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Characterization of the microbial and biochemical profile of the different segments of the digestive tract in horses given two distinct diets

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Abstract

A first group of three horses was given diet 1 (D1) allowing 1180 g per 100 kg body weight (BW) of a pelleted food rich in fibre (P1) and 556 g per 100 kg BW of straw during a 20-day period to allow for adaptation. A second group of four horses were given diet 2 (D2) allowing 1180 g per 100 kg BW of a pelleted food rich in cereals (P2) and 1000 g per 100 kg BW of meadow hay during the same period. Digesta was collected from the stomach, duodenum, jejunum, ileum, caecum, right ventral colon, left ventral colon, left dorsal colon, right dorsal colon, and small colon, and faeces were collected under general anaesthesia 2·5 h after the ingestion of the morning pelleted meal. The concentration of total anaerobic, cellulolytic and lactic acid-utilizing bacteria, lactobacilli and streptococci were determined in all these segments except for the duodenum, left ventral colon, right dorsal colon and small colon. D-/L-*lactic acid, volatile fatty acids and pH were measured in all anatomic segments of the digestive tract (from stomach to small colon). The caecal concentration of total anaerobic bacteria was the lowest (7·9 5 10^7 colony-forming units (c. f. u.) per ml), whereas that of the stomach was the highest (1·4 5 10^9 c. f. u. per ml) (P < 0·001). Cellulolytic bacteria did not exceed 3·0 5 10^2 c. f. u. per ml in the ante-caecal segments whereas in the hindgut the average concentration was 5·3 × 10^5 c. f. u. per ml (P < 0·001). Likewise, VFA concentrations were also greater in the large intestine (on average, 96·3 mmol/l* v. *8·8 mmol/l in the ante-caecal segments) (P < 0·001), confirming the limited extent of fibre degradation in these ante-caecal segments. Lactobacilli, streptococci and lactate-utilizing bacteria colonized all the digestive tract; the stomach and the small intestine tended to host the greatest numbers of these bacteria, which suggests a high interference of micro-organisms with the digestion of readily fermentable carbohydrates. Compared with the other ante-caecal segments, the stomach ecosystem seemed the most affected by the composition of the last pelleted meal ingested : the concentrations of lactobacilli and lactate-utilizing bacteria were higher (P < 0·05) with P2. The lower concentration of* D-/L-*lactate with P2 (P < 0·05) was concomitant with a greater proportion of propionate (P < 0·05), probably related to a greater fermentation of lactate. In the large intestine of horses given D2, cellulolytic bacteria tended to be lower, whereas VFA concentrations were higher (P < 0·05). The lower [NDF/starch] ratio of D2 was probably less propitious for the proliferation of cellulolytic bacteria but was compensated by the higher cellulose intake brought by the hay.*

Keywords: *digestive tract, fermentation, horses, intestinal micro-organisms, nutrition physiology.*

Introduction

Most studies on the equine digestive ecosystem are focused on the hindgut with the aim of describing and understanding the mechanisms involved in forage degradation. The caecum, considered as the main site of microbial activity, is generally compared with the rumen of ruminants. On the other hand, the colon is often neglected but when it is studied, it is considered as a single entity despite its different anatomo-physiological segments (Hintz *et al.,* 1971; Kern *et al.,* 1974). Later studies have highlighted the implication of the right ventral colon ecosystem in forage degradation (Bertone *et al.,* 1989; Drogoul *et al.,* 1995) and its sensitiveness to high starch diets (de Fombelle *et al.,* 2001). Diet characteristics were shown to influence both the caecal and right ventral colonic ecosystems (Hintz *et al.,* 1971; Kern *et al.,* 1973; Julliand *et al.,* 2001; Medina *et al.,* 2002). However, the ecosystems of the colon lower parts remain unexplored. Concerning the ante-caecal ecosystem, few studies have been conducted and are essentially descriptive; the stomach and small intestine of the horse have been shown to host an abundant microflora (Alexander and Davies, 1963; Kern *et al.,* 1974; Mackie and Wilkins, 1988). To our knowledge, the nutritional implication of these micro-organisms has not yet been explored in horses, but could be substantial in view of the involvement of the ileal microflora in the degradation of starch in dogs (Murray *et al.,* 2001).

This study was set to describe the microbial population and its end-products in the different anatomic segments of the digestive tract in the horse, from the stomach to the ascending colon. We also assessed the effect of two distinct diets on the ecosystems along the digestive tract. Our results should enable establishment of some data on the function of digestive micro-organisms in horses and their nutritional consequences on feeding habits.

Material and methods

Animals, management and diets

Seven adult geldings (average weight 364 (s.e. 38) kg), were maintained in indoor individual free-stalls, bedded with shavings (Doulit™), thus enabling an entire control of food consumption. They were watered *ad libitum* from automatic waterers. In order to destroy a wide rage of gastro-intestinal parasites, the horses were wormed with a double dose of pyrantel (Strongid™, Laboratoire Pfizer, Orsay, France) followed a week later with a dose of ivermectin (Eqvalan™, Laboratoire Merial, Lyon, France) 15 days prior to the beginning of the experiment (Herd, 1992; Hutchens *et al.,* 1999).

The horses were allotted into two groups (G1 : no. = 3 horses and G2 : no. = 4 horses); each group was assigned a specific diet : G1 was given diet 1 (D1), chosen to mimic common French stable feeding habits; D1 was made of a pelleted food rich in fibre (P1) and straw (Table 1). The amount of food was set to meet maintenance energy requirements according to French recommendations (0·038 UFC per kg $M^{0.75}$) (Martin-Rosset *et al.,* 1994). Straw was chosen for its bulk effect and to mimic voluntary intake of straw by horses kept on this type of bedding. G2 was given diet 2 (D2) which copies a typical diet given to performance horses; D2 was

Table 1 *Description of diets offered* (*dry-matter basis*)

	Diet 1			Diet 2		
	Pelleted food (P1)	Straw	Mean total composition	Pelleted food (P2)	Hay	Mean total composition
Main constituents of pellets (g/kg)						
Cereals	389			780		
Forage	456			60		
Chemical composition (g/kg)						
Starch	211	ND	142	416	ND	233
Crude protein	120	51	98	134	85	112
Ash	66	47	60	61	91	74
Neutral-detergent fibre (NDF)	404	786	528	229	643	428
Acid-detergent fibre (ADF)	242	490	323	108	373	236
Acid-detergent lignin (ADL)	44	58	49	22	52	44
NDF/starch	1·82		3·71	0·55		1·84
NDF-ADF (hemicellulose)			205			192
ADF-ADL (cellulose)			274			192

ND = not determined.

Table 2 *Daily feeding programme of each diet tested*

Schedule	Diet 1†	Diet 2†
08: 00 h	393 g of pellet	750 g of pellet
10: 00 h	278 g of straw	—
12: 00 h	393 g of pellet	1000 g of meadow hay
16: 00 h	278 g of straw	—
18: 00 h	393 g of pellet	430 g of pellet
Daily pellet intake (DM basis)	1050 g/100 kg BW	1082 g/100 kg BW
Daily forage intake (DM basis)	506 g/100 kg BW	848 g/100 kg BW
Morning starch intake (DM basis)	74 g/100 kg BW	286 g/100 kg BW

† DM = dry matter. Amount as fed per 100 kg body weight (BW) except where indicated.

made of a pelleted food rich in cereals (P2) and meadow hay (Table 1). The amount of food was set to provide 286 g of starch per 100 kg body weight (BW) in the morning meal which is above the level of intake shown to be only partially digested in the pre-caecal part of the digestive tract (Kienzle, 1994). Meadow hay was chosen to balance the fibre deficiency of the pelleted food and its level of intake was close to common feeding habits in stables. For both diets, pelleted foods were given at the same daily level of intake (i.e. 1180 g as fed per 100 kg BW). The feeding patterns were different for each diet (Table 2) and forage distribution was set to prevent any possible interaction with the pelleted meal. All horses were given 20 days to adapt to their diet.

Preparation of horses and digesta sampling

This project was conducted under license from the department of Health and Animal Care of the French Veterinary Authority (no. A21002). The day before digesta was collected, all horses were entirely washed and their abdomen and flanks were clipped. On the day digesta was collected, the horses' abdomen was washed carefully and shaved along the white line after the morning pelleted meal, and, 2 h later, they were tranquillized with acepromazine (Vétranquil®, SANOFI Santé Nutrition Animale, Libourne, France), and laid down with guaiphenesin (Myolaxin™, Laboratoire Coophavet, Ancenis, France) on dorsal decubitus, with their legs securely tied. General anaesthesia was induced by pentobarbital (pentobarbital sodique™, SANOFI Santé Nutrition Animale, Libourne, France). The white line was incised to exteriorize the digestive tract. The different anatomic segments of the digestive tract were identified, isolated with a ligature at both ends, and removed to allow digesta sampling. Horses were then euthanased with an overdose of pentobarbital (Doléthal®, Laboratoire Vétoquinol S. A., Lure, France).

For microbial analyses, a sample of faeces (FAE) was collected in the rectum before anaesthesia, and about 200 ml of digesta were collected, after anaesthesia, from the stomach (STO), jejunum (JEJ), ileum (ILE), caecum (CEC), right ventral colon (RVC) and left dorsal colon (LDC) in a pre-identified sterile CO_2-saturated flask. Each sample was resaturated with

Table 3 *Mean time (h) elapsed between the ingestion of morning meal (ML), inducing of anaesthesia (A) and collection (C) of the digestive tract anatomic part, for two groups of horses*

	Group 1 (no. = 3 horses)		Group 2 (no. = 4 horses)	
	ML/C	A/C	ML/C	A/C
Stomach	4: 30	2: 05	4: 52	2: 20
Duodenum	4: 12	2: 02	4: 42	2: 10
Jejunum	3: 47	1: 36	4: 04	1: 32
Ileum	3: 48	1: 23	4: 05	1: 33
Caecum	4: 09	1: 59	4: 41	2: 09
Right ventral colon	4: 15	1: 55	4: 42	2: 10
Left ventral colon	3: 13	0: 48	3: 43	1: 11
Left dorsal colon	3: 12	0: 46	3: 43	1: 11
Right dorsal colon	4: 15	1: 55	4: 46	2: 14
Small colon	3: 32	1: 06	4: 04	1: 31

CO_2 after collection and all samples were held at 38ºC in a water bath between collection and inoculation. Ten-fold dilution series were prepared under O_2-free CO_2 in an anaerobic mineral solution (Bryant and Burkey, 1953) for inoculation on specific media presented below.

For biochemical analyses, about 10 ml of digesta from each anatomic segment (STO, duodenum (DUO), JEJ, ILE, CEC, RVC, left ventral colon (LVC), LDC, right dorsal colon (RDC) and small colon (SMC)) were filtered (Blutex 100µm). Then two subsamples of the filtered intestinal contents were immediately frozen (–20ºC) for determination of D- and L-lactate (1 ml) and volatile fatty acids (VFA) [1 ml together with a preservative (0·1 ml mixture of 5% H_3PO_4 + 1% $HgCl_2$)].

Microbial analyses

Total viable anaerobic bacteria counts were determined with a modified non-selective medium with an O_2-free CO_2 gas phase in anaerobic roll tubes (Leeddle and Hespell, 1980; Julliand *et al.*, 1999). Concentrations were determined after a 96-h incubation at 38ºC, from four replicate roll tubes prepared at dilutions representing, for each anatomic segment sampled, 10^{-6}, 10^{-7}, 10^{-8} ml of digestive content.

Lactic acid-utilizing bacteria counts were determined on a selective medium (Mackie and Heath, 1979) in anaerobic roll tubes with an O_2-free CO_2 gas phase. The number of viable bacteria was determined after a 96-h incubation at 38ºC, as the average of colony counts from four replicate roll tubes prepared from dilutions representing either 10^{-5}, 10^{-6}, 10^{-7} ml of digestive content from STO, JEJ and ILE, or 10^{-4}, 10^{-5}, 10^{-6} ml of intestinal content from CEC, RVC, LDC and FAE.

Cellulolytic bacteria were counted on a modified broth medium containing a strip (10 5 80 mm) of filter paper (Whatman no. º1) as the sole cellulolytic substrate (Halliwell and Bryant, 1963; Baruc *et al.*, 1983; Julliand *et al.*, 1999). The concentration of cellulolytic bacteria was taken as the most probable number of MacGrady on four tubes inoculated from the dilution representing either 10^{-1}, 10^{-2}, 10^{-3} ml of content from STO, JEJ and ILE, or 10^{-5}, 10^{-6}, 10^{-7} ml of content from CEC, RVC, LDC and FAE and then incubated for 15 days at 38ºC.

Streptococci spp. were enumerated on a bile-esculin-azide agar medium (BK158HA, Biokar diagnostics, Beauvais, France). The number of viable bacteria was determined after a 48-h incubation at 38ºC, as the average of colony counts from three replicate Petri plates prepared from dilutions representing either 10^{-4}, 10^{-5}, 10^{-6} ml of caecal content, or 10^{-5}, 10^{-6}, 10^{-7} ml of content from all other compartments.

Lactobacilli spp. were enumerated on a Rogosa agar medium (BK033, Biokar Diagnostics, Beauvais, France) as described above for *Streptococci* spp.

Biochemical analyses

Digesta pH was measured with an electronic pH-meter (MP 120, Mettler, Toledo, Spain) immediately after the sample was collected. L- and D-lactic acid were assayed with an enzymatic reaction procedure (D-lactic acid/L-lactic acid Enzymatic BioAnalysis/ Food analysis kit, cat. no. 1112821, Boehringer Mannheim/R-Biopharm, Darmstadt, Germany) quantified spectrophometrically at 340 nm (MRX revelation, Dynatech Laboratories, Guyancourt, France). VFA concentrations were assayed by gas-liquid chromatography (Gas chromatograph model 437 A, United technologies Packard, Zurich, Switzerland) (Jouany, 1982). A ratio of acetate plus butyrate to propionate [(C2 + C4)/C3] was calculated according to Sauvant *et al.* (1994).

Statistical analysis

An analysis of variance with the GLM procedure of SAS version 8·2 was done to evaluate the variables' response (microbial counts, pH, VFA, D- and L-lactic acid) to the diet, to the anatomic segment of the digestive tract and to the diet 5 anatomic segment of the digestive tract interaction. The model included horse, nested in diet, as a randomized effect. Logarithmic transformations were performed on microbial counts before statistical analysis. Least-square means were calculated for all variables and separated using pairwise *t* tests (Pdiff option of SAS) and the significance threshold for all tests was set at $P < 0{\cdot}05$.

Results

Anaesthesia was induced an average of 2·5 h after the morning pelleted meal. The chronological removal of the anatomic segments of the digestive tract and the sampling were identical for each horse, starting with the left ventral and dorsal colon and finishing with the stomach. Digesta was collected from the isolated anatomic segments between 3 : 12 and 4:52 h after the morning meal (Table 3). Thus, the retention of digesta lasted 46 min at the least and 2:33 h at the most after the induction of the anaesthesia (Table 3).

Concentrations of micro-organisms (*Table 4*)

Concentrations of total anaerobic bacteria in every anatomic segment explored ranged from $7{\cdot}4\ 5\ 10^7$ to $3{\cdot}7\ 5\ 10^9$ colony-forming units (c.f.u.) per ml

Table 4 *Microbial concentration in the different parts of the digestive tract of horses given two distinct diets*

	Anatomic part of the digestive tract†								Significance‡		
	STO	JEJ	ILE	CEC	RVC	LDC	FAE	s.e.	D	P	D×P
Total anaerobic bacteria (log_{10} c. f. u. per ml)											
Diet 1 (no. = 3)§	$9{\cdot}1^{A;\,d,e}$	$8{\cdot}7^{A;\,c,d}$	$7{\cdot}9^{A;\,a,b}$	$7{\cdot}6^{A;\,a}$	$8{\cdot}1^{A;\,b}$	$9{\cdot}1^{A;\,e}$	$8{\cdot}6^{A;\,c}$	0·43		***	***
Diet 2 (no. = 4)	$9{\cdot}0^{A;\,d}$	$8{\cdot}6^{A;\,c,e}$	$8{\cdot}8^{B;\,d,c}$	$7{\cdot}7^{A;\,a}$	$8{\cdot}9^{B;\,d,c}$	$8{\cdot}1^{B;\,b}$	$8{\cdot}3^{A;\,b,e}$				
Cellulolytic bacteria (log_{10} c. f. u. per ml)											
Diet 1 (no. = 3)	$1{\cdot}4^{A;\,a}$	$1{\cdot}8^{A;\,a}$	$1{\cdot}7^{A;\,a}$	$6{\cdot}0^{A;\,b}$	$6{\cdot}0^{A;\,b}$	$5{\cdot}6^{A;\,b}$	$5{\cdot}2^{A;\,b}$	0·50	**	***	
Diet 2 (no. = 4)	$1{\cdot}0^{A;\,a}$	$0{\cdot}9^{B;\,a}$	$1{\cdot}0^{A;\,a}$	$5{\cdot}0^{B;\,b,c}$	$5{\cdot}5^{A;\,c}$	$4{\cdot}7^{B;\,b}$	$5{\cdot}0^{A;\,b,c}$				
Lactobacilli (log_{10} c. f. u. per ml)											
Diet 1 (no. = 3)	$7{\cdot}8^{A;\,b}$	$6{\cdot}5^{A;\,a}$	$6{\cdot}4^{A;\,a}$	$6{\cdot}2^{A;\,a}$	$6{\cdot}5^{A;\,a}$	$7{\cdot}4^{A;\,b}$	$7{\cdot}5^{A;\,b}$	0·49		***	**
Diet 2 (no. = 4)	$8{\cdot}4^{B;\,d}$	$6{\cdot}8^{A;\,b}$	$7{\cdot}2^{B;\,b,c}$	$6{\cdot}1^{A;\,a}$	$7{\cdot}0^{A;\,b}$	$7{\cdot}0^{A\,;\,b}$	$7{\cdot}4^{A;\,c}$				
Streptococci (log_{10} c. f. u. per ml)											
Diet 1 (no. = 3)	$7{\cdot}3^{A;\,b}$	$8{\cdot}1^{A;\,c}$	$7{\cdot}4^{A;\,b,c}$	$6{\cdot}2^{A;\,a}$	$7{\cdot}1^{A;\,b}$	$7{\cdot}4^{A;\,b}$	$7{\cdot}4^{A;\,b}$	0·60		***	*
Diet 2 (no. = 4)	$7{\cdot}5^{A;\,c}$	$7{\cdot}5^{A;\,c,d}$	$8{\cdot}0^{B;\,d}$	$6{\cdot}3^{A;\,a}$	$7{\cdot}6^{A;\,c,d}$	$6{\cdot}9^{A;\,b}$	$7{\cdot}8^{A;\,c,d}$				
Lactate-utilizing bacteria (log_{10} c. f. u. per ml)											
Diet 1 (no. = 3)	$6{\cdot}8^{A;\,c}$	$6{\cdot}7^{A;\,c,b}$	$6{\cdot}5^{A;\,b,c,d}$	$6{\cdot}1^{A;\,a,d}$	$5{\cdot}8^{A;\,a}$	$6{\cdot}5^{A;\,b,c,d}$	$6{\cdot}3^{A;\,b,d}$	0·53		***	***
Diet 2 (no. = 4)	$7{\cdot}6^{B;\,e}$	$6{\cdot}5^{A;\,c}$	$7{\cdot}2^{B;\,d}$	$5{\cdot}6^{B;\,a}$	$6{\cdot}0^{A;\,b}$	$5{\cdot}9^{B;\,a,b}$	$6{\cdot}2^{A;\,b,c}$				

† Values are least-square means. STO = stomach; JEJ = jejunum; ILE = ileum; CEC = caecum; RVC = right ventral colon; LDC = left dorsal colon; FAE = faeces.
‡ Significance of effects of the diet (D), the anatomic part of the digestive tract (P) and of the interaction (D×P).
§ no. = number of animals on each diet.
$^{A, B}$ Values within an item and a column are different if superscript differs ($P < 0{\cdot}05$).
^{a,b,c,d,e} Values within a row are different if superscript differs ($P < 0{\cdot}05$).

whatever the diet. Concentrations were different according to their anatomical location ($P < 0{\cdot}001$) and a significant interaction between diet and anatomical segment ($P < 0{\cdot}001$) was found. With both diets, total anaerobic bacteria concentrations were significantly lower in the caecum than in the other parts of the digestive tract ($P < 0{\cdot}001$). In those horses given the high cereal diet (D2) the ante-caecal concentrations tended to be slightly higher than in the hindgut, whereas, in those given the high forage diet (D1), they were more homogenous all along the digestive tract.

Concentrations of cellulolytic bacteria differed according to their anatomical location ($P < 0{\cdot}001$). With both diets, pre-caecal concentrations did not exceed $3{\cdot}0\ 5\ 10^2$ c. f. u. per ml, whilst they ranged from $5{\cdot}9\ 5\ 10^4$ to $1{\cdot}6\ 5\ 10^6$ c. f. u. per ml in the large intestine. Regardless of the anatomic segment, concentrations of cellulolytic bacteria were higher in horses given D1 than in those given D2 ($P < 0{\cdot}01$).

Concentrations of lactobacilli and streptococci differed according to their anatomical location ($P < 0{\cdot}001$) and a significant interaction between diet and anatomical segment was noticed ($P < 0{\cdot}01$ and $P < 0{\cdot}05$, for lactobacilli and streptococci respectively). For both microbial populations and both diets, caecal concentrations were lower than in the other anatomic segments ($P < 0{\cdot}01$). With both diets, concentrations of lactobacilli were highest in the stomach ($P < 0{\cdot}001$) whereas streptococci were more evenly distributed along the digestive tract. A diet effect could only be detected in the ante-caecal tract : the concentration of lactobacilli was highest in the stomach and the ileum of horses given D2 ($P < 0{\cdot}05$ and $P < 0{\cdot}001$, respectively); the concentration of streptococci was highest in the ileum with D2 ($P < 0{\cdot}05$).

Concentrations of lactate-utilizing bacteria were different depending on their anatomical location ($P < 0{\cdot}001$) and a significant interaction between diet and anatomical segment was noticed ($P < 0{\cdot}001$). Regardless of the diet, the highest concentration of lactate-utilizing bacteria was detected in the stomach ($P < 0{\cdot}05$). In horses given D2, concentrations in lactate-utilizers were higher in the stomach and the

Table 5 *pH and* D*-/*L*-lactate concentrations in the different parts of the digestive tract of horses given two distinct diets*

	Anatomic part of the digestive tract †											Significance‡		
	STO	DUO	JEJ	ILE	CEC	RVC	LVC	LDC	RDC	SMC	s.e.	D	P	D×P
pH														
Diet 1 (no. = 3)§	4·5$^{A;a}$	5·9$^{A;b}$	6·9$^{A;d}$	7·2$^{A;e}$	6·2$^{A;b}$	6·2$^{A;b}$	6·6$^{A;c,d}$	6·6$^{A;c}$	6·2$^{A;b}$	6·2$^{A;b}$	0·3		***	***
Diet 2 (no. = 4)	5·1$^{B;a}$	6·6$^{B;c}$	7·2$^{A;d}$	7·4$^{A;d}$	6·4$^{B;c}$	6·5$^{A;c}$	6·4$^{A;c}$	6·4$^{A;c}$	6·1$^{A;c,b}$	5·8$^{B;b}$				
D-Lactate (mmol/l)														
Diet 1 (no. = 3)	1·5$^{A;b}$	0·4$^{A;a,c}$	0·0$^{A;a}$	0·0$^{A;a}$	0·2$^{A;a}$	0·2$^{A;a,c}$	0·3$^{A;a,c}$	1·3$^{A;b,d}$	0·9$^{A;b,c,d}$	0·5$^{A;a,d}$	0·5		***	***
Diet 2 (no. = 4)	0·5$^{B;a,b}$	0·2$^{A;a}$	0·0$^{A;a}$	0·0$^{A;a}$	0·1$^{A;a}$	0·1$^{A;a}$	0·0$^{A;a}$	1·4$^{A;c}$	1·0$^{A;b,c}$	2·7$^{B;d}$				
L-Lactate (mmol/l)														
Diet 1 (no. = 3)	3·0$^{A;c}$	0·8$^{A;b}$	0·5$^{A;a,b}$	0·5$^{A;b,c}$	0·1$^{A;a}$	0·1$^{A;a,c}$	0·2$^{A;a,c}$	0·7$^{A;b,d}$	0·6$^{A;b,d}$	0·4$^{A;a,d}$	0·2		***	***
Diet 2 (no. = 4)	0·7$^{B;b}$	0·4$^{A;b}$	0·4$^{A;a,b}$	0·5$^{A;b}$	0·1$^{A;a}$	0·0$^{A;a}$	0·0$^{A;a}$	0·7$^{A;b}$	0·5$^{A;b}$	1·2$^{B;c}$				
Ratio L/D														
Diet 1 (no. = 3)	4·2$^{A;b,c}$	6·7$^{A;c}$	14·2$^{A;d}$	12·3$^{A;d}$	0·6$^{A;a}$	0·7$^{A;a,b}$	0·5$^{A;a,b}$	0·6$^{A;a,b}$	0·6$^{A;a,b}$	0·7$^{A;a,b}$	2·4		***	
Diet 2 (no. = 4)	2·2$^{A;a}$	6·1$^{A;b}$	10·4$^{A;c}$	9·3$^{A;b,c}$	0·8$^{A;a}$	0·5$^{A;a}$	0·7$^{A;a}$	0·5$^{A;a}$	0·5$^{A;a}$	0·3$^{A;a}$				

† Values are least-square means. STO = stomach; DUO = duodenum; JEJ = jejunum; ILE = ileum; CEC = caecum; RVC = right ventral colon; LVC = left ventral colon; LDC = left dorsal colon; RDC = right dorsal colon; SMC = small colon.
‡ ,§ ^{A,B,a,b,c,d,e} See Table 4 footnotes.

ileum ($P < 0{\cdot}001$ and $P < 0{\cdot}01$, respectively) and lower in the caecum and the left dorsal colon ($P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively) than in those given D1.

Biochemical parameters

The pH differed according to the anatomical location ($P < 0{\cdot}001$) and a significant interaction between diet and anatomical segment was noticed ($P < 0{\cdot}001$) (Table 5). With both diets, the lowest values were measured in the stomach ($P < 0{\cdot}001$) and the highest were measured in the jejuno-ileum ($P < 0{\cdot}001$). The pH values ranged from an average 6·1 to 6·6 in the different parts of the hindgut except in the small colon of horses given D2, which was more acid.

Concentrations of D- and L-lactate varied according to their anatomical location ($P < 0{\cdot}001$) and a significant interaction between diet and anatomical segment was noticed ($P < 0{\cdot}001$) (Table 5). With both diets, the lowest total lactate concentrations (data not shown) were measured in the jejuno-ileum, caecum and ventral colon; values ranged from 0·2 to 0·9 mmol/l with no significant differences. Variations of D- and L-lactate concentrations were related both to the diet and to the anatomic segment in interaction. However, the respective changes in the L-lactate/D-lactate ratio were similar on the two diets and only the anatomic segment affected this ratio ($P < 0{\cdot}001$) : regardless of the concentrations, the relative proportion of L-lactate was greater in the foregut and that of D-lactate was greater in the hindgut. Horses given D1, had D- and L-lactate concentrations that were higher in the stomach ($P < 0{\cdot}05$) and lower in the small colon ($P < 0{\cdot}001$) than horses given D2.

Total VFA concentrations (Table 6) were related to the anatomic segment of the digestive tract ($P < 0{\cdot}001$) and to the diet ($P < 0{\cdot}001$). In addition, the interaction between the diet and the anatomic segment had a significant effect on results ($P < 0{\cdot}001$). With both diets, the lowest concentrations were measured in the small intestine, with no difference between the stomach and the different parts of the small intestine ($P > 0{\cdot}05$), and the highest concentrations were found in the hindgut ($P < 0{\cdot}05$). Variations in total VFA concentrations in the different parts of the hindgut were different from one diet to the other in horses given D1, VFA concentrations decreased significantly from the right ventral colon to the small colon ($P < 0{\cdot}001$) whereas concentrations remained fairly constant throughout the large intestine in horses given D2. All along the digestive tract, total VFA concentrations were numerically greater in horses given D2, with significant differences from horses given D1 in all parts of the large intestine ($P < 0{\cdot}05$).

For every single VFA both the anatomic segment of the digestive tract ($P < 0{\cdot}001$) and the diet ($P < 0{\cdot}05$) affected its concentration. With both diets, ante-caecal concentrations were always significantly lower than in the hindgut. Furthermore, the concentration of each VFA was always higher, sometimes significantly, in horses given D2.

Changes in acetate and valerate concentrations in the large intestine were similar to those described for total VFA. Propionate and butyrate concentrations varied identically in the hindgut of the two groups : concentrations of propionate and butyrate were respectively higher in the caecum and ventral

Table 6 *Volatile fatty acid (VFA) concentrations in the different parts of the digestive tract of horses given two distinct diets*

	Anatomic part of the digestive tract†											Significance‡		
	STO	DUO	JEJ	ILE	CEC	RVC	LVC	LDC	RDC	SMC	s.e.	D	P	D×P
Total VFA (mmol/l)														
Diet 1 (no. = 3)§	10·1$^{A;a}$	4·9$^{A;a}$	3·6$^{A;a}$	4·5$^{A;a}$	82·6$^{A;d}$	89·1$^{A;d}$	75·5$^{A;c,d}$	56·5$^{A;c}$	73·4$^{A;c,d}$	34·5$^{A;b}$	12·9	***	***	***
Diet 2 (no. = 4)	19·5$^{A;a}$	6·8$^{A;a}$	9·0$^{A;a}$	9·2$^{A;a}$	121·8$^{B;c}$	122·8$^{B;b,c}$	117·4$^{B;b,c}$	103·3$^{B;b}$	121·7$^{B;c}$	117·1$^{B;b,c}$				
Acetate (mmol/l)														
Diet 1 (no. = 3)	9·7$^{A;a}$	4·6$^{A;a}$	3·4$^{A;a}$	3·5$^{A;a}$	54·1$^{A;d}$	56·7$^{A;d}$	50·0$^{A;c,d}$	40·6$^{A;c}$	55·3$^{A;c,d}$	25·3$^{A;b}$	9·0	***	***	***
Diet 2 (no. = 4)	15·7$^{A;a}$	5·4$^{A;a}$	6·1$^{A;a}$	6·4$^{A;a}$	81·3$^{B;b,c}$	79·4$^{A;b,c}$	77·9$^{B;b,c}$	75·8$^{B;b}$	90·1$^{B;c}$	87·7$^{B;b,c}$				
Propionate (mmol/l)														
Diet 1 (no. = 3)	0·1$^{A;a}$	0·1$^{A;a}$	0·1$^{A;a}$	0·5$^{A;a,b}$	19·7$^{A;e}$	18·3$^{A;e}$	15·3$^{A;d,e}$	9·1$^{A;c}$	10·5$^{A;c,d}$	6·0$^{A;b}$	3·3	*	***	
Diet 2 (no. = 4)	1·5$^{A;a}$	0·5$^{A;a}$	0·9$^{A;a}$	1·1$^{A;a}$	28·4$^{B;c}$	25·2$^{B;c}$	23·8$^{B;c}$	14·6$^{B;b}$	16·1$^{B;b}$	15·0$^{B;b}$				
Iso-butyrate (mmol/l)														
Diet 1 (no. = 3)	0·1$^{A;a,b}$	0·1$^{A;a}$	0·0$^{A;a}$	0·0$^{A;a}$	0·5$^{A;b}$	1·3$^{A;c}$	1·3$^{A;c}$	1·4$^{A;c}$	1·5$^{A;c}$	0·6$^{A;b}$	0·3	**	***	***
Diet 2 (no. = 4)	0·7$^{B;b}$	0·1$^{A;a}$	0·4$^{A;a,b}$	0·2$^{A;a}$	0·7$^{A;b}$	2·6$^{B;c,d}$	2·3$^{B;c}$	2·3$^{B;c}$	2·8$^{B;d}$	2·9$^{B;d}$				
Butyrate (mmol/l)														
Diet 1 (no. = 3)	0·1$^{A;a}$	0·1$^{A;a}$	0·1$^{A;a}$	0·4$^{A;a}$	7·3$^{A;c}$	10·6$^{A;d}$	7·1$^{A;c}$	3·3$^{A;b}$	3·8$^{A;b}$	1·7$^{A;a,b}$	1·5	*	***	
Diet 2 (no. = 4)	1·2$^{A;a}$	0·5$^{A;a}$	1·2$^{A;a}$	0·9$^{A;a}$	9·4$^{B;c,d}$	12·1$^{B;e}$	10·5$^{B;d,e}$	6·7$^{B;b}$	8·1$^{B;b,c}$	6·7$^{B;b}$				
Iso-valerate (mmol/l)														
Diet 1 (no. = 3)	0·0$^{A;a}$	0·0$^{A;a}$	0·0$^{A;a}$	0·0$^{A;a}$	0·5$^{A;a}$	1·4$^{A;b}$	1·3$^{A;b,c}$	1·4$^{A;b}$	1·7$^{A;b}$	0·6$^{A;a,c}$	0·5	*	***	***
Diet 2 (no. = 4)	0·2$^{A;a}$	0·3$^{A;a}$	0·3$^{A;a}$	0·4$^{A;a}$	1·1$^{B;b}$	2·3$^{B;c,d}$	1·8$^{B;c}$	2·8$^{B;d,e}$	3·2$^{B;e}$	3·6$^{B;e}$				
Valerate (mmol/l)														
Diet 1 (no. = 3)	0·0$^{A;a}$	0·0$^{A;a,b}$	0·0$^{A;a,b}$	0·0$^{A;a,b}$	0·5$^{A;b,c}$	0·8$^{A;d}$	0·7$^{A;c,d}$	0·6$^{A;c,d}$	0·7$^{A;d}$	0·3$^{A;b}$	0·2	*	***	**
Diet 2 (no. = 4)	0·2$^{A;a}$	0·1$^{A;a}$	0·1$^{A;a}$	0·2$^{A;a}$	0·8$^{B;b}$	1·3$^{B;d,e}$	1·0$^{B;b,c,e}$	1·0$^{B;c}$	1·4$^{B;d}$	1·2$^{B;c,d}$				
(C2 + C4)/C3‖														
Diet 1 (no. = 3)	85·1$^{A;a}$	48·3$^{A;b}$	40·5$^{A;b}$	22·3$^{A;b,c}$	3·3$^{A;c}$	3·7$^{A;c}$	3·8$^{A;c}$	5·0$^{A;c}$	5·7$^{A;c}$	4·4$^{A;c}$	15·7		***	**
Diet 2 (no. = 4)	14·4$^{B;a,b}$	25·7$^{A;a}$	11·9$^{B;a,b}$	14·1$^{A;a,b}$	3·2$^{A;b}$	4·4$^{A;a,b}$	4·4$^{A;a,b}$	5·9$^{A;a,b}$	6·1$^{A;a,b}$	8·5$^{A;a,b}$				

† See Table 5 footnote.
‡, §, $^{A,B,\ a.,b,c,d,e}$ See Table 4 footnotes.
‖ Ratio of acetate plus butyrate to propionate.

colon than in the dorsal colon and small colon ($P < 0·05$). Iso-butyrate and iso-valerate also varied in a similar way : concentrations were greater in the right dorsal colon and small colon than in the upper parts of the large intestine ($P < 0·05$).

The ratio [(C2 + C4)/C3] differed according to the anatomical location ($P < 0·001$) and a significant interaction between diet and anatomical segment was noticed ($P < 0·001$). With both diets, the ratio ranged from 11·9 to 85·1 in the stomach and small intestine and from 3·2 to 6·1 in the large intestine. The ratio calculated in the dorsal and small colon was higher, though not significantly higher, than in the caecum and ventral colon. A significant diet effect could only be detected in the stomach and the jejunum where the ratio was lower in horses given D2.

Discussion

Describing microbial and fermentative profiles simultaneously for each anatomic segment of the digestive tract and for two distinct diets brought new knowledge of the digestive ecosystem physiology of the horse. Furthermore, the functional characterization of the micro-organisms allowed us to formulate several hypotheses regarding the nutritional implication of this ecosystem. We considered that what occurred in the ante-caecal segments was directly related to the morning pelleted meal, whereas the microbial profile of the hindgut was probably more a reflection of the whole daily diet. When 2·5 h after the meal, the digestive transit is stopped, only a limited proportion of that meal has probably reached the hindgut. We therefore assumed that it could not have affected the microbial population yet, but could have affected the biochemical profile (Medina *et al.*, 2002).

Of all the pre-caecal segments of the digestive tract, the stomach is the one most studied and its pH is particularly well documented. Our values ranged from 4·0 to 5·9 pH units. They were consistent with results collected on sacrificed horses (Kern *et al.*, 1974; Wolter and Chaabouni, 1979; Morris *et al.*, 2002) but slightly below those recorded in gastric-cannulated horses 4 and 5 h after the ingestion of a pelleted meal (pH 6·5 to 6·0) (Healy *et al.*, 1995). Gastric pH results were hard to interpret as they may

vary greatly not only from one section of the stomach to another — the pylorus being the most acid (Kern *et al.*, 1974; Morris *et al.*, 2002) — but even in the same section (range from 1·8 to 6·2 pH units; Murray and Schusser, 1993). Such variations could suggest that microbial and fermentative profiles vary within the stomach, as put forward by Morris *et al.* (2002). We, therefore, had to consider that our results only gave an idea of the average composition of the gastric content. Regarding the effect of the last pelleted meal ingested, gastric pH was significantly more acid with the high fibre pellet (P1). Saliva production being related to meal size (Meyer *et al.*, 1986), the smaller volume of P1 induced a lower production of saliva and its buffering effect was therefore certainly lower than with the ingestion of P2. Furthermore, the smaller size of the meal together with the greater fibre content of P1 might have led to a greater extent of gastric emptying. In consequence, the amount of the remaining pelleted meal was reduced which allowed an easier mix of the ingesta with the gastric secretion of HCl, hence a more acid pH. Nevertheless, these results show the stomach's ability to be colonized by various micro-organisms.

In our study, gastric concentrations of total anaerobic bacteria were slightly above the concentrations presented by Kern *et al.* (1974): $1{\cdot}4\ 5\ 10^9$ *v.* $1{\cdot}0\ 5\ 10^8$ c. f. u. per ml. Microbial populations related to starch and highly fermentable carbohydrates were predominant in the gastric microflora : we measured concentrations of lactobacilli and streptococci in a similar range to those presented by Alexander and Davies (1963). Our concentrations of lactate-utilizing bacteria were similar to Alexander and Davies' concentrations of *Veillonella gazogenes* ($6\ 5\ 10^8$ c. f. u. per ml), a lactate fermenting bacterium (Alexander *et al.*, 1952). Gastric emptying, together with the concomitant activity of lactate-producing and lactate-utilizing bacteria, led to a total lactate concentration ranging from 0·7 to 5·4 mmol/l which, though below the minimum 12·7 mmol/l reported by Alexander and Davies (1963) and Morris *et al.* (2002), was consistent with other results (Kern *et al.*, 1974; Wolter and Chaabouni, 1979; Healy *et al.*, 1995). The lower concentration of D-lactate than of L-lactate might be related to a selective activity of the lactate-utilizing bacteria. In the rumen of cows given high concentrate diets, for instance, most of the lactate that is fermented into VFA is by *Megasphera elsdenii*, but only 16% of L-lactate against 75% of D-lactate was converted into propionic acid (Counotte *et al.*, 1983). Whether *M. elsdenii* is present in the stomach of horses remains to be proven. Although with both diets, the concentrations of total anaerobic bacteria were identical in the stomach, the proportion of lactobacilli and lactate-utilizing bacteria was greater in horses given the high cereal pellet (P2). As previously reported in the caecum by Garner *et al.* (1978) and Goodson *et al.* (1988), the higher intake of starch supplied by P2 might have promoted a proliferation of lactobacilli. By contrast, there was no significant effect of the diet on the concentration of streptococci. This might be due to the low gastric pH. In the rumen, *Streptococcus bovis* was shown to decline below pH 5 (Espinasse *et al.*, 1995). The concentration of total lactate was greater in the stomach of horses given P1, and this, together with mechanisms discussed earlier, might contribute to lowering the gastric pH. In animals given the high starch pellet (P2), the lower concentration of lactate, and the proportion of lactate-utilizing bacteria in the stomach (8·3 times greater), suggested that more lactate was fermented into VFA. This higher fermentation of lactate was confirmed by the significantly lower [(C2 + C4)/C3] ratio in horses given P2. That ratio was indeed affected by the higher proportion of propionate in the stomach of horses given the high starch pellet ($P < 0{\cdot}05$) (data not shown). This is consistent with results in the rumen where lactate is mainly converted to propionate when cows are given high concentrate diets.

Our concentrations of cellulolytic bacteria in the stomach were slightly below those reported by Kern *et al.* (1974) ($2\ 5\ 10^1$ *v.* $2\ 5\ 10^2$ c. f. u. per ml) and they did not exceed $3{\cdot}0\ 5\ 10^2$ c. f. u. per ml in the jejuno-ileum, which was consistent with the range of concentrations measured by Kern *et al.* (1974). Both studies agree that forage degradation in the pre-caecal section of the digestive tract is probably extremely low. This hypothesis is confirmed by the low VFA concentrations reported in our results and in previous literature (Alexander and Davies, 1963; Kern *et al.*, 1974; Morris *et al.*, 2002). The decreasing production of VFA from the stomach to the small intestine and its ultimate absorption through the intestinal wall should be investigated further.

Concentrations of total anaerobic bacteria were lower in the small intestine than in the stomach. As in ruminants, the dilution of the chyme with intestinal secretions and the occurrence of some proteolysis might explain this observation. Nevertheless, our concentrations remained relatively high and ranged from $8{\cdot}5\ 5\ 10^7$ to $1{\cdot}4\ 5\ 10^9$ c. f. u. per ml in the jejunum, which is consistent with previous data (Mackie and Wilkins, 1988; Kollarczik *et al.*, 1992). In the ileum, concentrations of total anaerobic bacteria ranged from $7{\cdot}0\ 5\ 10^6$ to $3{\cdot}6\ 5\ 10^9$ and were also consistent with literature (Kern *et al.*, 1974; Mackie and Wilkins, 1988). Lactate-utilizing bacteria and lactobacilli concentrations were similar (on average

$6{\cdot}0\ 5\ 10^7$ and $2{\cdot}9\ 5\ 10^7$, respectively) but lower than previous data showing respective concentrations of $6{\cdot}0\ 5\ 10^8$ (Alexander and Davies, 1963) and $3{\cdot}3\ 5\ 10^8$ (Kollarczik *et al.*, 1992).

The concentrations of lactate-utilizing bacteria and lactobacilli decreased from the stomach to the small intestine whereas streptococci counts increased. In addition, streptococci became more numerous in the small intestine than lactobacilli, resulting in an increase in the streptococci/lactobacilli ratio (from 0·5 in the stomach, to 4·7 in the jejunuo-ileum). Unlike in the stomach, the higher pH in the small intestine was probably more propitious for streptococci growth. Concentrations of lactate decreased constantly from the duodenum to the ileum, and the average small intestine concentration was lower than in the stomach. This was consistent with previous data (Wolter and Chaabouni, 1979). The L-/ D-lactate ratio increased from the stomach to the small intestine. Assuming that lactate absorption through the intestinal wall is equivalent for both isomers, it is likely that this L-lactate predominance is related to modifications of the microflora fermentative activities. In contrast, ileal microbial communities were affected by the last pelleted meal ingested: concentrations of total anaerobic bacteria were greater in the ileum of horses given P2, and these changes were positively related to those of lactobacilli, streptococci and lactate-utilizing bacteria. It was likely that the amount of starch that reached the ileum in horses given the high forage pellet (P1) was not sufficient to promote the growth of bacteria involved in starch degradation. Beyond the lower starch intake, the type of starch composing the pellets was probably different in P1 compared with P2. It is known that the amylose/amylopectin ratio strongly influences the speed and extent of starch digestion (Duprat *et al.*, 1980). If the starch from P1 was better hydrolysed by the horses' enzymes, the substrate available for microbial growth and metabolism was then limited in the ileum and lower compartments of the gut.

On average, total anaerobic bacteria concentrations were lowest in the caecum compared with the other digestive sections. This reinforces the idea that the ecosystems of each section should be investigated further to understand its respective function and ultimate consequences for the host. We enumerated an average of $7{\cdot}9\ 5\ 10^7$ c. f. u. of total anaerobic bacteria per ml of caecal content, which was consistent with the range of concentrations reported in literature (from $1{\cdot}8\ 5\ 10^7$ up to $2{\cdot}6\ 5\ 10^9$ c. f. u. per ml) (Kern *et al.*, 1973 and 1974; Mackie and Wilkins, 1988; Julliand *et al.*, 2001; Medina *et al.*, 2002). The average of total anaerobic bacteria counts for both diets was not significantly different between the different parts of the colon, but it is clear that variations within the colon had to be considered for each diet tested. It is well established that the microbial profiles of the caecum and right ventral colon are directly related to the diet composition (Kern *et al.*, 1973; Julliand *et al.*, 2001; Medina *et al.*, 2002). Our results suggest that diet characteristics have a direct influence on the microbial development in each part of the large intestine.

Unlike the pre-caecal segments, the hindgut is entirely colonized by high concentrations of intensely active cellulolytic bacteria, as shown by the high concentrations of VFA. The proportion of cellulolytic bacteria among total anaerobes appeared to be greater in the caecum than in the lower parts of the hindgut, and confirmed this blind pouch as probably the most propitious for cellulolysis. But the decrease in proportion of cellulolytic bacteria in the lower parts of the colon might be due to the reduction of cellulose in the digesta. We hypothesize that the particles entering the dorsal colon have a lower parietal polysaccharide content. The retention time of particles above 1 cm long might indeed be prolonged in the ventral colon due to the pelvic flexure (Dellow (1982) from Hume and Sakagushi (1991)). However, concentrations of cellulolytic bacteria were not truly modified within the large intestine and changes in VFA concentrations along the colon could hardly be related to cellulolytic bacteria counts. Again, the diet has a strong influence both on cellulolytic bacteria and VFA concentrations. Although only significant for the caecum and the left dorsal colon, concentrations of cellulolytic bacteria were highest in the hindgut of horses given D1. Our diets differed in many points but two main characteristics might have acted together : the proportion of cellulose (found in the difference [ADF-ADL]), which was 8·2 points higher in D1 than in D2, promoted growth of cellulolytic bacteria, while the higher proportion of starch in D2 had a depressive effect on their development. In contrast, horses given D2 showed total VFA concentrations significantly greater for all segments of the large intestine. Though both diets brought the same amount of NDF (822 g and 827 g per 100 kg BW, respectively for D1 and D2), the quality of the fibre in D2 was probably better and promoted the enzymatic activity of cellulolytic bacteria more, thus leading to a larger production of VFA. Besides, the limited amount of forage offered with D1 might also explain the regular decrease of VFA production along the hindgut to reach its minimum in the small colon. But one should not ignore that, with both diets, and in spite of the differences highlighted above, the VFA profile in the large intestine was identical. It is likely

that the large amount of hay provided in D2 inhibited the negative effects of the starch on the hindgut ecosystem. Although cellulolytic bacteria were less numerous, their activity was stimulated by the amount of substrate, maintaining the ecosystem balance. Compared to D1, the energetic concentration of D2 was greater, not only due to the higher starch concentration but also to the greater yield of VFA from the fibre constituents.

On average, concentrations of lactobacilli, streptococci (= starch-utilizing bacteria) and lactate-utilizing bacteria tended to be lower in the caecum than in any other part of the digestive tract, which suggests that the caecal ecosystem is less exposed to rapidly fermentable carbohydrates than the lower parts of the hindgut. This is consistent with previous data reporting that soluble carbohydrates and undigested starch flowed very quickly through the caecum and had a greater impact on the colonic flora than on caecal flora (de Fombelle *et al.,* 2001). This is confirmed by the direct relation of the concentrations of the right ventral colon micro-organisms to the starch content of the diet: lactobacilli and lactate-utilizing bacteria increased significantly from the caecum to the right ventral colon in horses given the high starch diet (D2), whereas they remained unchanged in horses given D1. The amplitude of the streptococci increase from the caecum to the RVC was greater in horses given D2. It is likely that D2 promoted both starch- and lactate-utilizing bacteria, though no significant effect of the diet was detected within the different anatomic sections.

Although the total lactate concentration remained unchanged in the small intestine, caecum and ventral colon, the respective production of D- and L-lactate varied significantly : L-lactate predominated in the small intestine, but both isomers were in similar proportion in the caecum and the colon, which suggests a production-utilization balance. The average D- and L-lactate concentrations within the large intestine were increased at least 4·7-fold from the left ventral colon to the left dorsal colon. This greater lactate concentration was concomitant with a decrease in propionate leading to a two-point increase in the [(C2 + C4)/C3] ratio. This suggests a decrease in the enzymatic activity of lactate fermenting bacteria. Although the total amount of starch provided in D2 was 2-fold that in D1, no difference was noticed in D- and L-lactate concentrations. When the digestive transit stopped, a limited amount of soluble carbohydrate had reached the hindgut. Consequently, if, when the digesta was collected in the hindgut, starch had been fermented into lactate, lactate-utilizing bacteria might have had time to convert that lactate into VFA.

This trial suggested that the microbial degradation of food in the stomach of horses, and in particular that of readily fermentable carbohydrates, should be investigated further, in order to assess its consequences on the energetic promotion of the diet. Furthermore, the impact of foods and feeding practices on the gastric microbial fermentation should be all the more studied because they may lead to the production of organic acids, which can be a cause of gastric ulcers. Regarding the hindgut, fibre degradation occurred mainly in the caecum, but the involvement of each anatomic segment of the colon has been proven, particularly when a large amount of fibre is supplied. The stability of pH and VFA profiles, together with the large VFA production, showed that the decrease in the [NDF/starch]-ratio had no negative impact on the hindgut ecosystem. This suggests that when large amounts of starch are used to increase the energetic density of a diet, a significant amount of good quality fibre should be maintained. In feeding 1 kg of hay per 100 kg BW, no sign of microbial disturbance was noticed, but further studies should indicate the most appropriate level of intake, to prevent digestive disorders caused by microbial dysfunction in the hindgut.

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References

Alexander, F. and Davies, E. 1963. Production and fermentation of lactate by bacteria in the alimentary canal of the horse and pig. *Journal of Comparative Pathology* **73:** 1-8.

Alexander, F., MacPherson, M. and Oxford, A. E. 1952. Fermentative activities of some members of the normal coccal flora of the horses large intestine. *Journal of Comparative Pathology* **62:** 252-259.

Baruc, J. C., Dawson, K. A. and Baker, J. P. 1983. The characterization and nitrogen metabolism of equine caecal bacteria. *Proceedings of the eighth equine nutrition and physiology symposium*, pp. 151-156. University of Kentucky, USA.

Bertone, A. L., Ralston, S. L. and Stashak, T. S. 1989. Fiber digestion and voluntary intake in horses after adaptation to extensive large-colon resection. *American Journal of Veterinary Research* **50:** 1628-1632.

Bryant, M. P. and Burkey, L. A. 1953. Cultural methods and some characteristics of the more numerous groups of bacteria in the bovine rumen. *Journal of Dairy Science* **36:** 205-217.

Counotte, G. H. M., Lankhorst, A. and Prins, R. A. 1983. Role of DL-lactic acid as an intermediate in rumen metabolism of dairy cows. *Journal of Animal Science* **56:** 1222-1235.

Drogoul, C., Faurie, F. and Tisserand, J. L. 1995. Estimation of the contribution of the pony's colon in fibre digestion: a methodological approach. *Annales de Zootechnie* **44:** 182.

Duprat, F., Gallant, D., Guilbot, A., Mercier, C. and Robin, J. P. 1980. L'amidon. In *Les polymères végétaux, polymères pariétaux et alimentaires non azotés* (ed. B. Montiès), pp. 176-231. Gauthier-Villards.

Espinasse, J., Kuiper, R. and Schelcher, F. 1995. Physiopathologie du complexe gastrique. In *Nutrition des ruminants domestiques — ingestion et digestion* (ed. R. Jarrige, Y. Ruckebusch, C. Demarquilly, M. -H. Farce and M. Journet), pp. 805-852. INRA, Paris.

Fombelle, A. de, Julliand, V., Drogoul, C. and Jacotot, E. 2001. Feeding and microbial disorder. 1. Effects of an abrupt incorporation of two levels of barley in a hay diet on microbial profiles and activities. *Journal of Equine Veterinary Science* **21:** 439-444.

Garner, H. E., Moore, J. N., Johnson, J. H., Clark, L., Amend, J. F., Tritschler, L. G. and Coffman, J. R. 1978. Changes in the caecal flora associated with the onset of laminitis. *Equine Veterinary Journal* **10:** 249-252.

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

Halliwell, G. and Bryant, M. P. 1963. The cellulolytic activity of pure culture strains of bacteria from the rumen of cattle. *Journal of Generic Microbiology* **32:** 441-448.

Healy, H. P., Siciliano, P. D. and Lawrence, L. M. 1995. Effect of concentrate form on blood and gastric fluid variables in ponies. *Journal of Equine Veterinary Science* **15:** 423-428.

Herd, R. 1992. Choosing the optimal equine anthelminthic. *Veterinary Medicine* **87:** 232-239.

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

Hume, I. D. and Sakagushi, E. 1991. Patterns of digesta flow and digestion in foregut and hindgut fermenters. *Proceedings of the seventh international symposium on ruminant physiology,* pp. 427-451.

Hutchens, D. E., Paul, A. J. and di Pietro, J. A. 1999. Treatment and control of gastrointestinal parasites. *Veterinary Clinics of North America: Equine Practice* **15:** 561-573.

Jouany, J. P. 1982. Volatile fatty acid and alcohol determination in digestive contents, silage juices, bacterial cultures and anaerobic fermentor contents. *Sciences des Aliments* **2:** 131-144.

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

Julliand, V., Vaux, A. de, Millet, L. and Fonty, G. 1999. Identification of *Ruminococcus flavefaciens* as the predominant celluloytic bacterial species of the eqine cecum. *Applied and Environmental Microbiology* **65:** 3788-3741.

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

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

Kienzle, E. 1994. Small intestinal digestion of starch in the horse. *Revue de Médecine Vétérinaire* **145:** 199-204.

Kollarczik, B., Enders, C., Friedrich, M. and Gedek, B. 1992. Effect of diet composition on microbial spectrum in the jejunum of horses. *1. Europäische Konferenz über die Ernährung des Pferdes-Physiologie und Pathologie des Verdauungskanals-Konsequenzen für die Ernährung*, pp. 49-54. Pferdeheilkunde, Hannover.

Leeddle, J. A. Z. and Hespell, R. E. 1980. Differential carbohydrate media and anaerobic replica plating techniques in delineating carbohydrate-utilizing subgroups in rumen bacteria populations. *Applied and Environmental Microbiology* **34:** 709-719.

Mackie, R. I. and Heath, A. B. 1979. Enumeration and isolation of lactate-utilizing bacteria from the rumen of sheep. *Applied and Environmental Microbiology* **38:** 416-421.

Mackie, R. I. and Wilkins, C. A. 1988. Enumeration of anaerobic bacterial microflora of the equine gastrointestinal tract. *Applied and Environmental Microbiology* **54:** 2155-2160.

Martin-Rosset, W., Vermorel, M., Doreau, M., Tisserand, J. L. and Andrieu, J. 1994. The French horse feed evaluation systems and recommended allowances for energy and protein. *Livestock Production Science* **40:** 36-56.

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

Meyer, H., Coenen, M. and Probst, D. 1986. Beiträge zur verdauungsphysiologie des pferdes 14. Futtereinspeichelung und -passage im kopfdarm des pferdes. *Journal of Animal Physiology and Animal Nutrition* **56:** 171-183.

Morris, V. K., Girvan, M., Hill, J. and Ball, A. S. 2002. Microbial diversity in the fore stomach of the horse. *Proceedings of the 53rd European Association for Animal Production meeting, Cairo, Egypt*, p. 260.

Murray, M. J. and Schusser, G. F. 1993. Measurement of 24-h gastric pH using an indwelling pH electrode in horses unfed, fed and treated with ranitidine. *Equine Veterinary Journal* **25:** 417-421.

Murray, S. M., Flickinger, E. A., Patil, A. R., Merchen, N. R., Brent Jr, J. L. and Fahey Jr, G. C. 2001. *In vitro* fermentation characteristics of native and processed cereal

grains and potato starch using ileal chyme from dogs. *Journal of Animal Science* **79:** 435-444.

Sauvant, D., Chapoutot, P. and Archimède, H. 1994. La digestion des amidons par les ruminants et ses conséquences. *INRA Productions Animales* **7:** 115-124.

Wolter, R. and Chaabouni, A. 1979. Étude de la digestion de l'amidon chez le cheval par analyse du contenu digestif après abattage. *Revue de Médecine Vétérinaire* **130:** 1345-1357.

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