Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2020:65
Faculty of Veterinary Medicine and Animal Science

Free faecal liquid in horses

Faecal composition and associations with feeding and management

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SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

DOCTORAL THESIS

Uppsala 2021

Acta Universitatis agriculturae Sueciae
2020:65

Cover: A pile of horse faeces in pasture grass and a horse grazing in the background (photo: Carin Wrange).

ISSN 1652-6880
ISBN (print version) 978-91-7760-648-2
ISBN (electronic version) 978-91-7760-649-9

Uppsala
Print: SLU Service/Repro, Uppsala 2021

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Abstract

Free faecal liquid (FFL) is a condition where horses show two-phase characteristics of faeces, with one solid phase (faecal balls) and one liquid phase (only free liquid). Causes of this condition are unknown. The aim of this thesis was to characterize horses with FFL, investigate feeding and management factors suggested to be associated with presence of FFL, and to compare faecal composition in horses with (case) and without (control) FFL. Characterisation of horses with FFL showed that all types (*e.g*. breeds, ages, disciplines performed) of horses could be affected. No specific feeding or management factors were overrepresented among FFL horses. Changes in forage feeding were reported to result in reduced signs of FFL. A comparably high incidence of previous colic was reported in horses showing FFL. Faecal bacterial composition was in general similar, but some specific low abundant bacterial taxa differed between case and control horses. Case horses had a lower concentration and proportion of lactic acid, and lower water holding capacity of faecal compounds, compared with controls. Case horses were reported to be fed lower average amounts of straw, and a higher proportion and amount of concentrates in the diet, compared with controls. Case horses were also reported to have lower daily intake of digestible crude protein and neutral detergent fibre and higher daily intake of starch and water soluble carbohydrates compared with control horses. Overall, this thesis shows that feeding and management may be important for the occurrence of FFL. Further studies are required to establish the clinical relevance of low-abundant taxa in faeces and the impact of specific feed components on FFL.

Keywords: Bacteria, diarrhoea, equine, faeces, free faecal water syndrome, management, microbiota, nutrition

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Fri fekal vätska hos hästar

Sammanfattning

Fri fekal vätska (FFV) är ett tillstånd där hästar uppvisar tvåfasindelad träck med en fast fas (typiska träckbollar) och en vätskefas (endast fri vätska). Orsakerna till FFV är inte kända. Syftet med denna avhandling var därför att karaktärisera hästar med FFV, undersöka utfodrings- och skötselfaktorer, samt att jämföra träckens sammansättning hos hästar med (fall) och utan (kontroll) FFV. Karaktärisering av hästar med FFV visade att alla typer av hästar (raser, åldrar, användningsområden etc.) kunde vara drabbade. Inga specifika utfodrings- eller skötselfaktorer var överrepresenterade hos hästar med FFV. Förändringar i vallfoderutfodring rapporterades resultera i minskad förekomst av FFV. En jämförelsevis hög förekomst av tidigare kolik rapporterades hos hästar med FFV. Fekal bakteriesammansättning hos fall- och kontrollhästar var generellt lika, men vissa specifika bakterietaxa med låg förekomst skiljde sig åt i relativ förekomst mellan fall och kontrollhästar. Fallhästarna hade en lägre koncentration och andel mjölksyra och en lägre vattenhållande förmåga i träckens komponenter jämfört med kontrollhästarna. Fallhästarna utfodrades med lägre genomsnittlig mängd halm och högre andel och mängd kraftfoder i foderstaten jämfört med kontrollhästarna. Fallhästar hade ett lägre dagligt intag av smältbart råprotein och fibrer, men ett högre dagligt intag av stärkelse och lättlösliga kolhydrater jämfört med kontrollhästarna. Sammanfattningsvis visade resultaten att utfodring och skötsel kan vara av betydelse för förekomsten av FFV. Ytterligare studier krävs för att fastställa den kliniska relevansen av de bakterietaxa som återfanns i låg förekomst hos hästar med FFV, liksom inverkan av specifika foderkomponenter på FFV.

Nyckelord: Diarré, fritt fekalt vatten syndrom, häst, mikrobiota, nutrition, skötsel, träck

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Dedication

Till mamma, tack för allt ♡

Contents

List of publications

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

I. Lindroth, K.M., Johansen, A., Båverud, V., Dicksved, J., Lindberg, J.E. & Müller, C.E. (2020). Differential defecation of solid and liquid phases in horses – descriptive survey. *Animals* 10 (1), 76.

II. Lindroth, K.M., Dicksved, J., Pelve, E. & Müller, C.E. Faecal bacterial composition in horses with and without free faecal liquid – a case control study (submitted).

III. Lindroth, K.M., Dicksved, J., Vervuert, I. & Müller, C.E. Chemical composition and physical characteristics in faeces from horses with and without free faecal liquid – two case-control studies (manuscript).

IV. Lindroth, K.M., Johansen, A., Lindberg, J.E. & Müller, C.E. Feeding and management of horses with and without free faecal liquid – a case-control study (manuscript).

Paper I is reproduced with the permission of the publisher.

The contribution of Katrin Lindroth to the papers included in this thesis was as follows:

I. Was responsible for data collection and management during the experiment. Analysed the data and performed the statistical analyses. Performed data management, interpreted the data and wrote the manuscript together with the co-authors. Corresponded with the journal and revised the article under supervision.

II. Was responsible for organisation and sampling management during the experiment. Performed the practical preparations for analysis, carried out the DNA extraction. Analysed the data and performed the statistical analyses. Performed data management, bioinformatics and biostatistical analyses, and interpreted the data together with the co-authors. Wrote the manuscript with regular input from the co-authors. Corresponded with the journal and revised the article under supervision.

III. Was responsible for organisation and sampling management during the experiment. Performed the practical preparations for analysis. Analysed the data and performed the statistical analyses. Performed data management and interpreted the data together with the co-authors. Wrote the manuscript with regular input from the co-authors.

IV. Was responsible for organisation and sampling management during the experiment. Performed the practical preparations for analysis. Analysed the data and performed the statistical analyses. Performed data management and interpreted the data together with the co-authors. Wrote the manuscript with regular input from the co-authors.

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Abbreviations and definitions

16SrRNA	Component of the small subunit of the prokaryotic ribosomal ribonucleic acid with size unit 16S
α- diversity	A calculated metric that account for the number and evenness of Genera, Family, OTU, etc. within a sample
β- diversity	A calculated metric that account for the number and evenness of Genera, Families, OTU, etc. between samples
ADF	Acid detergent fibre
Bacterial taxonomy	Domain – phylum – class – order – family – genus – species – subspecies
BCS	Body condition score
BW	Body weight
(C2+C4)/C3	Ratio between sum of acetic (C2) and butyric acid (C4) to propionic acid (C3)
CDS	Chronic diarrhoea syndrome
CP	Crude protein
dCP	Digestible crude protein
DM	Dry matter
Dysbiosis	Disruption of the microbial community in the gastrointestinal tract or faeces
FAS	Faecal appearance score

FFL	Free faecal liquid
FFW	Free faecal water
FFWS	Free faecal water syndrome
FMT	Faecal microbiota transplantation
GIT	Gastrointestinal tract
IBD	Inflammatory bowel disease
LAB	Lactic acid bacteria
ME	Metabolisable energy
ME_h	Metabolisable energy for horses
Microbiota	Groups of microorganisms (bacteria, yeasts, viruses, fungi *etc*.) that share a common living space.
NDF	Neutral detergent fibre
NSC	Non structural carbohydrates
OM	Organic matter
OTU	Operational Taxonomic Unit
rRNA	Ribosomal ribonucleic acid
Richness	Quantifies how many types (of Genera, families, OTUs…) the dataset contain
SCFA	Short chain fatty acids (including acetic, propionic, butyric, valeric and lactic acid)
VFA	Volatile fatty acids (including acetic, propionic, butyric and valeric acid)
WSC	Water soluble carbohydrates

1. Introduction

1.1 Free faecal liquid

Free faecal liquid (FFL), also referred to in the literature as free faecal water (FFW) or free faecal water syndrome (FFWS), is a condition where horses show two-phase characteristics of faeces, with one solid phase (typical faecal balls) and one liquid phase (only free liquid). The liquid phase may be voided immediately before, during or after, or on a separate occasion from, defecation of the solid phase (Kienzle *et al*., 2016; Valle *et al*., 2013), sometimes together with excess gas. Affected horses may show discomfort when voiding faeces or only the liquid phase, by extensive tail swishing and nervous trampling with the hindlegs (Kienzle *et al*., 2016). The welfare of affected horses may be compromised, as it has been reported that in severe cases there is constant presence of faecal liquid around the anus and on the inside of the hindlegs (Figure 1). This and frequent cleaning may result in skin lesions and dermatitis (Ertelt & Gehlen, 2015). Frequent cleaning to a satisfactory standard is required according to the Swedish Board of Agriculture regulations and general guidelines for horse keeping (SJVFVS 2018: 49) (SJV, 2018).

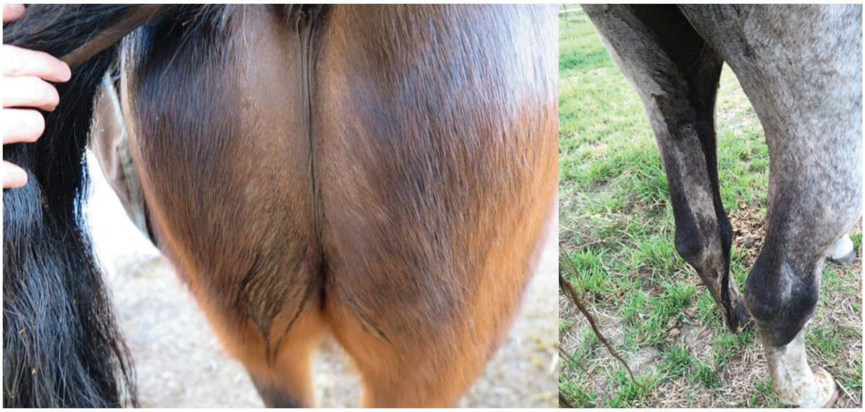

Figure 1. Free faecal liquid below the anus and on the inside of the hindlegs in affected horses with (left) a mild case (photo: C.E. Müller) and (right) a more severe case (photo: D. Ross) of free faecal liquid.

Information on FFL is scarce and there are only a few publications and case reports available in the literature. The condition has been described in horses in Germany (Kienzle *et al*., 2016; Ertelt & Gehlen, 2015; Zehnder, 2009), Italy (Valle *et al*., 2013) and Switzerland (Schoster *et al*., 2020). However, there is no information on causes or prevalence of FFL or whether it is associated with other conditions in horses. Presence of FFL continuously throughout the year has been reported (Zehnder, 2009), as has intermittent occurrence, and the duration of an FFL episode can vary from days to weeks, months or several years (Kienzle *et al*., 2016; Valle *et al*., 2013; Zehnder, 2009).

Clinical examination of horses with FFL symptoms have not revealed any signs of apparent dehydration, pyrexia, weight loss (Kienzle *et al*., 2016; Valle *et al*., 2013) or presence of equine gastric ulcer syndrome (Schoster *et al*., 2020). Serum protein profile, consisting of total protein, albumin, α-1 globulin, α-2-globulin, β-1 globulin, β-2 globulin and γ-globulin, is reported to be similar in horses with FFL and matched control horses, indicating no signs of ongoing inflammation in FFL horses (Kienzle *et al*., 2016). In a limited German study, examination of faeces samples for endoparasites yielded similar results for horses with and without FFL (Zehnder, 2009). Therefore, other factors are suggested to cause FFL, including both intrinsic and external factors.

2. Background

2.1 Diarrhoea in horses

Normal equine faeces take the form of semi-solid faecal balls, while hard dry faecal balls and soft "cowpat" faeces with watery consistency are regarded as representing gastrointestinal disturbances in horses (Mair, 2002). Diarrhoea is usually defined as increased defecation frequency of faeces containing higher content of water and lower content of dry matter compared with normally observed in healthy horses (Oliver & Staempfli, 2006; Hillyer, 2004; Thomas *et al*., 2003; Radostits *et al*., 2000). The nature of diarrhoea can be acute or chronic. Acute diarrhoea is defined as clinical signs of diarrhoea presenting for less than seven days, while chronic diarrhoea persists for at least 7-14 days (Mair, 2002). Underlying pathological causes of acute diarrhoea are mostly colonic microbiota disturbances, resulting in pathogen overgrowth and gastrointestinal motility alterations, intestinal fluid losses and electrolyte and acid-base imbalances (Chapman, 2009; McGorum & Pirie, 2009; Båverud, 2004). Depending on the initial cause of chronic diarrhoea, the condition can be present for several weeks to months, often intermittently with relapses with varying faecal texture from "cowpat" consistency to watery diarrhoea (Valle *et al*., 2013; Mair, 2002). Chronic diarrhoea is suggested to be associated with large intestine (caecum and colon) disease caused by physical damage to the caecal or colonic wall or physiological disturbances in caecal or colonic function. However, causes of chronic diarrhoea in horses are poorly investigated, mainly due to historic lack of appropriate diagnostic tools (Mair *et al*., 2013; Mair, 2002; Staempfli *et al*., 1993). A definitive diagnosis of the cause of chronic diarrhoea is reported to be achieved in only 60-70% of cases, and in many of these it only becomes apparent at *post-mortem* examination (Mair, 2002).

Diarrhoea can be present in horses suffering from stomach ulceration, colitis or impaction of the large intestine (Clarke *et al*., 1990). Cases of chronic watery diarrhoea without any other clinical signs indicative of disease are referred to as chronic diarrhoea syndrome (CDS) in the literature (Manahan, 1970). During episodes of CDS, horses show a sudden onset of watery diarrhoea, with little or no rise in rectal temperature (Manahan, 1970). In addition, faeces are voided at frequent intervals with varying characteristics and texture (Manahan, 1970). There are many similarities between descriptions of CDS and FFL, but also differences, as horses with CDS are reported to show dehydration and weight loss (Manahan, 1970), which were not present in horses with FFL (Kienzle *et al.,* 2016; Valle *et al.,* 2013).

2.2 Causes of diarrhoea

Although FFL and diarrhoea do not represent exactly the same condition, the similarity of the conditions makes it interesting to review briefly the factors causing diarrhoea, as they may also be implicated in FFL. Common noninflammatory causes of diarrhoea are often related to alteration of microbial function in the hindgut (microbial dysbiosis), or physiological disturbances of hindgut function such as motility abnormalities in the caecum and large colon of the horse (Mair *et al*., 2013; Moreau, 2011; Naylor & Dunkel, 2009). Inflammatory causes of chronic diarrhoea could be due to a variety of mechanisms, including mucosal damage, increased secretion across the gastrointestinal wall and osmotic overload of the luminal contents (Naylor & Dunkel, 2009).

2.2.1 Microbial dysbiosis

A functional gastrointestinal microbiota is crucial for maintaining equine health and welfare. Microbial dysbiosis (defined as disruption of the microbial community in the gastrointestinal tract) has been demonstrated in faeces from horses with diarrhoea (McKinney *et al*., 2020; Rodriguez *et al*., 2015; Costa *et al*., 2012), colitis, hindgut acidosis (Costa *et al*., 2012) and *post-partum* colic (Weese *et al*., 2015) compared with healthy controls. Colitis, hindgut acidosis and colic may include diarrhoea as one of several symptoms. Horses with diarrhoea are reported to have lower faecal microbial biodiversity, including both lower bacterial richness (number of different species) and evenness (distribution of species) compared with healthy control horses (McKinney *et al*., 2020; Rodriguez *et al*., 2015; Costa *et al*., 2012). The relative abundance of Bacteroidetes was higher and it was the most abundant phylum, followed by Firmicutes, in diarrhoeic horses, while Firmicutes is the most abundant phylum in healthy horses (Costa *et al.*, 2012). Lower variation in faecal microbiota composition (beta diversity) and increased abundance of Fusobacteria have been found in horses with diarrhoea compared with non-diarrhoeic horses (McKinney *et al*., 2020; Costa *et al*., 2012). Increased relative abundance of *Fusobacterium* spp. and *Lactobacillus* spp. has also been found in horses with colitis, compared with horses without colitis, in studies investigating microbiota composition in caecal and colonic content and in intestinal mucosal tissue (Arroyo *et al*., 2020). In the same study, microbial dysbiosis was found at all different sites of the intestinal tract.

2.2.2 Motility disturbances

Motility abnormalities have been identified in horses with chronic diarrhoea and FFL, without other physiological disturbances of colonic function (Valle *et al*., 2013). Increased intestinal motility may enhance the severity of diarrhoea through increased passage rate of the digesta through the hindgut (Mair, 2002). Motility disturbances have also been reported in horses suffering from inflammatory bowel disease (IBD), in which diarrhoea is one of the major clinical signs (Mair, 2002; Clarke *et al*., 1990). Increased propulsive activity in the colon has been shown in horses with colitis, leading to shortened intestinal transit times and increased frequency of defecation (Mair *et al*., 2013). When increased motility is combined with passive and active fluid secretion into the intestinal lumen, this may result in increased faecal fluid content and output associated with increased faecal volume and frequency of defecation. The primary stimuli for hypermotility are inflammatory by nature and consist of chemical, mechanical and functional signals related to injury or dysfunction of the gastrointestinal mucosa following infection or irritation. The resulting increase in propulsion and decrease in digesta passage rate are primarily mediated by the enteric nervous system (Hansen, 2015; Jones & Blikslager, 2002).

2.2.3 Increased osmolality

Fluid loss into the gut can occur through increased osmotic concentration in the intestinal lumen (Mair *et al*., 2013; Naylor & Dunkel, 2009). In osmotic diarrhoea, the osmotic force of specific particles or substances pulls water and ions into the intestinal lumen. When short-chain fatty acids (SCFA) are produced in quantities exceeding the capacity for their absorption in the colon, they are accumulated, which may result in osmotic diarrhoea (Zeyner *et al*., 2004). Increased production of volatile fatty acids (VFA) has been reported in faeces from horses with chronic diarrhoea (Reed *et al*., 2004; Minder *et al*., 1980), indicating for a possible cause of excess faecal water. If the microbial community in the large intestine is not well-functioning, the proportions of SCFA may be changed. This may also influence water resorption in the gut, as SCFA are involved in both sodium and water uptake in the large intestine. In particular, accumulation of lactic acid may be involved, as it attracts water, which may lead to osmotic diarrhoea (Bailey *et al*., 2002; Jones & Spier, 1998).

2.2.4 Inflammatory bowel disease

Equine inflammatory bowel disease (IBD) has been discussed as a possible cause of the clinical symptoms shown in horses with FFL. Horses with IBD often show colic, weight loss and/or diarrhoea, along with less common symptoms such as anaemia, subcutaneous oedema and dermatitis (Olofsson, 2016). The underlying causes of IBD are unknown, but an excessively active immune system expressed as a delayed hypersensitivity reaction (with dominant T-cells) is suspected to play a major role in this condition (Olofsson, 2016). The term IBD includes several different main types, based on their clinical symptoms and pathological changes (Olofsson, 2016; Schumacher *et al*., 2000). Weight loss has been shown in 95-100%, diarrhoea in 30-63% and colic in 4-20% of clinically examined horses with IBD (Schumacher *et al*., 2000). In another study, 78% of horses with IBD were found to show weight loss without having a reduced appetite (Boshuizen *et al*., 2018). Weight loss has not been reported in horses with FFL to date.

2.2.5 Pathogens and toxins

Infections with *Clostridium perfringens* and *Clostridioides difficile* (Chapman, 2009; Båverud, 2004) are commonly implicated in equine diarrhoeic syndromes (Schoster *et al*., 2012; Weese *et al*., 2006; Hillyer, 2004; Wilson, 2001; Rolfe, 2000; Songer, 1996; Wierup, 1977). Infection with *C. difficile* is considered to be one of the most important causes of enterocolitis in horses (Weese *et al*., 2006; Magdesian *et al*., 2002; Weese *et al*., 1999; Båverud *et al*., 1998; Teale & Naylor, 1998; Jones *et al*., 1998; Båverud *et al*., 1997), where clinical signs including diarrhoea, pyrexia, mild colic and dehydration are common (Weese *et al*., 2006). The bacterial species *C. perfringens* is a common member of the enteric flora of healthy horses, with disease associated with increased numbers of the bacteria (Schoster *et al*., 2012; Songer, 1996). The pathogenic properties of the two species depend on the production of bacterial toxins, with *C. difficile* producing primarily toxin A and B and *C. perfringens* producing more than 15 different enterotoxins (Songer, 1996). Type A and B toxins produced have been shown to cause intestinal fluid secretion and inflammation. The enterotoxins produced by *C. perfringens* impair fluid and electrolyte absorption followed by increasing intestinal inflammation and damage to the epithelium, leading to development of secretory diarrhoea (Songer, 1996).

Abnormal faecal characteristics (soft, cow-pie or loose texture) and diarrhoea (acute or chronic) has also been reported as a common clinical sign in horses with enteric disease caused by infections with equine coronavirus (ECoV) and *Salmonella* spp. (Manship *et al*., 2019), but chronic parasitism (c*yathostomosis*, large strongyle infection and other enteric parasites) (Peregrine *et al*., 2014; Love *et al*., 1999; Owen & Slocombe, 1985; Drudge, 1979) and equine proliferative enteropathy (due to *Lawsonia intracellularis* infection) (Frazer, 2008; Lavoie *et al*., 2000) have also been associated with presence of chronic diarrhoea. While *Salmonella* spp. is a well-established cause of pyrexia and enteric disease, equine coronavirus has emerged quite recently as an agent isolated in association with outbreaks of fever and enteric disease in adult horses (Fielding *et al*., 2015; Oue *et al*., 2013; Oue *et al*., 2011). Common clinical signs of ECoV and enteric *Salmonellosis* include anorexia and colic in addition to pyrexia and diarrhoea (Manship *et al*., 2019; Kim *et al*., 2001; Traub-Dargatz *et al*., 1990), which has also been reported in horses infected with intestinal parasites and *L. intracellularis* (Peregrine *et al*., 2014; Lavoie *et al*., 2000). These clinical signs have to date not been reported in horses with FFL.

2.2.6 Sand ingestion

Ingestion of sand may cause mucosal irritation in the intestinal lumen, potentially leading to mechanical obstruction and motility disorder, as well as mucosal inflammation and damage (Mair, 2002; Clarke *et al*., 1990; Colahan, 1987; McIntyre, 1917). Accumulation of ingested sand, resulting in constant irritation of the intestinal lumen, is reported to reduce the absorptive surface area of the intestine in horses, leading to chronic diarrhoea (Bertone *et al*., 1988; Ramey & Reinerston, 1984), including loose and watery faeces (Niinistö *et al*., 2019), colic (Kilcoyne *et al*., 2017) and colitis (Hillyer, 2002). Ingestion of sand has been associated with specific feeding and management practices, as horses may accidently ingest sand mixed with feed when fed on the ground in sand or gravel paddocks (Hansson, 2002; Weise & Lieb, 2001) or when grazing pasture on sandy soils (Hansson, 2002).

2.3 Factors suggested to be associated with presence of free faecal liquid

Although the number of studies on FFL in horses is scarce and the aetiology of FFL is unknown, several factors have been suggested to cause FFL. These factors include both external nutrition- and management-related factors and intrinsic horse-related factors.

2.3.1 Nutritional factors

Nutrition-related factors suggested to cause FFL include high amounts of lucerne (*Medicago sativa*) in the diet, diets rich in concentrates with a high concentration of non structural carbohydrates and drinking cold water (Gerstner & Liesegang, 2018; Kienzle *et al*., 2016; Valle *et al*., 2013; Zehnder, 2009). Feeding wrapped forages (such as silage[1] and haylage[2]) instead of hay has also been suggested to cause FFL (Kienzle *et al*., 2016), and the term "haylage intolerance" is used by horse owners and veterinarians. However, no systematic investigations of associations between presence of FFL and feeding wrapped forages, high amounts of alfalfa, concentrate-rich diets or drinking cold water have been performed.

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[1]Dry matter content <500 g per kg.

[2]Dry matter content >500–840 g per kg.

Horses with and without FFL were investigated in a study where the aim was to identify feeding- and management-related factors associated with FFL (Kienzle *et al*., 2016). No nutritional factors were found to be associated with presence of FFL. The majority of horses were fed concentrates (mean, 1.5 kg/d; range, 0–4 kg; dry matter, ~ 86%) and all horses with FFL had been fed grass hay as their main forage the whole year, with only a few of these horses (7%, n=3) being fed silage or haylage in addition to hay during winter.

Diets with a high inclusion of concentrates (4.55 kg oats fed every 12 hours) have however been reported to result in changed characteristics of digesta and faeces (Lopes *et al*., 2004). Two-phase separation (one liquid and one solid phase) of both digesta and faeces was observed in that study when horses were fed hay with inclusion of oats, but when the horses were fed hay only, faecal balls were well-formed and with no separation in liquid and solid phases (Lopes *et al*., 2004). Faeces of horses fed hay and oats were less formed and the liquid phase had noticeable gas bubbles and was more viscous compared with faeces of horses fed hay only (Lopes *et al*., 2004).

In a case study of a 12-year-old horse in Italy with a history of chronic diarrhoea and persistent FFL for the previous four years (Valle *et al*., 2013), diet changes were made in attempts to resolve the condition. Rebalancing the diet to be consistent with the theoretical nutritional requirements of the horse, replacing half the daily forage (long-stemmed hay) with ground and pelleted meadow hay, avoiding an excess of water soluble carbohydrates (WSC) from both forage and concentrate and increasing the number of daily feedings from four to six resulted in absence of FFL within a few days (Valle *et al*., 2013). However, in addition to the nutritional changes, the horse in question was treated for 15 days with sulfasalazine (an anti-rheumatic drug used in human medicine (FASS, 2019)), making it difficult to evaluate which of the interventions resolved the condition. The same horse had relapses of FFL in conjunction with increasing the proportion of long-stemmed hay and decreasing the amount of ground and pelleted hay, and when a change in forage batch was performed (Valle *et al*., 2013). Both relapses were resolved by dietary changes and treatment with sulfasalazine, as in the initial FFL episode (Valle *et al*., 2013).

2.3.2 Management factors

Inadequate parasite control (resulting in high intestinal parasitic load in horses) is suggested to be a management-related factor involved in the aetiology of FFL. However, similar anthelminthic routines have been reported for horses with and without FFL (Kienzle *et al*., 2016). In addition, parasitological examination for detection of helminth eggs and larvae in faeces samples in that study showed no difference between horses with and without FFL for small strongylids (*Cyathostomins* spp.) or tapeworm (*Anoplocephala perfoliata*). Similar results were reported in a recent study (Schoster *et al*., 2020), where similar faecal egg counts were found in horses with and without FFL.

Dental problems, resulting in impaired mastication of feed, have also been suggested as a cause of FFL. However, clinical dental examinations of FFL-affected horses have not shown any abnormalities in the oral cavity in teeth or in function of mastication (Valle *et al*., 2013; Zehnder, 2009). In addition, the oral cavity was examined at similar frequencies in horses with and without FFL (Zehnder, 2009).

The effect of stress on gut peristalsis is well-documented in several animal species, and horses are known to increase their frequency and fluidity of defecation in response to stressful situations (Goymann & Wingfield, 2004). In the case study on a FFL-affected horse in Italy by Valle *et al*. (2013), FFL was reported to reoccur during stressful events such as changes in stable management (related to a new stable manager and new routines). In the German study by Kienzle *et al*. (2016), horses with FFL were reported to be more than four times more likely not to defend their feed against other horses compared with horses without FFL. That study also found that the risk of FFL was 17-fold higher in horses considered to have a low social rank position compared with horses with a high position (Kienzle *et al*., 2016). The social hierarchy of horses depends on the group composition, the number of horses kept together and the availability of resources (*e.g.* feed access) (Goymann & Wingfield, 2004). Related management factors may therefore be implicated in the presence FFL.

2.3.3 Intrinsic factors

Intrinsic factors, such as gender, breed and coat colour, have been suggested as factors of importance for the presence of FFL in horses, while age is reported not to differ between horses with and without FFL (Kienzle *et al*., 2016). Breed, gender and coat colour were investigated as risk factors for FFL by Kienzle *et al*. (2016), who found that geldings had a three-fold higher risk of displaying FFL than mares in a limited population of horses. The study also found that paint horses were at four times higher risk of exhibiting FFL than horses with other coat colours (Kienzle *et al*., 2016). It is known that grey horses may show gastrointestinal disturbances, especially at higher age, due to presence of melanoma tumours within the gastrointestinal tract (Hofmanová *et al*., 2015), but no such association has been reported in paint horses.

Another intrinsic factor suggested to be involved in the aetiology of FFL is microbial hindgut dysbiosis (Schoster *et al*., 2020; Kienzle *et al*., 2016). Microbiota composition in faeces of horses with and without FFL has been investigated in a case-control study in Switzerland (Schoster *et al*., 2020), where horses were sampled on two occasions and faecal microbiota composition was analysed using high-throughput sequencing. The results showed similar general composition and distribution of the bacterial community in case and control horses, but relative abundance of specific bacterial taxa (*e.g. Verrumicrobia*, *Clostridia*, *Treponema, Treponema 2*, *Ruminococcus*) differed between case and control horses within different sampling occasions (Schoster *et al*., 2020).

2.4 Impact of different feeds on gastrointestinal health and faecal composition

As several nutritional factors are suggested causes of FFL, it is of interest to review briefly current knowledge on the impact of different feeds and feeding strategies on digestion, gastrointestinal health and faecal composition in horses. Horses are adapted to a grazing and browsing existence where they select forage plants containing large amounts of water and structural carbohydrates. Compared with forages, grains and many concentrates contain higher concentrations of non structural carbohydrates (NSC) such as starch, which horses have limited capacity to digest in the small intestine, and which may cause hindgut disturbances (Hintz & Cymbaluk, 1994).

2.4.1 Feeding of wrapped forages and hay

Forage conservation method may result in biochemical and microbial differences between hay, haylage and silage, such as higher concentration of lactic acid, VFA and lactic acid bacteria (LAB) and lower pH in silage compared with haylage and hay (Müller *et al*., 2008). However, in a study where all forage types were harvested from the same ley and at the same stage of plant maturity, these differences produced similar responses in microbial and biochemical composition in the equine hindgut and faeces in horses fed the forages (Muhonen *et al*., 2009; Müller *et al*., 2008). Similarly, Miyaji *et al*. (2008) found that total faecal VFA concentration and apparent digestibility of dry matter (DM), organic matter (OM), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were similar for hay and silage.

2.4.2 Feed ration composition

Forage-only diets are reported to result in more stable faecal microbial communities with lower relative abundance of LAB compared with diets with inclusion of concentrates (35 % starch) (Dougal *et al*., 2014; Willing *et al*., 2009). Inclusion of concentrates in the diet (3-3.5 kg/d) has been shown to result in a decreased proportion of fibrolytic bacteria belonging to the bacterial genus *Fibrobacter* spp. and the *Ruminococcaceae* family, while members of the *Bacillus–Lactobacillus–Streptococcus* group increased in abundance, compared to a forage-based diet in colon of horses (Daly *et al*., 2012). A traditional forage-concentrate diet resulted in higher relative abundance of bacteria belonging to *Clostridiaceae* family and presence of *Lactobacillus ruminis* in equine faeces, in comparison to a forage-only diet (Willing *et al*., 2009). The type of bacteria present also affect the proportions of fermentation products, where acetate is the dominant VFA produced by equine gut microbes (Hussein *et al*., 2004; Argenzio *et al*., 1974a; Hintz *et al*., 1971). Diet composition (among other factors) influence faecal SCFA concentrations, as well as the ratio between the sum of acetate and butyrate over propionate (C2+C4)/C3), where the latter is generally lower with higher starch content in the diet (Swyers *et al*., 2008; Hussein *et al*., 2004). These variables are therefore of interest when studying equine GIT health and function.

2.4.3 Specific feed components

Feeding starch-rich feeds (*e.g.* grains and commercial mixtures containing ≥ 300 g starch per kg DM) is a well-known risk factor for increased production of lactic acid in the large intestine (de Fombelle *et al*., 2003; Julliand *et al*., 2001). High amounts of starch, >0.4% of BW per meal (Potter *et al*., 1992;

Roberts, 1975), are not well digested in the small intestine of the horse due to comparatively low amylase secretion and fast passage of digesta (de Fombelle *et al*., 2003; Kienzle *et al*., 1997). Starch may therefore escape enzymatic digestion in the small intestine and enter the large intestine, where it is rapidly fermented. This rapid fermentation results in increased lactic acid production and lowering of the pH (Hintz *et al*., 1971). An acidic environment favours rapid proliferation of lactic acid-producing bacteria, resulting in further enhanced lactic acid production and a further decline in pH, which causes hindgut acidosis, laminitis, colic and stomach ulcers (Al Jassim & Andrews, 2009; Julliand *et al*., 2001). High abundance of LAB in the equine hindgut has been reported in experiments in which laminitis was induced using high oral doses of oligofructose resulting in hindgut dysfunction (Milinovich *et al*., 2008). A high inclusion of concentrates in the diet (2.5-5 kg/ day) is also known to be associated with gastrointestinal dysfunctions such as hindgut acidosis, colic and diarrhoea (Kaya *et al*., 2009; Cohen *et al*., 1999; Tinker *et al*., 1997, 1994; Reeves *et al*., 1996; Cohen *et al*., 1995; Garner *et al*., 1978: Garner *et al*., 1977; Roberts, 1975; Hintz *et al*., 1971). Even comparatively low amounts of concentrates (2.5-5 kg fed daily) are reported to increase the risk of gastrointestinal disturbances such as colic, compared with feeding less than 2.5 kg concentrates daily (Hudson *et al*., 2001; Tinker *et al*., 1997, 1994).

2.5 Treatment of horses with free faecal liquid

Although causes of FFL are unknown, various treatments have been evaluated or suggested to reduce or eliminate the condition. Pro- and prebiotics have been used with moderate, transient or no success to reduce the incidence of FFL (Kienzle *et al*., 2016). In a case study in Italy by Valle *et al*. (2013), it was found that using prebiotics such as fructo-oligosaccharides or inulin and probiotics including several unspecified strains of *Lactobacillus* spp. was not effective in reducing FFL. When using fresh baker's yeast (*Saccharomyces cerevisiae*) at a dosage of 100 g/day, the faecal balls returned to normal texture, but free faecal water was still present (Valle *et al*., 2013). Other yeast probiotics (*Saccharomyces boulardii*) are reported to both reduce the time to recover from acute enterocolitis in horses (Desrochers *et al*., 2005), and not to reduce the time to recover in horses with antibiotic-induced diarrhoea (Boyle *et al*., 2013). In general, the efficacy and mechanisms of probiotics in horses have been studied very sparsely (Schoster, 2018), and studies on probiotics as a remedy specifically for FFL are at present unavailable in the literature. Another feed supplement, consisting of montmorillonite bentonite, whey, hops and absinthe extract, was used in a study by Gerstner & Liesegang (2018) to reduce the presence of FFL. However, the supplement was only provided to healthy horses (without FFL) and no effect on faecal chemical composition was observed (Gerstner & Liesegang, 2018).

Faecal microbiota transplantation (FMT) has been tested as a clinical treatment for horses with FFL (Laustsen *et al*., 2018). During FMT, a solution of faecal matter from a healthy donor is administered into the gastrointestinal tract of a recipient (*i.e.* horse with FFL), in order to improve the gut microbial composition (Cammarota *et al*., 2017). This method has been used for treatment of diarrhoea in horses for several decades, but procedures and effects are poorly described in the literature (Mullen *et al*., 2018; Mullen *et al*., 2014). However, a recent study investigated the efficacy of FMT as a treatment for horses with FFL (Laustsen *et al*., 2018). The results showed that faecal texture in horses with FFL improved 14 days *post* FMT, from a faecal severity score of 3.5 to 2.3 (where 0 = no symptoms and 4 = maximum severity), and remained improved for the entire study period of 164 days (Laustsen *et al*., 2018). However, only 10 horses with FFL were included in the study, and although the horses improved with the FMT treatment they were not free of symptom. This suggests that FMT can be used as a treatment to reduce the presence of FFL in some cases, but not in all. Further studies are required to evaluate the effect of FMT on the presence of FFL.

3. Objectives

The overall aim of this PhD-project was to investigate associations between feeding and management factors and presence of free faecal liquid in horses, and to compare faecal composition in horses with and without free faecal liquid. The general goal was to enhance the knowledge of factors of importance for the aetiology of the condition.

Specific objectives were to:

1. Characterise horses with free faecal liquid and map the overall management and feeding strategies of affected horses.
2. Compare faecal bacteria composition in horses with and without free faecal liquid.
3. Compare chemical composition and physical characteristics of faeces from horses with and without free faecal liquid.
4. Compare feeding and management strategies in horses with and without free faecal liquid.

4. Materials and methods

This chapter provides a brief summary of the materials and methods used in the studies on which this thesis is based. More detailed descriptions can found in the individual papers (I-IV).

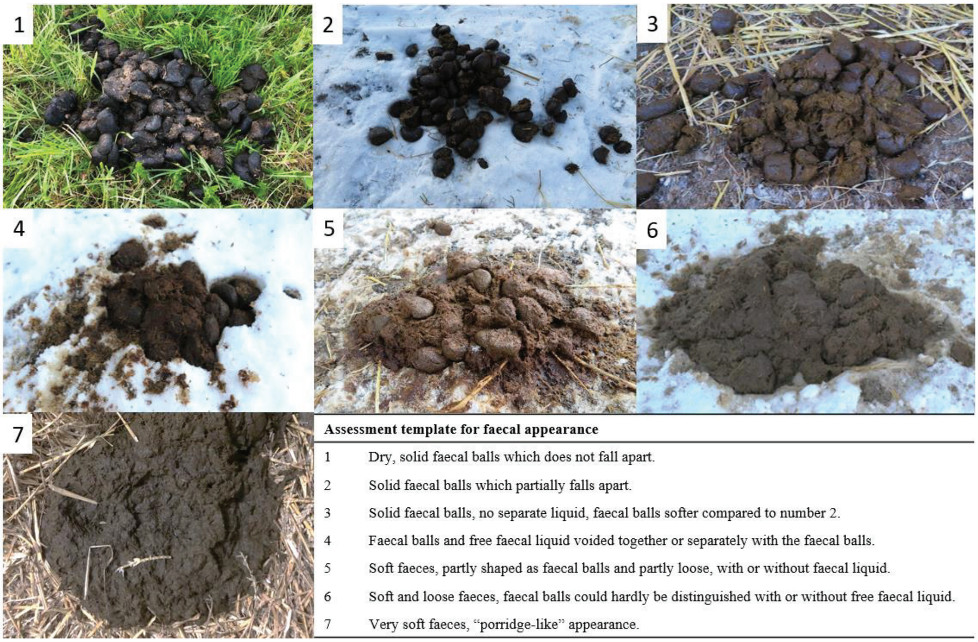
4.1 Study design

The study reported in Paper I comprised an online survey lasting for one year. Papers II-IV were based on a case-control study lasting for six months and with three sampling periods for forage and faeces. In addition, a German horse population including case and control horses, from which faeces samples were collected on one occasion, was included in Paper III.

4.2 Horses

All horses were recruited to the studies through advertisements in web-based channels connected to the Department of Animal Nutrition and Management at the Swedish University of Agricultural Sciences (SLU). All horses in paper II-IV were privately owned and stabled in Sweden and Norway (Figure 2), and in paper III an additional sub-group of privately owned horses stabled in Germany was included. The definition of a horse with free faecal liquid (FFL) was a horse showing two-phase characteristics of faeces (one solid and one liquid phase), and the definition of a control horse was a horse showing only a solid phase and no separate liquid phase of their faeces (Figure 3). Detailed descriptions and inclusion criteria for horses are given in each paper. In paper I, participation was open to all horse-owners in Sweden and Norway that owned or had responsibility for a horse showing FFL. In paper II-IV, the first 30 Swedish and the first 20 Norwegian horse-owners that volunteered to participate and had horses fulfilling all inclusion criteria (also comprising access to a healthy control horse on the same farm) were included in the study. In the German sub-study (paper III), the horses were recruited from the clientele of an equine clinic (Pferdeklinik An der Rennbahn, Iffezheim, Germany), and the farms were located in the south part of Germany (n=32).

Figure 2. Location of the farms in Sweden (n=30) and Norway (n=20) keeping the case and control horse pairs included in Papers II-IV.



Assessment template for faecal appearance	
1	Dry, solid faecal balls which does not fall apart.
2	Solid faecal balls which partially falls apart.
3	Solid faecal balls, no separate liquid, faecal balls softer compared to number 2.
4	Faecal balls and free faecal liquid voided together or separately with the faecal balls.
5	Soft faeces, partly shaped as faecal balls and partly loose, with or without faecal liquid.
6	Soft and loose faeces, faecal balls could hardly be distinguished with or without free faecal liquid.
7	Very soft faeces, “porridge-like” appearance.

Figure 3. Template used for assessment of faecal appearance score (FAS). Photograph 1 and 7 by the author, and 2-6 by C.E. Müller.

4.3 Data collection

4.3.1 Paper I

The online survey in paper I was open from March 2016 to March 2017 and was available in both the Swedish and Norwegian language. The survey contained questions on horse characteristics, training, current feeding and management, presence of FFL and previous history of gastrointestinal tract (GIT) disease. The complete survey responses, translated to English, are accessible in the Appendix (Survey). All data were handled according to EU General Data Protection Regulation.

4.3.2 Papers II-IV

In the Swedish-Norwegian sub-study in paper II-IV, information on horse characteristics, training, feeding, management, presence of FFL and previous history of GIT disease was collected using the same online survey as in Paper I. Additional questions on whether the horse showed signs of FFL or not was also asked for. In the German sub-study in paper III, information on horse characteristics and type and amount of feeds fed was obtained in an on-site interview before the start of the study. Horse body weight was measured using a transportable scale.

4.4 Faeces sample collection and analysis (papers II and III)

4.4.1 Sample collection

In the Swedish-Norwegian sub-study (paper III), non-invasive methods were used for collection of faeces samples. Faeces samples were collected three separate sampling periods, starting in October 2016 and ending in March 2017. Horse owners performed the sampling after receiving detailed instructions and sampling materials. Instructions included sampling freshly voided faeces (within one minute of defecation) and only sampling a part of the faeces that had not touched the ground or floor. Case and control horses on the same farm was sampled the same day. Samples were sent to the Laboratory at the Department of Animal Nutrition and Management, SLU, Uppsala. If the number of days between sampling and arrival to the laboratory exceeded four, the sample was discarded and the horse owner was asked to collect new samples. Accepted samples were first evaluated for faecal appearance score (FAS, 1 to 7, described in paper II) and then sub-sampled for analysis of microbiota composition and targeted detection of clostridia by cultivation, before being processed for analysis of chemical composition and physical characteristics. In the German sub-study in paper III, faecal samples were collected once from all horses by rectal sampling performed by a veterinarian. Case and control horses on the same farm were sampled on the same day. Faeces samples were stored at 4°C until analysis. On the collection day, all horses underwent a clinical health check performed by the veterinarian.

4.4.2 Chemical analysis

In the Swedish-Norwegian sub-study in paper III, faecal samples were analysed for DM, ash, sodium (Na), potassium (K), calcium (Ca), phosphorous (P), magnesium (Mg), sulphur (S), ammonia-N, osmolality, pH and short-chain fatty acids (SCFA). In the German sub-study, faecal samples were analysed for DM, ash, pH, K, Mg and SCFA. In both sub-studies total SCFA was calculated as the sum of acetic, propionic, *i*- and *n*-butyric, *i*- and *n*- valeric and lactic acid concentrations, and the ratio of (C2+C4)/C3 was calculated as sum of acetic and *i*- and *n*-butyric acid over propionic acid concentrations.

4.4.3 Physical characteristics

For analysis of physical characteristics in faecal samples from the Swedish-Norwegian sub-study (paper III), faecal liquid was centrifuged and the supernatant was used for measurement of osmolality. Water holding capacity in faeces was measured by mixing dried and milled faecal samples with distilled water that were left to sediment after which the volume of absorbed water in solid phases was calculated as ml per g dried faeces. Faeces with high water holding capacity absorbed all added water (Figure 4a) while faeces with lower water holding capacity had a clearly visible unabsorbed water phase (Figure 4b). The amount of free liquid and sand in faecal samples was determined by centrifugation of diluted samples and determination of volumes of sand and supernatant (Figure 5).

In paper III, faecal samples from both sub-studies were analysed for particle size distribution by wet sieving using sieve mesh sizes 8 mm, 4 mm, 2 mm, and 1 mm.

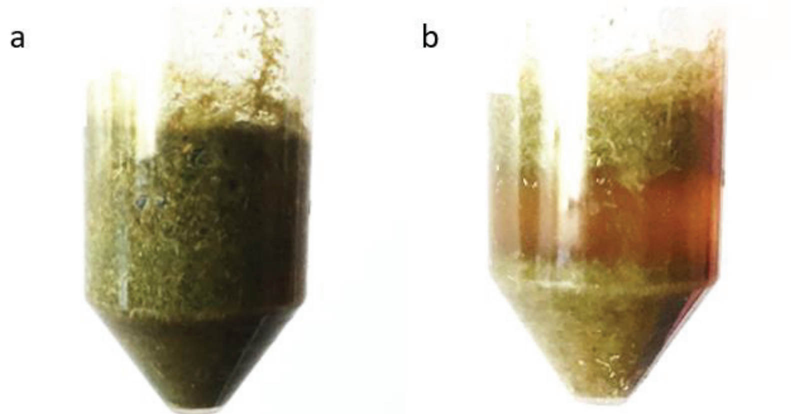


Figure 4. Faeces with high (a) and low (b) water holding capacity after sedimentation. Photographs by the author.

Figure 5. Faeces with presence of sand in the bottom of the test tube, after centrifugation. Photograph by the author.

4.4.4 Faecal bacterial analysis

Sub-samples of faeces for analysis of bacterial composition were stored at −80°C until isolation of DNA. Extraction of DNA was performed using an extraction robot (BioRobot). The DNA quantification and quality were assessed using a Qubit Fluorometer. Extracted DNA was sent to Novogene (Tianjin, China) for generation of 16S rRNA gene amplicon libraries and sequencing. The regions amplified and sequenced were V3-V4 regions of 16S rRNA. The amplicon library was sequenced on an Illumina HiSeq 2500 platform, generating 250 bp paired-end reads which were then merged and filtered. The reads were clustered and OTUs (Operational Taxonomic Units) generated based on 97% sequence homology and classified taxonomically.

4.4.5 Analysis of *Clostridioides difficile* and *Clostridium perfringens*

Detection of *C. difficile* and *C. perfringens* was performed by cultivation of fresh faecal samples at the National Veterinary Institute (SVA, Uppsala, Sweden). Cultures for *C. perfringens* were made on fastidious anaerobe (FA) agar and incubated anaerobically at 37°C for two days. Heat selection (65°C water bath for 30 min) were performed for detection of spores. Cultures for *C. difficile* were made from faecal material streaked on selective agar medium.

4.5 Forage sample collection and analysis (Paper IV)

4.5.1 Sample collection

Forage samples were collected at the same three sampling periods as faecal sampling by the horse owners. Samples were sent by postal service to the Laboratory at the Department of Animal Nutrition and Management, SLU, Uppsala. If the number of days between sampling and arrival exceeded four, the sample was discarded and the horse owners were requested to collect new forage samples.

4.5.2 Chemical analysis

Forage samples were analysed for content of dry matter (DM), CP, ash and content of macro minerals (Ca, P, Mg, Na, K and S), Ammonia-N, pH and concentrations of SCFA, ethanol and 2,3-butanediol were determined with HPLC. Concentrations of glucose, fructose, sucrose, and fructans were analysed enzymatically-spectrophotometrically and summed to water soluble carbohydrates (WSC). Content of neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin was analysed. Content of metabolisable energy (MJ ME) was estimated from *in vitro* digestibility of organic matter, and the content of ME for horses (ME_h) and digestible CP (dCP) was calculated as described in paper IV.

4.6 Statistical analysis

4.6.1 Survey study (Paper I)

In Paper I, statistical analysis was performed using SAS version 9.4 (Statistical Analysis System Institute Inc., Cary, NC, USA). For continuous variables, minimum, maximum, quartiles Q1, Q2 and Q3, mean and standard deviation were calculated. Analysis were performed using a Chi^2-test (with expected model). Level of significance was set at $p<0.05$.

4.6.2 Faecal bacterial composition in the case-control study (Paper II)

Data processing of faecal bacterial composition was performed in R (R Foundation for Statistical Computing, Vienna, Austria) and PAST (reference). All analyses of faecal bacterial composition were performed on genera level. Case and control groups were compared for horse characteristics using a Chi²-test. For age, case and control horses were compared using a Wilcoxon matched-pairs signed rank test.

Alpha diversity indices were estimated using the Shannon diversity index (H). Comparisons between case and control horses and categorical metadata (including sampling period (SP)) were analysed. Matched-pairs test were used for comparisons between case and control horses.

Beta diversity was visualised using Principal coordinate analysis (PCoA) based on Bray Curtis metrics, and the clustering pattern was statistically evaluated using one way-ANOSIM analysis. Univariate analysis was used to assess if specific taxa differed in relative abundance between case and control horses. The univariate analyses were performed on a filtered data set excluding genera that were present in <20% of the samples or having a mean relative abundance <0.2 %. Differences between case and control horses were tested within each sampling period. Statistical differences were accepted at $p < 0.05$ and the Benjamini-Hochberg (B-H) procedure was used to adjust for multiple comparisons. FDR-adjusted p-values were presented as q-values.

4.6.3 Faecal chemical composition and physical characteristics in the case-control study (Paper III)

All statistical analyses were performed using SAS (Statistical Analysis System Institute Inc., Cary, NC, USA). Comparisons between case and control horses were performed using the generalised linear mixed model procedure with effect of sampling period and interactions between case/control and sampling periods included, as well as repeated measurements on horses, and with farm included as a random effect. An additional comparison between the Swedish- Norwegian and German sub-study was performed using the generalized linear mixed model procedure. Differences where $p \leq 0.05$ were regarded as statistically different.

4.6.4 Feeding and management in the case-control study (Paper IV)

Statistical analysis was performed using SAS (Statistical Analysis System Institute Inc., Cary, NC, USA). Basic information on the horses, type of feeds, and feeding practices and management were compared between case and control horses using a Chi^2 test. For the chemical composition of wrapped forages, the minimum, maximum, median, average and standard deviation were calculated. Daily intake of different feeds and of specific feed components were compared between case and control horses using the generalised linear mixed model procedure with farm (ID) included as a random effect. Differences were accepted as statistically significant at $p<0.05$.

5. Main results

This section summarises the main results reported in Papers I-IV. Detailed results can be found in the individual papers.

5.1 Horse characteristics

Data from 339 FFL horses were studied in Paper I. A large variety of horses of different breeds, age and coat colours, and used for different training disciplines, were represented among FFL horses, showing that all types of horse can be affected by this condition. Similar horse characteristics were present among horses in the case-control study (Papers II-IV), with no difference between case and control horses. Horses with FFL in Paper I were found to have a previous colic incidence of 23%, while in the case-control study (Papers II-IV) the colic incidence was similar (20 % for case and 24 % for control horses). Apart from presence of FFL in horses in Paper I, other major clinical signs reported during episodes of FFL included distinct irritation during defecation, bloated abdomen and colic.

5.2 Faecal bacterial composition (Paper II)

Faecal bacterial composition described as alpha- and beta diversity were similar in case and control horses, indicating similar number and distribution of bacteria within samples, and similar composition of bacterial community between samples. The similarity in beta diversity between case and control horses is illustrated in Figure 6. Sampling period (SP) was found to be important for faecal microbiota composition. When comparing faecal microbiota composition between case and control horses within each sampling period, 14 low- abundance genera differed in at least one SP. In two of three SP (and with a tendency in the third SP), the genus *Alloprevotella* spp. was more abundant in case horses compared with control horses, whereas lower relative abundance of the genus *Bacillus* spp. was found for case horses compared with control horses within all SP (Table 1). The other twelve genera differed between case and control horses in only one SP. All horses tested negative for *C. difficile* and *C. perfringens* by culture of faeces.

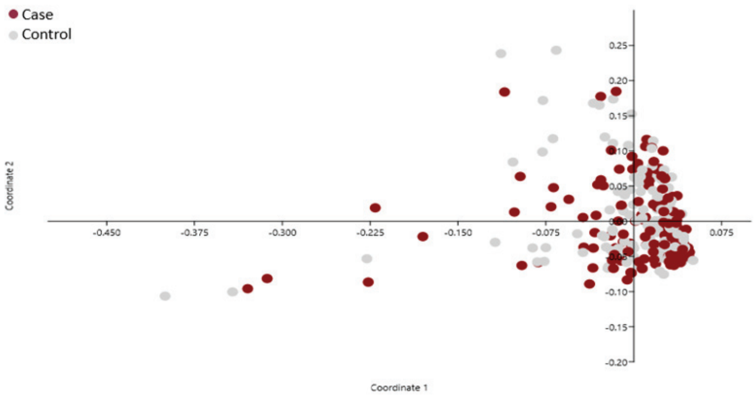


Figure 6. Principal coordinate analysis (PCoA) plot illustrating similar faecal bacterial composition for horses with (case, n=142) and without free faecal liquid (control, n=138).

Table 1. Mean relative abundance, % (±SD) of *Alloprevotella* spp. and *Bacillus* spp. in faecal samples of horses with free faecal liquid (case) and horses without free faecal liquid (control) within each sampling period (SP 1-3). False discovery rate (FDR) adjusted p-values are presented as *q*-values

Genera	SP1 Case (±SD)	SP1 Control (±SD)	SP1 *q*-value	SP2 Case (±SD)	SP2 Control (±SD)	SP2 *q*-value	SP3 Case (±SD)	SP3 Control (±SD)	SP3 *q*-value
Alloprevotella	1.0 (0.96)	0.62 (0.43)	0.04	1.20 (0.97)	0.84 (0.42)	0.02	0.85 (1.05)	0.54 (0.38)	0.08
Bacillus	0.17 (0.23)	0.23 (0.19)	0.01	0.19 (0.16)	0.31 (0.37)	0.03	0.16 (0.19)	0.25 (0.27)	0.03

5.3 Faecal chemical composition and physical characteristics (Paper II-III)

Differences in chemical composition and physical characteristics in faeces were found between case and control horses in the Swedish-Norwegian sub-study, but not in the German sub-study (paper III). In the Swedish-Norwegian sub-study, FAS was commonly 4 to 6 in case horses and 1 to 3 in control horses, with no overlap of scores between the horse groups. Concentration of faecal lactic acid and the proportion of lactic acid to total SCFA was lower in faeces from case compared to control horses Table 1). For other SCFAs, both concentration and proportion to total SCFA was similar in case and control horses. Case horses had lower water holding capacity in their faeces compared to control horses (Table 2). No interaction effects between case/control and sampling period (SP) or general, differences between SPs were present for any of the other measured variables.

5.4 Feeding and management

Feeding practices and management factors were similar for case and control horses. Feed changes, including changes from wrapped forage to hay, to pasture or to another batch of wrapped forage, were found to result in reduction or absence of FFL in a majority (but not all) of the horses studied (papers I and IV). Large variations in management practices were reported for horses with FFL (paper I and IV), but the range of variation was similar for case and control horses (paper IV). Horses with FFL were in general fed roughage-dominated feed rations (Papers I and IV), but were reported to be fed a higher proportion of concentrates in their total feed ration compared with control horses (Paper IV). Case and control horses in the German sub-study were fed similar proportions of concentrates in their diets (Paper III) and were fed hay as their main forage. The Swedish-Norwegian horses in Paper II-IV were fed wrapped forages (haylage or silage), with some horses also having inclusion of hay in the diet. The majority of horses were also reported to be fed some type of concentrate (Papers I and IV). The wrapped forages fed to the horses in Papers I-IV had a DM content ranging from 179 to 951 g/kg, and there was also large variation in chemical components (Paper IV). There was no difference in the reported amount of wrapped forages fed between case and control horses (Paper IV). However, case horses were fed lower amounts of straw and were fed twice as much concentrate (in kg DM per 100 kg BW and day) as the control horses. Daily intake (in g per 100 kg BW and day) of dCP and NDF was higher in control compared with case horses, whereas daily intake of starch and WSC was higher in case compared with control horses (Table 2).

Table 2. Chemical composition and physical characteristics of faeces and daily intake of specific feeds and feed components (per 100 kg body weight and day) for horses with (case) and without (control) free faecal liquid. SD = standard deviation

Paper	Variable	Case Mean± SD	Control Mean± SD	*p*-value
	In faeces:			
III[1]	Lactic acid, mmol/L	1.9±2.42	2.3±2.15	0.04
	Lactic acid, % of SCFA	5.1±5.89	7.2±6.71	0.04
	Water holding capacity, mL/g	6.2±2.08	6.9±2.27	0.03
	Daily intake of:			
IV[2]	Straw, kg DM	0.1±0.18	0.2±0.19	<0.0001
	Concentrates, kg DM	0.2±0.30	0.1±0.16	0.004
	Digestible crude protein, g	89±50.6	95±49.4	0.007
	Neutral detergent fibre, g	1005±524.7	1105±522.9	<0.0001
	Starch, g	19±28.9	17±26.0	0.004
	Water soluble carbohydrates, g	177±79.68	167±74.51	0.002
	Proportion of concentrate, % of diet	10±8.22	9±8.16	<0.0001

[1]Data from Tables 1 and 2 and Figures 1 and 2 in Paper III. SCFA: short-chain fatty acids).

[2]Data from Table 2 in Paper IV. DM: dry matter

6. General discussion

6.1 Horse characteristics

The majority of horses with FFL represented in the survey in Paper I were reported to be of warmblood type, had bay coat colour, were geldings, were on average 12 years old and were used for leisure riding. A similar distribution of horse characteristics has previously been described in comparable horse populations in both Norway (Heldt *et al*., 2018) and Sweden (Hartmann *et al*., 2017). This indicates that the population of horses with FFL in Paper I represented the general horse population and that almost any type of horse could be affected.

Among horses with FFL in Paper I, the proportion of geldings was larger than the proportion of mares or stallions. This is in agreement with findings in a previous study on FFL in horses in Germany, where a larger proportion of case horses compared with control horses were geldings (Kienzle *et al*., 2016). This could simply be due to it being more common to keep geldings than mares and stallions as leisure horses (Ross *et al*., 2018; Wylie *et al*., 2013). However, among horses in Papers II-IV the distribution of genders was similar for horses with and without FFL.

In Papers II-IV, a higher proportion of horses with FFL tended to be kept for company compared with control horses, as also reported in a previous study where the majority of horses with FFL were reported not to be ridden (Zehnder, 2009). This may indicate that reduced and/or absence of exercise is important for the development of FFL, or that presence of FFL affects the will or possibilities of horse owners to exercise the horse. Although horses with FFL in Papers I-IV were reported to have no symptoms of disease, such as pyrexia or weight loss, presence of FFL may still be perceived as a health disturbance in the horse and may cause the horse owner to refrain from exercising the horse.

Although the majority of horses with FFL in Paper I were reported not to show any clinical signs other than free faecal liquid, some horses were reported to have one or a combination of other clinical signs, including colic symptoms, bloated abdomen and irritation while voiding faeces. At present, it is not known whether different combinations of these symptoms are signs of different disturbances with different underlying causes or whether they reflect the severity of FFL. Another possibility is that FFL is a generic symptom of several different conditions of a similar nature.

Almost a quarter of horses with FFL in Paper I and in the case-control study (Papers II-IV) were reported to have a previous history of colic. This is a higher proportion compared to the colic incidence in the general horse population reported in previous studies, which ranged between 3.5% and 10.6% (Larsson & Müller, 2018; Hillyer *et al*., 2002; Traub-Dargatz *et al*., 2001; Tinker *et al*., 1997, 1994). It is also much higher than the 4.8% colic incidence reported within a German population of horses showing FFL (Zehnder, 2009). This could indicate that FFL and increased risk of colic are associated with each other, but whether FFL leads to colic, colic leads to FFL or there is a common background factor that increases the risk of both FFL and colic is currently not known. This result could also be affected by how colic diagnosed i.e. by a veterinarian or by the horse owner. Further investigation of the association between FFL and colic, and other gastrointestinal disturbances, is of interest.

6.2 Feeding and management strategies

Feeding practices and management factors, including *e.g.* number of feedings and time between feedings, type of housing system and paddock use, were similar in horses with and without FFL (Paper IV). Although no specific feeding practice or management factor was identified as important for presence of FFL in paper I or IV, it is possible that other feeding or management factors may be of relevance for the aetiology of FFL.

Feeding wrapped forages has been suggested as a possible cause of FFL (Kienzle *et al*., 2016). However, in Paper IV the case and control horses on each farm were fed the same wrapped forage. In addition, both case and control horses in the German sub-study in Paper III were fed hay as forage.

Further, horses displaying FFL were predominantly fed hay, with only a few horses also being fed wrapped forages during the winter (Kienzle *et al*., 2016). It is therefore unlikely that wrapped forage *per se* is a general cause of FFL in horses.

Wrapped forages generally differ from hay in DM content and, depending on the DM content in wrapped forages, also in pH and fermentation products such as lactic acid (Müller *et al.,* 2008). Studies investigating the influence of hay, haylage and silage (produced from the same grass crop) on the equine hindgut have shown that overall digestibility (Miyaji *et al.,* 2008) as well as microbial and biochemical composition in colon and faeces were similar in healthy horses (Muhonen *et al.,* 2009; Müller *et al*., 2008).

Changes in forage batch or forage type were reported by case horse owners in Papers I and IV to result in reduction or absence of FFL. Several factors may differ between different forage batches in addition to the forage conservation method, such as plant maturity at harvest, harvest number (*e.g*. primary and regrowth harvest), and botanical composition. Plant maturity at harvest is the dominant factor determining nutritive value and overall digestibility of forages for horses, and it may differ widely between different forage batches irrespective of the conservation method used (Müller, 2012; Särkijärvi *et al*., 2012; Ragnarsson & Lindberg, 2010, 2008). Plant maturity and harvest number affects *e.g.* NDF concentration and digestibility, due to a higher proportion of leaf to stem in younger plants and in regrowth (Ragnarsson & Lindberg, 2010, 2008). As changing forage from primary to regrowth harvest or to pasture was reported to reduce or eliminate FFL in several case horses, fibre content and digestibility of forage could be of interest for further study. The individual variation in fibre degrading hindgut microbes in horses is large (Salem *et al*., 2018; Proudman *et al*., 2015; Fernandes *et al*., 2014; Blackmore *et al*., 2013; Daly *et al*., 2012) and may result in higher or lower capacity of degradation of different fibres among individual horses (Edouard *et al*., 2008). This may in turn affect the water holding capacity of the digesta, as different fibres varies in their hydrophilic properties (Jones & Spier, 1998; Auffret *et al*., 1994; Clarke *et al*., 1990). In paper III, faeces from case horses had a lower water holding capacity compared to faeces from control horses. In paper II, FAS was 4-6 for case horses and 1-3 for control horses, also reflecting differences in water holding capacity in faeces. The faecal water holding capacity may be related to the type and amount of fibre present in the faeces, similar to what was described for digesta, which could in turn influence presence or absence of FFL. Very little is known about how different fibre fractions of forage plants influence the large intestinal function in horses (Brøkner *et al*., 2016), and this calls for further studies.

Only half of the horse owners in Paper IV reported that they had data on the nutritive content of their forage (through forage analysis reports), indicating that only half of the horse owners had possibilities to calculate feed rations and feed their horses according to their nutritional requirements. In the case report by Valle *et al*. (2013) one of the interventions that contributed to resolve FFL was recalculating the feed ration to fit the nutrient requirements of the horse. These changes included reducing the energy content in the feed ration by excluding concentrate feeds, decreasing the amount of long-stemmed forage and changing batch of forage, in combination with increased exercise.

One of the first steps for that can be performed on farm level for FFL-horses is therefore to have the forage analysed for its nutritional composition and use the analytical values for feed ration calculations. It would also improve the possibilities to evaluate responses in FFL horses at feed changes if the composition of the forages involved was known.

6.3 Feed ration composition

In Paper IV, only small differences were observed in type and amount of feed and in daily intake of specific nutrients between case and control horses. Control horses were fed a higher average amount of straw and lucerne compared with control horses, and the daily intake of NDF was higher in control compared with case horses. Lower intake of NDF could affect the microbiota of the horse, as the hindgut is inhabited mainly by fibrolytic bacteria that ferment fibre to SCFA (Daly *et al*., 2012; Hintz *et al*., 1971). Intake of fibre-rich feeds enables a steadier rate of production and absorption of nutrients and water in the intestine compared with intake of low-fibre feeds (Stevens & Hume, 1995; Clarke *et al*., 1990; Argenzio *et al*., 1974b). Although the difference in Paper IV in roughage inclusion in the diet between case and control horses was small, it may have been sufficiently large to affect the properties of faeces in the horses. The results in Paper IV showed that control horses were fed a diet higher in dCP compared with the case horses, which may have been due to the higher inclusion of brewer's yeast, lucerne and concentrates high in protein (NRC, 2007). However, the association between protein intake and presence of FFL, as well as gastrointestinal disturbances is not known. However, in Paper IV the control horses had higher daily intake of dCP than the case horses. The case and control horses were also similar in factors affecting their crude protein requirements (such as age and training intensity), so it is unlikely that FFL was associated with excessive dCP intake in Paper IV.

Case horses in Paper IV were fed a higher amount of concentrates per 100 kg BW in their diet compared to control horses. Concentrate proportion in the total feed ration differed but to a small extent (9.7% in case horses, 9.1% in control horses), and influenced daily total intake of starch and WSC which were higher in case than in control horses. Although case horses were reported to be fed a higher proportion of concentrate in the diet and to have higher daily intake of starch and WSC, they had a lower concentration of lactic acid and lower proportion of lactic acid to total SCFA in their faeces compared to control horses (Table 2). The lower faecal lactic acid concentration was unexpected as case horses were fed a higher proportion of concentrates in their diet compared to control horses. Changes in faecal chemical composition, including increased concentrations of lactic- and propionic acid, as well as decreased concentration of acetic acid, and low faecal pH (<6), have previously been associated with horses fed concentrates and hay compared with hay only (Grimm *et al*., 2017; da Veiga *et al*., 2005; Rowe *et al*., 1994), with abrupt inclusion of starch rich feeds in equine diets (de Fombelle *et al*., 2001; Argenzio *et al*., 1974a; Kern *et al*., 1973; Willard *et al*., 1977) and in horses where laminitis has been induced by creating hindgut acidosis through high oral doses of starch or oligofructose (Milinovich *et al*., 2008; Milinovich *et al*., 2007; Milinovich *et al*., 2006; van Eps & Pollitt, 2006; Rowe *et al*., 1994). In addition, faecal concentrations of acetic, butyric, propionic and valeric acid and total SCFA was similar in case and control horses, as well as proportions of individual SCFA to total SCFA and (C2+C4)/C3 ratio (Paper III). Faecal pH was similar in case and control horses (Paper III), and was within the range for healthy horses. As faecal pH and SCFA concentrations and proportions were similar in horses with and without FFL, the condition was not considered to be a sign of hindgut acidosis.

The data on feeding was provided by the horse owners, and different individuals may have interpreted the questions differently. This is a limitation of the study (paper IV), as respondents may have reported what was fed or what was actually consumed by the horse. The same applies to daily intake of straw, as a number of horses were reported to have straw as bedding material and could have ingested more straw than the reported amount. Controlled feeding studies are difficult to perform with privately owned horses, but would be needed in order to further investigate the feed related factors that were associated with FFL in the current study.

6.4 Faecal bacterial composition

In horses with gastrointestinal diseases such as diarrhoea (Rodriguez *et al*., 2015), colitis (Costa *et al*., 2012) and *post-partum* colic (Weese *et al*., 2015), faecal bacterial composition has differed in comparison with healthy control horses. These differences include lower faecal bacterial diversity, lower bacterial richness and evenness compared with control horses (Elzinga *et al*., 2016; Rodriguez *et al*., 2015). When comparing the faecal bacterial composition in case and control horses in Paper II, no such differences were detected. The results in Paper II were in agreement with findings in a Swiss study (Schoster *et al*., 2020), where horses with and without FFL had similar faecal bacterial composition. However, sampling period was found to affect bacterial composition in Paper II and also in the Swiss study (Schoster *et al*., 2020). Season of the year has previously been reported to influence faecal microbiota composition by influencing both alpha and beta diversity (Salem *et al*., 2018; Dougal *et al*., 2017; Steelman *et al*., 2012). Therefore faecal bacterial composition was compared between case and control horses within each sampling period in paper II, to remove sampling period as a confounding factor. The results from this analysis revealed a difference in relative abundance for 14 genera between case and control horses for at least one sampling period (paper II). These results were similar to findings reported by Schoster *et al*. (2020), where differences in specific bacterial taxa in faeces from horses with and without FFL were present. However, the specific taxa found in horses with and without FFL by Schoster *et al*. (2020) were not similar to the specific taxa found in case and control horses in paper II. As an example, Schoster *et al*. (2020), found the relative abundance of

Treponema 2 (and *Treponema* spp.) to be lower in horses with compared to without FFL, but in paper II *Treponema* 2 was present in similar relative abundance in case and control horses. These differences could be due to a number of factors such as horse diets (Kristoffersen *et al*., 2016; Daly *et al*., 2012; Willing *et al*., 2009), season of year (Salem *et al*., 2018; Dougal *et al*., 2017; Steelman *et al*., 2012) and analytical methods, among others. Both studies used 16S Illumina sequencing, but may have differed in sample preparation, filtration of data etc., which could have influenced the results. Time from sampling to analysis also differed between the studies, and this has been shown to influence regrowth of microorganisms in equine faecal samples (Beckers *et al*., 2017). Due to the study design and the widespread geographic location of the farms in paper II, it was not possible to achieve the exact same number of days between sampling and arrival at laboratory for all farms. However, faecal samples from each case-control pair of horses were taken and sent on the same day, resulting in similar treatment of samples from case and control horses.

In paper II, case horses had a higher relative abundance of *Alloprevotella* spp. in two SPs and a lower relative abundance of *Bacillus* spp. in all sampling occasions compared to control horses. *Alloprevotella* spp. has previously been detected in the faecal microbiota of healthy horses (Plancade *et al*., 2019), but has not been considered as a part of the core microbiota of horses (defined as being present in 99.9% of individuals with a relative abundance of at least 0.001%). The genera *Bacillus* spp. is considered part of the normal intestinal microbiota in mammals (Alou *et al*., 2016; Lopetuso *et al*., 2016) and has shown to be a predominant genera in faecal samples from healthy horses (Costa *et al*., 2012). The bacterial genera *Bacillus* spp. is also part of what has been referred to as the Bacillus-Lactobacillus-Streptococcus group, which is reported to increase in relative abundance in horses adapted to a diet consisting of hay/ haylage (6 kg/day) and concentrates (2-2.5 kg commercial müsli and 1 kg barley/ day) compared to horses fed a grass-only diet (Daly *et al*., 2012). The role of *Alloprevotella* spp. and *Bacillus* spp. in equine health and disease is not well known and needs to be further studied. Studies on faecal bacterial composition, as in Paper II, are not designed to identify new pathogenic species, but can indicate bacterial species that are of specific interest for further studies. In this thesis (Papers II and IV), case horses were fed higher proportions of concentrates than control horses, but had lower relative abundance of *Bacillus* spp. and lower faecal lactic acid concentration than control horses. This indicates that knowledge gaps are still present in how hindgut microbial composition (including bacterial composition) affects the pattern of hindgut fermentation products, and in how feed ration composition interacts with the individual gastrointestinal microbiota in different horses.

A reason for not detecting any general differences in faecal bacterial composition between case and control horses (paper II) could simply be that faecal (and hindgut) bacterial composition is not important in all, or any, cases of FFL. In Paper I, affected horses showed variation in both type and number of clinical symptoms, in addition to two-phase characteristics of faeces, during episodes of FFL. As FFL is a condition described quite recently, little is known about it and it is possible that several different underlying causes may result in the same clinical symptom *i.e.* FFL.

If microbial dysbiosis was present in horses with FFL, corresponding findings in faecal fermentation patters should also have been present, as reported for *e.g.* horses with chronic diarrhoea where faecal concentration of acetic acid was lower and concentration of *i*-butyric acid and total VFA was higher compared to in healthy horses (Merritt & Smith, 1980). It may also be possible that faeces samples are not ideal for detecting microbial dysbiosis within the gastrointestinal tract. Previous studies comparing microbial composition in different gastrointestinal compartments of the horse have shown that, compared with the colon microbiota, faeces samples are less likely to represent the microbiota composition within the caecum and the small intestine (Ericsson *et al*., 2016; Costa *et al*., 2015; Dougal *et al*., 2013, Dougal *et al*., 2012; Al Jassim & Andrews, 2009). If FFL is a condition that primarily affects or has its origin in the caecum, small intestine or in intestinal mucosal tissue, faeces samples may not be sufficient for detecting microbial dysbiosis. It might be that there is a local colonisation of a specific bacteria or other microbes in the intestinal mucosa in horses with FFL that could not be investigated due to low abundance in their faeces. Another reason could be that there is a difference in microbial function between faeces from horses with and without FFL. Functional profiling would require additional analysis to the 16S amplicon sequencing, such as shotgun metagenomics as it examines all metagenomic DNA, while 16S amplicon sequencing only examines 16S rRNA genes (Laudadio *et al*., 2019).

7. Conclusions and practical implications

From the results of the present study, it can be concluded that all types of horses can be affected by free faecal liquid (FFL). Feeding and management of FFL-horses were similar to feeding and management in other horse populations. Many, but not all horses with FFL were reported to show reduced or absent signs of FFL when changes in forage feeding was performed. Colic incidence was comparably high in general in the studied horse populations.

Faecal bacterial microbiota composition was generally similar in horses with and without FFL, but relative abundance of *Alloprevotella* spp. was higher and *Bacillus* spp. lower in case compared to control horses and this difference was independent of sampling period.

Horses with FFL had lower concentration and proportion of lactic acid in faecal samples compared to horses without FFL, despite having similar faecal pH and being fed higher proportions of concentrates as well as higher amounts of starch and water soluble carbohydrates in their daily feed rations. Horses with FFL had lower water holding capacity in their faeces compared to horses without FFL, and were fed lower amounts of straw and neutral detergent fibre in their daily diets. This could indicate differences in fibre hydrophilic properties in faeces (and possible digesta) between horses with and without FFL.

These findings could aid in further studies of causes of FFL, as well as in advice for owners of horses with FFL and/or for veterinarians encountering FFL-horses in their practice. The practical implications of this study includes that it may be justified to provide FFL horses with feed rations high in roughage, to try a change in forage type or -batch as well as to provide access to pasture in order to reduce or eliminate the presence of FFL.

8. Future perspectives

In future studies, it would be of interest to conduct more detailed investigations on the response of FFL horses to controlled changes in feeding. As such studies are difficult to perform on privately-owned horses, a starting point could be *in vitro* fermentation studies using faeces from horses with and without FFL as inoculants and testing several well-characterised roughages varying in plant maturity, cut number, and botanical and chemical composition. Analysis of the resulting fermentation products may provide further insights into the dynamic interactions between different forage types and microbial fermentation.

To increase knowledge of the equine gastrointestinal tract, it would also be of interest to use motility capsules to measure gastrointestinal pressure, pH and temperature, in order to assess gastric emptying and total transit time. This could provide valuable information on the gastrointestinal tract of horses with gastrointestinal disturbances, by detecting and localising transit abnormalities to a specific gastrointestinal region.

It would also be of interest to continue studying the faecal microbiota composition to establish the clinical significance of some of the low-abundance bacterial taxa groups that differed between horses with and without FFL. This could be studied for horses showing different types of clinical symptoms during episodes of FFL and horses showing differences in production and defecation (variation in severity), but also for horses suffering from colic and hindgut dysbiosis. Analysis of faecal microbiota composition may also be combined with functional profiling of microbiota by performing shotgun metagenomics. In addition to bacterial composition, it would also be interesting to include and investigate other eukaryotes (*e.g.* plants, fungi) and viruses as this has not been studied in horses with FFL.

It would also be of interest to study gene expression patterns in response to specific nutrients (nutrigenomics) and individual effects of nutrients on individual horse phenotypes. As clinical signs in the development of gastrointestinal disease can be vague, these analyses could highlight the problem at an early stage and allow action to be taken before the symptoms become more severe. The phenotypes investigated could be based on differences in clinical signs expressed during episodes of FFL and on responses on different treatments such as specific feed changes. The effect of gene expression patterns in response to specific nutrients might help explain why specific nutritional components cause problems, or influence the nutritional requirements, in certain individuals.

Another area of interest for FFL that is often mentioned but less researched is feed hygiene. It is possible that horses with FFL have a higher sensitivity towards forages with higher counts of certain microbes or levels of toxins. However, the knowledge of associations between microbial composition of forage and the equine gastrointestinal health is scarce. Performing such studies would also be challenging as it requires intentional feeding of feeds with impaired hygienic quality and risk of causing disease in the horses.

References

Al Jassim, R.A. & Andrews, F.M. (2009). The bacterial community of the horse gastrointestinal tract and its relation to fermentative acidosis, laminitis, colic, and stomach ulcers. *Veterinary Clinics: Equine Practice*. 25(2), pp. 199-215.

Argenzio, R.A., Southworth, M. & Stevens, C.E. (1974a). Sites of organic acid production and absorption in the equine gastrointestinal tract. *American Journal of Physiology*. 226, pp. 1043–1050.

Argenzio, R.A., Lowe, J.E., Pickard, D.W. & Stevens, C. E. (1974b). Digesta passage and water exchange in the equine large intestine. *American Journal of Physiology-Legacy Content*. 226, pp. 1035-1042.

Arroyo, L.G., Rossi, L., Santos, B.P., Gomez, D.E., Surette, M.G. & Costa, M.C. (2020). Luminal and Mucosal Microbiota of the Cecum and Large Colon of Healthy and Diarrheic Horses. *Animals*. 10, pp. 1403.

Auffret, A., Ralet, M.C., Guillon, F., Barry, J.L. & Thibault, J.F. (1994). Effect of grinding and experimental conditions on the measurement of hydration properties of dietary fibres. *LWT-Food Science and Technology*. *27*(2), pp. 166-172.

Bailey, S.R., Rycroft, A. & Elliott, J. (2002). Production of amines in equine cecal contents in an in vitro model of carbohydrate overload. *Journal of animal science*. 80(10), pp. 2656-2662.

Beckers, K.F., Schulz, C.J. & Childers, G.W. (2017). Rapid regrowth and detection of microbial contaminants in equine fecal microbiome samples. *Plos One*, 12(11), pp. e0187044.

Bertone, J.J., Traub-Dargatz, J.L., Wrigley, R.W. (1988). Diarrhea associated with sand in the gastrointestinal tract of horses. *Journal of the American Veterinary Medical Association*. 193(12), pp. 1409-1412.

Blackmore, T.M., Dugdale, A., Argo, C.M., Curtis, G., Pinloche, E., Harris, P.A. & McEwan, N.R. (2013). Strong stability and host specific bacterial community in faeces of ponies. *PloS one*. 8(9), pp. e75079.

Boshuizen, B., Ploeg, M., Dewulf, J., Klooster, S., de Bruijn, M., Picavet, M.T. & Delesalle, C. (2018). Inflammatory bowel disease (IBD) in horses: a retrospective study exploring the value of different diagnostic approaches. *BMC veterinary research*. 14(1), pp. 21.

Boyle, A.G., Magdesian, K.G., Gallop, R., Sigdel, S. & Durando, M.M. (2013). Saccharomyces boulardii viability and efficacy in horses with antimicrobial-induced diarrhoea. *Veterinary Record*. 172(5), pp. 128-128.

Brøkner, C., Austbø, D., Næsset, J.A., Blache, D., Knudsen, K.B. & Tauson, A.H. (2016). Metabolic response to dietary fibre composition in horses. *Animal*. 10(7), pp. 1155-1163.

Båverud, V. (2004). Clostridium difficile diarrhea: infection control in horses. *The Veterinary Clinics of North America: Equine Practice*. 20(3), pp. 615-630.

Båverud, V., Franklin, A., Gunnarsson, A., Gustafsson, A. & Hellander-Edman, A. (1998). Clostridium difficile associated with acute colitis in mares when their foals are treated with erythromycin and rifampicin for Rhodococcus equi pneumonia. *Equine veterinary journal*. 30(6), pp. 482-488.

Båverud, V., Gustafsson, A., Franklin, A., Lindholm, A. & Gunnarsson, A. (1997). Clostridium difficile associated with acute colitis in mature horses treated with antibiotics. *Equine veterinary journal*. 29(4), pp. 279-284.

Cammarota, G., Ianiro, G., Tilg, H., Rajilić-Stojanović, M., Kump, P., Satokari, R. & Gasbarrini, A. (2017). European consensus conference on faecal microbiota transplantation in clinical practice. *Gut*, 66(4), pp. 569-580.

Chapman, A.M. (2009). Acute diarrhea in hospitalized horses. *The Veterinary Clinics of North America: Equine Practice*. 25(2), pp. 363-380.

Clarke, L.L., Roberts, M.C. & Argenzio, R.A. (1990). Feeding and digestive problems in horses: physiologic responses to a concentrated meal. *The Veterinary Clinics of North America: Equine Practice*. 6(2), pp. 433-450.

Cohen, N.D., Gibbs, P.G. & Woods, A.M. (1999). Dietary and other management factors associated with colic in horses. *Journal of the American Veterinary Medical Association*. 215, pp. 53-60.

Cohen, N.D., Matejka, P.L., Honnas, C.M. & Hooper R.N. (1995). Case-control study of the association between various management factors and development of colic in horses. *Journal of the American Veterinary Medical Association.* 206, pp. 667-673.

Colahan, P. 1987, Sand colic. In: Robinson, N.E (eds) *Current therapy in equine medicine*. Saunders, Philadelphia, pp. 55-58,

Costa, M.C., Arroyo, L.G., Allen-Vercoe, E., Stämpfli, H.R., Kim, P.T., Sturgeon, A. & Weese, J.S. (2012). Comparison of the fecal microbiota of healthy horses and horses with colitis by high throughput sequencing of the V3-V5 region of the 16S rRNA gene. *PLoS One*. 7(7), pp. e41484.

Costa, M.C., Silva, G., Ramos, R.V., Staempfli, H.R., Arroyo, L.G., Kim, P. & Weese, J.S. (2015). Characterization and comparison of the bacterial microbiota in different gastrointestinal tract compartments in horses. *The Veterinary Journal*. 205(1), pp. 74-80.

Daly, K., Proudman, C.J., Duncan, S.H., Flint, H.J., Dyer, J. & Shirazi-Beechey, S. P. (2012). Alterations in microbiota and fermentation products in equine large intestine in response to dietary variation and intestinal disease. *British Journal of Nutrition*. 107(7), pp. 989-995.

da Veiga, L., Chaucheyras-Durand, F. & Julliand, V. (2005). Comparative study of colon and faeces microbial communities and activities in horses fed a high starch diet. *Pferdeheilkunde*. *21*, pp. 45.

de Fombelle, A., Julliand. V., Drogoul, C. & Jacotot, E. (2001). Feeding and microbial disorders in horse: 1 Effects of an abrupt incorporation of two levels of barley in a hay diet on microbial profile and activites. *Journal of Equine Veterinary Science.* 21, pp. 439–445.

de Fombelle, A., Varloud, M., Goachet, A. G., Jacotot, E., Philippeau, C., Drogoul, C. & Julliand, V. (2003). Characterization of the microbial and biochemical profile of the different segments of the digestive tract in horses given two distinct diets. *Animal Science*. 77(2), pp. 293-304.

Desrochers, A.M., Dolente, B.A., Roy, M.F., Boston, R. & Carlisle, S. (2005). Efficacy of Saccharomyces boulardii for treatment of horses with acute enterocolitis. *Journal of the American Veterinary Medical Association*. 227, pp. 954-959.

Dougal, K., de la Fuente, G., Harris, P.A., Girdwood, S.E., Pinloche, E., Geor, R.J. & Newbold, C. J. (2014). Characterisation of the faecal bacterial community in adult and elderly horses fed a high fibre, high oil or high starch diet using 454 pyrosequencing. *PloS one*. 9(2), pp. e87424.

Dougal, K., Harris, P.A., Girdwood, S.E., Creevey, C.J., Curtis, G.C., Barfoot, C.F. & Newbold, C.J. (2017). Changes in the total fecal bacterial population in individual horses maintained on a restricted diet over 6 weeks. *Frontiers in microbiology*. 8, pp. 1502.

Dougal, K., Harris, P.A., Edwards, A., Pachebat, J.A., Blackmore, T.M., Worgan, H.J. & Newbold, C.J. (2012). A comparison of the microbiome and the metabolome of different regions of the equine hindgut. *FEMS microbiology ecology*. 82(3), pp. 642-652.

Dougal, K., de la Fuente, G., Harris, P.A., Girdwood, S.E., Pinloche, E., & Newbold, C. J. (2013). Identification of a core bacterial community within the large intestine of the horse. *PloS one*. 8(10), pp. e77660.

Drudge, J.H. (1979). Clinical aspects of Strongylus vulgaris infection in the horse. Emphasis on diagnosis, chemotherapy, and prophylaxis. *The Veterinary Clinics of North America. Large Animal Practice*. 1(2), pp.251-265.

Edouard, N., Fleurance, G., Martin-Rosset, W., Duncan, P., Dulphy, J.P., Grange, S. & Gordon, I.J. (2008). Voluntary intake and digestibility in horses: effect of forage quality with emphasis on individual variability. *Animal: an international journal of animal bioscience*. *2*(10), pp. 1526.

Ericsson, A.C., Johnson, P.J., Lopes, M.A., Perry, S.C. & Lanter, H.R. (2016). A microbiological map of the healthy equine gastrointestinal tract. *PloS one*. 11(11), pp. e0166523.

Ertelt, A. & Gehlen, H. (2015). Free fecal water in the horse-an unsolved problem. *Pferdeheilkunde*. 31(3), pp. 261-268.

FASS (2019-12-19) sulfasalazine, läkemedelsinformation. https://www.fass.se/LIF/product?userType=2&nplId=20040607006962 [2016-07-08]

Fernandes, K.A., Kittelmann, S., Rogers, C.W., Gee, E.K., Bolwell, C.F., Bermingham, E.N., & Thomas, D.G. (2014). Faecal microbiota of forage-fed horses in New Zealand and the population dynamics of microbial communities following dietary change. *PloS one*. 9(11), pp. e112846.

Fielding, C.L., Higgins, J.K. & Higgins, J.C. (2015). Disease associated with equine coronavirus infection and high case fatality rate. *Journal of Veterinary Internal Medicine*. 29, pp. 307-310.

Frazer, M. L. (2008). Lawsonia intracellularis infection in horses: 2005–2007. *Journal of veterinary internal medicine*. 22(5), pp. 1243-1248.

Garner, H.E., Hutcheson, D.P., Coffman, J.R., Hahn, A.W. & Salem, C. (1977). Lactic acidosis: A factor associated with equine laminitis. *Journal of Animal Science.* 45, pp. 1037–1041.

Garner, H.E., Moore, J.N., Johnson, J.H., Clark, L., Amend, J.F., Tritschler, L.G., Coffman, J.R., Sprouse, R.F., Hutcheson, D.P. & Salem, C.A. (1978). Changes in the caecal flora associated with the onset of laminitis. *Equine Veterinary Journal*. 10, pp. 249-252.

Gerstner, K. & Liesegang, A. (2018). Effect of a montmorillonite-bentonite-based product on faecal parameters of horses. *Journal of animal physiology and animal nutrition*. 102, pp. 43-46.

Goymann, W. & Wingfield, J. C. (2004). Allostatic load, social status and stress hormones: the costs of social status matter. *Animal Behaviour*. 67(3), pp. 591-602.

Grimm, P., Philippeau, C. & Julliand, V. (2017). Faecal parameters as biomarkers of the equine hindgut microbial ecosystem under dietary change. *Animal*. 11(7), pp. 1136-1145.

Hansen, N.C., Avershina, E., Mydland, L.T., Næsset, J.A., Austbø, D., Moen, B. & Rudi, K. (2015). High nutrient availability reduces the diversity and stability of the equine caecal microbiota. *Microbial ecology in health and disease*, 26(1), pp. 27216.

Hartmann, E., Bøe, K.E., Jørgensen, G.H.M., Mejdell, C.M. & Dahlborn, K. (2017). Management of horses with focus on blanketing and clipping practices reported by members of the Swedish and Norwegian equestrian community. *Journal of Animal Science*. 95(3), pp. 1104-1117.

Heldt, T., Macuchova, Z., Alnyme, O., Andersson, H. (2018). Socio-Economic Effects of the Equine Industry. Falun: Dalarna University. (Report 2018:04). Available online: https://hastnaringen-i-siffror.se/files/Hästnäringens_samhällsekonomi_rapport2018_4_2.pdf (accessed on 5 November 2018). (In Swedish).

Hillyer, M. (2004). A practical approach to diarrhoea in the adult horse. *In practice.* 26(1), pp. 2-11.

Hillyer, M.H., Taylor, F.G.R., Proudman, C.J., Edwards, G.B., Smith, J.E., & French, N.P. (2002). Case control study to identify risk factors for simple colonic obstruction and distension colic in horses. *Equine veterinary journal*. 34(5), pp. 455-463.

Hintz, H.F., Argenzio, R.A. & Schryver, H.F. (1971). Digestion coefficients, blood glucose levels and molar percentage of volatile acids in intestinal fluid of ponies fed varying forage-grain ratios. *Journal of Animal Science*. 33(5), pp. 992-995.

Hintz, H.F. & Cymbaluk, N.F. (1994). Nutrition of the horse. *Annual review of nutrition*. 14(1), pp. 243-267.

Hofmanová, B., Vostrý, L., Majzlík, I., Vostrá-Vydrová, H. (2015). Characterization of greying, melanoma, and vitiligo quantitative inheritance in Old Kladruber horses. *Czech Journal of Animal Science*. 60, pp. 443–451.

Hudson, J.M., Cohen, N.D., Gibbs, P.G. & Thompson, J.A. (2001). Feeding practices associated with colic in horses. *Journal of the American Veterinary Medical Association*. 219(10), pp. 1419-1425.

Hussein, H.S., Vogedes, L.A. & Fernandez, G.C.J. (2004). Effects of cereal grain supplementation on apparent digestibility of nutrients and concentrations of fermentation end-products in the feces and serum of horses consuming alfalfa cubes. *Journal of Animal Science*. 82, pp.1986–1996

Jones, S.L. & Blikslager, A.T. (2002). Role of the enteric nervous system in the pathophysiology of secretory diarrhea. *Journal of veterinary internal medicine*. 16(3), pp. 222-228.

Jones, S.L. & Spier. S.T. (1998). Pathophysiology of colonic inflammation and diarrhea In Reed, S.M. &, Bayly, W.M. (eds) *Equine Internal Medicine.* Philadelphia, WB Saunders, 1998, pp. 678-679.

Julliand, V., De Fombelle, A., Drogoul, C. & Jacotot, E. (2001). Feeding and microbial disorders in horses: Part 3—Effects of three hay: grain ratios on microbial profile and activities. *Journal of Equine Veterinary Science*. 21(11), pp. 543-546.

Kaya, G., Sommerfeld-Stur, I. & Iben, C. (2009). Risk factors of colic in horses in Austria. *Journal of animal physiology and animal nutrition*. 93(3), pp. 339-349.

Kern, D.L., Slyter, L.L., Weaver, J.M., Leffel, E.C. & Samuelsons, G. (1973). Pony cecum vs. steer rumen: the effect of oats and hay on the microbial ecosystem. *Journal of Animal Science*. 37(2), pp. 463-469.

Kienzle, E., Zehnder, C., Pfister, K., Gerhards, H., Sauter-Louis, C. & Harris, P. (2016). Field study on risk factors for free fecal water in pleasure horses. *Journal of Equine Veterinary Science*. 44, pp. 32-36.

Kienzle, E., Pohlenz, J., & Radicke, S. (1997). Morphology of starch digestion in the horse. *Journal of Veterinary Medicine Series A*. 44(1-10), pp. 207-221.

Kilcoyne, I, Dechant, J.E., Spier, S.J., Spriet, M. & Nieto, J.E. (2017). Clinical findings and management of 153 horses with large colon sand accumulations. *Veterinary surgery*. 46(6), pp. 860-867.

Kim, L.M., Morley, P.S. & Traub-Dargatz, J.L. (2001). Factors associated with salmonella shedding among equine colic patients at a veterinary teaching hospital. *Journal of the American Veteterinary Medicine Association*. 218, pp. 740-748.

Kristoffersen, C., Jensen, R.B., Jensen, Avershina, E. Austbø, D.A., Tauson, A-H. & Rudi, K. (2016). Diet-dependent modular dynamic interactions of the equine cecal microbiota. *Microbes Environ*, 31, pp. 378-386

Larsson, A. & Müller, C.E. (2018). Owner reported management, feeding and nutrition-related health problems in Arabian horses in Sweden. *Livestock Science*. 215, pp. 30-40.

Laudadio. I., Fulci, V., Stronati, L. & Carissimi, C. (2019). Next-Generation Metagenomics: Methodological Challenges and Opportunities. *Omics a Journal of Integrative Biology*. 23(7), pp. 327-333.

Laustsen, L., Edwards, J.E., Smidt, H., van Doorn, D. & Lúthersson, N. (2018). "Assessment of faecal microbiota transplantation on horses suffering from free faecal water. Proceedings of the 9th European Equine Health & Nutrition Congress (EEHNC), pp. 8.

Lavoie, J.P., Drolet, R., Parsons, D., Leguillette, R., Sauvageau, R., Shapiro, J. & Gebhart, C.J. (2000). Equine proliferative enteropathy: a cause of weight loss, colic, diarrhoea and hypoproteinaemia in foals on three breeding farms in Canada. *Equine Veterinary Journal*. 32(5), pp. 418-425.

Lopes, M.A., White II, N.A., Crisman, M.V. & Ward, D. L. (2004). Effects of feeding large amounts of grain on colonic contents and feces in horses. *American journal of veterinary research*. 65(5), pp. 687-694.

Love, S., Murphy, D. & Mellor, D. (1999). Pathogenicity of cyathostome infection. *Veterinary parasitology*. 85(2-3), pp. 113-122.

Mair, T., Love, S., Schumacher, J., Smith, R.K. & Frazer, G. (2013). *Equine Medicine, Surgery and Reproduction*-E-Book. Elsevier Health Sciences, pp. 55.

Mair, T. (2002). Chronic diarrhea. T. Mair, T. Divers, N. Ducharme (Eds.), Manual of equine gastroenterology, Saunders, New York/London, pp. 427-446.

Magdesian, K.G., Hirsh, D.C., Jang, S.S., Hansen, L.M. & Madigan, J.E. (2002). Characterization of Clostridium difficile isolates from foals with diarrhea: 28 cases (1993–1997). *Journal of the American Veterinary Medical Association* 220(1), pp. 67-73.

Manahan, F.F. (1970). Diarrhoea in horses with particular reference to a chronic diarrhoea syndrome. *Australian veterinary journal.* 46, pp. 231-234.

Manship, A.J., Blikslager, A.T., & Elfenbein, J. R. (2019). Disease features of equine coronavirus and enteric salmonellosis are similar in horses. *Journal of veterinary internal medicine*. 33(2), pp. 912-917.

McGorum, B.C. & Pirie, R.S. (2009). Antimicrobial associated diarrhoea in the horse. Part 1: Overview, pathogenesis and risk factors. *Equine Veterinary Education*. 21(11), pp. 610-616.

McIntyre, G. (1917). Remarks on sand colic as met with in Egypt. *The Veterinary Journal (1900)*. 73(8), pp. 282-284.

McKinney, C.A., Oliveira, B.C.M., Bedenice, D., Paradis, M.R., Mazan, M., Sage, S., Sanchez, A. & Widmer, G. (2020). The fecal microbiota of healthy donor horses and geriatric recipients undergoing fecal microbial transplantation for the treatment of diarrhea. *PLoS one*. 15, pp. e0230148.

Merritt, A.M. & Smith, D.A. (1980). Osmolarity and volatile fatty acid content of feces from horses with chronic diarrhoea. *American journal of veterinary research*. 41, pp. 928- 931.

Milinovich, G.J., Trott, D.J., Burrell, P.C., van Eps, A.W., Thoefner, M.B. & Blackall, L.L. (2006). Changes in equine hindgut bacterial populations during oligofructose-induced laminitis. *Environmental microbiology*. 8, pp. 885–898.

Milinovich, G.J., Trott, D.J., Burrell, P.C., Croser, E.L., Al Jassim, R.A. & Morton, J.M. (2007). Fluorescence *in situ* hybridization analysis of hindgut bacteria associated with the development of equine laminitis. *Environmental microbiology.* 9, pp. 2090–2100.

Milinovich, G.J., Burrell, P.C., Pollitt, C.C., Klieve, A.V., Blackall, L.L., Ouwerkerk, D. & Trott, D.J. (2008). Microbial ecology of the equine hindgut during oligofructose-induced laminitis. *The ISME Journal. 2*(11), pp.1089-1100.

Minder, H.P., Merritt, A.M. & Chalupa, W. (1980). In vitro fermentation of faeces from normal and chronically diarrhoeal horses. *American journal of veterinary research* 41(4), pp. 564-567.

Miyaji, M., Ueda, K., Kobayashi, Y., Hata, H. & Kondo, S. (2008). Fiber digestion in various segments of the hindgut of horses fed grass hay or silage. *Animal science journal*. 79(3), pp. 339-346.

Moreau, P. (2011). Le diarree croniche nel cavallo adulto e nel puledro. *Veterinary Clinics: Practice Equine*. 13, pp. 5-19.

Muhonen. S., Julliand, V., Lindberg, J.E., Bertilsson, J. & Jansson, A. (2009). Effects on the equine colon ecosystem of grass silage and haylage diets after an abrupt change from hay. *Journal of animal science*. 87(7), pp. 2291-2298.

Mullen, K.R., Yasuda, K., Divers, T.J. & Weese, J.S. (2018). Equine faecal microbiota transplant: current knowledge, proposed guidelines and future directions. *Equine Veterinary Education*. 30(3), pp. 151-160.

Mullen, K.R., Yasuda, K., Divers, T.J. & Bicalho, R. (2014). Microbiota transplantation for equine colitis: revisiting an old treatment with new technology. *Abstract Programme*, 600.

Müller, C.E. (2012). Impact of harvest, preservation and storage conditions on forage quality. In: *Forages and grazing in horse nutrition*. Wageningen Academic Publishers, Wageningen. pp. 237-253.

Müller, C.E., Von Rosen, D. & Udén, P. (2008). Effect of forage conservation method on microbial flora and fermentation pattern in forage and in equine colon and faeces. *Livestock Science*. 119(1-3), pp. 116-128.

Naylor, R.J. & Dunkel, B. (2009). The treatment of diarrhoea in the adult horse. *Equine Veterinary Education*. 21(9), pp. 494-504.

Niinistö, K.E., Määttä, M.A., Ruohoniemi, M.O., Paulaniemi, M. & Raekallio, M.R. (2019) Owner-reported clinical signs and management-related factors in horses radiographed for intestinal sand accumulation. *Journal of Equine Veterinary Sciences*, pp. 45-52.

NRC, 2007. Nutrient Requirements for Horses. Sixth Revised Edition. National Research Council.

Oliver, O.E. & Staempfli, H. (2006). Acute diarrhoea in the adult horse: case example and review. *Veterinary Clinics: Equine Practice*, 22(1), pp. 73-84.

Olofsson, K. (2016). *Immunopathological aspects of equine inflammatory bowel disease* (Vol. 2016, No. 11).

Oue, Y., Morita, Y. & Kondo, T. (2013). Epidemic of equine coronavirus at Obihiro racecourse, Hokkaido, Japan in 2012. *Journal of Veterinary Medicine Science.* 75, pp.1261-1265.

Oue, Y., Ishihara, R. & Edamatsu, H. (2011). Isolation of an equine coronavirus from adult horses with pyrogenic and enteric disease and its antigenic and genomic characterization in comparison with the NC99 strain. *Veterinary Microbiology*. 150, pp. 41-48.

Owen, J. & Slocombe, D. Pathogenesis of helminths in equines. *Veterinary Parasitology*. 18(2), pp. 139-153.

Peregrine, A.S., Molento, M.B., Kaplan, R.M., & Nielsen, M.K. (2014). Anthelmintic resistance in important parasites of horses: does it really matter?. *Veterinary Parasitology*. 201(1-2), pp.1-8.

Potter, G.D., Arnold, F.F., Householder, D.D., Hansen, D.H. & Brown, K.M. (1992). Digestion of starch in the small or large intestine of the equine. *Pferdeheilkunde*, 1(4), pp. 107-111.

Proudman, C.J., Hunter, J.O., Darby, A.C., Escalona, E.E., Batty, C. & Turner, C. (2015). Characterisation of the faecal metabolome and microbiome of Thoroughbred racehorses. *Equine veterinary journal*. 47(5), pp, 580-586.

Radostits, O.M., Gay, C.C., Blood, D.C. & Hinchcliff, K.W. (2000). *Veterinary Medicine, Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses*. (9thedn). B. Saunders, pp. 144.

Ragnarsson, S. & Lindberg, J. E. (2008). Nutritional value of timothy haylage in Icelandic horses. *Livestock Science*. 113, pp. 202–208.

Ragnarsson, S. & Lindberg, J. E. (2010). Nutritional value of mixed grass haylage in Icelandic horses. *Livestock Science*. 131, pp. 83–87.

Ramey, D.W. & Reinertson, E.L. (1984). Sand induced diarrhea in a foal. *Journal of the American Veterinary Medical Association*. 185(5), pp. 537-538.

Reed, M. R., Bayly, W. M., Sellon, D. C. (2004). *Equine Internal Medicine*. Philadelphia, WB Saunders, pp. 608-611.

Reeves, M.J., Salman, M.D. & Smith, G. (1996). Risk factors for equine acute abdominal disease (colic): results from a multi-center case-control study. *Preventive Veterinary Medicine.* 26, pp. 285-301.

Roberts, M.C. (1975). Carbohydrate digestion and absorption studies in the horse. *Research Veterinary Science* 18, pp. 64–69.

Rodriguez, C., Taminiau, B., Brévers, B., Avesani, V., Van Broeck, J., Leroux, A. & Daube, G. (2015). Faecal microbiota characterisation of horses using 16 rdna barcoded pyrosequencing, and carriage rate of clostridium difficile at hospital admission. *BMC microbiology.* 15(1), pp. 181.

Rolfe, R.D. (2000). The role of probiotic cultures in the control of gastrointestinal health. *The Journal of nutrition.* 130(2), pp. 396-402.

Ross, S.E., Murray, J.K., & Roberts, V.L.H. (2018). Prevalence of headshaking within the equine population in the UK. *Equine veterinary journal*. 50(1), 73-78.

Rowe, J.B., Lees, M.J. & Pethick, D.W. (1994). Prevention of acidosis and laminitis associated with grain feeding in horses. *The Journal of nutrition*. *124*(12), pp. 2742-2744.

Salem, S.E., Maddox, T.W., Berg, A., Antczak, P., Ketley, J.M., Williams, N.J. & Archer, D.C. (2018). Variation in faecal microbiota in a group of horses managed at pasture over a 12-month period. *Scientific reports*. *8*(1), pp. 1-10.

Särkijärvi, S., Sormunen-Cristian, R., Heikkilä, T., Rinne, M. & Saastamoinen, M. (2012). Effect of grass species and cutting time on in vivo digestibility of silage by horses and sheep. *Livestock Science*. 144(3), pp. 230-239.

Schoster, A. (2018). Probiotic use in equine gastrointestinal disease. *Veterinary Clinics: Equine Practice*, 34(1), pp.13-24.

Schoster, A., Arroyo, L.G., Staempfli, H.R., Shewen, P.E. & Weese, J.S. (2012). Presence and molecular characterization of Clostridium difficile and Clostridium perfringensin intestinal compartments of healthy horses. *BMC Veterinary Research*. 8(1), pp. 94.

Schoster, A., Weese, J.S., Gerber, V. & Graubner, C. (2020). Dysbiosis is not present in horses with fecal water syndrome when compared to controls in spring and autumn. *Journal of veterinary internal medicine*, 34(4), pp.1614-1621.

Schumacher, J., Edwards, J.F. & Cohen, N.D. (2000). Chronic idiopathic inflammatory bowel diseases of the horse. *Journal of veterinary internal medicine*. 14(3), 258-265.

SJV. (2018). SJVFVS 2018:49. Föreskrifter och allmänna råd om hästhållning. Jönköping: Statens jordbruksverk (In Swedish).

Songer, J.G. (1996). Clostridial enteric diseases of domestic animals. *Clinical microbiology reviews*. 9(2), pp. 216.

Staempfli, H.R., Prescott, J.F. & Carman, R.J. (1993). Etiology and treatment of idiopathic colitis: recent studies. In Proceedings of the annual convention of the American Association of Equine Practitioners (USA).

Steelman, S.M., Chowdhary, B.P., Dowd, S., Suchodolski, J. & Janečka, J. E. (2012). Pyrosequencing of 16S rRNA genes in fecal samples reveals high diversity of hindgut microflora in horses and potential links to chronic laminitis. *BMC veterinary research*. 8(1), pp. 231.

Stevens, C.E. & Hume, I.D. (1998). Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. *Physiological reviews*. 78(2), pp. 393-427.

Swyers, K.L., Burk, A.O. & Hartsock, T.G. (2008) Effects of direct-fed microbial supplementation on digestibility and fermentation end-products in horses fed low- and high-starch concentrates. *Journal of Animal Science*. 86, pp. 2596–2608

Teale, C.J. & Naylor, R.D. (1998). Clostridium difficile infection in a horse. *The Veterinary record*. 142(2), pp. 47.

Thomas, P.D., Forbes, A., Green, J., Howdle, P., Long, R., Playford, R., Sheridan, M., Stevens, R., Valori, R., Walters, J., Addison, G.M., Hill, P. & Brydon, G. (2003). Guidelines for the investigation of chronic diarrhoea, 2nd edition, pp. 123.

Tinker, M. K., White, N. A., Lessard, P., Thatcher, C. D., Pelzer, K. D., Davis, B. & Carmel, D. K. (1997). Prospective study of equine colic risk factors. *Equine veterinary journal*. 29(6), pp. 454-458.

Tinker, M.K., White, N.A., Lessard, P., Thatcher, C.D., Pelzer, K.D., Davis, B. & Carmel, D.K. (1994) Nutritional risk factors for colic on horse farms: A prospective study. *Kenya Veterinarian.* 18, pp. 267-269.

Traub-Dargatz, J.L., Kopral, C.A., Seitzinger, A.H., Garber, L.P., Forde, K. & White, N.A. (2001). Estimate of the national incidence of and operation-level risk factors for colic among horses in the United States, spring 1998 to spring 1999. *Journal of the American Veterinary Medical Association*. 219(1), pp. 67-71.

Valle, E., Gandini, M. & Bergero, D. (2013). Management of chronic diarrhoea in an adult horse. J*ournal of equine veterinary science.* 33(2), pp. 130-135.

Van Eps, A.W. & Pollitt, C.C. (2006). Equine laminitis induced with oligofructose. *Equine Veterinary Journal*. 38(3), pp. 203-208.

Weese, J.S., Holcombe, S.J., Embertson, R.M., Kurtz, K.A., Roessner, H.A., Jalali, M. & Wismer, S.E. (2015). Changes in the faecal microbiota of mares precede the development of post-partum colic. *Equine veterinary journal.* 47(6), pp. 641-649.

Weese, J.S., Toxopeus, L. & Arroyo, L. (2006). Clostridium difficile associated diarrhoea in horses within the community: predictors, clinical presentation and outcome. *Equine Veterinary Journal*. 38, pp. 185-188.

Weese, J.S., Parsons, D.A. & Staempfli, H.R. (1999). Association of Clostridium difficile with enterocolitis and lactose intolerance in a foal. *Journal of the American Veterinary Medical Association*. 214(2), 229-32.

Weise, J. & Lieb, S. (2001). The effects of protein and energy deficiencies on voluntary sand intake and behaviour in the Horse. *Proceedings 17th Equine Nutrition and Physiology Symposium*, pp. 103.

Wierup, M. (1977). Equine intestinal clostridiosis. An acute disease in horses associated with high intestinal counts of Clostridium perfringens type A. *Acta Veterinaria Scandinavica. Supplementum*. (62), pp. 1.

Willard, J.G., Willard, J.C., Wolfram, S.A. & Baker, J.P. (1977). Effect of diet on cecal pH and feeding behaviour of horses. *Journal of animal science*. 45(1), pp. 87-93.

Willing, B., Vörös, A., Roos, S., Jones, C., Jansson, A. & Lindberg, J.E. (2009). Changes in faecal bacteria associated with concentrate and forage-only diets fed to horses in training. *Equine veterinary journal*, 41(9), pp. 908-914.

Wilson, W.D. (2001). Rational selection of antimicrobials for use in horses. *Equine Veterinary Journal*. 47, pp. 75-93.

Wylie, C.E., Ireland, J.L., Collins, S.N., Verheyen, K.L.P. & Newton, J.R. (2013). Demographics and management practices of horses and ponies in Great Britain: A cross-sectional study. *Research in veterinary science*. 95(2), pp. 410-417.

Zehnder, C. (2009). Feldstudie zu Risikofaktoren für den Absatz von freiem Kotwasser beim Freizeitpferd (Doctoral dissertation, lmu).

Zeyner, A., Geißler, C. & Dittrich, A. (2004). Effects of hay intake and feeding sequence on variables in faeces and faecal water (dry matter, pH value, organic acids, ammonia, buffering capacity) of horses. *Journal of animal physiology and animal nutrition.* 88(1-2), pp. 7-19.

Popular science summary

Horses with free faecal liquid (FFL) show two-phase characteristics of their faeces, a solid phase with more or less normally-shaped faecal balls and one liquid phase (free faecal liquid). The liquid phase can be voided together with, or completely separately from, the solid phase. Free faecal liquid can cause problems with contamination of the tail and hind legs and can lead to skin irritation around the anus and on the inside of the hind legs, due to the constant presence of faecal liquid on the skin and also to the need for frequent cleaning of the horse. Horses with FFL can also show discomfort and irritation during defecation, as indicated by frequent tail swishing and nervous trampling with the hindlegs from side to side. The presence of FFL can constitute a welfare problem for affected horses, even if disease symptoms such as fever or inflammation are not present in FFL horses.

There are currently no known causes of FFL. A common perception is that feeding of wrapped forages (haylage or silage) is a cause of FFL, while other feed-related factors and different management routines have also been suggested as possible causes. However, feeding routines, different feeds and management factors as contributing causes to FFL have not previously been systematically investigated in horses. Further, faecal chemical composition in horses with FFL has not been investigated previously, but could provide important information for identifying factors of relevance for presence of FFL. It is also unclear whether some horse types are more prone to develop FFL than others. The aim of the studies presented in this thesis was therefore to map feeding and management routines and characteristics of horses with FFL, and to compare feed ration composition, feeding routines, management factors and faecal composition in horses with and without FFL. The general goal of the studies was to provide increased knowledge of FFL that may aid to identify factors that are important for the presence of the condition.

Implementation

Two large-scale studies were performed, an overall survey directed at owners of horses with FFL and a case-control study where matched pairs of horses with (case) and without (control) FFL were examined in more detail. In the survey, owners of horses with FFL were asked about the characteristics, feeding and management of their horse and about the presence of FFL. In the case-control study, feed ration composition, feeding routines, management factors and faecal chemical, bacterial and physical composition were compared between 50 horses with and 50 horses without FFL.

Results from the survey

The survey, which included 339 horses, revealed that all types of horses can show FFL, and no special management or feeding routine was overrepresented in the population of FFL horses. The feed rations were dominated by roughage, with a relatively low proportion of concentrates. Several of the horse owners (but not all) also reported reduced or absent signs of FFL when changing from one batch of wrapped forage to another, changing from wrapped forage to hay, or changing from wrapped forage to pasture. The survey results showed that 23% of horses with FFL had a previous history of colic, which was a high colic incidence compared with that reported for other similar horse populations. This may indicate that FFL and increased risk of colic are associated with each other, and it is of interest to investigate further.

Results from the case-control study – nutrition and management

The results from the case-control study, which included 50 horses with and 50 horses without FFL, showed only small differences in the type and amount of different feeds fed to horses with and without FFL. Horses with FFL were fed lower amounts of straw and higher proportions of concentrate compared with control horses. When comparing total daily intake of different nutrients, it was found that horses with FFL had a higher intake of starch and sugar and a lower intake of fibre and digestible crude protein than horses without FFL. As found in the survey, some horse owners (but not all) reported reduced or absent signs of FFL when changing from one batch of wrapped forage to another, changing from wrapped forage to hay, or changing from harvested forage to pasture. The conclusion was therefore that it may be appropriate to feed FFL horses with a feed ration that contains only roughage and mineral feeds, and that changing the batch of forage and/or providing pasture can in some cases reduce the presence of FFL.

Results from the case-control study – faecal composition

Results from the case-control study showed that horses with FFL had a lower faecal concentration and proportion of lactic acid than horses without FFL, despite the fact that FFL horses were fed more starch and sugar per 100 kg body weight, which was expected to give the opposite result. In addition, the faecal chemical composition did not reflect the differences found in the composition of the feed ration between case and control horses, as other variables such as pH, acetate, propionate and butyrate were similar in horses with and without FFL. This could be due to differences in microbial composition in the large intestine of case and control horses. The faecal bacterial composition was examined using DNA sequencing and identification of species. The faecal bacterial composition was in general similar between case and control horses, but the genera *Alloprevotella* spp. was found in higher relative abundance, and the genera *Bacillus* spp. in lower relative abundance, in the faeces from case horses compared with control horses. The importance of these bacterial genera for the function and health of the gastrointestinal tract in horses is currently not known, so future studies involving equine intestinal microbiota in both health and disease should consider these genera.

Faeces from horses with FFL was found to have a lower water holding capacity than faeces from horses without FFL. The water holding capacity can depend on several factors, such as fibre types present in the digesta and faeces and their ability to absorb liquid. This could in turn be affected by both the type and amount of feed, and the individual ability of the horse hindgut microbiota to degrade fibres.

Conclusions

This thesis demonstrated that FFL could be present in all types of horses and that horses with FFL in some cases show reduced or absent signs of FFL on changing forage batch or on changing from forage to pasture. It also showed that faecal microbial and chemical composition are generally similar in case and control horses, but that case horses had lower faecal lactic acid concentration than control horses. Presence of FFL is therefore probably not a sign of microbial dysfunction in the large intestine. The differences that were present in faecal physical properties between case and control horses as well as the higher colic incidence in a population of horses with FFL are of interest to study further as factors of importance for the presence of FFL.

Populärvetenskaplig sammanfattning

Fri fekal vätska (FFV) hos häst innebär att hästen avger träck i två faser; en fast fas med mer eller mindre formade träckbollar, och en vätskefas (fri fekal vätska). Vätskefasen kan avges tillsammans med eller helt skilt från den fasta fasen. Fri fekal vätska kan skapa problem med nedsmutsning av svans och bakben och leda till hudirritation kring anus och på insidan av bakbenen, både på grund av ständig närvaro av fekal vätska på huden och på grund av frekvent rengöring av hästen. Hästar med FFV kan också uppvisa obehag och irritation vid träckavgång genom frekventa svansviftningar och nervöst trampande med bakbenen från sida till sida. Förekomst av FFV kan därmed utgöra ett välfärdsproblem för drabbade hästar, även om det inte finns några indikationer på att hästen uppvisar några sjukdomssymptom som feber eller inflammatoriska tillstånd.

Det finns i dagsläget inga kända orsaker till varför FFV uppkommer. En vanlig uppfattning är att utfodring med inplastat vallfoder (hösilage eller ensilage) är orsaken till FFV, men även andra utfodringsrelaterade faktorer har föreslagits liksom olika skötselrutiner. Det finns dock inga studier där utfodringsrutiner, fodermedel eller olika skötselfaktorer undersökts systematiskt som bidragande orsaker till FFV. Träckens sammansättning hos hästar med FFV har inte heller undersökts, något som kan ge viktig information för att vidare utröna vilka faktorer som är relevanta för förekomst av FFV. Det är inte heller känt om vissa typer av hästar är mer drabbade än andra. Syftet med studierna som presenteras i denna avhandling var därför att kartlägga utfodring och skötselrutiner samt egenskaper hos hästar med FFV, samt att jämföra foderstatens sammansättning, utfodringsrutiner, skötselfaktorer och träckens sammansättning hos hästar med och utan FFV. Det generella målet med studierna var att ge ökad kunskap om FFV för att kunna identifiera vilka faktorer som är av vikt för uppkomst av tillståndet.

Studiernas genomförande

Avhandlingen består av fyra vetenskapliga artiklar baserade på två studier; en övergripande enkätstudie riktad till ägare av hästar med FFV, och en fall-kontrollstudie där matchade par av hästar med (fall) och utan (kontroll) FFV undersöktes mer detaljerat. I enkätstudien fick ägare till hästar med FFV svara på frågor om hästarnas egenskaper, träning, utfodring och förekomsten av FFV. I fall-kontrollstudien jämfördes foderstatens sammansättning, utfodringsrutiner, skötselfaktorer och träckens kemiska, bakteriella och fysikaliska sammansättning mellan 50 hästar med och 50 hästar utan FFV.

Resultat - enkätstudie

I enkätstudien som omfattade 339 hästar framkom att alla typer av hästar kan uppvisa FFV, och att det inte fanns någon särskild skötsel- eller utfodringsrutin som var överrepresenterad hos dessa. Foderstaterna dominerades av grovfoder med förhållandevis låg andel kraftfoder. Flera av hästägarna (men inte alla) rapporterade också att hästens träck ”normaliserades” vid övergång från ett parti inplastat vallfoder till ett annat, vid övergång från inplastat vallfoder till hö, eller vid övergång från skördat vallfoder till bete. Resultaten från enkätstudien visade också att 23 % av hästarna med FFV hade en tidigare kolikhistorik, vilket är en förhållandevis hög andel i relation till kolikförekomsten i jämförbara hästpopulationer. Det kan tyda på att FFV och ökad risk för kolik är associerade med varandra och det finns därmed starka motiv att undersöka detta vidare.

Resultat – fall-kontrollstudie - utfodring och skötsel

Resultaten från fall-kontrollstudien som omfattade 50 hästar med och 50 hästar utan FFV visade endast små skillnader i typ och mängd av olika foder som hästar med och utan FFV utfodrades med. Hästarna med FFV utfodrades med något mindre halm och mer kraftfoder jämfört med kontrollhästarna. Vid jämförelse av det totala dagliga intaget av olika näringsämnen visade det sig att hästar med FFV hade ett högre intag av stärkelse och lättlösliga kolhydrater och ett lägre intag av fibrer och smältbart råprotein jämfört med hästarna utan FFV. I likhet med resultatet från enkätstudien rapporterade även flera av hästägarna (men inte alla) i denna delstudie att fallhästens träck ”normaliserades” vid exempelvis övergång från ett parti inplastat vallfoder till ett annat, vid övergång från inplastat vallfoder till hö, eller vid övergång från skördat vallfoder till bete. Slutsatsen från studien var därför att det kan vara befogat att utfodra hästar med FFV med en foderstat som innehåller endast grovfoder och eventuellt mineralfoder, samt att byte av vallfoderparti eller övergång till bete i vissa fall kan avhjälpa FFV.

Resultat – fall-kontrollstudie - träckens sammansättning

Resultaten från fall-kontrollstudien visade att hästarna med FFV hade ett lägre innehåll av laktat i träcken jämfört med hästarna utan FFV, trots att FFV-hästarna utfodrades med mer stärkelse och lättlösliga kolhydrater per 100 kg kroppsvikt, vilket borde ha gett ett omvänt resultat. Träckens biokemiska sammansättning speglade i övrigt inte heller de skillnader som fanns i foderstatens sammansättning mellan fall- och kontrollhästar, då övriga träckvariabler som pH, ättiksyra, propionsyra och smörsyra var lika hos hästar med och utan FFV. Detta skulle kunna bero på olika mikrobiell sammansättning i grovtarmen hos fall- och kontrollhästar. Den bakteriella sammansättningen i träcken från fall- och kontrollhästar undersöktes via DNA-sekvensering och identifiering av förekommande arter. Träckens bakteriella sammansättning var generellt sett mycket lika mellan fall- och kontrollhästar, men släktet *Alloprevotella* spp. fanns i högre och släktet *Bacillus* spp. i lägre relativ förekomst hos fall- jämfört med hos kontrollhästarna. Betydelsen av dessa bakteriesläkten för magtarmkanalens funktion och hälsa hos häst är i dagsläget inte känt, men vidare studier som omfattar hästars tarmmikrobiota i sjukdom och hälsa bör inkludera dessa.

Träcken från hästarna med FFV visade sig ha en lägre vätskebindande förmåga jämfört med träck från hästarna utan FFV. Träckens vätskebindande förmåga kan bero på flera olika faktorer, en av dessa är vilka fibrer, och deras förmåga att absorbera vätska, som finns i grovtarmen, vilket i sin tur kan påverkas av såväl vilka foder som utfodras och i vilka mängder, som hästars individuella förmåga till fibernedbrytning i grovtarmen.

Slutsatser

Studiens resultat visade att FFV drabbar alla olika typer av hästar och att hästar med FFV i vissa fall kan uppvisa “normaliserad” träck vid byte av vallfoderparti eller vid övergång till bete. Resultaten visade också att träckens mikrobiella och kemiska sammansättning överlag var lika mellan fall-och kontrollhästar, att träck från fallhästar hade lägre laktathalt än träck från kontrollhästar, och därmed att FFV sannolikt inte är ett tecken på mikrobiell dysfunktion i grovtarmen. De skillnader som uppvisats i träckens fysikaliska egenskaper mellan fall- och kontrollhästar liksom den högre kolikincidensen i en population av FFV-hästar är av intresse att undersöka vidare som faktorer av vikt för förekomst av FFV.

Acknowledgements

First, I'd like to acknowledge the **Swedish-Norwegian Foundation for Equine Research** for funding this project**.** Second, I'd like to acknowledge the **Department of Animal Nutrition and Management** for providing research facilities. The **Institute of Animal Nutrition, Nutrition Diseases and Dietetics**, at University of Leipzig is also greatly acknowledged. The **SLU fund for internationalisation of postgraduate studies (FUR)**, The **Royal Swedish Agricultural Academy (KSLA)** and **Ellen and Tage Westin Foundation for Agricultural Research** are gratefully acknowledged for their contributions to travels associated with the project. And last, but not least, my great appreciation to all **horse owners** (and their horses) for participating in the studies.

There are a number of people (supervisors, colleagues, friends and family) who have, in different ways, helped out during the work with this thesis, and to whom I would like to express my sincere gratitude:

Cecilia Müller, my main supervisor. Thank you for introducing me in the field of equine nutrition when I first started my education at Ultuna (9 years ago!). I am very grateful for all the things that I have learned from you throughout the years and especially during the work of this thesis. Thanks for always having time for my questions, for cheering me on during the project and for pointing me in the right direction when needed. You have made me believe in myself and my work, and I think that I have been very fortunate to have you as my main supervisor. Thank you for allowing me to be part of this project!

I would also like to give my greatest gratitude to my co-supervisors for having your support during the work of my thesis and the papers included, for believing in me and for all things you have taught me. Thank you **Jan-Erik** Lindberg for all the nice discussions about equine nutrition, for making me think outside the box and see things in a bigger perspective. Thank you **Johan** Dicksved for all the support, for taking the time to answer all my questions, having nice discussions and for introducing me to the super interesting field of microbiota. Thank you **Viveca** Båverud for all interesting discussions and for cheering me on during the project. I thought it was very nice to have your support, especially during the work with *Clostridia* which was a completely new field for me. Thank you **Ingrid** Vervuert for joining the supervisor group and for inviting me to work in Leipzig. It has been great to work with you having so much experience in equine nutrition, but also having experience in the specific subject of the thesis.

Also many thanks to the other co-authors for your contribution to the papers included in this thesis. **Astrid** Johansen for helping out with the organisation of sampling collection in Norway and for your valuable inputs regarding horse nutrition. **Erik** Pelve for introducing me to the world of bioinformatics.

I would also like to acknowledge the agricultural advisor **Eva** Dahlström who assisted in identifying farms and for having valuable inputs on the project.

Tack till alla **nuvarande och före detta arbetskamrater på HUV** för att jag har fått vara, och känt mig som, ”en i gänget”. Tack **labbet** för all hjälp med alla träck och foderanalyser och tack **administrationen** för svar på alla frågor, för all hjälp med datorstrul och för att ni även efter 10 000 gången förklarar hur en reseräkning fungerar med ett leende.

Tack **Ann-Sofie ”Fiffi”** Waxin **(och Lena)** för all hjälp med materiel under min tid i labbet och alla pratstunder.

Tack **Lise-Lotte** Fernström och **Sara** Frosth för att ni visade mig extraktions-roboten och för att jag fick ockupera ert labb då och då.

Tack så mycket **Claudia** von Brömssen och **Ulf** Olsson för svar på alla frågor och all hjälp med statistiken. Also, thanks to **Mikhail** Churakov for finally making me friends with R ☺.

Thank you **Mary** McAfee for helping to improve the language in this thesis.

Thank you **Carola, Janine, Dominique** for opening up your home and hearts to me when I came to Leipzig, making sure that I would not get lost on my bike, on the way between pubs or at the University.

Thanks to all present and former **PhD Students**! I am so happy to have been part of the PhD group at HUV and for all fun things we have done!

Tack mina vänner och doktorandkollegor **Lidija, Hanna, Sofia** och **Malin** för att ni dragit med mig på välbehövliga kafferaster på Brokers, för att jag alltid känt att jag kan höra av mig till er vara sig det gäller jobb eller inte, stort som smått. Tusen tack!

Tack **Anna,** min rumskompis och vän för alla pratstunder och för all peppning. Jag är så glad att vi hamnade i samma rum! Du är den mest positiva person jag vet och det smittar av sig! Det var ju även rätt praktiskt då vi kunde hjälpas åt att komma ihåg saker åt varandra ☺

Tack mina vänner och doktorandkollegor **Eliška** och **Thomas** (och såklart Tove) för alla roliga pluggstunder (som vi kämpade med bioinformatiken Thomas), grillkvällar, spontana kaffestunder hemma hos er och att ni alltid finns där!

Tack **Haldis** för allt roligt vi har varit med om tillsammans, allt skratt och långa samtal på kvällarna för att någon av oss behöver prata av oss eller för att diskutera R-koder och programmering.

Tack mina fina vänner **Natalie, Maria, Josefin** och **Joss** för ni alltid finns där, vad det än gäller! Speciellt stort tack till **Elise** för all hjälp och att du alltid får mig att skratta när jag som mest behöver det ☺ Tusen, tusen tack för allt!

Tack alla stallkompisar på **stall Åby** för all hjälp, och speciellt tack till **Ullis** som har hjälpt mig så mycket under denna tid med mina hästar (framförallt med Bacchus). Jag hade absolut inte gjort det bättre själv ☺.

Tack **Amoorina** och **Bacchus** för att jag oavsett vilket humör jag är på när jag åker till stallet, alltid kommer hem med ett stort leende på läpparna. Finns inte mycket som slår en hästkram! Tack **Figaro** för allt pluggsällskap genom alla år och för att du tvingat mig att ta pauser genom att omvandla mitt tangentbord till en skön sovplats.

Tack min älskade **familj** för att ni alltid trott på och stöttat mig, och speciellt tack till dig **mamma**! Jag skulle aldrig ha klarat detta utan dig. Du har ALLTID funnits där för mig oavsett vad. Jag har inte ord nog för att beskriva hur mycket du betyder för mig! Tack♡

Tack **Robert** för att du alltid får mig att stanna upp och vara i nuet. Denna resa hade varit så mycket svårare utan allt ditt stöd. Du är min bästa vän och min trygghet. Jag älskar dig ♡

Appendix

Survey

This survey were used for collect data from horse owners of horses with FFL in paper I. The same survey was used for data collection in paper II-IV, but with additional questions on whether the horse showed signs of FFL or not was also asked for. Bulleted points indicate the response of the questions and different response alternatives are comma-separated. Space was provided for alternative answers where necessary.

1. In which region is your horse stabled?
o Northern Sweden/Norway, Central Sweden/Norway, Southern Sweden/Norway

2. How old is your horse? (Years): ______

3. Which breed is your horse? *If crossbred, enter the breeds you know*
o Arabian, Angoloarabian, Thoroughbred, Swedish warm-blood (SWB), Standardbred, Cold-blood trotter, North Swedish draught-horse, Ardenneais, Gotland pony, Shetland pony, Connemara pony, New Forest pony, Welsh pony, Welsh Cob, Friesian horse, Haflinger, Quarter horse, Paint horse, Appaloosa, Tinker horse, Clydesdale, Shire horse, Icelandic horse, P.R. E (Pura Raza Española, Andalusian), Lusitano, Riding pony, Crossbred: ____________

4. Which gender is your horse?
o Mare, Gelding, Stallion

5. Is your horse born and bred in Sweden/Norway?
o Yes, Don’t know, No; imported from: _____________

6. What is the colour of your horse? *Choose the colour closest to the colour of your horse.*
o Grey, Bay, Black, Chestnut, Paint, Palomino/Isabelline, Leopard pattern, Buckskin, Cremello, Other: ________________

7. What is the withers height of your horse? *Type the answer in cm.*____________________________

8. What is the weight of your horse? *Type the answer in kg. Help: Approximate weight for different breeds: Shetland pony 100-200 kg, Gotland pony 150–250 kg, Icelandic horse 250–400 kg, Arabian horse 350–500 kg, Thoroughbred 400–600 kg, Standardbred 400–600 kg, Swedish warmblood (SWB) 450–700 kg, Ardenneais 700–900 kg.* ____________________

9. My horse is:
o An easy keeper (needs less feed than the average horse to maintain its body condition), A hard keeper (needs more feed than the average horse to maintain its body condition), A normal keeper.

10. The body condition score (BCS) of my horse is at the moment:
o 0, 1, 2, 3, 4, 5

11. I think my horse is (*Multiple responses possible)*:
o Calm, Nervous, Curious, Introvert, Happy, Tense, Alert, Lazy, Hardworking, Unwilling, Stressed, Uninterested, Active, Passive, Irritable, Angry

12. Which disciplines do you perform with your horse? *(Multiple responses are possible)*
o 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:_______

13. Which training intensity is consistent with your horse's training? *Choose one option*

o Very low (maximum 30 min/day, 1–3 days/week, mainly in walk), Low (e.g., leisure riding, 30–60 min per day, 4–7 days/week, all gaits), Medium (e.g., riding school, more intense leisure riding, all gaits), High (e.g., cross country, high-level show jumping, all gaits), Very high (Trotting and racing, high-level cross country training, endurance), Breaking in, Others:_______

14. My horse is kept as follows during winter:
o 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, Individual 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: _____________

15. How long does your horse spend outside in a paddock during wintertime? *Type your answer in number of hours per day*.____________
16. What type of paddock is your horse kept in during wintertime?
o Grass paddock with grass all year round (old grass during winter), Sand/Gravel, Soil/Clay,
Other type of paddock: ____________

17. Which bedding material(s) do you use in your horsebox/stall/loose housing system? *(Multiple responses are possible)*
o Straw, Shavings, Sawdust, Wood, Paper, Mix of sawdust and peat, Rubber mat, Raw sawdust, Straw pellets, Sawdust pellets, Other:_________________

18. My horse has access to water in the stable/loose housing system in the following way during winter;
o Frostless waterer, Frostless tub, Waterer, Tub, Bucket, Natural water sources, Other:___________

19. My horse has access to water in the paddock in the following way during winter;
o Frostless waterer, Frostless tub, Waterer, Tub, Bucket, Natural water sources, Other: ___________

20. Does the horse have access to a saltlick in the stable/loose housing system?
o Yes, No, Yes; and also gets extra salt in feed, Yes; and also gets extra salt in special water buckets, No; gets extra salt in feed instead, No; gets extra salt in special water buckets, Other_________________

21. Is your horse kept at pasture during summer?
(Meaning that the horse covers all or part of its nutritional requirements from grass)
o Yes; less than 4 weeks, Yes; 4–8 weeks, Yes; 8–12 weeks, Yes; longer than 12 weeks, No, Other: _______

22. Which type of pasture is your horse kept at during summer?
o Pasture established on cropland, Natural pasture, Forest, No pasture, Other type of pasture: ________

23. Does your horse have access to a saltlick while on pasture?
o Yes, No, Yes; and also gets extra salt in feed, Yes; and also gets extra salt in special water buckets, No; gets extra salt in feed instead, No; gets extra salt in special water buckets, My horse is not let out on pasture,
Other: _______________

24. My horse has access to water at the pasture in the following ways during summer;
o Frostless waterer, Frostless tub, Waterer, Tub, Bucket, Natural water sources, Other: ______________

25. Which of the following best describes your deworming routines?
o 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: ___________________

26. When was your horse last dewormed?
o I have never dewormed my horse, 0–3 months ago, 3–6 months ago, 6–12 months ago, >1 years ago, Other: _________________

27. Which roughage(s) is your horse fed at the moment? *Choose one or more options*.
o Small bale hay, Big bale hay, Loose hay, Big bale grass haylage (at least 50% DM), Small bale grass haylage (at least 50% DM), Big bale grass silage (under 50% DM), Small bale grass silage (under 50% DM), Straw, Lucerne, (pelleted) Lucerne (chopped), Other: ________________

28. Is the forage bought or produced on the farm?
o Bought, Produced on farm (but not by the owner), Produced on farm, by the owner, Other: _________

29. Is the forage analysed for its nutritive contents?
o Yes, No, Don't know______________________________

30. 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.
o Dry matter (%), Energy (MJ/kg DM), Digestible crude protein (g/kg DM), Ca (g/kg DM), P (g/kg DM), Mg (g/kg DM):___________

31. Do you feed your horse any concentrate (s)?
o Yes, No

32. Which type of concentrate(s) do you feed your horse? *Choose one or more options*.
o Oats, Barley, Molassed sugar beet pulp, Linseed/linseed cake, Soybean meal, Potato protein, Wheat bran, Vegetable oil, No concentrate(s), Other (write brand and type): ________________

33. Do your horse get any supplement feeds? (E.g. mineral feeds, vitamin feeds, herb supplements etc.)
o Yes, No

34. What type of supplemental feeds do you give your horse?
o Mineral feeds, Multivitamin feeds, B-Vitamin feeds, Selenium + E-vitamin additive, Garlic, Herbs, Do not feed any concentrate,
Other (specify brand and type): ______________________________

35. Which amounts (gram 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., “3 kg hay and 5 kg grass haylage”.
o Forage (including hay, grass haylage and glass silage) (kg/day), Concentrate (kg/day), Straw (kg/day), Lucerne (kg/day) Additional feeds (g/day)

36. How many times per day is your horse fed roughage?
o 0 times, 1 times, 2 times, 3 times, 4 times, >4 times, Free access

37. How many times per day is your horse fed concentrate?
o 0 times, 1 times, 2 times, 3 times, 4 times, >4 times, Free access

38. How many hours is it at the most between two feedings of roughage?
o 0–2 h, 2–4 h, 4–8 h, 8–12 h, >12 h, Free access, Don’t know

39. How is the forage fed in the paddock?
o Forage is not fed in the paddock, On the ground, In a feeding rack, In a haynet, In a tub or similar,
Other way: ________________

40. How do you store your forage? (If you feed your horse wrapped forage the question concerns opened bales).
o Indoors (stall, barn or similar), Outdoors (under roof), Outdoors (no roof),
Other: __________________

41. How do you store your concentrate feeds?
o In covered/closed container indoors, Uncovered/open container indoors, In paperbags/original package indoors, Do not feed concentrate, Other________________

42. Has your horse showed loose faeces when fed wrapped forages?
o Yes—generally loose faeces which looks like “cow pat”, Yes, solid faecal balls but also free faecal liquid—Yes, diarrhoea without solid faecal balls, No,
Other: ________________________________

43. If your horse has showed loose faeces when fed wrapped forages, has it become better or good when:
o 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: ___________

44. 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?
o 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):____________

45. Has your horse shown loose faeces when fed hay?

o 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: ___________

46. Has your horse showed any of the following signs during an episode of loose faeces when fed wrapped forages and/or hay? *Choose one or more options*
o My horse have never showed loose faeces when fed wrapped forages or hay, Colic, Skin problems (e.g., nodules and urticaria, but not summer eczema), Swollen legs not caused by training or injury, Bloated abdomen, Irritation while voiding faeces (swishing tail and/or trampling with hindlegs), None of the options,
Other: ____________________

47. Does your horse have a history of previous colic episodes?
o Yes, No, Don't know

48. Has your horse been examined and diagnosed with gastric ulcers by a veterinarian?
o Yes—my horse has been examined by a veterinarian and has been diagnosed with gastric ulcers, Yes—my horse has been examined by a veterinarian but has not been diagnosed with gastric ulcers, No—not examined, Don't know

49. Has your horse been treated by a veterinarian for any other diseases/conditions in the gastro-intestinal tract?
o No, Don't know, Yes—my horse has been treated for:____________

50. Does your horse show any of following behaviour?
o Crib biting, Wind sucking, Weaving, Box walking (walk around in the box in a repeated pattern), Self-biting (bites itself on the sides/flanks), Wood chewing (e.g., stable interior, fence, but not trees and bushes), Tongue rolling ("chewing on the tongue" in a repeated pattern, e.g., before feeding), My horse do not show any of the behaviours listed,
Other: ____________

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2020:65

This thesis investigated the faecal composition, feeding and management of horses with free faecal liquid (FFL). Results showed that horses with and without FFL in general had similar faecal bacterial composition, but differences in specific faecal bacterial taxa were present. Faecal lactate concentration was lower in horses with than without FFL, but other chemical components in faeces were similar. Type and amount of feeds and daily intake of nutrients differed between horses with and without FFL, but management was similar.

Katrin Lindroth received her postgraduate education at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences (SLU), and obtained her Master of Science degree in Agriculture (Animal Science) at the same university.

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Online publication of thesis summary: http://pub.epsilon.slu.se/

ISSN 1652-6880
ISBN (print version) 978-91-7760-648-2
ISBN (electronic version) 978-91-7760-433-4