Institut für Tierernährung

der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. med. vet. Dr. h. c. Marcel Wanner

Arbeit unter Leitung von PD Dr. med. vet. Annette Liesegang
und Dr. med. vet. Brigitta Wichert

Fermentation of six different forages in the semi-continuous fermentation technique Caesitec

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Judith Vosmer

Tierärztin
von Uslar, Deutschland

genehmigt auf Antrag von

PD Dr. med. vet. Annette Liesegang, Referentin

Prof. Dr. agr. habil. Annette Zeyner, Korreferentin

Zürich 2012

Meiner Familie
&
Sven gewidmet

Inhaltsverzeichnis

Fermentation of six different forages in the semi-continuous fermentation technique Caesitec

Judith Vosmer, 2011

Institut für Tierernährung der Vetsuisse-Fakultät Universität Zürich

sekretariat@vetphys.uzh.ch

Summary

The aim of the present study was to compare carbohydrate degradation of forages in horses' hindgut which store carbohydrates predominantly as fructan or primarily as starch. The effects of an abrupt change from a hay-based diet to a forage-based diet on the caecal flora were tested with the in vitro hindgut simulation technique "Caesitec".

Six trials with different forages (english ryegrass, tall fescue, grass mixture-horses, grass mixture-cows, lucerne, white clover) were conducted in the "Caesitec". During a four-day stabilisation period samples were taken once a day before loading the fermenters with hay. After diet change to forage-based feeding, samples were taken four times a day. Ammonia and pH-value were measured before and 1h, 2h and 6h after loading the "Caesitec". Gas formation was measured daily. Bacterial numbers, lactate and short chain fatty acids were measured at four time-points of each trial.

The grass mixtures contained the highest amounts of fructan. The pH-values were in the physiological range from pH 6 up to pH 7 (6.58 – 6.83) during all trials. Gas formation, anaerobic and aerobic bacterial numbers increased after diet change.

The maximum amount of fructan (3.75 g/ kg) in swiss pasture did not cause a permanent pathological change in the hindgut-flora.

Fermentation of six different forages in the semi-continuous fermentation technique Caesitec

J.Vosmer[1], A. Liesegang[1], M. Wanner[1], A. Zeyner[2], D. Suter[3], L. Hoelzle[4,5], B. Wichert[1]

[1]Institute of Animal Nutrition, Vetsuisse-Faculty University of Zurich, Winterthurerstr. 260, 8057 Zurich, Switzerland

[2]Chair for Nutrition Physiology and Animal Nutrition, University of Rostock, Rostock, Germany

[3]Research Station Agroscope Reckenholz-Tänikon ART, Zurich-Reckenholz, Switzerland

[4,5] Institute of Veterinary Bacteriology Vetsuisse-Faculty University of Zurich, Switzerland, Institute of Environmental and Animal Hygiene and Veterinary Medicine, University of Hohenheim, Stuttgart, Germany

Summary

The aim of the present study was to compare carbohydrate degradation of forages which store carbohydrates either predominantly as fructan or starch, in horses' hindgut. The effects of an abrupt change from hay-based feeding to green fodder-based feeding on the caecal flora were tested with the in vitro hindgut simulation technique "Caesitec".

Six trials with different forages (english ryegrass, tall fescue, grass mixture-horses, grass mixture-cows, lucerne, white clover) were conducted. During a four-day stabilisation period, samples were taken once a day before loading the fermenters with hay. After diet-change to forage-based feeding, samples were taken four times a day. Ammonia and pH-value were measured before and 1h, 2h and 6h after loading the "Caesitec". Gas formation was measured daily. Bacterial numbers, lactate and short chain fatty acids were detected at four time-points of each trial.

The grass mixtures contained the highest amounts of fructan. The pH-values were in the physiological range from pH 6 up to pH 7 (6.58 – 6.83) by feeding all forages. Gas formation, anaerobic and aerobic bacterial numbers increased after diet change from hay to any forage.

The maximum amount of fructan (3.75 g/kg) in swiss pasture did not cause a permanent pathological change in the hindgut-flora.

Key words: Horse, Carbohydrates, Caecum, Digestion, pH, in vitro digestion

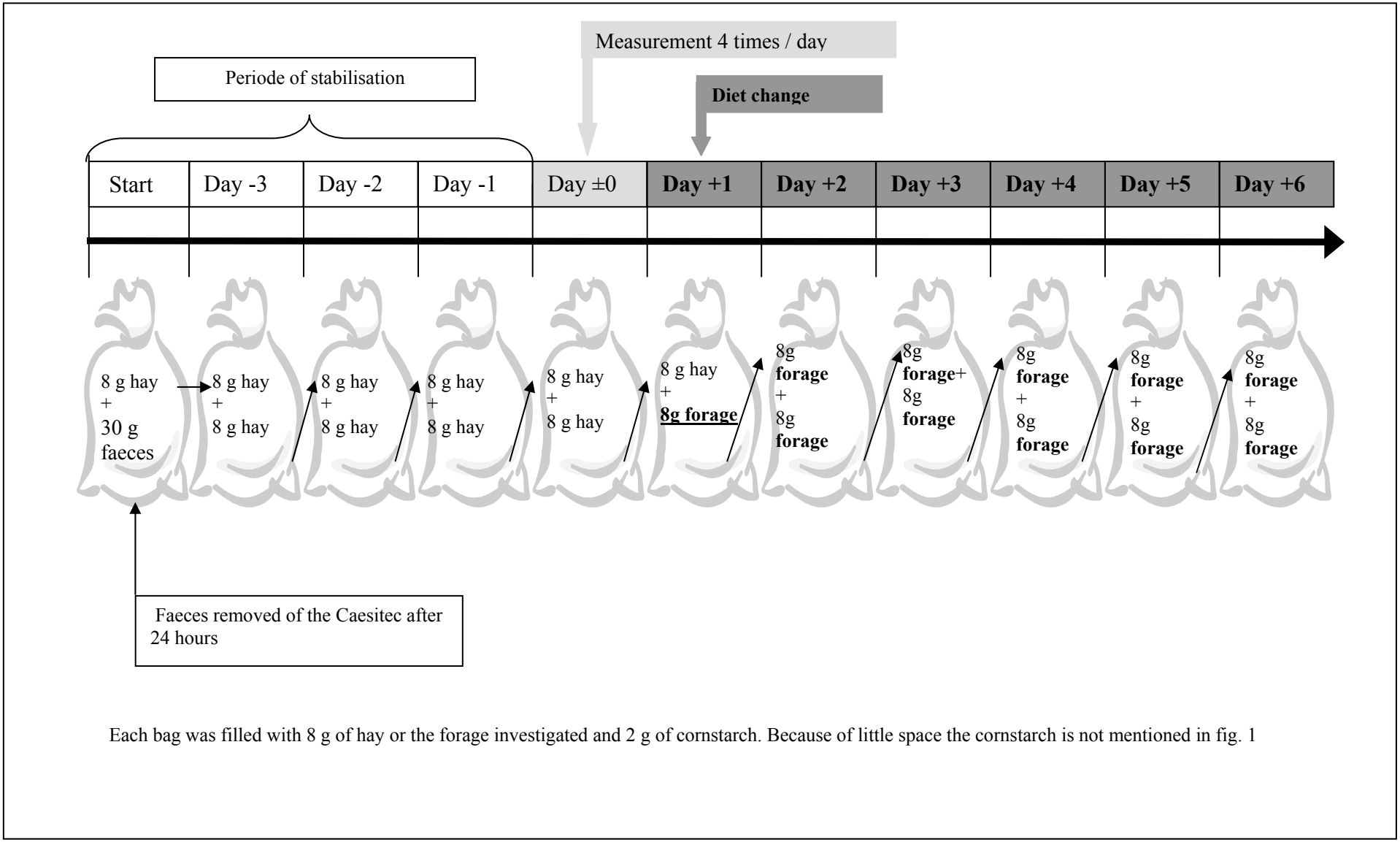
Introduction

An increase of rapidly fermentable carbohydrates in the diet of horses in contrast to a fiber-rich diet causes an increase of total viable bacterial numbers (Kern et al. 1973; Medina et al. 2001). This reaction is associated with an enforced formation of short chain fatty acids (Kern et al. 1973; Garner et al. 1975; Willard et al. 1977). Acidification of the intestinal content gives rise to acid tolerant lactobacilli. The strengthened production of lactate produced by the lactobacilli causes a more intense acidosis and enforces death of less acid tolerant bacteria. Due to changing environmental conditions in the hindgut, gram-negative bacteria decrease for the benefit of the gram-positive bacteria (Huntington and Pollitt 2002). As a response to the death of these bacteria, endotoxins of the cell wall are set free, which can now enter the peripheral blood circulation. Further some gram-positive bacteria build exotoxins. As a consequence, exotoxins and endotoxins can cause diseases like laminitis and colic (Mungall et

al. 2001; Bailey et al. 2004). In different studies it was shown that an alimentary overload of carbohydrates like starch or fructan can cause laminitis and colic (Garner et al. 1975; Pollitt and van Eps 2002). The clinical and histological signs of laminitis were triggered by oral intake of 7.5 g fructan/kg BW (Pollitt and van Eps 2002).
Longland et al. (1999) tested the concentration of fructans in grass (*Lolium perenne*) in Great Britain. They found seasonal maximum fructan concentrations of 400 g/ kg in dry matter (DM). These high concentrations of fructan on pasture and the results of the research of Pollitt and van Eps (2002) led to the theory that laminitis in horses could be caused by an increased intake of grass which contains high concentrations of fructan. A high amount of storage carbohydrates from grass which is not digested in the small intestine reaches the large intestine and is fermented intensively. Therefore, parameters such as composition of the gut flora, pH-value, concentration of ammonia as well as short chain fatty acids can be affected as described before.
The semi continuous system "Caesitec" simulates the environment and the physiological processes in the hindgut of horses. During the last few years, this technique has been considered as a proven and economic alternative to animal experiments (Dill et al. 2006; Zeyner et al. 2006ab, Dill et al. 2007; Zeyner et al. 2007; Müller et al. 2008; Kuhn 2009). Forages which are often grown on swiss pasture are different grasses, grass mixtures and legume. The interest of different forages lies in the storing of carbohydrates, precisely as fructan in comparison to starch and other sugars. In Switzerland most pasture actually used for gazing of horses was also used for dairy cattle in earlier days. The pasture contains high amounts of carbohydrates to cover the requirements for dairy production. But in contrast to dairy cattle, most horses need less energy and protein
Therefore in the present study, changes in the microbial flora and biochemical parameters of the hindgut of the horse after intake of different forages, varying in the concentration of rapidly fermentable carbohydrates, were analyzed. In addition, the aim of the present study was to demonstrate the characteristics of carbohydrate degradation of different forages in the hindgut of horses.

Materials and Methods

Six trials with different types of forages were conducted using the semi continuous fermentation technique "Caesitec" as it has been developed for equine caecum content or faeces as inoculums (Zeyner et al. 2006ab). The "Caesitec" comprised of six glass units (fermenters): fine–particles phase (liquid phase) and solide-particles phase (hay, forage). Each unit is simulating fermentation by hind gut microbes in horses. The fermenters were placed in water of 39 °C degrees. They were mechanically moved to simulate the peristaltics of the large intestine. In order to simulate physiological conditions an especially developed "Caesitec-buffer" (Zeyner et al. 2006a) was admitted to the fermenters continuously during the day. The experimental set up and the time points of sampling were adopted according to results of former and current studies (Caesitec: Dill et al. 2006, Engelmann et al. 2006, Zeyner et al. 2006ab, Dill et al. 2007, Engelmann et al. 2007, Zeyner et al. 2007, Müller et.al 2008, Engelmann et al. 2009, Müller et al. 2009, Janczyk et al. 2010 ; Rusitec: Kelly 1996).
Within the six trials english ryegrass [ER] (*Lolium perenne L.*), tall fescue [TF] (*Festuca arudinacaea*), a grass mixture for horses [GMH] (only grasses), a grass mixture for cows



Each bag was filled with 8 g of hay or the forage investigated and 2 g of cornstarch. Because of little space the cornstarch is not mentioned in fig. 1

Fig. 1: Timetable about the filling of the bags

[GMC] (grasses and clover), lucerne [LU] (*Medicago sativa L.*) and white clover [WC] (*Trifolium repens L.*) were tested. Each forage was grown as a monoculture and harvested on 5th Mai 2009 at 8.15 am.

Time flow

The detailed test procedure is given in Fig. 1. The faeces used for each trial were harvested in the morning of the "start-day" within a herd of Islandic horses which were fed with roughage only. Faeces were collected immediately after spontaneous defecation and stored under anaerobic conditions.

Sampling

From day –4 (start) until day –1 samples for measurement of pH-value and ammonia were taken once a day before changing the feedbags in the fermenters. Beginning on day 0 samples for these analyzes were taken four times a day (before and 1h, 2h and 6h after changing the feedbags). At each sampling-point, samples were taken out of the fine-particles phase of the fermenters by a venous catheter for horses. The material of every fermenter was analysed individually. Gas formation of each fermenter was measured once a day during the whole time of measurement for all forages. Concentration of fructan was analysed daily in forage samples of the feedbags after 48h of digestion in the fermenters. Because of small amount of material, samples of all fermenters were pooled. Fructan was also analysed in the original material of every forage. All analyzes were arranged twice. Starch was measured in original material of all forages. All analyzes were conducted twice. Forage samples for measurement of crude nutrients had to be pooled because of small amount of material (pool 1: fermenter 1, 3, 5 and pool 2: fermenter 2, 4, 6). Pool-samples taken during the period of stabilisation were mixed up to one sample. Pool-samples taken after diet-change were analysed once a day individually. All analyzes of crude nutrients were arranged twice. Lactate was measured at five sampling-points (see Tab.1). The material of each fermenter was tested individually and all analyzes were arranged twice. The experimental design for measurement of short chain fatty acids was identical to measurement of lactate. Samples for microbial analyzes were also taken five times during each trial (see Tab.1). Samples were also pooled because of small amount of material (pool 1: fermenter 1, 4; pool 2: fermenter 2, 5; pool 3: fermenter 3, 6). Four degrees of dilution of each pool were analysed for aerobe, anaerobe and lactic acid producing bacteria for all forages investigated. Samples incubated on Gassner-Agar were analysed for one degree of dilution. All analysis were repeated. A detailed schedule for the sampling of every parameter is given in Tab.1.

Tab. 1: General view of sampling

0h = sampling before changing the feedbags; 1h, 2h, 6h = sampling 1, 2 and 6 hours after changing the feedbags; pH = pH-value; NH3 = Ammonia; Gas = gas production; pa = proximate analysis out of the grass rests in the nylon bags; mibi = samples for microbial analysis; scfa = samples for short chain fatty acids analysis; lactate = samples for lactate determination

Time point	Day										
	- 4	**- 3**	**- 2**	**- 1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**
	Character of samples										
0h	pH, NH_3, Gas, pa	pH, NH_3, Gas,pa	pH, NH_3, Gas, pa	pH,NH_3, Gas, pa	pH,NH_3, Gas, pa	pH, rp, NH_3, Gas, pa mibi, scfa, lactate	pH,NH_3, Gas, pa	pH,NH_3, Gas, pa	pH,NH_3, Gas, pa	pH,NH_3, Gas, pa	pH, NH_3, Gas, pa, mibi, scfa, lactate
1h					pH, NH_3	pH, rp, NH_3, mibi, scfa, lactate	pH, NH_3	pH, NH_3	pH, NH_3	pH, NH_3	
2h					pH, NH_3	pH, NH_3	pH, NH_3	pH, NH_3	pH, NH_3	pH, NH_3	
6h					pH, NH_3	pH, NH_3, mibi, scfa, lactate	pH, NH_3, mibi, scfa, lactate	pH, NH_3	pH, NH_3	pH, NH_3	

Measurement of different parameters

The pH-value was tested in the fine-particles phase in the fermenters. It was measured by a Mettler Toledo pH-Meter connected with a Metrohm electrode (MA 130 pH/ Ionenmeter, Mettler Toledo, Greifensee, CH ; electrode 6.0234.100 by Metrohm, Herisau, CH). Ammonia was measured by a Metrohm pH-Meter connected with a Metrohm ammonia electrode (MA 130 pH/ Ionenmeter, Mettler Toledo, Greifensee, CH; electrode: ammonia selective gas electrode 6.0506.010, Metrohm, Herisau, CH). The gas produced in the fermenters was collected in gas tight bags daily. For the gas measurement, each gas bag was connected to a flask filled with water and the amount of gas was measured from the change of height of the water surface. For fructan measurement, forage samples were pre-treated and the concentration of fructan was measured by refraction index after passage of a prior column and a separating column of a HPLC (Shimadzu, Duisburg, Germany). Furthermore fructan was analysed in the lyophilised original material, as well as starch. Amylase (Thermamyl 120, Novo Nordisk A/S, Denmark) was used for enzymatic determination of starch (Schmidt et al 2005). The fermented forage samples of the feed bags as well as the original material of the forages were analysed for crude nutrients without any other extract according to the VDLUFA method (Naumann and Bassler 1997). The lactate concentration and concentration of SCFA were measured in the fine-particles phase in each fermenter, which were stored at – 20 °C until analysis. The lactate concentration was measured photometric with COBAS Mira (Roche Autoanalyzer, F. Hoffmann-La Roche Ltd., Basel, CH). For analytical determination the RANDOX Lactate-kit was used (Randox Laboratories Ltd., Crumlin, UK). The short chain fatty acids were measured with a gas chromatograph (Varian, Star 3400 CX Varian Medical Systems, Imaging Laboratory GmbH, Baden-Daettwil, CH) and the glass column was filled with GP 10% SP-1200/ 1% H_3PO_4 on 80/100 Chromosorb WAW (Supelco, (Nr.1-1965) Bellafonte, PA 16823, USA). For microbial analyses one ml of the fine-particles phase was taken and transferred into 10 ml PRAS-Medium. After mixing, samples were diluted up to 1:491520. 50µl of each dilution sample up to a dilution factor 1:122880 (1:15360, 1: 30720, 1:61440 and 1:122880) were transferred and incubated on MRS-Agar (Lactobacillus-Agar developed by de Man, Rogosa and Sharpe, Oxoid AG, Pratteln, CH). 50 µl of each dilution sample up 1:491520 (1:61440, 1:122880, 1:245760 and 1:491520) were carried over on blood agar (Oxoid AG, Pratteln, CH) and were incubated anaerobic. The same dilutions were also transferred on blood agar but were incubated aerobic. Samples diluted by the factor 1:10 were transferred and incubated on Gassner-Agar (Oxoid AG, Pratteln, CH). After 48 hours of incubation, the microbes were counted on all plates.

Statistic analysis

An analysis of variance (ANOVA) for repeated measures was performed with SYSTAT©11 (Systat Software, inc., Point Richamond, CA 94804-2028, USA). In addition, significant differences between the forages were calculated with the Kruskal-Wallis Test and significant differences between time during one trail with one forage were calculated by Wilcoxon-Test. Results are given as mean ± and standard error (Mean ± SE). The level of significance was $p<0.05$.

Results

pH-value

During the whole time of measurement, the pH-value was between 6.58 – 6.83. From day 0 to 2 the pH-value decreased after loading the **"**Caesitec". On day 0 the fall of the pH-value immediately after changing the diet was significant for the GMC, ER, WC and LU. On day 1 the fall was significant for the GMC and LU as well as the GMH and TF. Only the pH-value of ER and WC increased on day 1. On day 2 the pH-value decrease with the GMC was significant, only. Beginning with day 3 no change in the pH-value could be observed after loading the "Caesitec".

Ammonia

The concentration of ammonia measured in the present study was between 7.9 and 20.4 mmol/ l depending on the forage investigated. In general concentration of ammonia was higher during the trials when legumes were tested, especially with LU (20.4 mmol/ l). Over the measuring period, the ammonia concentration of WC and LU increased. The maximum and minimum concentrations of ammonia measured in each trial are given in Tab. 2.

Tab. 2: Maximum and minimum concentrations of ammonia in each trial of the forages investigated

(mmol/ l= millimol/ liter)

Forage	**data are given in mmol/l**	
	Minimum	**Maximum**
Grass mixture for horses	9.77	15.62
Grass mixture for cows	10.36	15.06
Tall fescue	9.83	14.87
English ryegrass	10.97	14.95
White clover	7.91	18.17
Lucerne	9.55	20.42

Gas development

Every day, 1681 ± 35.0 ml of gas were build in each fermenter. Gas development increased significantly after changing the diet from hay to any of the forages tested (Fig. 2). The highest amount of gas was measured when the GMC was tested.

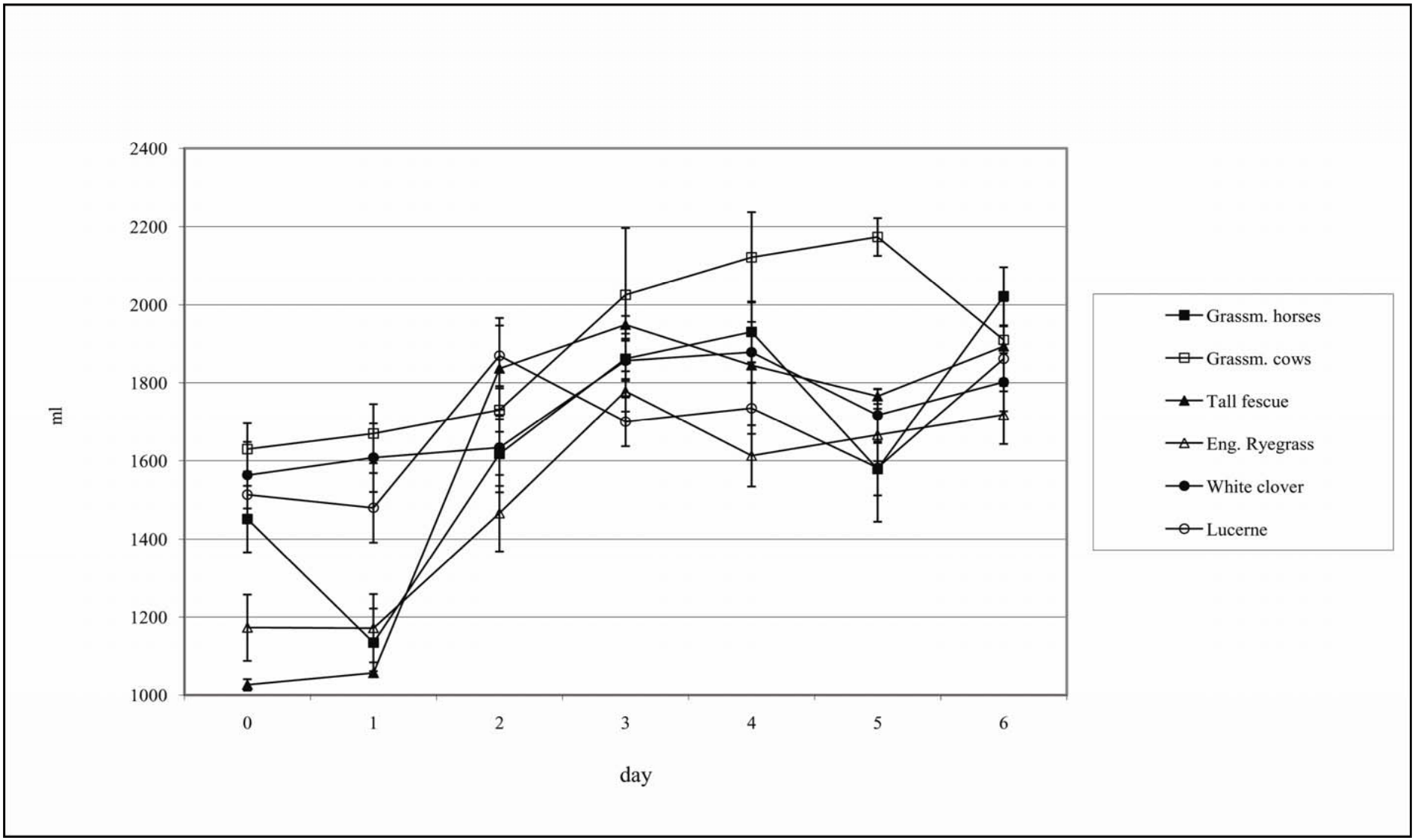


Fig. 2: Gas development of the forages investigated after changing the diet on day 1
0 = first day of sampling after period of stabilisation; 1 = day of diet change

Fructan

The two grass mixtures contained the highest concentrations of fructan of all the forages investigated as shown in Tab. 3. No fructan was detected in the legumes.

Tab.3: Concentration of fructan, starch, all storage carbohydrates as well as crude protein and crude fibre in forages investigated

(% i.DM) = percentage in dry mass **,** n.a. = no analysis

Forage	**Fructan** (% i.DM)	**Starch** (% i.DM)	**All storage carbohydrates** (% i.DM)	**Crude protein** (% i.DM)	**Crude fibre** (% i.DM)
Grass mixture horses	15.0	0.8	23.5	8.9	20.9
Grass mixture cows	12.2	0.4	20.7	10.9	22.6
Tall fescue	11.3	0.7	13.6	10.2	22.9
English ryegrass	7.0	0.3	21.5	8.6	23.9
White clover	0.0	1.2	8.2	24.3	20.4
Lucerne	0.0	n.a.	3.7	17.5	20.9

Starch

Concentration of starch in all forages tested was between 0.3 – 1.2 % i. DM. Detailed information are given in Tab. 3.

Proximate analysis

The values for crude protein for the grasses and grass mixtures were between 8.6 and 10.9 % i.DM. In samples of LU 24.3 % and in WC 17.5 % i.DM of crude protein were measured. In all forages rate of crude fibre was between 20.4 and 23.9 % i.DM. Detailed data are given in Tab. 3.

Lactate

Lactate was only reliably measured at time 1.1 and ranged between 0.0 – 1.8 mmol/ l (ø 1.0 mmol/ l ± 0.14 [D-lactat: ø 0.3 mmol/ l ± 0.05; L-lactate: ø 0.7 mmol/ l ± 0.1]). The highest concentration was measured during the LU trial.

Short chain fatty acids (SCFA)

The total concentration of SCFA was between 25.5 – 70.7 mmol/ l (ø 47.5 mmol/ l ± 2.3). The concentration of acetate ranged between 14.3 – 43.3 mmol/ l (ø 28.5 mmol/ l ± 1.5), of propionate between 7.2 – 17.9 mmol/ l (ø 11.7 mmol/ l ± 0.5) and of butyrate between 3.4 – 13.3 mmol/ l (ø 7.0 mmol/ l ± 0.5). Butyrat concentration increased significantly during day 1 in all trials.

Microbial samples

The number of aerobic bacteria was between 23 – 244 x 10^6 cfu during all trials and increased significantly one hour after changing the diet from hay to each of the forages investigated (Tab. 4). The maximum amount of bacteria was observed after changing the diet from hay to the GMC, the GMH, TF or WC.

Tab. 4: Aerobe Bacteria in the fine-particles phase

$*^{-}$ $*^{7}$= significant time differences within one forage species ($p < 0.05$); $^{a-j}$ = different characters show significant differences ($p < 0.05$)

°= explains day and hour of sampling, for example 1.6 = Day 1; 6 hours after changing feedbags

Forages	Time point°				
	1.0	1.1	1.6	2.6	6.0
	cfu x 10^6				
Grass mixture horses	91 ± 6.0 $^{b,d,f,g;}$ $*$	238 ± 25.9 $^{b,d,f;}$ $*^{1,}$ $*^{2}$	218 ± 23.9 $^{b,d,f;}$ $*^{1,}$ $*^{4}$	167 ± 13.2 $^{b,d,f,i;}$ $*^{1,}$ $*^{4}$	90 ± 6.5 $^{b,d,f,h,j;}$ $*^{3,}$ $*^{5}$
Grass mixture cows	165 ± 15.9 $^{b,d,f,h,j;}$ $*$	244 ± 16.2 $^{b,d,f;}$ $*^{1,}$ $*^{2}$	217 ± 13.8 $^{b,d,f;}$ $*^{4}$	223 ± 11.3 ^{b,d,} $^{f,h,j;}$ $*^{1,}$ $*^{4}$	43 ± 3.2 $^{b,c;}$ $*^{1,}$ $*^{3,}$ $*^{5}$
Tall fescue	99 ± 4.7 $^{b,d,f,j;}$ $*$	211 ± 2.1 $^{b,d;}$ $*^{1,}$ $*^{2}$	138 ± 7.1 $^{b,d,e;}$ $*^{1,}$ $*^{2,}$ $*^{4}$	87 ± 6.4 $^{b,e;}$ $*^{3,}$ $*^{5,}$ $*^{6}$	49 ± 5.2 $^{b,g;}$ $*^{1,}$ $*^{3,}$ $*^{5,}$ $*^{7}$
English ryegrass	64 ± 4.3 $^{b,e;}$ $*$	164 ± 11.2 $^{b,d,e;}$ $*^{1,}$ $*^{2}$	178 ± 26.5 $^{b,d;}$ $*^{1,}$ $*^{4}$	94 ± 10.7 $^{b,g;}$ $*^{1,}$ $*^{3,}$ $*^{5,}$ $*^{6}$	48 ± 6.1 $^{b,e;}$ $*^{1,}$ $*^{3,}$ $*^{5,}$ $*^{7}$
White clover	48 ± 8.7 $^{c;}$ $*$	79 ± 10.7 $^{c;}$ $*^{1,}$ $*^{2}$	55 ± 8.4 $^{a,c;}$ $*^{3,}$ $*^{4}$	38 ± 3.5 $^{a;}$ $*^{3,}$ $*^{4}$	23 ± 1.9 $^{a;}$ $*^{1,}$ $*^{3,}$ $*^{5}$
Lucerne	30 ± 2.3 $^{a;}$ $*$	42 ± 5.1 $^{a;}$ $*^{1,}$ $*^{2}$	52 ± 5.5 $^{a;}$ $*^{1,}$ $*^{3,}$ $*^{4}$	73 ± 7.0 $^{c;}$ $*^{1,}$ $*^{3,}$ $*^{5}$	53 ± 4.7 $^{b,d,i;}$ $*^{1,}$ $*^{5}$

The number of anaerobic bacteria also increased significantly one hour after diet change, apart from the trial with LU and GMC. In these trials the number of anaerobic bacteria increased between time point 1.1 to 1.6. During both trials highest amounts of bacteria were on time point 2.6, as well as during the trial conducted with TF. During the other trials, highest amounts of anaerobic bacteria were measured at point 6.0. The highest number of anaerobic bacteria overall was detected when LU was investigated, with a total of 1658 ± 154 x 10^6 cfu (see Tab. 5).

Tab. 5: Anaerobe Bacteria in the fine-particles phase

$*^{-}$ $*^{7}$= significant time differences within one forage species ($p < 0.05$); $^{a-i}$ = different characters show significant differences ($p < 0.05$)

°= explains day and hour of sampling, for example 1.6 = Day 1; 6 hours after changing feedbags

Forages	Time point°				
	1.0	1.1	1.6	2.6	6.0
	cfu x 10^6				
Grass mixture horses	178 ± 13.8 $^{b,e;}$ $*$	413 ± 67.7 $^{b,d;}$ $*^{1}$	339 ± 16.0 $^{b,d,e;}$ $*^{1,}$ $*^{2}$	351 ± 22.4 $^{b,d,e;}$ $*^{1,}$ $*^{2}$	541 ± 41.1 $^{b,d,f;}$ $*^{1,}$ $*^{3}$
Grass mixture cows	787 ± 82.5 $^{b,d,f,h,j;}$ $*$	431 ± 45.6 $^{b,d;}$ $*^{1,}$ $*^{2}$	890 ± 176.0 $^{b,d,f,h;}$ $*^{3,}$ $*^{4}$	1658 ± 153.6 $^{b,d,f,h,j;}$ $*^{1,}$ $*^{3,}$ $*^{5,}$ $*^{6}$	704 ± 50.3 $^{b,d,f,h;}$ $*^{3,}$ $*^{7}$
Tall fescue	268 ± 20.8 $^{b,d,f,g;}$ $*$	425 ± 27.5 $^{b,d;}$ $*^{1}$	465 ± 17.7 $^{b,d,g;}$ $*^{1,}$ $*^{2}$	467 ± 36.3 $^{b,d,g;}$ $*^{1,}$ $*^{2}$	313 ± 31.2 $^{e;}$ $*^{3}$
English ryegrass	71 ± 8.0 $^{a,c;}$ $*$	203 ± 9.4 $^{b,c;}$ $*^{1,}$ $*^{2}$	251 ± 18.9 $^{b,c;}$ $*^{1,}$ $*^{4}$	299 ± 13.2 $^{b,c;}$ $*^{1,}$ $*^{3,}$ $*^{5}$	331 ± 22.4 $^{b,e,g;}$ $*^{1,}$ $*^{3,}$ $*^{5}$
White clover	55 ± 8.8 $^{a;}$ $*$	124 ± 17.5 $^{a;}$ $*^{1,}$ $*^{2}$	108 ± 15.5 $^{a;}$ $*^{1}$ $*^{3,}$ $*^{5}$	146 ± 22.7 $^{a;}$ $*^{1,}$ $*^{5}$	233 ± 21.3 $^{a;}$ $*^{1,}$ $*^{3,}$ $*^{5}$
Lucerne	424 ± 48.4 $^{b,d,f,h,i;}$ $*$	402 ± 58.8 $^{b,d;}$ $*^{2}$	329 ± 76.0 $^{b,e,g;}$ $*^{4}$	1061 ± 123.4 $^{b,d,f,h,i;}$ $*^{3,}$ $*^{5,}$ $*^{6}$	253 ± 23.0 $^{a,c;}$ $*^{1,}$ $*^{3,}$ $*^{7}$

For the development of lactic acid producing bacteria, there was no constant pattern obvious for all forages. Noticeable were the significant high numbers of lactic acid producing bacteria during the trial with the GMC compared to the other trials. In contrast only very little numbers of bacteria were countable when WC and LU were tested (see Tab.6).

Tab. 6: Lactic acid producing bacteria in the fine-particles phase
$*^{1}$ - $*^{7}$= significant time differences within one forage species ($p < 0.05$); $^{a-j}$ = different characters show significant differences ($p < 0.05$)
°= explains day and hour of sampling, for example 1.6 = Day 1; 6 hours after changing feedbags

Forages	**Time point°**				
	1.0	**1.1**	**1.6**	**2.6**	**6.0**
	cfu x 10^6				
Grass mixture horses	$10 \pm 1.2^{b,d,e;\ *}$	$52 \pm 2.6^{b,d,f,i;\ *1,\ *2}$	$52 \pm 9.2^{b,d,f;\ *1,\ *4}$	$25 \pm 1.3^{b,d,e;\ *1,\ *3,\ *5,\ *6}$	$37 \pm 4.5^{b;\ *1,\ *3,\ *7}$
Grass mixture cows	$65 \pm 4.6^{b,d,f,h,j;\ *}$	$96 \pm 15.4^{b,d,f,h,j;\ *2}$	$86 \pm 2.9^{b,d,f,h;\ *1,\ *4}$	$163 \pm 10.0^{b,d,f,h,j;\ *1,\ *3,\ *5,\ *6}$	$42 \pm 3.9^{b,d;\ *1,\ *3,\ *5,\ *7}$
Tall fescue	$32 \pm 2.3^{b,d,f,h,i;\ *}$	$51 \pm 6.6^{b,d,f,g;\ *1,\ *2}$	$54 \pm 3.0^{b,d,f,g;\ *1,\ *4}$	$38 \pm 1.4^{b,d,f,h,i;\ *4,\ *5}$	$30 \pm 2.8^{b,c;\ *3,\ *5,\ *7}$
English ryegrass	$1 \pm 0.3^{a;\ *}$	$10 \pm 1.1^{a;\ *1,\ *2}$	$25 \pm 3.3^{b,e;\ *1,\ *3,\ *4}$	$14 \pm 1.6^{b,c;\ *1,\ *5,\ *6}$	$36 \pm 2.7^{b;\ *1,\ *3,\ *5,\ *7}$
White clover	$1 \pm 0.4^{a;\ *}$	$11 \pm 2.7^{a,c;\ *1,\ *2}$	$6 \pm 1.0^{a;\ *1\ *3,\ *4}$	$7 \pm 1.2^{a;\ *1,\ *6}$	$29 \pm 0.9^{b,c;\ *1,\ *3,\ *5,\ *7}$
Lucerne	$14 \pm 1.6^{b,d,f,g;\ *}$	$19 \pm 1.1^{b,e;\ *1,\ *2}$	$23 \pm 4.4^{b,c}$	$26 \pm 1.9^{b,d,g;\ *1,\ *3}$	$21 \pm 1.1^{a;\ *1}$

Discussion

The pH-value measured in the present study were always in the physiological range of pH 6 - 7 (Engelhardt and Breves 2000) as seen in other studies using the Caesitec (6.4 - 6.8) (Dill et al. 2006; Dill et al. 2007; Engelmann et al. 2007; Müller et al. 2008; Müller et al. 2009; Kuhn 2009). In studies in which caecum content was incubated with starch, inulin or fructan the pH-value declined to 5.1 - 5.3 (Bailey et al. 2002; Engelmann et al. 2007). The concentrations of measured ammonia in the present study in all trials were similar to the range of 1.2 – 23.7 mmol/ l measured by other investigators (Kern et al. 1974; Stott et al. 1983; Landes 1992; Medina et al. 2002; Engelmann et al. 2007; Kuhn 2009). A provocation with starch leads to a drop down to 11.8 – 12.0 mmol/ l (Müller et al. 2008). A provocation of caecum content with increasing amounts of inulin leads to a continuous drop of ammonia (Engelmann et al. 2007). Higher concentrations of ammonia during the trials when legume were tested are resulting from stronger degradation of protein, caused by higher concentrations of crude protein in LU and WC. In the present study the diet change from hay to any of the forages tested revealed a rise of gas production. That effect was also observed after an excessive starch feeding (Müller et al. 2008) and a chronic incubation of caecum content with fructan (Engelmann et al. 2007). During all studies the gas production increased after changing the diet. These findings confirm the assumption that excessive food intake and a sudden change in diet can lead to partial forage breakdown and incorrect fermentation, what was supposed as an increase of gas formation in the simulated large intestine. This can also be observed when horses have access to young grass, fresh clover, legume, lucerne, withered or heated forage (Meyer 1995; Dietz and Huskamp

2006). The concentrations of lactate detected during the different trials in the present study were comparable to other studies when hay based feeding was tested (Alexander and Davies 1963; Willard et al. 1977; de Fombelle et al. 2001; Medina et al. 2002; de Fombelle et al. 2003; Respondek et al. 2008). In other studies, where starch-rich diets were tested the lactate concentration was considerably higher (0.2 – 24.1 mmol/ l) (Willard et al. 1977; Medina et al. 2002; de Fombelle et al. 2003). One reason for the detectable lactate concentrations only one hour after changing the feedbag might be the fact, that there were only low concentrations of lactic acid that decreased rapidly and were below the limit of detection after six hours. However, Medina et al. (2002), observed a rapid increase of lactic acid after feeding with a maximum 3 hours after the meal and thereafter a continuous decrease was noticed. The concentration of SCFA measured in the present study was in the range of 18.2 – 115 mmol/ l, measured in the caecum and colon in former studies (Alexander and Davies 1963; Kern et al. 1974; Stott et al. 1983; Landes 1992; Dill et al. 2007; Müller et al. 2008; Respondek et al. 2008; Müller et al. 2009). Also the amounts of acetic acid and propionic acid were comparable to results of Kern et al. (1974), Medina et al. (2002), Veiga et al. (2005) and Respondek et al. (2008). In these studies acetic acid was between 20.6 – 72.8 mmol/ l and propionic acid was between 2.8 – 18.0 mmol/ l. Only maximum concentrations of butyric acid in the present study were out of the range. The obtained results of fructan concentrations in forages grown in Switzerland have been against expectations. ER as a fructan storing grass showed the lowest concentration of the fructan (6.95 % i.DM) of the grasses and grass mixtures and did not at all reach the maximum amounts of 400 g/ kg i.DM measured in Great Britain (Longland et al., 1999). In contrast the grass mixture for horses contained the highest concentration of fructan (14.99 i.DM). In other forage samples taken of swiss pasture the concentration of fructan was on average 37.6 g/ kg DM (Nater et al. 2007). The small amounts of fuctan in general might be caused by the warm and sunny weather up to 20.4 °C the two days before harvesting. Warm temperature as well as adequate humidity might have caused that carbohydrates were used for the growth of the plant instead of incorporating storage carbohydrates. The time of day when the forages were harvested as well as the location of growth might be further reasons for smaller fructan concentrations. Highest amounts of fructan were measured at noon and during the early evening hours by Cairns et al. (2002) and environmental conditions of countries and areas seem to influence the amount of fructan (Jeaungros et al. 2001).

To conclude, none of the forages tested in the present study contained concentrations of fructan, high enough to reach the trigger point of 7.5 g fructan/ kg BW to induce laminitis. Calculating with an intake of 10 kg dry mass/ 500 kg BW (Zeyner 1995) the ingestion of fructan was between 0.0 and 3.0 g/ kg BW depending on the forage. Crawford et al. (2007) fed ponies with a diet of chopped dried spring pasture and 3g per kg BW of inulin. Similar to our findings that there were only slight reactions in the “Caesitec”, the diet did not seem to cause laminitis. The measured concentration of starch (0.3 % i. DM) in ER was similar to the results of analysis (0.4 % i. DM,) conducted by Ojima and Isawa (1968). The concentration of WC (1.2 % i. DM) in the present study was below the result of Ojima and Isawa (1968) (4.2 % i. DM). In contrast the concentration of starch in TF (0.7 % i. DM) was considerably higher compared to the concentration (0.1% i. DM) measured by Ojima and Isawa (1968).The differences might refer to the surrounding conditions. For the grass mixtures tested are no reference values available.

Grasses, grass mixtures and legumes are generally classified as feeding stuff with only low concentrations of starch. Under physiological conditions starch is digested by the pancreatic amylase and brushborder-membrane-bound enzymes in the small intestine. Starch particles which have not been digested by the pre-cecal digestion will be degraded by microbial enzymes in the large intestine. An increasing in starch intake into the large intestine can lead to a change of micro flora and metabolism parameters (Kern et al. 1973, de Fombelle et al. 1999, Julliand et al. 2001, de Fombelle et al. 2003). Due to the fact that the forages tested in the present study are classified as feedstuff with low starch concentrations and the fact the large intestine is able to tolerate limited amounts of starch by microbial enzymes without causing a pathological change in the microflora, it can be supposed that the starch concentrations stored in the forages had no significant influence on the behavior of the micro flora. This expectation is supported by the fact that the amount of starch (2g starch/ fermenter/ day) loaded into the Caesitec every day (Fig. 1) was higher compared to the starch concentration measured in all forages. The accurately defined amount of starch simulates an established diet based on the knowledge of former studies (Dill et al. 2006, Engelmann et al. 2006, Zeyner et al. 2006b, Dill et al. 2007, Engelmann et al. 2007, Zeyner et al. 2007). It was noticeable that the concentration of crude protein in the GMH, GMC and WC was below the values given in the Swiss feed database (all data in % i.DM: GMH: 10.5 – 18.9; GMC: 14.4 – 22.9; WC: 21.8 – 26.0). The reason for this deviation might be the point of harvest. Depending on the species of forage the concentration of crude protein decreases during the first growth (Jeaungros et al. 2001; Kamphues et al. 2009). Further factors of influence are the manuring, the climate and the character of ground (Kamphues et al. 2009). A study conducted by Agroscope Switzerland showed prominent variations between different locations (Jeaungros et al. 2001). In consequence of low concentrations of crude protein, higher concentrations of crude fiber would have been expected. The reason, that crude fiber was in the range might have been influenced by the weather and the environmental conditions of the location. The increase in aerobic bacterial numbers is similar to the results reported by de Fombelle et al. (2001) when diet was changed from 100% hay to 70% hay + 30% barley. As well as observed in the present study the aerobe bacterial number remained on a higher level at 29-30 hours after diet change. Also similar effects were observed in the behavior of anaerobic bacteria. After diet change the number of anaerobic bacteria in the large intestine increased after 29 (de Fombelle et al. 2001), respectively 30 (present study) hours. The numbers counted in all trials was in the range ($10^{7.6}$ - $10^{9.7}$) reported in other studies (Kern et al. 1973; Kern et al. 1974; Goodson et al. 1988; de Fombelle et al. 2003; Veiga et al. 2005). The number of lactic acid producing bacteria was in the range reported by de Fombelle (2001). The unbalanced behavior of the lactic acid producing bacteria can not be explained at the moment, but there might be a link to the production of lactate. Overall the results underline that an abrupt change in diet modifies the counts and activities of some microbial communities in the caecum. Against expectations the grass mixture especially composed for horses contained the highest concentrations of fructan. Also the amount of fructan and other storage carbohydrates does not entail a permanent change in the microflora of the hindgut in horse. Nevertheless further studies should be done to prove the influence and importance of the environmental conditions and the date and time of harvest. Furthermore the pre-digestion of the stomage and small intestine should be regarded and incorporated in the experimental set-up.

References:

Alexander, F.; Davies, M.E., 1963: Production and fermentation of lactate by bacteria in the alimentary canal of the horse and pig. *J. Comp. Pathol.* **73**, 1-8.

Bailey, S.R.; Marr, C.M.; Elliott, J., 2004: Current research and theories on the pathogenesis of acute laminitis in the horse. *Vet. J.* **167**, 129-142.

Bailey, S.R.; Rycroft, A.; Elliot, J., 2002: Production of amines in equine cecal contents in an in vitro model of carbohydrate overload. *J. Anim. Sci.* **80**, 2656-2662.

Cairns, A.J.; Cookson, A.; Thomas, B.J.; Turner, L.B., 2002: Starch metabolism in fructan-grasses: patterns of starch accumulation in excised leaves of Lolium temulentum (L.). *J. Plant Physiol.* **159**, 293-305.

Crawford, C.; Sepulveda, M.F.; Elliott, J.M.; Harris, P.A.; Bailey, S.R., 2007: Dietary fructan carbohydrate increases amine production in the equine large intestine: implications for pasture-associated laminitis. *J. Anim. Sci.* **85**, 2949-2958.

Dietz, O.; Huskamp, B., 2006: *Handbuch Pferdepraxis*, 3. Auflage, Enke Verlag, Stuttgart, Germany.

Dill, B.; Engelmann, W.; Markuske, K.D.; Zeyner, A., 2006: Suitability of equine caecum content as inoculum in a modified "Rumen Simulation Technique" - Preliminary results. in *Proc. Soc. Nutr. Physiol.2006* **15**, 163.

Dill, B.; Engelmann, W.; Markuske, K.D.; Zeyner, A., 2007: Comparison of equine caecum content and faeces as inocula in a modified 'Rumen Simulation Technique'. in *Proc. Soc. Nutr. Physiol.2007* **16**, 73.

Engelhardt, W.V.; Breves, G., 2005: *Physiologie der Haustiere*, 2. Auflage, Enke Verlag, Stuttgart, Germany.

Engelmann, W.; Dill, B.; Markuske, K.D.; Zeyner, A., 2006: Comparison of equine caecum content and faeces as inocula in modified “Hohenheim Gas test” with different substrates. in *Proc. Soc. Nutr. Physiol.2006* **15**, 162.

Engelmann, W.; Dill, B.; Markuske, K.D.; Aschenbach, J.; Zeyner, A., 2007: Investigations on chronic incubationof equine caecum content with fructan in a modified "Rumen Simulation Technique". in *Proc. Soc. Nutr. Physiol. 2007* **16**, 74.

Engelmann, W.; Zeyner, A.; Markuske, K.D.; Aschenbach, J., 2009: HPLC analysis of histamine and other biogenic amines in digesta cultures. *Chromatographia* **70**, 1207-1213.

de Fombelle, A.; Julliand, V.; Drogoul, C.; Jacotot, E., 2001: Feeding and microbial disorders in horses: 1 - Effects of an abrupt incorporation of two levels of barley in a hay diet on microbial profile and activities. *J. Equine Vet. Sci.* **21**, 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. *J. Anim. Sci.* **77**, 293-304.

Garner, H.E.; Coffman, J.R.; Hahn, A.W.; Hutcheson, D.P.; Tumbleson, M.E., 1975: Equine laminitis of alimentary origin: an experimental model. *Am. J. Vet. Research* **36**, 441-444.

Goodson, J.; Tyznik, W.J.; Cline, J.H.; Dehority, B.A., 1988: Effect of an abrupt diet change from hay to concentrate on microbial numbers and physical-environment in the cecum of the pony. *Appl. Environ. Microbiol.* **54**, 1946-1950.

Huntington, P.; Pollit, C.C., 2002: Nutrition and the Equine Foot. in *Proc. 2002 Equine Nutr. Con. Kentucky Equine Res.*, 149-162.

Janczyk, P.; Engelmann, W.; Dill, B.; Souffrant, W.B.; Markuske, K.D.; Aschenbach, J.; Zeyner, A., 2010: Changes in the microbial population caused by supplementation of inulin-like fructans in the Caesitec-system. in *ESVCN Proc. 14th Congr. Europ. Soc. Vet. Comp. Nutr.,* Zürich, Switzerland, 30.

.

Jeaungros, B.; Scehovic, J.; Schubiger, F.X.; Lehmann, J.; Daccord, R.; Arrigo, Y., 2001: Nährwert von Wiesenpflanzen: Trockensubstanz-, Rohprotein- und Zuckergehalte. *Agrarforschung* **8**, 79-86.

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. *J. Equine Vet Sci.* **21**, 543-546.

Kamphues, J.; Kienzle, E.; Coenen, M.; Pallauf, J.; Wanner, M.; Simon, O.; Zentek, J., 2009: *Supplemente zu Vorlesungen und Übungen in der Tierernährung*, 11. Auflage, M. & H. Schaper, Hannover, Germany.

Kern, D.L.; Slyter, L.L.; Weaver, J.M.; Leffel, E.C.; Samuelson, G., 1973: Pony cecum vs. steer rumen: the effect of oats and hay on the microbial ecosystem. *J. Anim. Sci.* **37**, 463-469.

Kern, D.L.; Slyter, L.L.; Leffel, E.C.; Weaver, J.M.; Oltjen, R.R., 1974: Ponies vs. steers: microbial and chemical characteristics of intestinal ingesta. *J. Anim. Sci.* **38**, 559-564.

Kuhn, M., 2009: In-vitro-Untersuchungen zum Einfluss von Erythromycin und Nahrungsreduktion auf mikrobielle Stoffwechselleistungen im Caecum des Pferdes. Tierärztliche Hochschule Hannover, Institut für Tierernährung, Hannover, Germany.

Landes, E., 1992: Amylaseaktivität und Konzentration organischer Säuren im Jejunum- und Caecumchymus des Pferdes nach Hafer- und Maisfütterung. Tierärztliche Hochschule Hannover, Institut für Tierernährung, Hannover, Germany.

Longland, A.C.; Cairns, J.; Humphreys, M.O., 1999: Seasonal and diurnal changes in fructan concentration in Lolium perenne: Implications for the gazing management of equines pre-disposed for laminitis. in *16. Equine Nutrition and Physiology Society*, 258-259.

Medina, B.; Girard, I.D.; Jacotot, E.; Julliand, V., 2002: Effect of a preparation of Saccharomyces cerevisiae on microbial profiles and fermentation patterns in the large intestine of horses fed a high fiber or a high starch diet. *J. Anim. Sci.* **80**, 2600-2609.

Meyer, H., 1995: *Pferdefütterung*, 3. aktualisierte Auflage, Blackwell Wissenschaftsverlag, Berlin, Germany.

Müller, A.-M.; Gall, D.; Bremer, S.; Zeyner, A., 2008: Suitability of differently harvested and prepared equine faeces as inoculum in the semi-continuous fermentation technique Caesitec. in *ESVCN Proc. 12th Congr. European Soc. Vet. Comp. Nutr.*, Wien, Austria, 117.

Müller A.-M.; Gall, D.; Bremer, S.; Romanowski, K.; Zeyner, A., 2009: Effects of the preservation of equine faeces as inoculum on fermentation patterns in the semi-continuous fermentation technique Caesitec. in *ESVCN Proc. 13th Congr. Europ. Soc. Vet. Comp. Nutr.*, Oristano, Italy, 160.

Mungall, B.A.; Kyaw-Tanner, M.; Pollitt, C.C., 2001: In vitro evidence for a bacterial pathogenesis of equine laminitis. *Vet. Microbiol.* **79**, 209-223.

Nater, S.; Wanner, M.; Wichert, B., 2007: Nutrient content and adequacy of roughage for horse nutrition: An investigation under swiss conditions. *Schweiz. Arch. Tierheilk.* **149**, 103-109.

Naumann, C.; Bassler, R., 1997: *Die chemische Untersuchung von Futtermitteln.* in: *Methodenbuch*, Band III, 3. VDLUFA Verlag, Darmstadt, Germany.

Pollitt, C.C.; van Eps, A.W., 2002: Equine laminitis: A new induction model based on alimentary overload with fructan. in *Aust. Equine Vet. Assoc. Bain-Fallon Memorial Lectures*, Australia, 96-97.

Respondek, F.; Goachet, A.G.; Julliand, V., 2008: Effects of dietary short-chain fructooligosaccharides on the intestinal microflora of horses subjected to a sudden change in diet. *J. Anim. Sci.* **86**, 316-323.

Stott, N.M.; Mitchell, G.E.; Dawson, K.A.; Baker, J.P., 1983: Technique for the continiuous invitro culture of equine caecal microorganisme. *J. Anim. Sci.* **56**, 1340-1344.

Veiga, L.D.; Chaucheyras-Durand, F.; Julliand, V., 2005: Comperative study of colon and faeces microbial communitis and activities in horses fed a high starch diet. *Pferdeheilk.* **21**, 45-46.

Willard, J.G.; Willard, J.C.; Wolfram, S.A.; Baker, J.P., 1977: Effect of diet on cecal pH and feeding-behavior of horses. *J. Anim. Sci.* **45**, 87-93.

Zeyner, A., 1995: *Diätetik beim Pferd*, Enke Verlag, Stuttgart, Germany.

Zeyner, A.; Dill, B., 2006a: Suitability of equine caecum content as inoculums in a modified "rumen simulation technique". in *ESVCN Proc.10th Congr. Europ. Soc. Vet. Comp. Nutr.,* Nantes, France, 127.

Zeyner A.; Engelmann W.; Dill B.; Markuske, K.D., 2006b: Equine caecum content and faeces as inocula in a modified „Hohenheim das test" with hay, maize and a combination of both. in *ESVCN Proc. 10th Congr. Europ. Soc. Vet. Comp. Nutr.,* Nantes, France, 218.

Zeyner A.; Engelmann W.; Dill B.; Markuske, K.D.; Aschenbach, J., 2007: Effects of slowly increasing fructan load on equine caecum content in a semi-continuous in vitro technique (Caesitec). in *ESVCN Proc 11th Congr. Europ. Soc. Vet. Comp. Nutr.,* Leipzig, Italy, 160.

Danksagung

Meinen beiden Projektleiterinnen **Dr. Brigitta Wichert** und **PD Dr. Annette Liesegang** für das Überlassen des Themas und die nette fachliche Betreuung während der Doktorarbeit.

Der Korreferentin **Frau Prof. Dr. Annette Zeyner** und ihrem gesamten **Team in Rostock** für die großartige Unterstützung und schnelle Auswertung unserer Analysen, sowie die Übernahme der Korrektur.

Ines Mittner und **Barbara Schneider** für die allzeit tatkräftige körperliche und zeitweise auch seelische Unterstützung während der langen Labortage.

Sina Barth und **Dr. Sarah Prohaska** für die immer nette Unterstützung im Labor der Veterinärbakteriologie.

Dr. Daniel Suter und **Hansueli Hirschi** für die fachliche Hilfe beim Anbau und der Ernte der Versuchsgräser, sowie die Anfertigung des Papers.

PD Dr. Ludwig Hölzle und **Prof. Dr. Max Wittenbrink** für die fachliche Unterstützung im Bereich Mikrobiologie.

Prof. Dr. Dr. Marcel Wanner für die Finanzierung meiner Doktorandenstelle.

Meinen tollen Kollegen **Carola Kaulfers, Julia Trossen, Kerstin Siedler, Martina Signer, Sara Weilenmann** und **Thomas Häring**. Danke für die tolle Zeit mit euch in der Schweiz und die viele unvergessliche Momente.

Meinen Kollegen **Mirjam, Ina** und **Dominik** für die moralische Unterstützung und das Übernehmen von Terminen und Diensten, wenn mal wieder eine Abgabefrist für das Paper bedenklich nahe rückte.

Gabriela Eger und **Rita Kant** für die gute Zusammenarbeit und die Hilfe im bürokratischen Dschungel der Uni.

Meinen tollen Freundinnen **Tini, Kristina** und **Anne** die sich geduldig alles angehört haben und mich immer und jederzeit unterstützt haben und mich manchmal auch auf den Boden der Tatsachen zurückgeholt haben.

Meiner Familie die mich während meines Studiums und auch während der Doktorarbeit immer unterstützt hat und immer an mich geglaubt hat.

Sven, dem ich nicht genug danken kann, weil er einfach mein Fels in der Brandung ist. Danke, dass Du ungezählte Wochenenden mit mir im Labor gestanden hast. Danke, dass Du mich immer wieder aufgebaut hast, wenn der Caesi mal wieder gebockt hat. Danke, für so grandiose Ideen wie ein Picknick im Labor.

Curriculum Vitae

Name	Judith, Vosmer
Geburtsdatum	04.12.1981
Geburtsort	Uslar
Nationalität	deutsch

1988 – 1992	Grundschule Uslar, Uslar
1992 – 1994	Orientierungsstufe Uslar, Uslar
1994 – 2001	Gymnasium Uslar, Uslar
2001	gymnasialer Abschluss, *Abitur*
2001 – 2008	Studium der Veterinärmedizin an der Tierärztlichen Hochschule Hannover, Deutschland
2008	Staatsexamen an der Tierärztlichen Hochschule Hannover, Deutschland
2008 – 2010	Assistentin und Doktorandin, Institut für Tierernährung, Vetsuisse Fakultät Zürich, Zürich, Schweiz
Seit 2010	Assistenztierärztin in der Tierarztpraxis Dr. D. Roeder, Bensheim, Deutschland