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Final report

Haylage ”intolerance” in horses?

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Part 1: Detailed summary

”Hösilageintolerans” är ett begrepp som ofta används för att beskriva förekomsten av tvåfasindelad träck hos hästar. Träcken är då indelad i en fast fas och en vätskefas, där den sistnämnda kan skapa problem med nedsmutsning och hudirritation kring anus och på insidan av hästens bakben. En vanlig uppfattning är att utfodring med inplastat vallfoder (hösilage) är orsaken till förekomst av fri fekal vätska (FFV), men det finns inga systematiska studier där utfodringens roll undersökts. Det är inte heller känt om vissa typer av hästar är mer drabbade än andra, eller om olika skötselrutiner inverkar på förekomsten av FFV. Syftet med studien var därför att jämföra utfodring, skötselrutiner, individuella egenskaper hos hästen, foderstatens sammansättning och träckens egenskaper hos hästar med och utan FFV, för att om möjligt identifiera faktorer inom dessa områden som är associerade med FFV. Studien bestod av två delar; en översiktlig enkätstudie riktad till hästägare med en häst med FFV, och en fall-kontrollstudie där matchade par av fall- och kontrollhästar undersöktes mer detaljerat. I enkätstudien, som omfattade 339 hästar, framkom att alla typer av hästar kan vara drabbade av FFV, och att det inte fanns någon särskild skötsel- eller utfodringsrutin som var överrepresenterad. Foderstaterna dominerades av grovfoder med förhållandevis låg andel kraftfoder. Ungefär 60 % av respondenterna rapporterade att hästens träck ”normaliserades” vid en övergång från inplastat vallfoder till hö, nästan 50 % rapporterade detsamma vid en övergång från skördat vallfoder till bete, och ca 20 % rapporterade motsvarande vid byte från ett parti inplastat vallfoder till ett annat. Begreppet “hösilageintolerans” är alltså inte korrekt och beskriver inte någon generell orsak till FFV. Resultaten visade också att 23 % av hästarna hade en tidigare kolikhistorik, vilket är en förhållandevis hög andel jämfört med kolikförekomsten i andra jämförbara hästpopulationer. Det tyder på att förekomst av FFV och ökad risk för kolik kan ha ett samband, vilket är ny kunskap.

I fall-kontrollstudien jämfördes utfodrings- och skötselfaktorer liksom foderstatens och träckens sammansättning mellan hästar med och utan FFV (totalt 50 matchade hästpar). Resultaten visade att fallhästarna utfodrades med något mindre halm och något mer kraftfoder jämfört med kontrollhästarna. Skillnaderna var små men statistiskt säkerställda. Trots detta avspeglade träckens biokemiska sammansättning inte skillnaderna i foderstaternas sammansättning som förväntat, då fallhästarnas träcksammansättning mer likande den som uppkommer då hästar utfodras med högre andel grovfoder. Detta skulle kunna förklaras av skillnader i tarmmikrobiotan mellan fall- och kontrollhästar. Fallhästarna hade också en tendens till högre koncentration av sand i träcken jämfört med kontrollhästarna. Inga tydliga förklaringar till detta påträffades i utfodrings- och skötselrutiner, men det skulle kunna bero på skillnader i ät- eller betesbeteende som kan medföra ett större eller mindre

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intag av sand via foder eller bete. Det är av intresse för förekomst av FFV, eftersom förekomst av sand/grus i grovtarmen tidigare har associerats med kronisk diarré hos hästar. Den näringsmässiga och hygieniska kvaliteten i de vallfoder som utfodrades till fall- och kontrollhästar (varje par utfodrades med samma vallfoder) föranledde inga anmärkningar med undantag för några enstaka foderprov där antalet jästsvampar och klostridiesporer var ovanligt högt. Betydelsen av detta för förekomst av FFV är oklar. Studiens resultat har påvisat att utfodring med inplastat vallfoder inte verkar vara en generell orsak till FFV, men för drabbade hästar kan det vara befogat att prova att byta vallfoderparti. Resultaten understryker också vikten av fortsatta studier på hur hästens grovtarmsjäsning och -funktion påverkas av olika fodermedel, för att om möjligt förebygga uppkomst av störningar som FFV och kolik. För sådana studier är användning av metoder som beskriver grovtarmens funktion på mer än ett sätt av överordnad betydelse då resultaten i den här studien inte kunde förklaras av enbart t ex mikrobiotans sammansättning.

Part 2: Main report

Background and objective

Traditionally hay has been the main forage source in equine nutrition, but during the last 15 to 20 years wrapped forages such as silage (300-500 g dry matter (DM)/kg) and haylage (>500-840 g DM/kg) has partly or fully replaced hay. Data from Sweden estimates that approximately half of the horse-owners use wrapped forages and the other half hay (Müller, 2018), but the proportion varies with *e.g.* weather at harvest every year, and a large proportion of horses are fed both forage types overlapping. In Norway, about 70 % of the horse-owners feed hay and 90 % feed wrapped forages, also overlapping (Müller, 2018). There are advantages and disadvantages with both forage types in equine feeding, largely depending on management and storage issues that may affect the hygienic quality of the forage. Previous research have shown that equine digestion of silage, haylage and hay is similar and that the conservation method of the forage does not seem to affect microbial or biochemical composition in the equine hindgut (Müller et al., 2008; Müller, 2018). However, during the last approximate 10 years, a condition known as “haylage-intolerance” in Sweden, has gained attention (Valle et al., 2013; Kienzle et al., 2016). “Haylage intolerance” manifests as two-phase faeces with one solid phase (faecal balls) and one liquid phase. The liquid phase may be voided together with the solid phase or separately, and sometimes together with excessive gas. Affected horses may show discomfort when voiding faeces by extensive tail swishing and/or nervous trampling with hindlegs, but do not show other signs of illness such as pyrexia, inappetence, weight loss etc. The constant presence of faecal liquid around the anus and on the inside of the hindlegs (Figure 1) may however result in skin lesions, and the welfare of affected horses can be questioned. The condition also causes additional work with cleaning affected horses several times daily. The cause of “haylage intolerance” is not known, although one or more feed components is suspected (Valle et al., 2013). Individual horse factors such as coat colour and temperament/rank order has been reported as being of importance for the condition (Kienzle et al., 2016). However, no studies exist where a systematic mapping of feeding, management, horse characteristics, associated health problems or faecal composition of affected and unaffected horses has been performed. The aim of this study was therefore to:

a) map the presence of “haylage intolerance” in Sweden and Norway through an Internet survey (Survey study)

b) Map and compare feeding and management including hygienic and nutritive composition of wrapped forages used for affected and unaffected horses in Sweden and Norway (Case-control study)

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c) Map and compare faecal microbial, biochemical and physical composition in affected and unaffected horses in Sweden and Norway (Case-control study)

Figure 1. Faecal liquid below anus and on inside of hindlegs in affected horses (mild case to the left, more severe case to the right). Photo: C. Müller (left) and D. Ross (right) ©.

Material and methods

Survey study

An online survey directed to owners of horses showing "haylage intolerance" when fed wrapped forages was performed in Sweden and Norway. The survey was created using the tool Netigate (Netigate, Stockholm) and was advertised through the websites of the Department of Animal Nutrition and Management, SLU (http://www.slu.se/sv/institutioner/husdjurens-utfodring-vard/), Hästsverige (www.hastsverige.se), and Norsk Hestesenter (http://www.nhest.no/). The survey was open from March year 2016 to March year 2017, and was available in both the Swedish and Norwegian languages. The respondents answered 53 questions grouped into six main parts; (1) horse characteristics (age, gender, breed, colour, body condition score (Carroll and Huntington, 1988) and temperament); (2) training (discipline, intensity) and management (type of housing system, paddock use); (3) type and amount of feeds used (roughage, concentrates and feed supplements); (4) feeding strategies (number of feedings, time between feedings, use of feed racks etc, type of water source); (5) faecal appearance (presence of "haylage intolerance" and descriptions of clinical signs), changes in faecal appearance with changes in feeding and management, number of affected horses kept in the same housing system, and (6) previous history of gastro-intestinal diseases (e.g. gastric ulcers, colic). The inclusion criteria were that horses should be >2 years old, showing "haylage intolerance" when fed wrapped forages, have no other known gastro-intestinal disease at the time (*e.g.* gastric ulcers, colic) and not have been treated medically for the last three months. Survey data was analysed statistically with SAS (Statistical Analysis System Institute Inc., Cary, NC, USA) version 9.4 for Windows.

Case-control study

A case-control study was performed in 50 stables (30 in Sweden and 20 in Norway) with one horse pair in each stable. The case and the control horse in each matched pair were housed in the same stable and kept in the same or in adjacent paddocks, and were fed the same forage. All horse owners responded to the same questionnaire as used in the survey study to provide information about the horse, feeding and management. Horse owners also registered the faecal appearance of both case and control horses in a monthly "diary", and provided an analytical report on faecal egg count for intestinal parasites. Faeces from each horse was sampled three times (in October/November, in January/February, and in March/April) and each horse pair was sampled simultaneously. The forage was sampled at the same occasions as the faeces. All samples were sent to the Department of Animal Nutrition and Management, SLU for analysis of microbial and chemical composition with methods described by Müller *et al.* (2008; 2011). Faecal samples were also analysed for presence of sand (mm in test tube) and free liquid volume (ml supernatant) by centrifugation, and for particle size distribution according to Fritz *et al.* (2012). Particle size distribution was analysed at Institut für

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Tierernährung, Ernährungsschäden und Diätetik, Veterinärmedizinische Fakultät, Universität Leipzig, with the help of Dr Med Vet Ingrid Vervuert. Water holding capacity of faeces and forages was analysed as volume water absorbed by dried and milled samples and expressed as ml absorbed/g sample. In addition, faecal samples were analysed for presence of *Clostridium perfringens* and *Cl. difficile* by microbial cultivation at the National Veterinary Institute, Uppsala (Båverud et al., 2003), and for microbial composition by DNA-sequencing of the 16s rDNA using Next Generation Sequencing platform Illumina MiSeq at SciLife Lab, Sweden. Technical problems with markers in the DNA-sequencing was detected (for several different projects in the same analytical run at SciLife Lab), which made results unusable and therefore new samples were sent to Neogen Corporation, Hong Kong for sequencing. A protocol for DNA-extraction from equine faecal samples was developed and can be provided upon request.

All statistical analyses were performed using SAS (Statistical Analysis System Institute Inc., Cary, NC, USA) version 9.4 for Windows. Descriptive analysis and univariate/multivariate logistic regression analysis were performed for all variables from the questionnaire data sets using the PROC FREQ, PROC LOGISTIC, and PROC GLIMMIX procedures in SAS. Statistical evaluation of data from forage and faeces composition in the case-control study was performed with variance analysis using a matched t-test with repeated sampling. Means where $P < 0.05$ were regarded as statistically different. Missing values were treated as such in the statistical analysis. For comparisons of microbial composition in faeces from case and control horses a Principal Coordinate Analysis (PCoA) and an analysis for similarity between groups were performed using ANOSIM analysis in PAST. Univariate analysis was performed using R and the Mann-Whitney test. Additionally, a false discovery rate (FDR) using Benjamini-Hochberg test was performed to exclude bacteria with false positive P-values. Significant variables were then analysed in a multivariate model using generalized linear mixed models (GLMM) in SAS (version 9.4) were farm were included as random.

Results and discussion

Survey study

In total, 593 responses to the survey were obtained of which 339 fulfilled the inclusion criteria. The included responses comprised horses from a large variety of breeds, ages, geographic regions, stable systems and training disciplines, and horses of all coat colours and body condition scores were represented. Fifty-two percent of the respondents reported that it was only their horse in the stable that showed signs of FFL, while 48 percent reported that other horses in addition to their own showed FFL. Data on feeding showed that feed rations were dominated by roughage with low proportions of concentrates (Table 1). Management factors such as water source, type of bedding materials, deworming strategies and pasture access etc was comparable to what has been reported for other horse populations in the same geographic region (Lindroth & Müller, 2017; Larsson & Müller, 2018). About 60 % of the respondents reported “normalized” faecal appearance when changing from wrapped forage to hay, and 46 % reported the same when the horse was kept on pasture. Over 20 % of respondents reported “normalized” faecal appearance when changing from one to another batch or harvest of wrapped forage. These results showed that “haylage intolerance” could be present in any type of horse being kept in any type of system, and that the faecal appearance “normalized” in some horses when changing from wrapped forage to hay, but not in all. This means that the term “haylage intolerance” is incorrect and it has therefore been replaced with the term free faecal liquid (FFL) as it is a more correct description of the clinical signs. The data also showed that 23 % of the horses had had a previous history of colic, which was comparably high as general colic incidence has been reported to be below 10 % in other horse populations (e.g. Tinker *et*

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al., 1997; Traub-Dargatz *et al*., 2001) and around 5 % in another horse population in the same geographic area (Larsson & Müller, 2018). This implies that presence of FFL may indicate increased risk of colic, which is new knowledge.

Table 1. Minimum, maximum, quartiles, mean and standard deviation (SD) per 100 kg body weight and day for feeds fed to the horses in the survey study, and proportions of roughage and concentrates in the daily total feed ration (n=339)[1]. Calculated from data reported by respondents

Variable	No. of horses	Min	Q1	Q2	Q3	Max	Mean	SD
Total amount of roughage[1] kg DM	339	0.3	1.5	2.0	3.1	4.8	1.8	2.17
Hay, kg DM	97	0.1	0.2	0.2	0.3	0.5	0.2	0.06
Haylage, kg DM	118	0.2	1.7	2.0	2.3	4.8	2.0	0.67
Silage , kg DM	2	1.2	1.2	2.9	3.8	4.7	2.9	1.73
Straw, kg DM	50	0.1	0.2	0.3	0.6	2.3	0.4	0.45
Lucerne, kg DM	75	0.01	0.1	0.1	0.2	0.7	0.1	0.13
Roughage proportion of total feed ration (%)[2]	217	20	90	100	100	100	90	0.14
Total amount of concentrate, kg DM	257	0.01	0.1	0.2	0.3	1.0	0.2	0.18
Concentrate proportion of total feed ration (%)[2]	217	0	1	5	10	80	7	0.14
Mineral feeds, g	208	0.1	6.0	10.8	17.8	83.3	13.5	11.43

[1]Horses reported to have *ad libitum* access of roughage not included. [2]Horses reported to have roughage *ad libitum* with no concentrate feeding, and horses fed roughage in specified amounts were included. DM= dry matter, BW= body weight, Q1= first quartile (25%), Q2= second quartile (median), Q3= third quartile (75%).

Case-control study- individual factors and management

No management variables had P<0.05 in the univariate regression analysis, indicating that these factors did not differ between case and control horses. Individual horse factors such as gender, age, breed, coat colour, training discipline etc were similar among case and control horses, and could not explain the presence of FFL as has previously been suggested, where being a paint, being a gelding and being low in rank increased the risk of being an FFL-affected horse (Kienzle et al., 2016).

Case-control study - feeds and feeding

Comparisons of feeding of case and control horses showed that case horses were on average fed lower proportions of roughage and higher proportions of concentrates in their total feed ration compared to controls, but differences were small (Table 2). Case horses were also fed less straw and less NDF in the total feed ration compared to control horses (Table 2). Nutritive value of forages varied among participating farms (selected variables reported in Table 3), but were within the intervals reported in similar studies in this geographic region (Lindroth & Müller, 2017; Larsson & Müller, 2018). Microbial analysis of forages showed variation in all analysed microbial groups (Figure 2), however median values of yeast, moulds, enterobacteria and clostridial spores were below recommended maximum values in forages for horses (Jansson et al., 2011). Values in some samples greatly exceeded the recommended maximum values for these microbes. As both case and control horses were fed the same forages, it was not possible to draw any conclusions about the influence of forage microbial composition on presence of FFL from the data in this project. Further studies where forage microbial composition and its influence on the hindgut microbiota is under study will be required for such investigations.

Case-control study- faeces

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Comparisons of biochemical composition in faeces showed that case horses had higher (P<0.03) concentration of butyric (3.3 mmol/L) acid compared to control horses (2.8 mmol/L). Case horses also tended (P=0.09) to have higher faecal acetate concentration than control horses as well as higher total short chain fatty acid (SCFA) concentration (P=0.05). This resulted in a higher ratio of acetic + butyric acid to propionic acid in faeces from case compared to control horses (Figure 3), indicating a difference in the net products of hindgut fermentation and absorption. These differences were small but of interest as they were not aligned with the feeding data. Generally, a higher inclusion of concentrates in the diet results in higher concentrations of SCFA and lower acetate+butyrate:propionate ratio (*e.g.* Müller et al., 2008). Case horses were however fed diets with a slightly higher proportion of concentrates than control horses, but their faecal biochemical profile were the opposite. This result may be explained by the microbial composition in faeces in case and control horses.

Table 2. Minimum, maximum, mean and standard deviation (SD) per 100 kg body weight and day of selected feed ration variables for horses with and without FFL, as reported by the participants in the case-control study (n=100, 50 case and 50 control horses if not otherwise mentioned)

Variable	**Case**				**Control**				
	Mean	SD	Min	Max	Mean	SD	Min	Max	p-value
Haylage, kg DM	1.4	0.50	0.4	3.0	1.4	0.45	0.3	3.0	0.19
Straw, kg DM (n=7 case, n= 6 control)	1.5	1.17	0.4	4.2	1.7	0.84	0.8	3.4	0.04
Total NDF, kg	1005	413.1	173	2815	1035	421.9	246	2745	<0.001
Concentrate, kg DM[1] (n=27 case, n= 31 control)	0.19	0.194	0.01	1.00	0.16	0.127	0.01	0.50	0.04
Mineral feeds, g	20	0.9	1.8	40.0	20	0.9	1.0	40.0	0.89
Total feed ration kg DM	1.9	0.95	0.4	6.8	1.9	0.90	0.4	5.8	0.10
Proportion of roughage, % in total diet	93.9	7.7	57.0	100.0	93.5	7.8	52.1	100.0	<0.001
Proportion of concentrate, % in total diet (n= 27 case, n=31 control)	9.7	8.22	0.5	42.7	9.2	8.16	0.7	47.9	<0.001

[1]Concentrates include grains and commercial feed mixtures (e.g. müsli, pelleted concentrates etc).

Table 3. Chemical composition, concentration of metabolizable energy and pH in wrapped forages used for the horses in the case-control study. Mean, standard deviation (SD), minimum and maximum value of selected variables for 50 forage batches with three replicate samples for each forage batch

Variable	**Mean**	**SD**	**Min**	**Max**
Dry matter, g/kg	688.5	128.5	378.0	884.0
Metabolizable energy, MJ/kg DM	9.2	0.89	6.9	11.0
Digestible crude protein, g/kg DM	55	23.0	10	120
Neutral detergent fibre, g/kg DM	611	37.2	532	684
Acid detergent fibre, g/kg DM	335	34.5	292	486
Lignin, g/ kg DM	35	5.8	24	49
Water soluble carbohydrates, g/kg DM	104	32.7	30	165
Ash, g/kg DM	59	11.5	37	87
pH	5.40	0.321	4.20	5.90

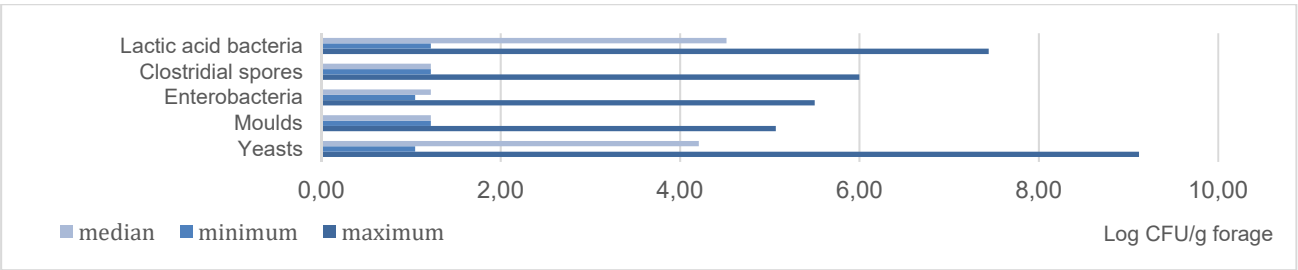

Figure 2. Microbial composition (minimum, median and maximum values) in log CFU/g in forage samples from 50 pairs of matched case and control horses in Sweden and Norway.

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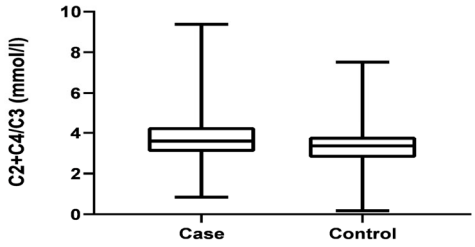

Figure 3. Ratio of acetic acid (C2) plus butyric acid (C4) to propionic acid (C3) in faecal samples from case and control horses (P=0.004).

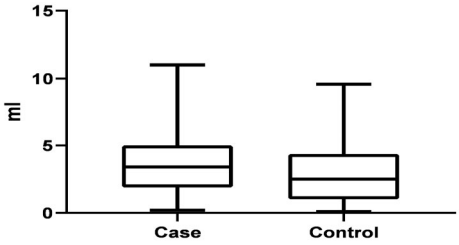

Figure 4. Volume (ml per 20 g faeces) of free liquid after centrifugation of faecal samples from case and control horses (P=0.02).

Case horses had higher (P=0.02) volumes of free liquid in their faeces as determined by centrifugation compared to controls (Figure 4). Also, absorptive capacity was higher in faecal samples from control (6.5 ml/g) compared to case (6 ml/g) horses (p=0.01). Faecal samples did not differ (P=0.36) in DM concentration between case (184 g/kg) and control (185 g/kg) horses, indicating that DM was not a suitable variable to describe the concentration of free liquid in faeces. Case horses tended (P= 0.06) to have higher faecal pH (6.6 vs 6.5) compared to control horses, however pH was within previously reported ranges for horses without clinical signs of hindgut disturbances (Müller *et al*., 2008). Particle size distribution did not differ between case and control horses. Case horses tended (P=0.08) to have higher volume of sand in faeces compared to control horses (0.10 vs. 0.04 mm/20 g sample). One explanation for this may be differences in soil ingestion between case and control horses. Previous studies (Husted *et al*., 2005) have reported differences in soil concentration in faeces in horses fed forages outdoors off the ground (higher faecal sand concentration) or in feed racks (lower faecal sand concentration). However, there were no differences in how case and control horses in the current study were fed their forages, and approximately 40 % of both case and control horses were fed forage on the ground in their paddocks. There may still be individual differences in soil ingestion between case and control horses that was not possible to detect with the current study design. Faecal sand concentration may be of further interest in finding causes of FFV as presence of sand in the large intestine has been associated with chronic diarrhoea and sand colic in horses (Bertone et al., 1988). No differences were found in lactate, propionate or valerate concentration, osmolality or ammonia-N in faeces from case and control horses. Also, no differences were found between case and control horses in anthelmintic routines or faecal egg counts. *Clostridium difficile* and *Cl. perfringens* were present only in one to two samples per sampling round, and then in samples from the same farm.

Microbial composition (relative abundance of species) and number of observed species in faeces from case and control horses did not differ in general when combining all three sampling rounds, however within sampling rounds, differences were present between case and control horses. These differences were present at phyla, family and general level (top 20 genera level reported in Figure 5). In all sampling rounds, Firmicutes dominated (51 to 57 %) followed by Bacteroidetes (22 to 30%) and Proteobacteria (4 to 14 %) with larger differences between sampling rounds than between case and control groups. These phyla have been reported as the most dominant also in other studies of the equine hindgut microbiota (*e.g*. Salem *et al*., 2018). Shannon diversity index showed that bacterial diversity differed between case and control horses in rounds 1 and 2 ($p<0.03$) but not in round 3 ($p<0.41$). Due to the limited space in this report, all differences in microbial composition cannot be reported here, but please see upcoming publications from the project.

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Faecal samples from the first sampling occasion in October/November were also used for analysis of Equine corona virus at the National Veterinary Institute (NVI) in a separate project financed by NVI. None of the samples had detectable presence of Equine corona virus.

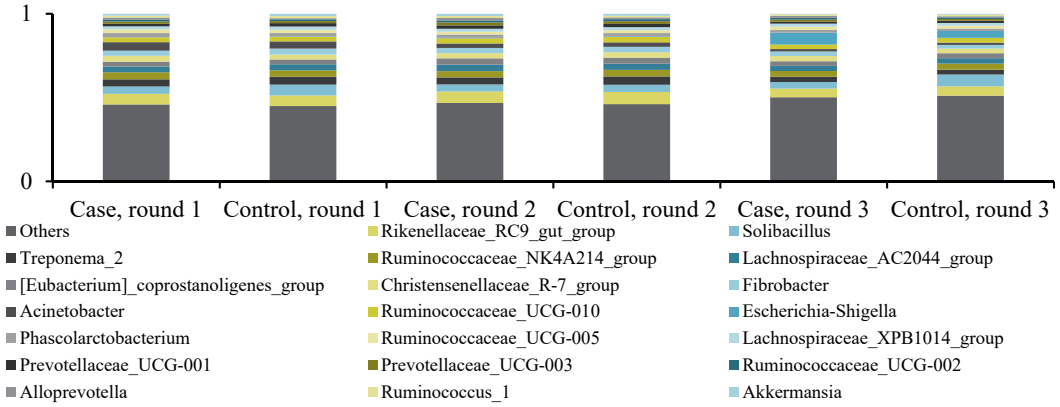

Figure 5. Average relative abundance of top 20 faecal bacteria on genera level in case and control horses in three sampling rounds.

Conclusions

In conclusion, the preliminary findings of this study showed that presence of FFL cannot be generally attributed to feeding wrapped forages such as haylage to horses. However, horses with FFL differed from controls in some hindgut variables, indicating that their hindgut may respond differently than control horses to similar feeding and management. It may be related to the individual hindgut microbiota composition of the horse, or other individual factors not identified in this study (*e.g.* soil ingestion behaviour). Further studies on FFL should therefore include hindgut functional variables in response to controlled feeding of different feedstuffs, and preferably also include ingestive behaviour in affected and unaffected horses.

Relevance for the practical horse sector including recommendations

With the current data, presence of free faecal liquid in horses does not seem to have any common feeding or management factor, and all horse types can be affected. There may be an increased risk of colic in horses showing FFL. For some horses, owners reported that a change in forage batch or keeping the horse at pasture may alleviate symptoms of FFL. It may therefore be worthwhile to try a change of forage batch and/or use as long pasture time as possible for horses with FFL. Further investigations of interactions between different forage/feed types and the equine hindgut are needed to enhance our understanding of the complex microbial hindgut ecosystem and its role in FFL (and colic). It is of highest relevance to the horse sector that different feeds are described accurately in relation to potential equine health risks to avoid costly health disturbances, but also to avoid situations where anecdotal “evidence” creates unjustified management difficulties in *e.g.* feeding routines. We need to continue with studies of equine gastrointestinal responses to different feeds to enhance our understanding of hindgut function in equines and by that also avoid feeding and management routines that may create health issues in horses.

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Part 3: Result dissemination

Scientific publications, *published*	Lindroth, K., Johansen, A., Båverud, V., Dicksved, J., Lindberg, J.E. and Müller, C.E. 2020. Differential Defecation of Solid and Liquid Phases in Horses—A Descriptive Survey. Animals 10, 76, doi: 0.3390/ani10010076
Scientific publications, *submitted*	
Scientific publications, *in manuscript*	-K.M. Lindroth, A. Johansen, V. Båverud, J. Dicksved, J. E. Lindberg, C.E. Müller. Nutrition and management associated with free faecal liquid in horses in a case-control study. Submission in April 2020 to Livestock Science. -K.M. Lindroth, I. Vervuert, A. Johansen, V. Båverud, J. Dicksved, J. E. Lindberg, C.E. Müller. Characterization of chemical and microbial composition in faeces from horses with and without free faecal liquid in a case-control study. In manuscript, submission in March 2020 to Livestock Science. -K.M. Lindroth, I. Vervuert, A. Johansen, C.E. Müller. Faecal particle size in horses with and without free faecal liquid – Sweden, Germany and Norway. In manuscript, submission in May 2020.
	- Gröndahl, G., Lindroth, K., Müller, C.E. et al., 2020. Presence of equine corona virus in faecal samples in a Swedish cohort. In preparation, submission during autumn 2020.
Conference publications/ presentations	-Lindroth, K., Lindberg, J.E., Müller, C.E. 2018. Free faecal liquid in horses- a survey of feeding routines and feedstuffs for affected horses. 9th European Workshop on Equine Nutrition, "Future Equine Nutrition", August 16-18, 2018, Uppsala, Sweden. pp.13. (oral presentation and abstract). -Lindroth, K., Johansen, A., Båverud V, Dicksved, J., Lindberg JE, Müller, C.E. 2020. Fri fekal vätska hos häst-relation till utfodring med hösilage? Vallkonferens 4-5 februari 2020. Sveriges Lantbruksuniversitet, Uppsala. -Planned: Lindroth, K.M., Lindberg, J.E. Müller, C.E. 2020. Free faecal liquid in horses and possible associations with nutritional factors (preliminary title). Abstract to 10th European Workshop on Equine Nutrition, Cirencester UK, 19-21st August 2020. Abstract submission 31st March 2020. -K.M. Lindroth, C.E. Müller. Microbial composition in wrapped forages for horses (effects of repeated sampling and sample submission time). Submission 31 March 2020 to 10th European Workshop on Equine Nutrition, Cirencester UK, 19-21st August 2020 (Conference with special issue proceedings in J Equine vet Sci)

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	-Planned: Lindroth, K.M. et al. 2020. Fri fekal vätska hos häst. Hippocampusdagen 2020 at SLU, October 2020.
Other publications, *media etc*	Fri fekal vätska hos häst – kartläggning av foder. 2018-09-02. News on Hästsverige. Link: https://hastsverige.se/nyheter/fri-fekal-vatska-hos-hast-kartlaggning-av-foder/ Varför lös träck vid utfodring? 2019-04-14. News on Hästsverige. Link: https://hastsverige.se/nyheter/varfor-los-track-vid-utfodring/
	Johansen, A. og Hustad, K. 2016. Stor undersøkelse: Hester som ikke tåler høyensilasje. www.nhest.no; Publisert 29.09.2016. Johansen, A., Lindroth, K. & Müller, C.E. 2018. Høyensilasje er ikke eneste årsak til fri fekal væske (FFV) hos hest. Hestesport, medlemsblad for Norges Rytterforbund, 6 (56) 2018, s. 42-43.
	"Hösilageintolerans utreds" 2017-07-06 Equipage: https://www.equipage.se/hosilageintolerans-utreds/ "Rinnigt träck av hösilage?" 2019-04-16 Equipage: https://www.equipage.se/rinnigt-track-av-hosilage/ "Det senaste om rinnigt träck" 2019-05-25. Equipage: https://www.equipage.se/det-senaste-om-rinnigt-track/ "Hästarna har ständigt kontaminerade bakben". Röök, I. Hippson (printed) nr 3, 2019, page 15. Planned: PhD dissertation of Katrin Lindroth, January 2021. In conjunction with the dissertation we will have a seminar on the subject equine nutrition and gastrointestinal health with presentations from evaluation committee and opponent at SLU, Uppsala.
Oral communication, *to horse sector, students etc.*	"Får hästar diarré av hösilage?" by Cecilia Müller. 2019-03-05. Oral presentation for members of Svenska Vallföreningen at Jällaskolan, Uppsala. Link to information about the event: https://hastsverige.se/nyheter/far-hastar-diarre-av-hosilage/ "Fri fekal vätska hos häst" by Katrin Lindroth. 2019-03-05. Oral presentation for members of Svenska Vallföreningen at Jällaskolan, Uppsala. "Fri fekal vätska hos häst associerad med utfodring och vård" by Katrin Lindroth. Recorded Research lecture in course HV0147 Hästens utfodring (Horse feeding). The part on Free faecal liquid in horses starts at time 32.50 in the movie: http://spectare.ucl.slu.se/vhfak/2017/huv/digestion_naringsbehov.html Recorded 2017-08-31. The movie has been used in the course during 2017 and 2018, and will be renewed in 2020. Free faecal liquid in horses. 2018-11-21. Oral presentation by C. Müller at Equine Researchers meeting at SLU. Planned: In October 2020, we will have a seminar open to the public at Norsk hestesenter/Bjerke where the results from this project will be presented.
	Under construction: popular science report in Swedish, will be published at www.hastsverige.se and on research blog: https://blogg.slu.se/utfodring-och-halsa-hos-hast/ Planned date for publication late April 2020.
Student theses	Ygland, H. Supervisor Cecilia Müller. 2018. Characterization of horses with free fecal liquid – nutrition and management. Master Thesis 2018:60. Swedish University of Agricultural Sciences, Uppsala, Sweden. Link: https://stud.epsilon.slu.se/13599/ Kangas, P. Supervisor Cecilia Müller. 2019. Free faecal liquid in horses – chemical composition of faeces in cases and controls. Master thesis, University of Helsinki, Department of Agricultural Sciences, Animal Nutrition. Link: https://helda.helsinki.fi/handle/10138/303025 Rönnqvist, E. Supervisors Cecilia Müller and Katrin Lindroth. 2019. Free faecal liquid in horses – associations with nutritional factors. Master Thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden. Ongoing, finished in December 2019.
Other	Research blog on hindgut disturbances in horses: https://blogg.slu.se/utfodring-och-halsa-hos-hast/ (over 14 000 views)

Please note that since the date of this application, themes for two conferences (International Silage Conference and Nordic Feed Science Conference) where we had planned to present results has changed and no longer cover equine nutrition. We have therefore presented our research when other opportunities for it has been given, and therefore there is also a delay in presentation of this research at conferences. Please also note that in the application, we wrote that scientific publication of results may take place first after project ending, as the publication process may be long for some journals within Animal Science and Veterinary medicine.

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