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Final report

The transition from winter-feeding to spring pasture - Metabolic responses related to diet and health in horses

Project number: H-19-47-484

Project period: 2020-03-01 to 2020-12-31

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Part 1: Detailed summary

Formålet med prosjektet:
Overgangen fra vinterfôring til beite innebærer en betydelig diettendring som kan skade hestens helse. Sykdommer som kolikk samt insulin-dysregulering og beiteassosiert laminitt er relatert til diettendringer og beiteinntak. Det finnes imidlertid lite informasjon om de metabolske responsene fra hester på beite. Målet med dette prosjektet er å undersøke hvordan overgangen fra vinterfôring til vårbeite påvirker konsentrasjonen av blodsukker og -insulin samt det mikrobielle miljøet i hestens baktarm. Dette prosjektet vil bidra med viktig kunnskap om overgangsfôring av hester, resultater som vil bli brukt i forebygging og behandling av sykdommer som kolikk, insulin-dysregulering og laminitt.

Forsøket i prosjektet:
Prosjektet ble inndelt i 3 perioder, en periode kun med grovfôr, en periode hvor grovfôr mengden ble redusert og mengden av gress steg, og en periode hvor hestene nesten kun fikk gress. Der ble innsamlet blod- og blindtarms prøver hver fjerde dag i forsøket, for å undersøke de metabolske responsene fra hester på grovfôr og beite.

Resultat fra prosjektet:
Der ble brukt striper av gress og begrenset med tid på beite for å kontrollere mengde av gress hestene spiste. Hestene kunne spise relativet store mengder gress på de 2 x 3 timer de var på beite. Der var høyere glukose og insulin konsentrasjoner når hestene fik høyensilage end gress hvilket ikke var forventet. Endringene i baktarmen til hestene ble påvirket av fôr og der var også individuelle forskjeller mellom hestene.

Perspektiver fra prosjektet:
Striper med gress kan anvendes som en måte hvorpå man kan kontrollere inntaket av gress (og energi) på hester på beite. Flere studier bør undersøke forskjellige gresser og beiteregimer, for at finne egnet løsninger til hester med lavt eller høyt energiforbruk.

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Part 2: Main report (max. 10 pages)

Introduction

The aim of the project was to investigate how the transition from winter-feeding to spring pasture affects the metabolic responses in horses. This transition involves a considerable diet change that might compromise the health of horses. The objective of the project is to investigate how the transition from winter-feeding to pasture affects blood glucose-insulin concentrations and the microbial environment in the hindgut of the horse. We expect to develop new knowledge beyond the current state of the art, and the results from this project will be used for management of horses on pasture and in the prevention of diseases like colic and laminitis.

Material and methods

Four caecum-cannulated horses were used to investigate the metabolic responses related to the transition from winter-feeding to pasture. In a longitudinal study, we conduct several observations on the same horses over time.

The experiment is divided into three periods:
- *1) a hay only period (day 0 to 8) reflecting winter-feeding*
- *2) a transition period (day 9 to 20) reflecting a diet where the proportion of forage decreases and grazing increases*
- *3) a grazing only period (day 21 to 28) reflecting horses on pasture (see illustration in Figure 1).*

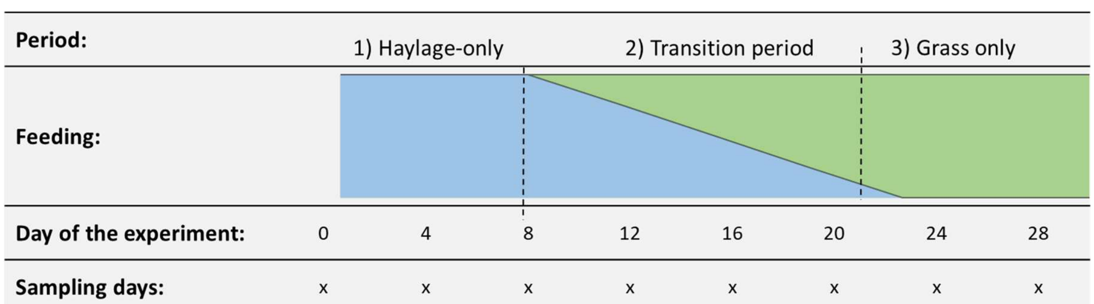

Figure 1: Illustration of the experimental design with the three periods, transition in feeding and a timeline showing days with collection of blood and caecal samples from the horses.

Horses are fed individually throughout the study. Feed intake is easy to control in period one, where the horses are on a hay-only diet. In period two and three, the horses are allowed access to pasture, and strip grazing was used to control and measure daily pasture intake. Each day the horses were allowed access to a new strip of grass for a specified time period. This period increases during period 2, as illustrated in Figure 1. The remaining grass was cut each day after grazing and the residual was compared to the amount of grass from a strip of control pasture without any grazing horses (Figure 2). Furthermore, hay and grass samples from every fourth day were analyzed for nutritional composition including the individual carbohydrate fractions (fiber (the good), sugar (the bad) and fructans (the ugly)) to estimate nutrient intake.

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Nutrient intake was related to the metabolic responses measured in the blood and in the hindgut. Blood and caecal contents were sampled at 12 every fourth day, when we expected the largest fluctuations in the measured parameters. On day eight (end of period 1) and on day 32 (end of period 3) the horses was fed a standardized ration of either 3 kg dry matter of hay or grass. On day 8 and 32, blood and caecal contents were sampled before and each hour after feeding the standardized ration for 8 hours.

The analysis was divided into two work-packaged based on the metabolism taking place in the small intestine (work-package 1) and in the hindgut (work package 2). Different laboratories at NMBU analyzed the collected samples. Blood samples were analyzed for glucose, insulin, triglycerides, and urea. The microbiota composition was assessed using 16S rDNA gene amplification analysis. To identify and visualize metabolically active populations within the caecal microbiome.

Figure 2: Grass intake was measured by using strip-grazing, where the horses were allowed access to a new strip of grass each day. The residuals were cut, collected, and analyzed.

Results and discussion

Feed intake and nutrient content of haylage and grass

Feed intake in the three periods are shown in Figure 3 and the amount of grass harvested on day 12 to 28 and the dry matter (g/kg) and nutrient content (g/kg DM) of grass and haylage fed to the horses is presented in Table 1. Intake of haylage in period 1 was stable and decreased in the transition period, whereas grass intake increased in the transition period and was stable in the third period. The weather was warm during the transition period, and it was decided to divide the grazing into two 3-hour blocks from 8 to 11(morning grazing) and 20 to 23 (evening grazing). In the period between 11 and 20 the horses were in their stables or in a gravel paddock.

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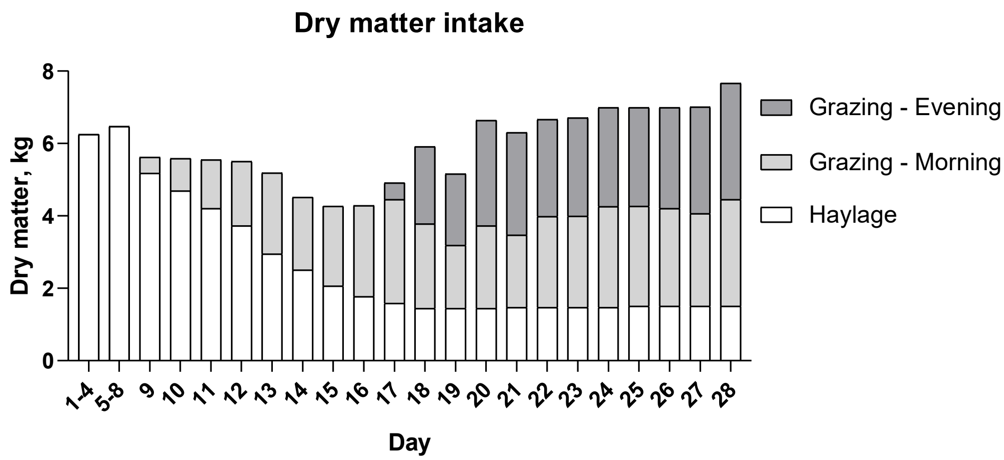

Figure 3: Feed intake of each horse (kg dry matter/per day) in the three periods

As it can be seen in table 1, the grass yield increased over time as the grass matured from 0.316 to 0.542 kg DM/m^2. The developmental stage of the grass did also affect the nutrient content, and fiber (aNDFom) content increased from 545 to 636 g/kg DM, and protein decreased from 158 to 101 g/kg DM. The sugar (total water soluble carbohydrates, WSC) content varied, and fructans were in general low.

Table 1. Amount of grass harvested on day 12 to 28 and the dry matter (g/kg) and nutrient content (g/kg DM) of grass and haylage fed to the horses

	12 (n=3)	16 (n=3)	20 (n=3)	24 (n=3)	28 (n=3)	Haylage (N=6)	SEM	P-value
Grass. kg/m^2	1.393bc	1.618ab	1.511bc	1.294c	1.805a	-	0.089	0.012
Grass. kg DM/m^2	0.316d	0.394c	0.459b	0.473b	0.542a	-	0.015	<0.001
Dry matter	227d	243d	305c	367b	301c	757a	12.7	<0.001
Ash	64a	65a	65a	59b	53c	58b	1.60	<0.001
aNDFom	545e	578d	607c	620bc	636b	659a	7.67	<0.001
ADFom	357	321	314	323	335	315	11.70	0.1197
Crude protein	158a	156a	141a	115b	101b	84b	6.90	<0.001
Total WSC	123a	93b	73c	99b	102b	105b	5.42	<0.001
Glucose	18d	17de	22c	28b	13e	36a	1.12	<0.001
Fructose	13b	11b	12b	13b	10b	60a	1.58	<0.001
Sucrose	70a	42c	28d	49bc	54b	3e	3.72	<0.001
Fructans	22a	24a	11b	9b	24a	5c	0.97	<0.001

From the daily dry matter intake (figure 3) and the nutrient content in the haylage and grass (Table 1) it was possible to calculate the daily nutrient intake (data not shown) and the nutrient intake from the morning meal. A profile of the metablic responeses were measured on day 8 and 28 before and after the morning meal of haylage or grass.

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Table 2. Estimated dry matter and nutrient intake (grams) on day 4 to 28 of the meal based on chemical composition of grass and haylage fed to the horses

	4	8	12	16	20	24	28
Kg dry matter	1.56	1.62	2.60	3.25	3.00	3.52	3.70
Aah	88	93	163	204	185	202	201
aNDFom	1027	1065	1509	1908	1805	2150	2307
ADF	506	558	901	1058	955	1070	1229
Protein	128	133	355	490	432	417	394
Total WSC	174	180	299	303	224	370	397
Glucose	60	59	60	66	82	109	63
Fructose	100	102	67	62	65	81	74
Sucrose	5	7	127	119	59	162	186
Fructans	10	12	45	56	17	18	74

The DM and nutrient intake of the morning meal was in general higher when the horses were grazing than when fed the haylage, and this is expected to affect the metabolic responses. Furthermore, within the 2 x 3 hours grazing, the horses consumed grass corresponding to the feed intake in period 1. The energy content in this amount of grass was enough for the horses to maintain body weight. However, if the horses were allowed more time they would probably have eaten more and been in a positive energy balance gaining weight over time. This illustrate the difficulties of keeping horses on pasture and still controlling body weight.

The metabolic responses in the blood

Nutrient intake form haylage (day 8) or grass (day 28) affected the metabolic responses (glucose, insulin, triglycerides, and urea) in the blood as illustrated in Figure 4.

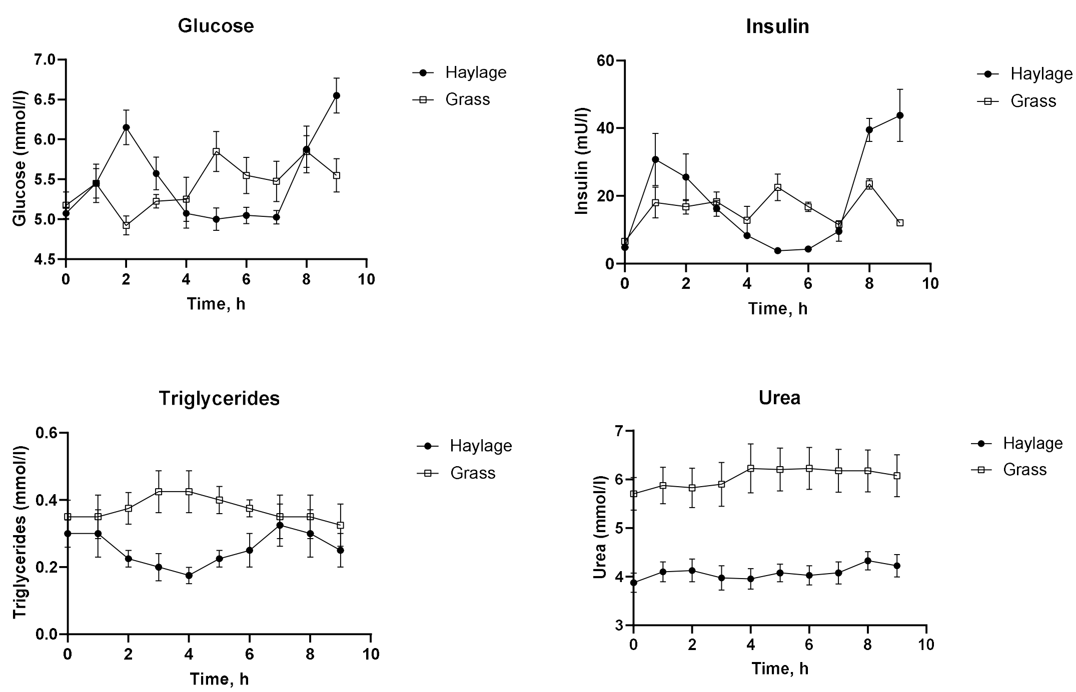

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Figure 4: Nutrient intake form haylage (day 8) or grass (day 28) affected the metabolic responses (glucose, insulin, triglycerides, and urea) in the blood.

Blood glucose and insulin followed each other (insulin increased when glucose increased), and interestingly the highest values were measured when the horses were fed haylage compared to grass (Figure 4). This was unexpected as the WSC intake was higher when the horses were fed grass on day 28 (Table 2). One explanation could be a faster intake of haylage (and WSC) than grass, resulting larger fluctuations in blood glucose and insulin. However, this requires further studies to clarify the effects of different forage and grass types on glucose insulin response. This is highly relevant in relation to feeding horses with insulin dysregulation prone to laminitis. The difference in triglycerides is not clear, but the higher concentrations of urea could be explained by the higher intake of protein when the horses were eating grass than haylage (Table 3).

The metabolic responses in the cecum

Nutrient intake form haylage (day 8) or grass (day 28) affected the metabolic responses (pH, total short-chained fatty acids (SCFA), acetate and propionate) in the cecum as illustrated in Figure 5.

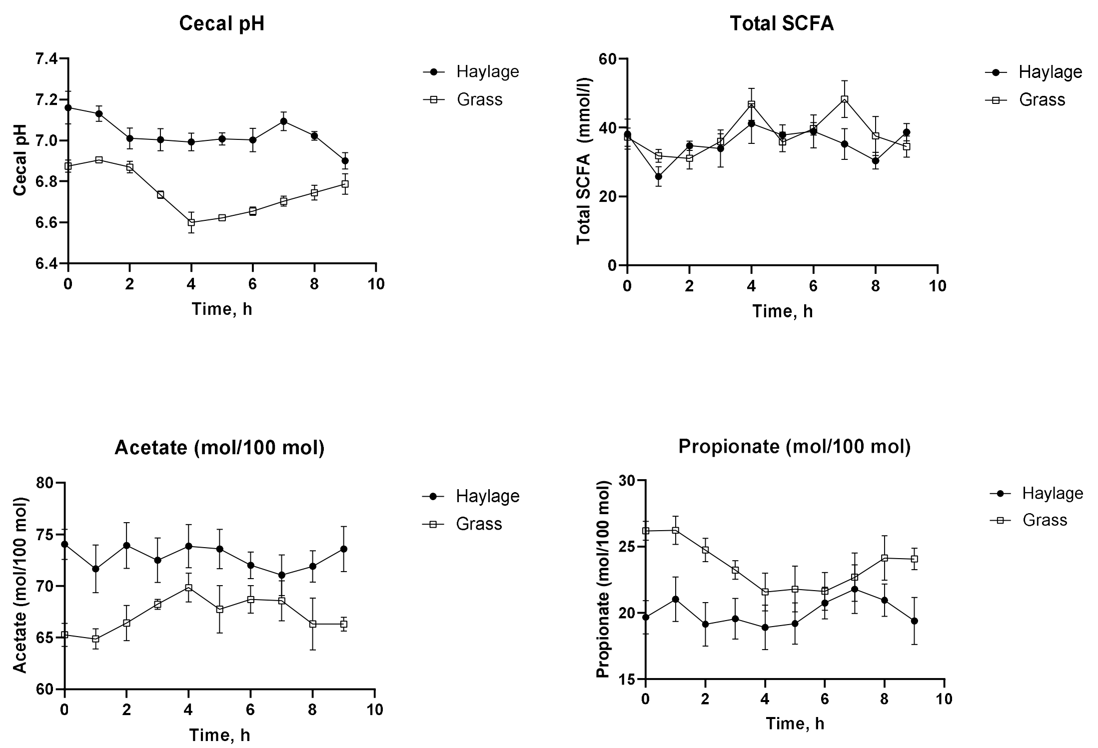

Figure 5: Nutrient intake form haylage (day 8) or grass (day 28) affected the metabolic responses (pH, total short-chained fatty acids (SCFA), acetate and propionate) in the cecum.

Cecal pH was lower when the horses were eating grass compared to haylage, however there was no clear difference in the concentration of total SCFA (Figure 5). It can be difficult to say if it is the differences in DM or nutrient intake, or a combination of both. Analysis of lactate was not included in this study, it can be speculated that the concentration of lactate was higher when the horses were eating grass causing the lower pH, and lactate analysis should

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be included in future studies. The Proportions of individual SCFA (acetate, propionate, butyrate, valerate, iso-butyrate, iso-valerate) gives an indication of the fermentation in the cecum (Figure 5, not all individual SCFA are shown). There were differences in the proportions of acetate and propionate, indicating differences in fermentation when horses are eating hay or grass. There were no clear signs of disturbances in the fermentation in the cecum during the transition period, and pH did not reach critical low values.

The responses in the cecal microbiota

The microbiota data needs further data processing, but preliminary results are shown in Figure 6 and 7. Identification of samples with similar microbial communities where done using multivariate statistics (PCA), where the distance between the sample dots signifies similarity; the closer the samples are, the more similar microbial composition they have. A PCA plot illustrating one of the horses is shown in Figure 6, and the transition from haylage to grass affects the microbial environment in the cecum.

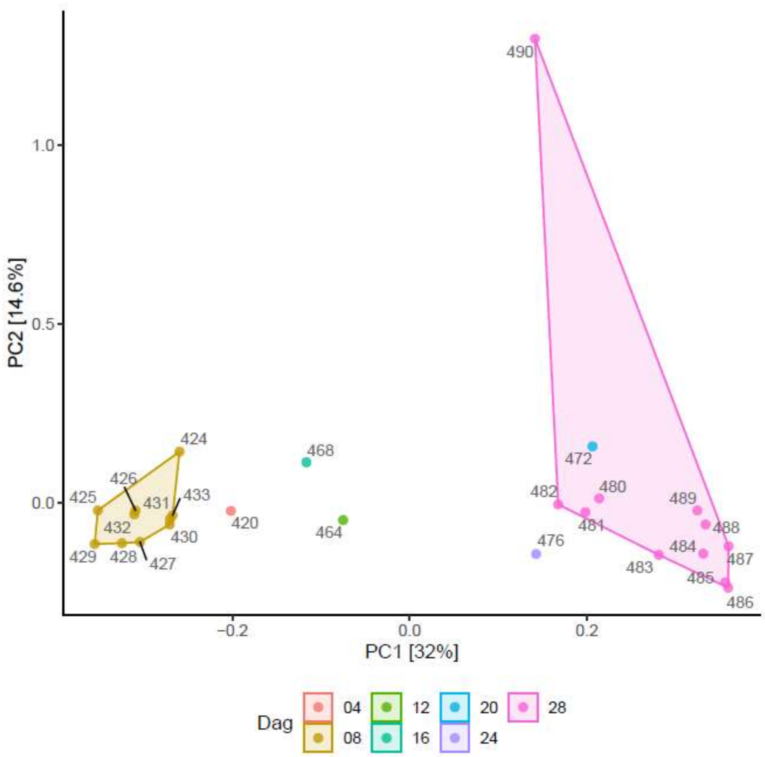

Figure 6: Identification of samples with similar microbial communities where done using multivariate statistics (PCA). Each point represents the microbial community in a specific sample from one horse. The distance between the sample dots signifies similarity; the closer the samples are, the more similar microbial composition they have.

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A PCA plot illustrating the four horses is shown in Figure 7. All samples from one horse has similar colour, and there are clear individual differences as each horse cluster separately despite alle horses are fed the same diets. These individual differences are interesting in relation to the health and disease in horses in general. Further analysis will b done, where data on pH and SCFAs are combined with microbiota data.

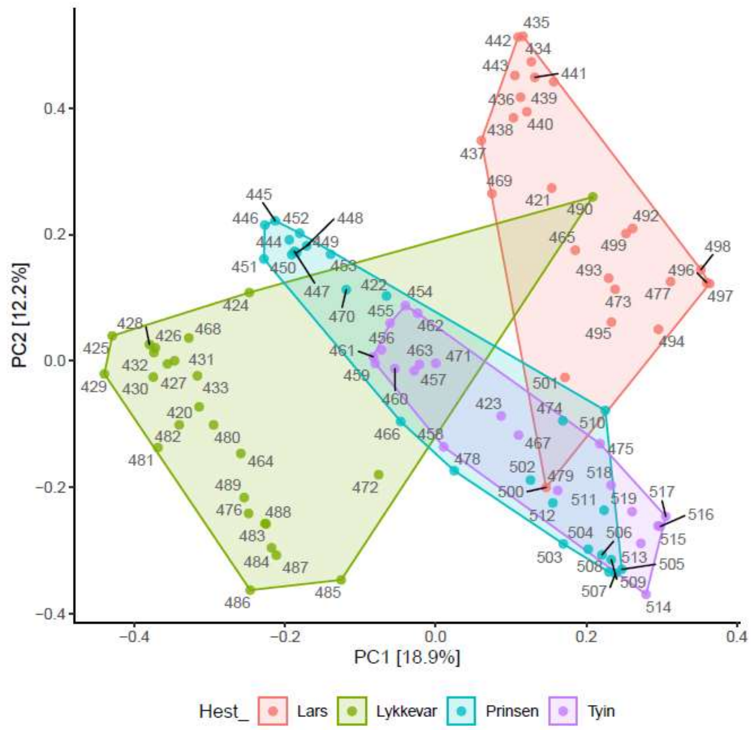

Figure 7: Identification of samples with similar microbial communities where done using multivariate statistics (PCA). Each point represents the microbial community in a specific sample from all horses, colours indicate each individual horse. The distance between the sample dots signifies similarity; the closer the samples are, the more similar microbial composition they have.

Conclusions

In conclusion, this project showed that strip-grazing and restricted time can be used to control feed intake on pasture. However, the horses consumed a relatively large amount of dry matter. Interestingly, the highest peaks in blood glucose and insulin was measured when the horses consumed haylage compared to grass. We measured metabolic changes in the cecum of the horses, and it was affected both by diet and individual horses. The transition period and grazing system used in the study can be used as a guideline for horses on pasture.

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Relevance for the practical horse sector incl. recommendations

This project has provided new insight into the transition from winter-feeding to spring pasture and the effects on the metabolic responses in horses. This transition involves a considerable diet change that might compromise the health of some horses. The amount of grass and the nutrient content changed during the project period as the grass matured. We demonstrated that strip grazing with restricted grass and time, is one way to control feed intake in horses on pasture. We quantified the amount of grass the horses consumed within the 2 x 3 hours, and the horses consumed enough dry matter to fulfill their energy requirements. This should be considered when horses are on pasture for a limited or longer time, as energy intake might excee3d the requirements and lead to obesity and increase the risk for laminitis. Further studies should investigate the effect different grass species and different grazing systems on feed and nutrient intake as well as metabolic responses in horses. Different grass species have different nutritive value and palatability, that might affect the metabolic responses in differently and this could be of benefit for horses with both high and low energy requirements.

The gradual transition used in the present study did not have any negative impact on the gastrointestinal health of the horses, and the period used in this project could be recommended as a guideline. However, other transitions could be relevant depending on time on pasture, the amount and composition of the pasture. Interestingly, the blood glucose and insulin had higher peaks when fed haylage compared to pasture. The metabolic responses in blood needs further investigations to clarify the blood glucose and insulin responses o different forages and grasses. The difference measured in microbiota and the fermentation products in the cecum showed both diet and individual differences. Future studies could include fecal as well as cecal samples, as fecal samples are easier to collect from a larger number of animals. Hence, fecal samples can then potentially be used to screen for a specific microbiota composition related to health and decease.

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Part 3: Result dissemination

State all result dissemination from the financed project into the appropriate section, including information as indicated. Additional rows can be added to the table.

Scientific publications, *published*	*Author(s), year, title, journal, Vol, No, pp. (doi/link if applicable)*
Scientific publications, *submitted*	*Author(s), title*
Scientific publications, *manuscript*	*Müller C, Pope P, Jensen RB: Metabolic responses related to the transition from winter-feeding to spring pasture in horses.*
Conference publications/ presentations	*Round table discussion on how to handle the insulin dysfunctional horse at EEHNC 25th March 2021 online conference, where our results were used to show the large variation in WSC in different grasses.*
Other publications, *media etc.*	*Velg riktig grovfôr til hesten, 27th of October 2020, Felleskjøpet webinar, https://www.youtube.com/watch?v=WlRhSzr7n2E*
	www.forskning.no - Hesten kan bli syk av å skifte beite - Mandag 11. januar 2021
	NMBU YouTube channel and news: https://www.nmbu.no/fakultet/biovit/om/aktuelt/node/42081(Published 11. januar 2021)
	www.tølt.nu - Viktig forskning:Hva skjer inni hesten din?Overgang mellom vinterfôring og beite: https://xn--tlt-0na.no/hva-skjer-inni-hesten-din/19.1072
	www.nhest.no - Hva skjer inne i hesten i overgangen mellom vinterfôring og beite? Forskning Finansiert av Stiftelsen Hâstforskning - https://nhest.custompublish.com/hva-skjer-inne-i-hesten-i-overgangen-mellom-vinterforing-og-beite.6359470-467706.html
	www.tgn.no - Hva skjer inne i hesten i overgangen mellom vinterfôring og beite? - https://tgn.no/artikkel/hva-skjer-inne-i-hesten-i-overgangen-mellom-vinterforing-og-beite/
Oral communication, *to horse sector, students etc.*	***Courses at NMBU:*** *Horse nutrition HFH255* *Grazing Ecology and Management HFX205*
Student theses	
Other	

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Link to YouTube webinar: [*https://www.youtube.com/watch?v=WlRhSzr7n2E*](https://www.youtube.com/watch?v=WlRhSzr7n2E)

Link to YouTube video: [*https://www.youtube.com/watch?v=UpGaOLsIcg8&t=4s*](https://www.youtube.com/watch?v=UpGaOLsIcg8&t=4s)

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