were suitable. In these cases, the respondents were asked to specify it by writing their own individual answer. In some cases, the option “other” was marked but without any added clarification. In those cases, no further specification was possible and was then left as the option “other”. In one of the questions where the horse owners were asked to report what type/types of concentrates their horses were fed, 38 different commercial concentrates were reported and therefore they were categorized as “commercial concentrates” with no further classification. Some of the horses were reported to have free access of straw in the diet, and since it was hard to estimate an actual intake of straw in these cases they were excluded in the calculations of daily intake of straw per 100 kg BW.

9.4.1. Additional variables created from the collected data

New variables were created for feed intake and intake of ME_h and nutrients daily. Calculations of forage DM intake per day was calculated as intake of kg forage/day * (DM of forage in percent)/100. This product was then divided with the given BW for each individual horse to estimate kg DM forage intake per 100 kg BW and day. Further, several horse owners stated that their horses had free access to forage. To be able to estimate forage intake in kg DM/100 kg BW, an estimated consumption of 3 kg DM/ 100 kg BW was used (Jansson *et al.* (2011).

Three new variables were created to estimate total daily intakes of ME, dCP, and NDF per 100 kg BW. This was done by the following calculations:

Total daily intake of ME_h = MJ ME_h/kg DM forage/day + MJ ME_h/kg DM concentrate/day

Total daily intake of dCP = g dCP/kg DM forage/day + g dCP/kg DM concentrate/day

Total daily intake of NDF = g NDF/kg DM forage/day + g NDF/kg DM concentrate/day

To calculate the proportion of concentrates in the daily total feed ration, a new variable was created by using the following formula: Daily intake of kg DM concentrate/ (daily intake of kg DM concentrate + daily intake of kg DM of roughage). Seventy-two horses in total were confirmed to eat concentrates of which it was possible to detect the amount of concentrates fed each day in 65 of these horses.

9.5 Statistical analysis

All data was processed and analysed in the statistical program SAS (Statistical Analysis System) version 9.4 for Windows. A descriptive analysis was performed for all feed related variables by using the PROC FREQ procedure. For further analysis a univariate logistic regression analysis was performed using the PROC GLIMMIX and PROC LOGISTIC procedure (Olsson, 2002).

10. Results

10.1. Descriptive statistics

10.1.1. Faecal characteristics of case and control horses

All control horses were reported to defecate typical horse faeces, and all case horses were reported to have varying degrees of FFL (reported elsewhere).

10.1.2. Type of roughage

Big bale haylage was the most common forage fed to case and control horses (59 percent, n=29). Big bale silage was the second most common forage fed to case (22 percent, n= 11) and control (27 percent, n=13) horses. Inclusion of hay (small or big bale) in the diet was reported for case (24 percent, n=12) and control (29 percent, n=14) horses. Lucerne, chopped or pelleted, was included for case (22 percent, n=11) and control (16 percent, n=8) horses. Feeding of straw was reported for both case (20 percent, n=10) and control horses (24 percent, n=12) (Figure 1).

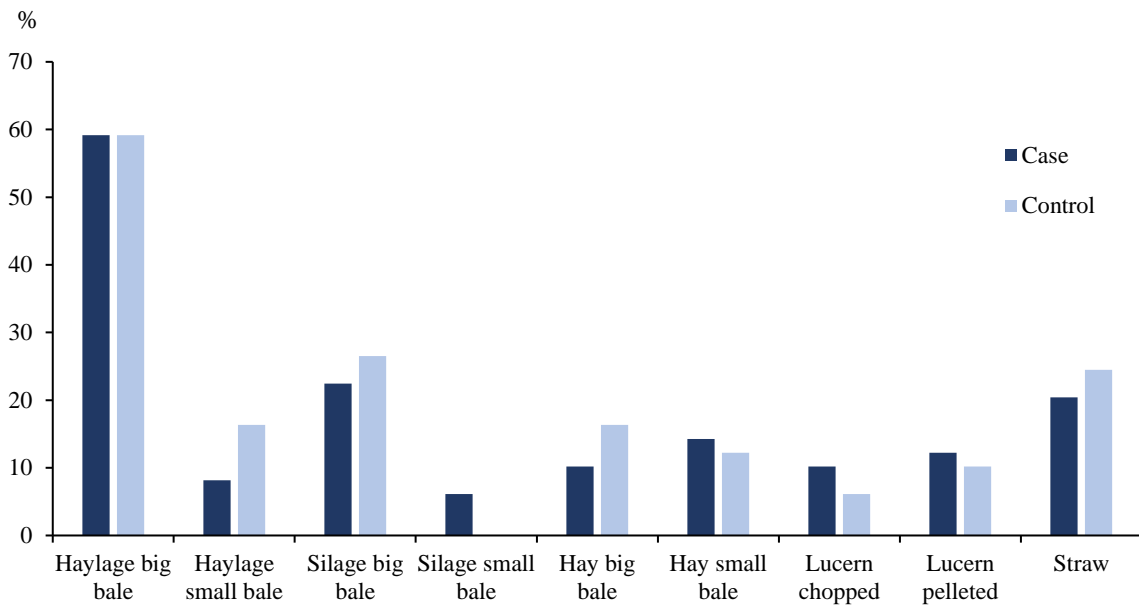


Figure 1. Type of roughage fed to case and control horses in the study. The total sum for case and control horses exceeds one hundred percent as the horses could be fed with more than one forage at the same time.

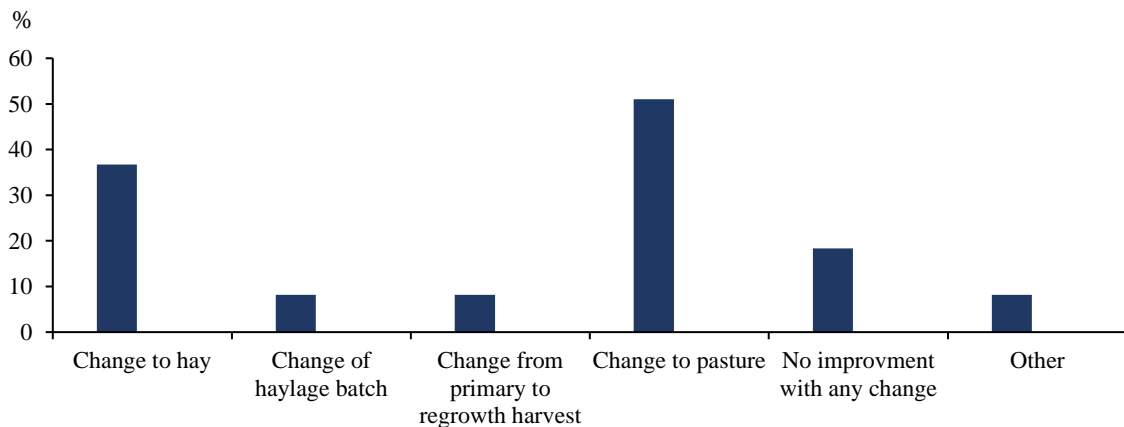


Figure 2. Changes in forage types reported to result in a decline in display of free faecal liquid and/or cowpat-like faeces, case horses only. Bar “other” contain unspecified changes in feeding. All changes were made from wrapped forage. All respondents had not tried all changes reported in the figure.

A change in the forage type used was reported to result in a decline in signs of FFL and/or cowpat like faeces in case horses (Figure 2). Changing from wrapped forage to pasture was reported to result in declined FFL signs for over half of the case horses, while 37 % were reported to improve when changing from wrapped forage to hay. No control horses were reported to have a change in faecal characteristics when changing forage type.

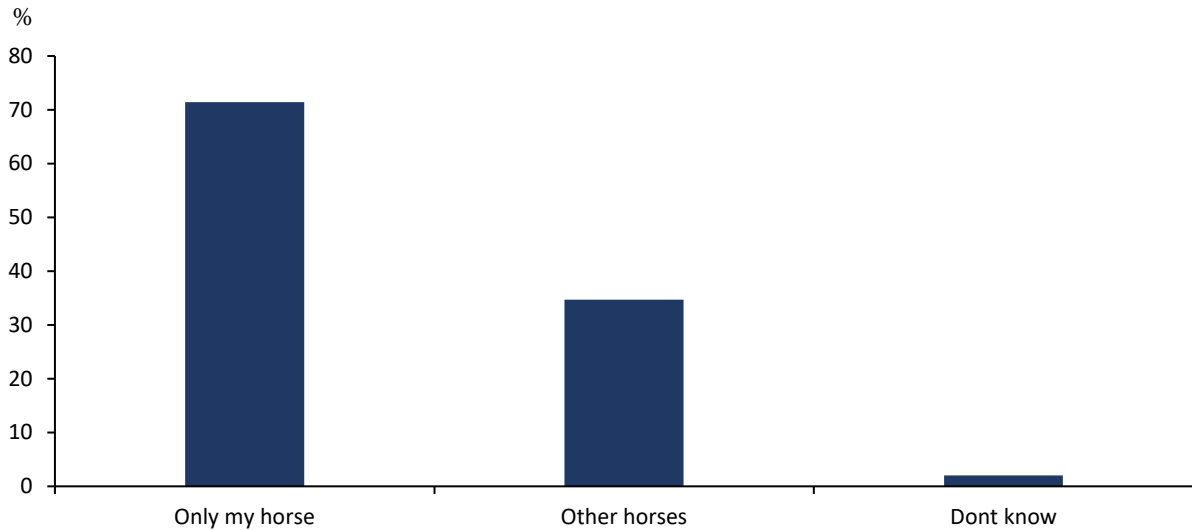


Figure 3. Respondents reports on the number of horses in the same stable that were fed the same wrapped forage and displayed FFL and/or cowpat-like faeces.

Seventy-one percent (n=35) of the case horses were reported to be the only horse in the stable displaying FFL and/or cowpat faeces when fed the same wrapped forage as other horses in the stable. Thirty five percent (n=17) of case horses had one or several other horses in the same stable that displayed FFL and/or cowpat-like faeces (Figure 3).

10.1.3. Type of concentrates

Commercial concentrates were the most common concentrate type fed to both case (53 percent, n=26) and control (57 percent, n=28) horses. Feeding molassed sugar beet pulp was reported for similar proportions of case (29 percent, n=14) and control (24 percent, n=12) horses. Twenty-two percent (n=11) of the case horses and 29 percent (n=14) of control horses were not fed any concentrates. The column other contains cases where a specific feed material was given for one/few horses or if the option other was filled in without any further specification (Figure 4).

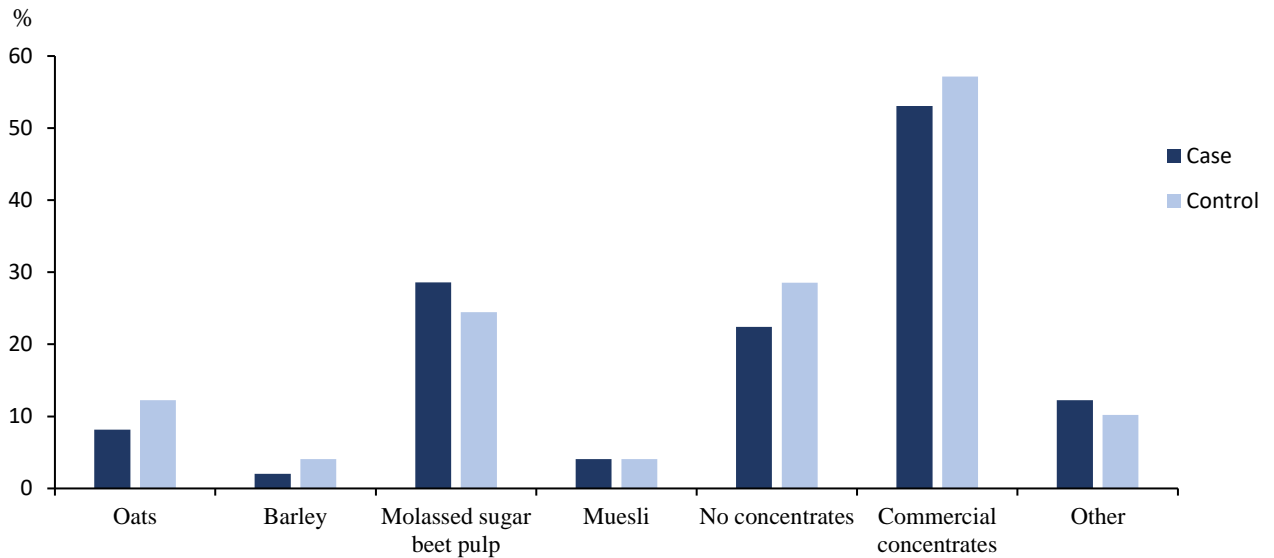


Figure 4. Type of concentrates fed to case and control horses in the study.

10.1.4. Type of supplements

Different mineral feeds were the most common supplements fed to both case (51 %, n=25) and control horses (53 %, n=26) (Figure 5). Supplementation with b-vitamin to the horses in the study was reported for 22 % of case (n=11) and 4 % of control horses (n=2). Three percent (n=3) of all horses were reported not to be fed any supplements and comprised control horses only. The column other contains cases where a specific supplement was given for one/few horses or if the option other was filled in without any further specification (Figure 5).

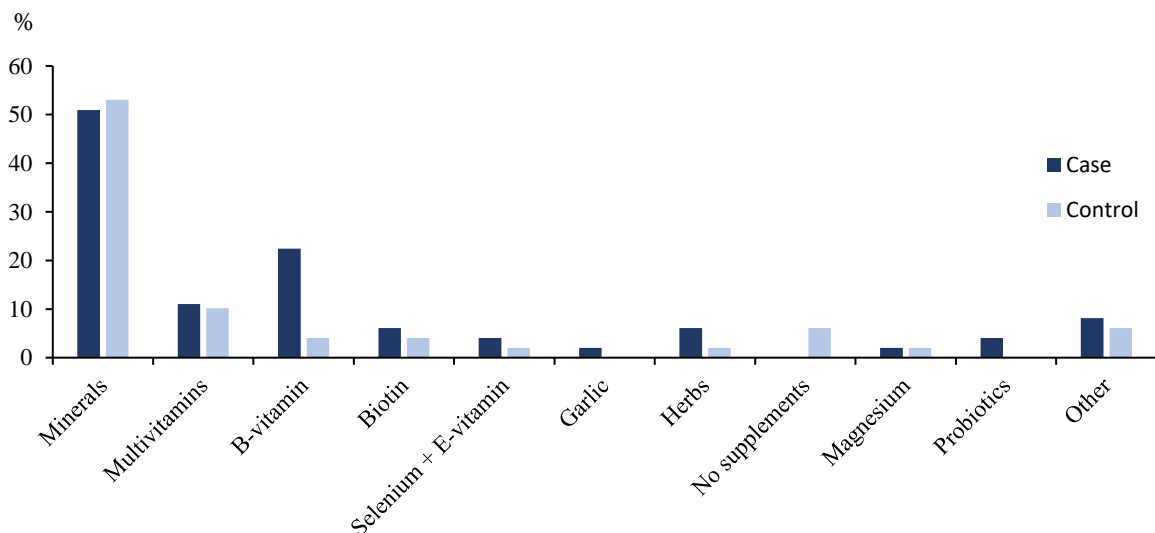


Figure 5. Type of supplement feeds fed to case and control horses in the study.

10.1.5 Feeding routines for roughage

Free access to forage was reported for 29 % of control (n=14) and 24 % of case (n=12) horses. Feeding of forages four times/day was reported for 31 % (n=15) of case and 29 % (n=14) of

control horses (Figure 6). Feeding of forage less than three times daily was reported for 18 percent (n=9) of case and 14 percent (n=7) of control horses. Eight to twelve hours between feeding occasions of forage was the single most common for both case (31 percent, n= 15) and control horses (27 percent, n=13) (Figure 7).

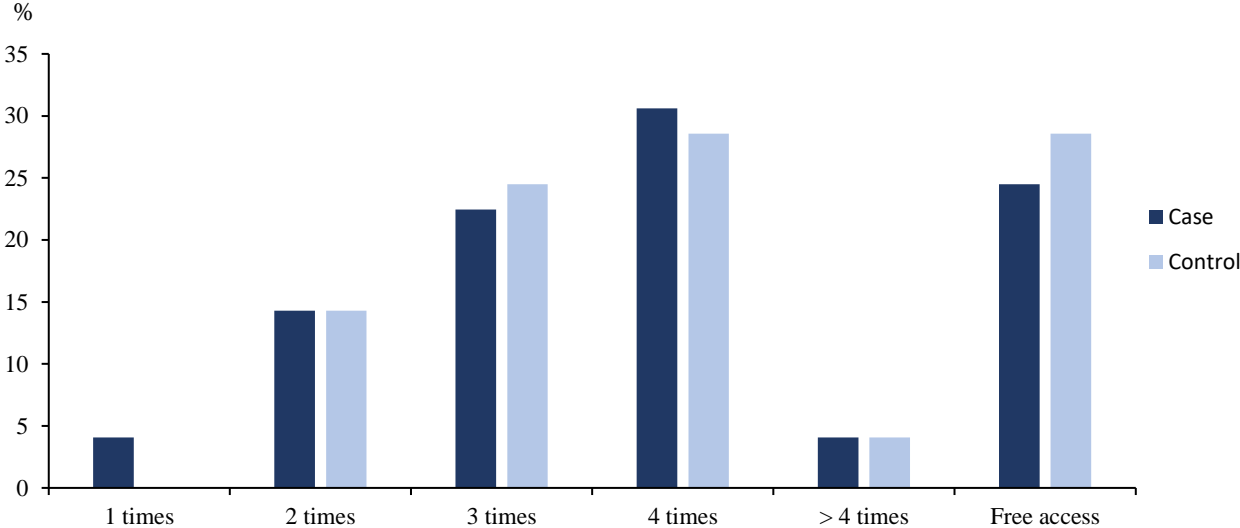


Figure 6. Number of feedings/day of forages to case and control horses in the study.

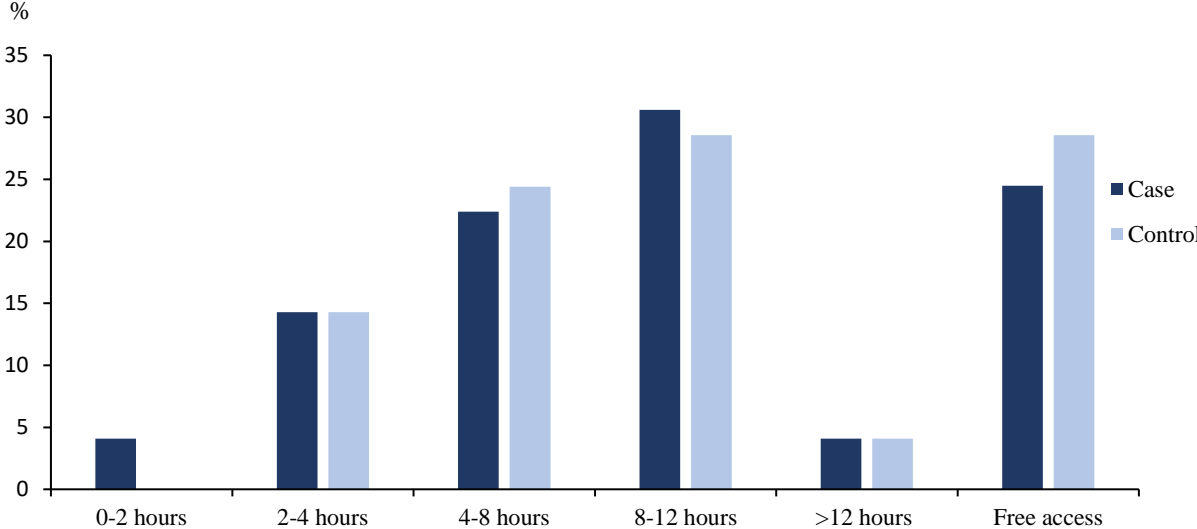


Figure 7. Number of hours in between feedings of forages for horses in the study.

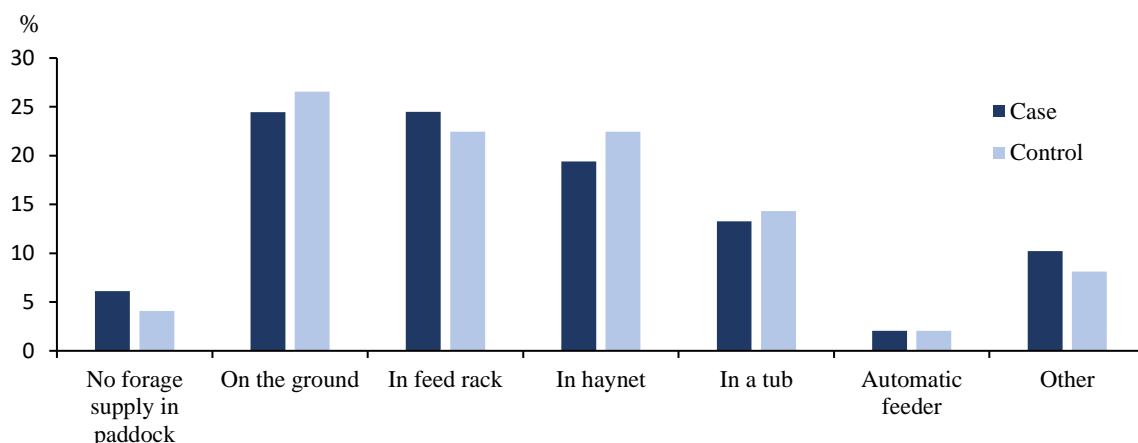


Figure 8. Method for providing forage in their paddocks to case and control horses in the study.

About one fourth of both case (24 percent, n=12) and control horses (27 percent, n=13) were fed forage on the ground in the paddock. Forage was provided in a feed rack for 24% (n=12) of case and 22 % (n=11) of control horses. Twenty % (n=10) of case and Twenty-two % (n=11) of control horses were fed forage in a hay-net in the paddock. The column other contain cases where the option other was marked but without no further specification (Figure 8).

10.1.6. Feeding routines for concentrates

Feeding concentrates one time/day was reported for 45 % of case (n=22) and 37 % of control horses (n=18). The remaining horses were fed concentrates more than one time daily with similar proportions among cases and controls (Figure 9).

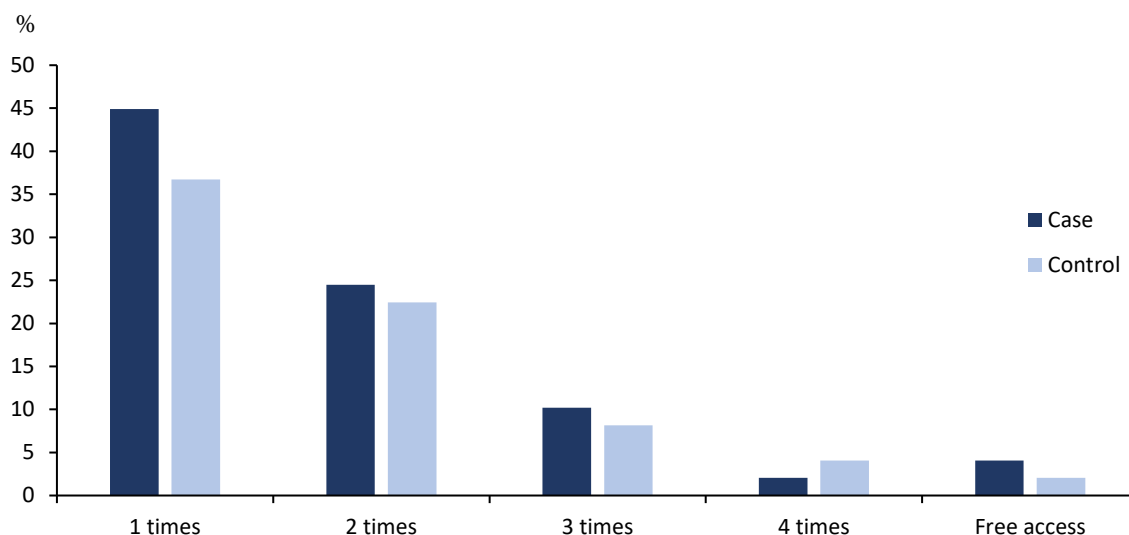


Figure 9. Number of concentrate feedings/day to case and control horses in the study.

10.1.7. Salt supply

Provision of salt through a saltlick in the stable was most common for both case (84 percent, n=41) and control horses (86 percent, n=42) (Figure 10). When horses were kept at pasture

provision of salt through a saltlick was reported for 77 % of case (n=38) and 73 % of control horses (n=36) (Figure 11).

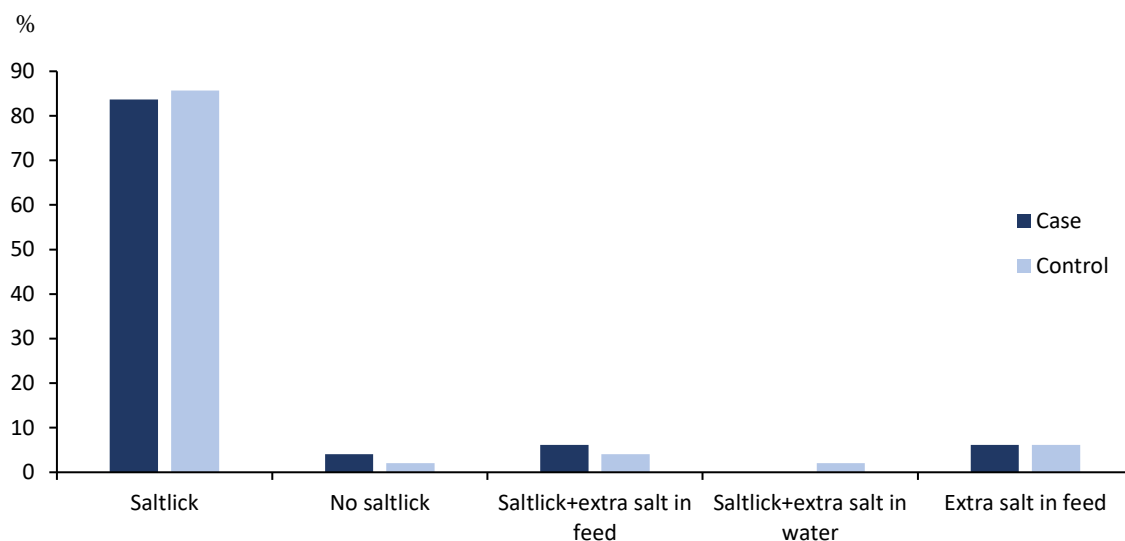


Figure 10. Method to supply salt in the stable for case and control horses in the study.

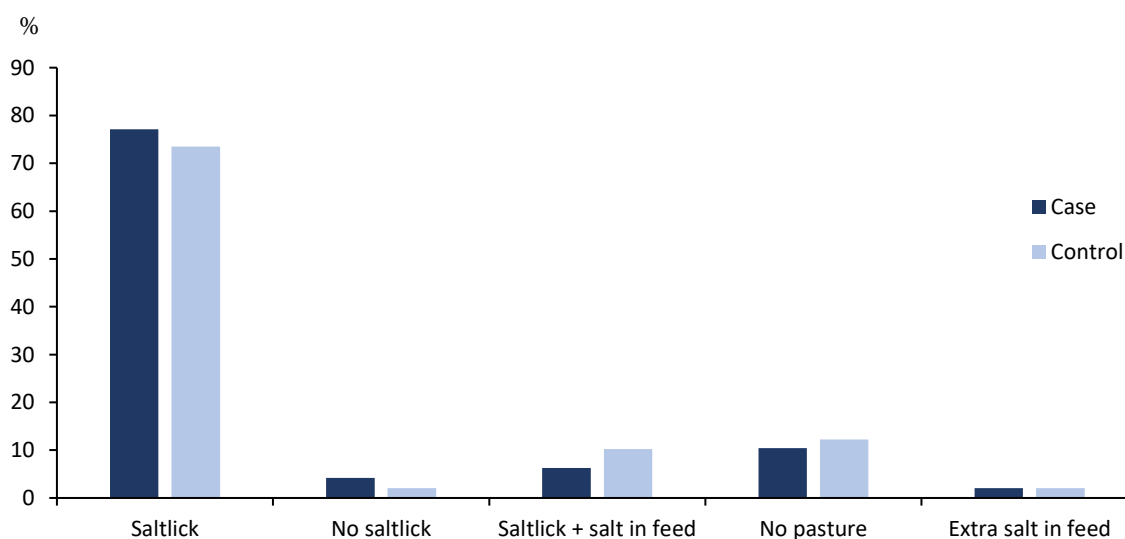


Figure 11. Method to supply salt on pasture for case and control horses in the study.

10.1.8. Type of pasture and time spent on pasture

Keeping their horse on pasture for 12 weeks or longer was reported for both case (49 percent, n=24) and control horses (45 percent, n=22). Twelve percent of case (n=6) and 14 % of control horses (n=7) were reported to not be kept on pasture at all (Figure 12).

Keeping their horse on a natural pasture was most common for both case (57 %, n=28) and control horses (53 %, n=26). The second most common pasture type was pasture on arable land for both case (37 percent, n=18) and control (29 percent, n=14) horses. The column other

contain cases where the option other was marked but without no further specification (Figure 13).

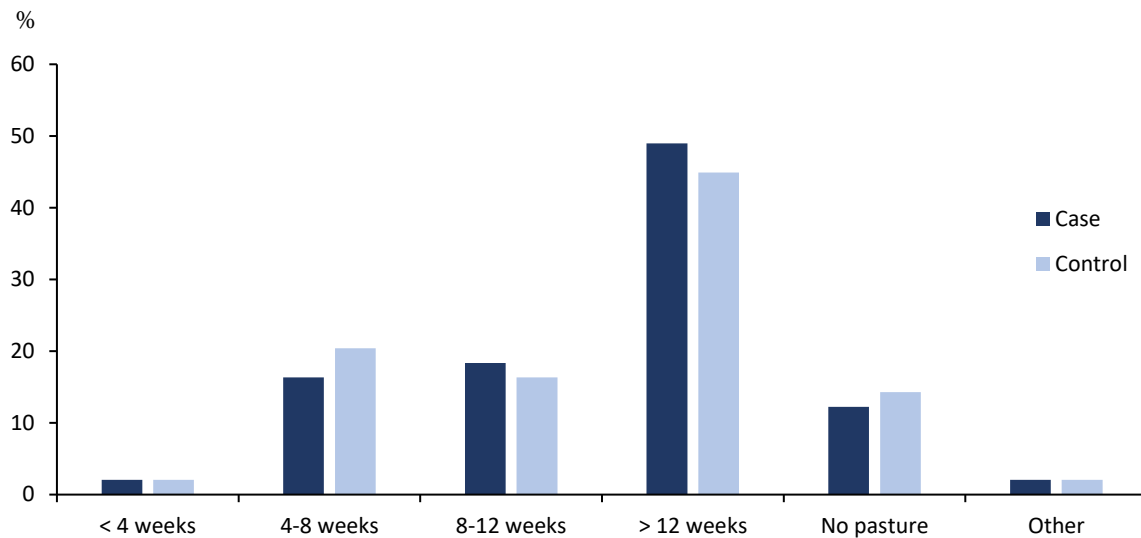


Figure 12. Time spent on pasture for case and control horses in the study.

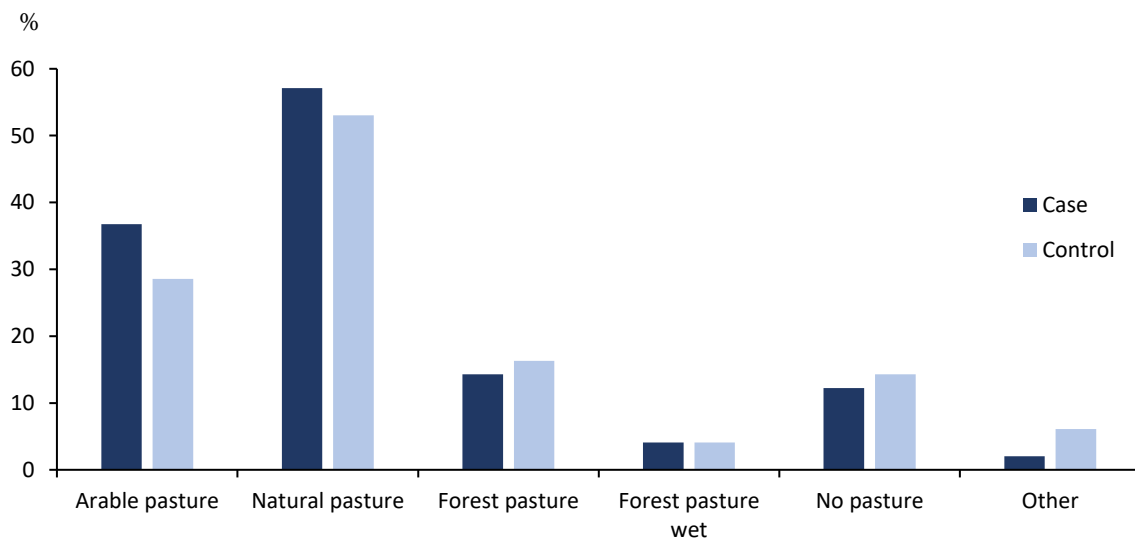


Figure 13. Type of pasture for case and control horses in the study.

10.1.9. Storage of feed

Most of the respondents for both case (51 percent, n=25) and control horses (53 percent, n=26) reported that the forage mainly fed to the horses in the study was stored indoors. Similarly, concentrates fed to the horses were stored indoors and covered for most of both case (53 percent, n=26) and control horses (57 percent, n=28).

10.1.10. Forage properties

Forty percent (n=39) of the participating horse pairs reported that the forage they were using was analysed for its nutritive content. Forty-two percent (n=41) reported that the forage was not analysed, and 18 percent (n=18) reported that they did not know if the forage was analysed or not. The majority of each horse-pair reported that the forage fed to their horses was bought and not produced on the farm or by the horse owner. Basic analyses were performed on the 49 different feed samples to identify the DM content and the nutrient content of MJ Me_h, NDF, and dCP. Mean value, standard deviation (SD), minimum (Min), Median, and maximum (Max) for the different feed values are found in Table 2.

Table 2. Average with standard deviation (SD), minimum, median and maximum concentration of dry matter (DM), metabolisable energy (ME_h), digestible crude protein (dCP), and neutral detergent fibre (NDF) per kg dry matter in forage samples from 49 farms. The forage on each farm was sampled three times

Variable	Mean	SD	Min	Median	Max
DM, g/kg	690	114.0	420	710	880
ME _h , MJ/kg DM	9.2	0.91	6.9	9.4	11.1
NDF, g/kg DM	611	36.8	532	609	684
dCP, g/kg DM	44	19.4	6	41	96

10.2 Univariate analysis

The variables used in the univariate model included type and amount of kg DM concentrates and kg DM forage per 100 kg BW, average daily intake of MJ ME_h, g dCP and g NDF per 100kg BW and proportion of concentrate (in % of total diet) for case and control horses. Average amount of different feedstuffs (Table 3) and the average daily intake of MJ ME_h, g dCP, or g NDF per 100 kg BW and day (Table 4) did not differ between case and control horses. There were no differences in reported intake of kg DM of forage per 100 kg BW or the proportion of concentrates in the total feed ration between case and control horses (Table 3).

Table 3. Average daily allowance of different feedstuffs per 100 kg bodyweight and day for case and control horses, and proportion of concentrates in total ration. SD=standard deviation

Feedstuff	Case		Control		p-value
	Mean	SD	Mean	SD	
Commercial concentrates (kg)	0.08	0.137	0.07	0.117	0.7
Haylage (kg DM)	2.13	1.105	2.23	1.179	0.6
Lucerne (kg)	0.03	0.065	0.02	0.053	0.5
Minerals (g)	12.07	11.796	12.99	15.513	0.7
Molassed sugar beet pulp (kg)	0.04	0.125	0.03	0.103	0.5
Straw (kg)	0.12	0.219	0.09	0.169	0.4
Total amount of concentrates (kg)	0.12	0.132	0.12	0.129	0.7
Proportion of concentrates in total ration (%)	7.24	5.789	7.29	5.696	0.9
Total DM (kg)	2.40	1.097	2.46	1.232	0.8

Table 4. Average daily allowance of metabolisable energy (MJ ME_h), digestible crude protein (g dCP), and neutral detergent fibre (g NDF) per 100 kg bodyweight and day for case and control horses

Total daily allowance per 100 kg BW	Case horses				Control horses				P-value
	Mean	SD	Min	Max	Mean	SD	Min	Max	
MJ ME _h	20.1	7.31	6.0	37.5	20.8	8.31	5.5	46.6	0.6
g NDF	1304	476.9	606	2397	1336	565.6	300	2940	0.8
g dCP	103	70.4	20	289	110	67.7	19	289	0.6

11. Discussion

11.1 Reported intake of different feedstuffs

11.1.1 Roughages

No differences in the daily intake of roughage was seen between case and control horses. Each pair of case and control horses were fed haylage as a basic feed where more than half of both case and control horses were reported to be fed with big bale haylage. Forages as silage and hay were included in the diet for both case and control horses, and about one fifth of the horses (both cases and controls) were also reported to be fed lucerne and straw. In another study on horses with FFL (Kienzle *et al.*, 2016), the horses were mainly fed hay all year round and only 7% were fed silage or haylage. No detailed information about the feed rations of the horses was included, and no control horses were included for comparison of feed rations (Kienzle *et al.*, 2016). However, the results of the current study together with results reported

by Kienzle et al. (2016) shows that forage type (hay or wrapped forages) is not a cause of FFL in horses in general. In the current study, approximately the same proportion of case as control horses were kept at pasture for similar periods. In another study (Zehnder *et al.*, 2009) it was found that horses with FFL was kept at pasture to a higher extent than control horses. This may be explained by the reports in the current study that changes from feeding wrapped forage to pasture was associated with a decline in presence of FFL in case horses. If the horse is not displaying FFL while at pasture, the horse owner may be inclined to keep the horse at pasture for as long time as possible.

11.1.2 Concentrates

There were no differences in the reported daily intake of concentrates between case and control horses and nor did the horses have remarkably high proportion of concentrates in their daily feed rations. Additionally, the number of horses with concentrates included in their diet was fairly similar between case and control horses. The most common concentrate type fed to horses in this study was commercial concentrates for both case and control horses. There was a large variation among type of commercial concentrates. Type and amount of concentrates included in the diet could be of importance for FFL considering the low small intestinal digestibility of starch and the inclusion of starch-rich feeds in many concentrates. Starch may be fermented to lactate in the hindgut, and an increased amount of lactate in the equine GIT has been reported to result in soft and unformed faeces (Rowe *et al.*, 1994). Further on, Lopes et al. (2004) found that horses fed hay and grains differed from horses fed only the same hay in faecal appearance. Horses fed hay and grains had less formed faeces and had a clear separation of the faeces in two phases where the liquid phase had noticeable gas bubbles and was more viscous, compared to faeces of horses fed hay only (Lopes et al., 2004). The concentration and small intestinal digestibility of starch in commercial concentrates may differ greatly (Richards *et al.*, 2006) why it is difficult to draw conclusions regarding possible impacts on FFL presence from commercial concentrates used for the horses in the current study.

11.2 Feed changes

It is well known that feed changes should be made successively to enable adaptation of the intestinal microbes to the new feed. In a previous study where a horse displayed problems with FFL in conjunction with diarrhoea, the owner of the horse reported an increase of faecal liquid when the horse was subjected to abrupt feed changes between two different batches of hay (Valle *et al.*, 2009). In the current study the participants were asked to report whether their horse improved or not when changing forage. A change from wrapped forage to pasture was reported to diminish FFL signs in half of the case horses and seemed to be the most effective change. Changing from wrapped forage to hay was reported to improve the condition for a little over one third of the case horses. However, no information was given on how the feed changes were performed (gradually or abruptly).

11.3 Calculated daily intake

11.3.1 Daily intake of dry matter

According to the feeding recommendations from SLU by Jansson *et al.* 2013 the horse should be supplied with 1.5-2.0 kg DM of forage per 100 kg BW and day, and no less than 1.0 kg DM of forage per 100 kg BW and day. In the current study there were no differences in the calculated intake of kg DM of haylage per 100 kg BW and day between case (mean: 2.13) and control (mean: 2.23). This indicates that a difference in the intake of kg DM of forage is not the cause of FFL in affected horses. Neither was there any difference in the calculated intake of total kg DM per 100 kg BW and day between case and control. Case and control horses were both fed forages at least 3 to 4 times daily. Both groups were fed forages within 8-12 hours. As each pair of case and control horses were housed in the same stable, and thereby kept under similar conditions, it is not surprising that feed management between case and control horses followed similar patterns. The proportion of kg DM concentrates in the total daily feed ration might be of importance as previous studies has shown that both the microbial community and their metabolites may be affected by starch rich cereals and thus changing the milieu in the bowel of the horse (Miwa *et al.*, 1997; De Fombelle *et al.*, 2001). However, in the current study, no difference in the calculated proportion of kg DM of concentrates in the daily total feed ration was seen between case (7.24 %) and control horses (7.29 %).

11.3.2 Daily intake of metabolisable energy

No differences in the calculated daily intake of MJ ME_h per 100 kg BW was found between case and control horses. Studies regarding the energy intake in horses and whether it affects the faecal characteristics is scarce.

11.3.3 Daily intake of neutral detergent fibre

The calculated daily intake of g NDF per 100 kg BW/day was similar between case and control horses. The daily intake of g NDF is an interesting nutritional factor in more than one aspect. For instance, ingestion of a lot of coarse feed with a high NDF content could increase digesta passage rate (Van Weyenberg and Sales, 2006) where a rapid transit time could impair fibre digestion with a depressed efficiency in reabsorbing water, Na, and K ions (Frape, 2010). Likewise, a lower intake of NDF may indicate a low intake of structural carbohydrates which could affect the microbiota of the horse, as the hindgut is inhabited mainly by fibrolytic bacteria that ferment fibre to short-chain fatty acids (SCFA) (Hintz *et al.*, 1971; Daly *et al.*, 2001). Further, apart from being an important energy source, SCFA (butyrate) also have a role in maintaining gut health and controlling colonic tissue homeostasis (Cuff *et al.*, 2005; Daly and Shirazi-Beechey, 2006). Also, chewing fibrous feed entails an enhanced saliva production with buffering effects in the GIT of the horse, and during a decreased intake of fibre a higher risk of colonic acidosis and/or colic may be present (Cuff *et al.*, 2005; Daly and Shirazi-Beechey, 2006). However, as the calculated daily intake of g NDF per 100 kg BW was similar between case and control horses, this does not seem to affect the appearance of FFL.

11.3.4 Daily intake of crude protein

The focus in this study regarding associations of protein intake and effects on faecal characteristics has been based on the consequences of overfeeding of protein. This is due to that previous studies have described and found associations between overfeeding of protein and

changes in the faecal DM content (Connysson *et al.*, 2006; Muhonen *et al.*, 2008). In the current study, no differences in the daily intake of g dCP per 100 kg BW was present between case and control horses. In the study by Connysson *et al.* (2006) where horses were fed either a high amount of g dCP (323 ± 12 g dCP/100 kg BW) in comparison to a recommended g dCP intake (216 ± 8 g dCP/100 kg BW) the former diet resulted in a lower DM content in faeces (faecal DM, 19.5 ± 0.6 % vs. faecal DM, 20.9 ± 0.6 %). However, a direct comparison between the results of changes in the DM of faeces found by Connysson *et al.* (2006) and the results in the current study is not possible as FFL horses have a clear separation of the solid and fluid phase of faeces, while such differences were not reported by Connysson *et al.* (2006). Further, the change in faecal DM found by Connysson *et al.* (2006) could be due to a higher intake of g dCP compared to horses in this study. This theory is consistent with the results reported by Muhonen *et al.* (2008a), where no differences were seen in faecal DM when the protein intake was equal to or lower than 202 g dCP/100 kg BW. Horses with hyperammonemia (HA) have been reported to show watery diarrhoea (Sharkey *et al.*, 2006; Stickle *et al.*, 2006; Desrochers *et al.*, 2003; Peek *et al.*, 1997). A considerable difference between the horses stated with HA in comparison to horses displaying FFL, is that horses with HA were clearly physically affected by the condition, where some horses become very ill (Desrochers *et al.*, 2003; Stickle *et al.*, 2006).

11.4. Experimental design

11.4.1 Strengths and limitations of the case-control study

When the aim of a study is to compare differences in nutrition between case and control horses, a matched case-control study is to prefer, as surrounding factors are similar for the horses in each pair. In this study each pair of case and control were housed in the same stable where management was similar. Each pair of horses were also fed the same forage. Further, this type of study enables data to be collected from a larger group of horses and also provides data directly from reality. However, this type of study may also result in certain sources of error. The owners of the horses in the study were asked to fill in a survey to describe their horse feeding and feeds used for their horses. This means that some uncertainties are inherent, as different respondents may have interpreted the questions differently. Another issue in analysing differences in daily intakes of nutrients among these horses is the information given on actual feed intake. That is, whether the horses consume the actual amount of feed they are supplied with, and/or if the respondents have entered correct values for feed intake. Further, some horses had free access to forage, and for these a standardized value of a daily intake of 3 kg DM per 100 kg BW was calculated according to Jansson *et al.* (2011). However, in some cases horses (especially ponies) can eat as much as 5 kg DM per 100 kg BW (Jansson *et al.*, 2011). It might have been better to exclude horses with free access from this calculation to have a more accurate value of the daily intake of NDF. On the other hand, several horses in the study had free access to forage, and exclusion of these would have resulted in fewer horses in the calculated value. Another source of error might be the daily intake of straw. For example, some horses with access to straw as bedding material can ingest more straw than the reported amount given as a feed. In the question of the daily intake of straw in the survey, it might have been interpreted differently among the participants. For instance, some may have calculated the actual feed intake from straw, whereas others might have estimated a possible daily intake from the bedding material. These above mentioned and potential sources of error could have affected the outcome of the calculations, which in turn could affect differences of ingested nutrients in g per 100 kg BW/day of MJ ME_h, dCP, NDF and kg DM between case

and control horses. Further, these calculations should also be regarded as a rough estimate as the BW of the horses was given by the horse owner and could be both measured using a scale or estimated from body measurements. This is a risk with this type of study, which would be easier to avoid in more controlled studies with the possibility to measure exact intake of nutrients. However, such a study would probably include much fewer horses.

12. Conclusions

The results from the current study showed that case and control horses were fed very similarly. Therefore, the results suggests that feed ration composition and feeding strategies do not play a major role in cases of FFL, as long as the feed rations and feeding strategies are kept within the boundaries presented in this study. The results of this study does however not exclude other nutritional factors as causes for FFL in horses.

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15. Appendix 1

“Haylage intolerance” in horses – a questionnaire survey from the Swedish agriculture university

This survey is aimed for you who have a horse which do not tolerate feeding with wrapped forages and react with a change in faecal characteristics, in particular horses with free faecal liquid in the faeces, in addition to faecal balls. It usually means that the tail and hindlegs of the horse are constantly contaminated with faeces and/or faecal liquid. We try to find out as much as possible about these horses, partly to find out what distinguishes these horses from those who do not react in the same way when fed the same forage, and if possible identify and exclude a number of different factors. The survey is a part of a larger study founded by The Swedish-Norwegian Foundation for Equine Research. The results will be reported on the website: <http://www.slu.se/sv/instutioner/husdjurens-utfodring-varld/> The contact person for this study is Cecilia.Muller@slu.se The answers of the questionnaire are used for research only and will not be handed over to any other part. In the final report all answers will be anonymous and it will not be possible to identify any horse or horse-owner. You start the survey by clicking on the arrow in the right corner. Thank you for your participation!

1. Is your horse a case- or control horse?

- Case horse
- Control horse

2. In which region is your horse stabled?

- Northern Sweden/Norway

- Central of Sweden/Norway
- Southern Sweden/Norway

3. How old is your horse (years)?

4. Which breed is your horse? If crossbred, name the breeds you know

- Arabian horse
- Angloarabian
- Thoroughbred
- Warm-blooded riding horse
- Standardbred horse
- Cold-blooded trotter horse
- North Swedish draught horse
- Ardenneais horse
- Gotland pony
- Shetland pony
- Connemara
- New forest pony
- Welsh pony
- Welsh cob
- Friesian horse
- Haflinger
- Quarter horse
- Paint horse
- Appaloosa
- Tinker horse
- Clydesdale
- Shire horse
- Icelandic horse
- PRE (Pura Raza Española)
- Lusitano
- Riding pony

Crossbreed:

5. Which gender is your horse?

- Mare
- Gelding
- Stallion

6. Is your horse born and bred in Sweden/ Norway?

- Yes
- Don't know
- No; imported from: _____

7. What colour is your horse?

- Grey
- Bay
- Black
- Chestnut
- Paint
- Palomino/Isabelline
- Leopard pattern
- Buckskin
- Cremello
- Other: _____

8. What is the withers height of your horse? Type the answer in cm.

9. What is the weight of your horse? Type the answer in kg.

Help: Proximate weight for different breeds: Shetland pony 100-200kg, Gotland pony 150-250kg, Icelandic horse 250-400, Arabian horse 350-500kg, Thoroughbred 400-600kg, Standardbred 400-600kg, Warm-blood riding horse 450-700kg, Ardenneais horse 700-900kg.

10. My horse is:

- An easy-keeper (Needs less feed than an average horse to keep a sufficiently low body condition)
- A normal-keeper
- A hard-keeper (Needs more feed than an average horse to keep a sufficiently high body condition)

11. The body condition score (BCS) of my horse is at the moment:

- 0
- 1
- 2

- 3
- 4
- 5

12. I think my horse is (Multiple responses possible):

- Calm
- Nervous
- Curious
- Introvert
- Happy
- Tense
- Alert
- Lazy
- Hard working
- Unwilling
- Stressed
- Uninterested
- Active
- Passive
- Irritable
- Angry

13. Which disciplines do you perform with your horse (Multiple responses are possible)?

- Dressage
- Show jumping
- Cross country
- Leisure riding
- Riding school
- Breeding show
- Breeding
- Western
- Working equitation
- Endurance
- Racing
- Trotting
- Pet/ Company
- Academic art of Riding
- Jousting/ Mounted archery
- Natural horsemanship/ Liberty
- Breaking in
- Other: _____

14. Which training intensity is consistent with your horse's training?

Choose one option

- Very low (e.g. maximum 30 min/day, 1-3 times/week, mostly walk)
- Low (e.g. leisure riding, about 30 min/day, 4-7 days/week, all gaits)
- Medium (e.g. riding school, some leisure riding, all gaits)
- High (e.g. medium level eventing, some racehorses, high level jumping, all gaits)
- Very high (racehorses, elite level eventing, endurance competition)
- Breaking in
- Others: _____

15. My horse is kept in the following way during the winter period:

- Individual box at night, paddock with other horses during daytime
- Individual box at night, alone in paddock during daytime
- Individual tied up stall during night, paddock with other horses during daytime
- Housed in tied up stall during night, alone in paddock during daytime
- Group housing during night, paddock with other horses during daytime
- Group housing during night, alone in paddock during daytime
- Loose housing system with other horses
- Kept alone in a loose housing system
- Other: _____

16. For how long is your horse kept outside in a paddock during winter time?

Type your answer in number of hours per day.

17. What type of paddock is your horse kept in during wintertime?

- Grass paddock covered with grass all year around (old grass during winter)
- Sand/gravel
- Soil/clay
- Other type of paddock: _____

18. Which bedding material(s) do you use in your horse box/ stable/ loose housing system?

- Straw
- Shavings
- Sawdust
- Peat
- Paper
- Mix of peat and shavings
- Rubber mat
- Raw sawdust
- Straw pellets
- Sawdust pellets
- Other: _____

19. My horse has access to water in the stable/loose housing system in the following way during winter:

- Frostless waterer
- Frostless tub
- Waterer
- Tub
- Bucket
- Natural water sources
- Other: _____

20. Do your horse have access to a saltlick in the stable/loose housing system?

- Yes
- No
- Yes, and also get extra salt in feed
- Yes, and also get extra salt in water
- No, get extra salt in feed instead
- No, get extra salt in water instead
- Other: _____

21. My horse has access to water in the paddock in the following way during the winter:

- Frostless waterer
- Frostless tub
- Waterer
- Tub
- Bucket
- Natural water source
- Other: _____

22. Is your horse kept on pasture during summer? (Meaning that the horse covers all or part of its nutritional requirements from grass).

- Yes, less than 4 weeks
- Yes 4-8 weeks
- Yes, 8-12 weeks
- Yes, longer than 12 weeks
- No
- Other: _____

23. Which type of pasture is your horse kept at during summer?

- Pasture established on cropland

- Natural pasture
- Forest
- No pasture
- Other type of pasture: _____

24. Do your horse have access to a saltlick while on pasture?

- Yes
- No
- Yes, and also get extra salt in feed
- Yes, and also get extra salt in water
- No, get extra salt in feed instead
- No, get extra salt in special water buckets instead
- My horse is not let out on pasture
- Other: _____

25. My horse has access to water at the pasture in the following ways during summer?

- Frostless waterer
- Frostless tub
- Waterer
- Tub
- Bucket
- Natural water sources
- Other _____

26. Which of following describes your deworming routines?

- The horse is dewormed one or several times/year The horse is dewormed regularly at least once a year
- The horse is dewormed when decided by the owner
- The horse is dewormed when needed based on faecal egg count at least once a year
- The horse is dewormed when needed based on a faecal analysis less than once a year
- The horse is not dewormed due to parasite free pastures
- The horse is not dewormed due to parasite free pastures as it has not been grazed by horses/ donkeys for several years
- The horse is not dewormed, Other: _____

27. When was your horse last dewormed?

- I have never dewormed my horse
- 0-3 months ago
- 3-6 months ago
- 6-12 months ago

- >1 years ago
- Other: _____

28. Which roughage(s) is your horse fed at the moment? Choose one or more options.

- Small bale hay
- Large bale hay
- Loose hay
- Big bale haylage (at least 50% DM)
- Small bale haylage (at least 50% DM)
- Big bale silage (less than 50% DM)
- Small bale silage (less than 50% DM)
- Straw
- Lucerne (pelleted)
- Lucerne (chopped)
- Other: _____

29. Is the forage bought or produced on the farm?

- Bought
- Produced on the farm (but not by the owner)
- Produced on the farm, by the owner
- Other: _____

30. Is the forage analysed for its nutritive contents?

- Yes
- No
- Don't know

31. What is the nutritional content of the forage? Please fill in the values per kg dry matter for the forage that you use at the moment.

- Dry matter (%)
 - _____
 - _____
 - _____
 - _____

- Energy (MJ/kg DM)
 - _____
 - _____
 - _____

-
-
- Digestible protein (g/kg DM)

- Calcium (g/kg DM)

- Phosphorus (g/kg DM)

- Magnesium (g/kg DM)

32. Do you feed your horse any concentrate (s)?

- Yes
- No

33. What type of concentrate do you feed your horse?

- Oats
- Barley
- Molassed sugar beet pulp
- Linseed/linseed cake
- Soybean meal
- Potato protein

- Wheat bran
- Vegetable oil
- No concentrate
- Other (write brand and type): _____

34. Is your horse fed any supplemental feeds? (E.g. mineral feeds, vitamin feeds, herb supplements etc.)

- Yes
- No

35. What type of supplemental feeds do you give your horse?

- Mineral feeds
- Multivitamin feeds
- B-Vitamin feeds
- Selenium+ Vitamin E additive
- Garlic
- Herbs
- Do not feed any supplemental feeds
- Other (specify brand and type): _____

36. Which amounts (g or kg) of feed is your horse fed per day?

Write 0 in the box if your horse is not fed that type of feed. If your horse is fed several types of feeds in the same category, write type of feed and specific amount for each type of feed e.g. “3kg hay and 5kg haylage”.

Forage (including hay, haylage, silage) (kg/day)

Concentrates (kg/day)

Straw (kg/day)

Lucerne (kg/day)

Additional feeds (g/day)

37. How many times per day is your horse fed roughage?

- 0 times
- 1 times

- 2 times
- 3 times
- 4 times
- >4 times
- Free access

38. How many times per day is your horse fed concentrate?

- 0 times
- 1 times
- 2 times
- 3 times
- 4 times
- >4 times
- Free access

39. How many hours is it at the most between two feedings of roughage?

- 0-2 hours
- 2-4 hours
- 4-8 hours
- 8-12 hours
- >12 hours
- Free access
- Don't know

40. How is the forage fed in the paddock?

- Forage is not fed in the paddock
- On the ground
- In a feeding rack
- In a hay net
- In a tub or similar
- Other way: _____

41. How do you store your forage? (If you feed your horse wrapped forage the question concern opened bales)

- Indoors (stall, barn or similar)
- Outdoors (under roof)
- Outdoors (no roof)
- Other: _____

42. How do you store your concentrate feeds?

- In covered/closed container indoors
- In uncovered/open container indoors
- In paper bags/original package indoors
- Do not feed concentrate
- Other: _____

43. Has your horse showed loose faeces when fed wrapped forages?

- Yes- generally loose faeces which looks like “cowpat”
- Yes, solid faecal balls but also free faecal liquid
- Yes, diarrhea without solid faecal balls
- No
- Other: _____

44. If your horse has shown loose faeces when fed wrapped forages, has it become better or good when:

- Changing from wrapped forages to hay
- Changing from one batch of grass haylage to another batch of grass haylage
- Change from primary harvest to regrowth harvest
- Change from wrapped forages to pasture
- No improvement with any tried change
- Worsened condition with any tried change
- My horse have never had any problems with loose faeces when fed wrapped forages,
- Other: _____

45. If your horse has shown loose faeces when fed wrapped forages, have other horses in your stable fed the same forage also shown loose faeces?

- No- only my horse
- Yes- several horses
- My horse have never had any problem with loose faeces when fed wrapped forages
- Don't know
- If “yes”, write the number of horses (e.g. 2 out of 10): _____

46. Have your horse shown loose faeces when fed hay?

- Yes- generally loose faeces who looks like “cow pat”
- Yes- solid faecal balls but also free faecal liquid
- Yes- diarrhoea without solid faecal balls
- No

- Other: _____

47. Has your horse shown any of the following signs during an episode of loose faeces and feeding of conserved forages and/or hay? Choose one or more options

- My horse have never showed loose faeces when fed wrapped forages or hay
- Colic
- Skin problems (e.g. lumps or urticaria)
- Swollen legs not caused by training or injury
- Bloated abdomen
- Irritation while voiding faeces (swishing tail and/ or trampling with hind legs)
- None of the options
- Other: _____

48. Do your horse have a history of previous history of colic?

- Yes
- No
- Don't know

49. Have your horse been examined and diagnosed with gastric ulcers by a veterinarian?

- Yes, my horse has been examined and has been diagnosed with gastric ulcers
- Yes, my horse has been examined but has not been diagnosed with gastric ulcers
- No-not examined
- Don't know

50. Have your horse been treated by a veterinarian for any other diseases/ conditions in the gastro-intestinal tract?

- No
- Don't know
- Yes, my horse has been treated for: _____

51. Do your horse show any of following behaviour:

- Crib-biting
- Wind sucking
- Weaving
- Box walking (walk around in the box in a repeated pattern)
- Selfbiting (Bites itself on the sides/ flanks)
- Wood chewing (e.g. stable interior, fence)
- Tongue rolling ("chews on the tongue" in a repeated pattern, e.g. before feeding)

- My horse do not show any of behaviours listed
- Other: _____

52. Do you have any one thoughts of what you believe is the cause of free faecal liquid in your horse?

53. Is there something else you think we need to know about the horse? E.g. if it has a normal chewing function, is treated with pharmaceutical etc.
