Comparative study of colon and faeces microbial communities and activities in horses fed a high starch diet

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Introduction

Under current practices, athletic horses receive massive quantities of readily fermentable carbohydrates (RFC) in one meal. The overload of RFC non digested in the foregut enters the large intestine and can cause severe digestive diseases like colics, laminitis or enterotoxemia. It was shown in fistulated animals that these disturbances were connected to a pH decrease and an increase of lactic acid concentration in the colon, due to fermentative activities of the autochthonous microflora. In addition to modifications in the fermentation pattern, important changes in numbers of some bacterial populations in the colon have been reported: counts of cellulolytics decreased whereas that of Streptococci, Lactobacilli and lactate utilisers increased. It is not known if the same changes would affect the faeces. In other monogastric animals and in humans, it has been established that faeces can reflect, on a microbiological and biochemical point of view, the distal colon. Therefore, the objective of our work was to compare colon and faeces microbial ecosystems of horses fed a high starch diet, in order to allow future utilisation of faeces as a marker to study changes of the microbial balance in horses hindgut.

Material and Methods

Four gelding (average live body weight (BW) of 464 kg) fitted with a right ventral colon cannula were housed in individual free stalls, bedded with shavings. They had free access to water. They received a daily diet of 70% hay and 30% concentrate feed (35% barley + 15% commercial pellets). The morning meal consisted of 50% hay and 50% and provided a minimum of 200g/100 kg BW of starch. The diet was calculated to cover horses needs. After 14 days adaptation to the diet, faecal and colonic contents were collected five hours after the morning meal. Colonic content were collected by gravity through the cannula and faecal samples were taken manually in the rectum. Both samples were placed immediately into CO_2 saturated bottles. pH and redox potential were instantly measured. Subsamples were prepared and frozen for further determination of VFA by gas-liquid chromatography and lactate by spectrophotometry. Usual microbiology techniques were used to enumerate total anaerobic bacteria, cellulolytic bacteria, Streptococci, Lactobacilli, and lactate-utilising bacteria. Logarithmic transformations were performed on microbial counts before statistical analysis. An analysis of variance with the GLM procedure of SAS v8.2 was done to evaluate the variables' response (microbial counts, pH, redox potential, VFA, D- and L-lactic acid). The model included horse as an randomised effect, and compartment. Least square means were calculated for all variables and separated using pairwise t-tests (P_{diff} option of SAS) and the significance threshold for all tests was set at $P<0.05$.

Results

Counts of total anaerobes, lactic-acid utilisers and Streptococci were higher in the faeces than in the colon. No statistical difference were observed for the Lactobacilli and cellulolytics concentrations. The values of redox potential were more reducing in the colon than in the faeces. The concentration of total VFA as well as acetate and propiontate and that of D- and L-lactate were lower in the faeces than in the colon.

Table 1 Different microbiological and biochemical parameters measured in colon and faeces of horses.

				Compartment		
Parameter	Units	r2	SD	Colon	Faeces	Effects (1)
Total anaerobes	Log_{10} cfu/mL	0.97	0.7	7.6^a	8.3^b	***
Lactic-acid utilisers	Log_{10} cfu/mL	0.97	0.5	6.9^a	7.5^b	***
Lactobacilli	Log_{10} cfu/mL	0.58	0.8	6.0	6.4	
Streptococci	Log_{10} cfu/mL	0.96	0.7	6.7^a	7.7^b	***
Cellulolytics	Log_{10} mpn/mL	0.35	0.9	4.3	4.6	
pH		0.69	0.3	6.6	6.6	
Redox potential	mV	0.89	153.9	–488.0 ^{a}	–226.1 ^{b}	***
Acetate	mmol/L	0.88	17.8	56.0^a	34.6^b	**
Propionate	mmol/L	0.81	4.8	15.9^a	9.5^b	**
Isobutyrate	mmol/L	0.89	0.6	0.8	0.8	
Butyrate	mmol/L	0.67	2.6	5.7	3.4	
Iso Valetrate	mmol/L	0.85	0.9	1.0	0.9	
Valerate	mmol/L	0.61	0.5	0.7	0.5	
Total VFA	mmol/L	0.85	24.6	80.0^a	49.6^b	**
D-Lactate	mmol/L	0.89	1.2	2.2^a	0.7^b	*
L-Lactate	mmol/L	0.68	0.8	1.6^a	0.3^b	*
Total Lactate	mmolL	0.88	1.8	3.8^a	1.0^b	*

r2: of the statistic model; SD: standard deviation; numbers in each line with different letter superscript differ significantly $P<0.05$; (1) *** $P<0.001$, ** $P<0.01$, $P<0.05$

Discussion

Our results underlined both similarities and differences in term of levels and activities between faecal and colonic bacterial communities. Considering total anaerobes and lactate utilizers counts, we measured an increase of five-fold and four-fold respectively in the faeces compared to the colon. This cannot be explained only by the dry matter increase of the intestinal content from the right ventral colon (15.8%) to the rectum (23.4%) (*Varloud* et al. 2004) due to the water absorption occuring along the colon. Therefore bacterial

growth probably occurs within and downstream the right ventral colon, depending on the substrates flowing from the upper tract. Regarding total anaerobes, previous results appeared controversial. *De Fombelle* et al. (2003) measured a higher concentration in the right ventral colon (8.9^{log10} cfu/g of content) than in the faeces (8.3^{log10} cfu/g) with a diet composed of a pelleted concentrate rich in cereals (providing 286 g of starch/100kg BW in the morning meal) and hay. On the contrary, with a diet composed of a pelleted concentrate rich in fibre (providing 74 g of starch/100kg BW in the morning meal) and straw, these latter authors found 8.6 ^{log10} cfu/g in the faecal content versus 8.1 log10 cfu/g in the colonic content. This was in accordance with *Julliand* and *Goachet* (2005) who enumerated higher counts of total anaerobes in the faecal content (8.3 and 8.7^{log10} cfu/g of content) compared to the colonic content (7.8 and 8.2^{log10} cfu/g of content) with diets composed of hay only or hay plus a pelleted concentrate rich in fibre (providing 45 g of starch / 100kg BW in the morning meal).

As for lactate utilisers, the increase we noted was in accordance with previous results (*de Fombelle* et al. 2003; *Julliand* and *Goachet* 2005). Interestingly, the representation of lactate utilisers amongst total strictly anaerobes remained similar in the colon and in the faeces (15 and 17%, respectively).
Similarly to total anaerobes and lactate-utilisers, counts of Lactobacilli and Streptococci were also more numerous in the faeces, increasing two- and eight fold, respectively. The increase of Lactobacilli density did not appear to be statistically significant and was mainly explained by the difference of dry matter. However previous results had shown a significant increase of Lactobacilli density in the faecal content (*de Fombelle* et al. 2003; *Julliand* and *Goachet* 2005). The important increase of Streptococci counts we measured was in accordance with *Julliand* and *Goachet* (2005) but disagreed with *de Fombelle* et al. (2003) who found no change. The concentration of lactate, higher in the faecal content than in the colonic content, was probably the result of the more important increase of lactate producing bacteria than lactate utilising bacteria in the faeces.

We measured no difference in the concentrations of cellulolytic bacteria between the colon and the faeces, in accordance with previous results (*de Fombelle* et al. 2003, *Julliand* and *Goachet* 2005). Our cellulolytic counts appeared on the whole very low compared to those found in the literature (*Mackie* et *Wilkins* 1988, *Medina* et al. 2002, *de Fombelle* et al. 2003, *Julliand* and *Goachet* 2005). This drop was related to the feeding conditions we chose in our experiment in order to destabilize the colonic ecosystem: we set a quantity of concentrate in the morning meal that exceeded the limit of prececal digestion (*Kienzle* 1994). Our low counts of cellulolytic bacteria in the colon were consistent with the lower concentrations of VFA we noted compared to results from the literature (*Medina* et al. 2002, *de Fombelle* et al. 2003). Total VFA, acetate and propionate concentration were lower in faecal than in colonic content. The cellulolytic bacteria that are strictly anaerobic probably have less favorable conditions in the rectum than in the colon. The Redox potential we measured was indeed a lot higher in the colon (-488 mV) than in the rectum (-226 mV); also, nutrients that flow to the rectum are in small quantities and mainly undigestible.

Conclusions

In relation with a different environment (lower redox potential), the faecal microflora differed from the colonic microflora both quantitatively (higher concentration of total anaerobes, lactate utilisers and streptococci) and by its activity (lower concentration of total VFA and lactate). However, the microbial communities and the activities we investigated remained comparable. Further experiments are needed to compare the microbial biodiversity of the two ecosystems. Also, a better knowledge about the reaction of faecal and colonic communities to differents effects (such as the impact of the diet, of the transport, etc.) is essential before concluding if faeces are appropriate markers to study the horse colonic ecosystem.

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