Random changes in the heifer rumen in bacterial community structure, physico-chemical and fermentation parameters, and *in vitro* fiber degradation

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The variability over time of several main ruminal characteristics was studied in heifers over 15 consecutive weeks. Three heifers were assigned to a low-fiber diet (27% NDF) and three to a high-fiber diet (44% NDF). The physico-chemical (pH and redox potential) and fermentation (volatile fatty acids and ammonia contents) parameters were determined on 1 day per week for 15 consecutive weeks. On the same days the bacterial community structure was studied using a molecular fingerprint technique and the ruminal fiber degradation was studied by *in vitro* incubation of a withdrawn ruminal content sample. Numerous random changes were observed from week to week for all physico-chemical and fermentative parameters and *in vitro* fiber degradation. The redox potential was the only parameter to show a significant interaction between diet and week. Except for the ammonia content, the amplitudes of fluctuations observed were higher for the low-fiber diet. The bacterial community structure did not differ between diets or weeks. The *in vitro* fiber degradation was similar for both diets, with numerous random changes throughout the study. The findings of this study indicated that most of the parameters of the ruminal ecosystem had time-related changes with random fluctuations around a mean value which reflect an unstable equilibrium. This conclusion was valid for both low- and high-fiber diets.

1. Introduction

The ruminal ecosystem fluctuates in terms of microbial composition and environmental parameters according to dietary supplies. In adult animals the changes in the environmental parameters of the rumen due to dietary composition have been well documented (Calsamiglia et al., 2008; Cantalapeidra-Hijar et al., 2009). The bacterial community composition was studied for a disturbed state generated by dietary changes (Goad et al., 1998; Tajima et al., 2000). To well interpret the changes observed after a disturbance, the ruminal ecosystem stability must be characterized without disturbance. Only few data were available in the literature. The bacterial community structure of the cow rumen evolved in a short-term study (3 weeks) while the physico-chemical and fermentation parameters did not differ (Michelland et al., 2009b). These data need to be confirmed for a longer time period.

The study of the ruminal ecosystem requires consideration of the microbial community, its activity and its environment. Bacteria represent the majority of the microbial

Table 1
Ingredients and chemical composition of the two experimental diets.

	Low-fiber diet	High-fiber diet	SE
Ingredients (% of DM[a])			
Dehydrated alfalfa	–	50.2	–
Corn silage	40.2	–	–
Wheat straw	–	20.8	–
Ground corn	43.4	25.5	–
Soybean meal	13.2	–	–
Minerals[b]	3.2	3.5	–
Nutrient analysis			
Dry matter (%)	66.3	88.4	1.1
Organic matter (% of DM)	96.4	93.0	0.9
NDF(% of DM)	26.7	44.0	0.3
CP (% of DM)	13.3	14.1	0.6
Starch (% of DM)	44.5	18.5	0.5
NE_L (Mcal/kg DM)	1.73	1.75	0.01

[a] DM = dry matter.
[b] Contained (per kg of premix, DM basis): 90 g of P, 90 g of Ca, 100 g of Mg, 18 g of Na, 8 g of Zn, 4 g of Mn, 120 mg of I, 100 mg of Co, 30 mg of Se, 1.6 g of Cu, 800 000 IU of vitamin A, 200 000 IU of vitamin D3, 10 g of vitamin E, 10 mg of β carotene, 150 mg of vitamin B1, and 2000 mg of vitamin C. Ferophosphat® VLT.

Table 2
Covering rate of the animal requirements at the beginning and the end of trial according to the diet.[a]

	Low-fiber diet		High-fiber diet	
Time of trial	Beginning	End	Beginning	End
Covering rate of (%)				
Net energy	136	99	110	100
Protein	150	145	120	110

[a] Animal requirements and supplies by dietary intake were calculated according to Jarrige (1989). See Calculation in Material and methods section for more details.

community in terms of biomass and fermentative activity (Lin et al., 1997). This current work studied the time-related changes of the ruminal ecosystem. The physico-chemical and fermentative parameters, *in vitro* fiber degradation and the bacterial community structure in the heifer rumen were studied over a longer time period (15 consecutive weeks) than usual. Moreover this study tested the effect of the dietary composition of both diets (27 *vs* 44% of fiber in DM) on the time-related changes of the ruminal ecosystem, and the interaction between dietary composition and sampling week.

2. Material and methods

2.1. Animals, experimental design and diets

The experiment was carried out at the experimental farm of the Ecole Nationale d'Agronomie de Toulouse (Pouchar-rammet, France). Six Holstein heifers (20 ± 2 months, 482 ± 46 kg) without previous acidosis were used in this study. They were fitted with a dorsal rumen cannula 2 months before the trial in accordance with Animal Care Guidelines (Galyean, 2010). They were not pregnant at any time during the study. They were randomly assigned to one of the two groups and fed either a low- or high-fiber diet (Table 1). They were kept in individual pens with *ad libitum* access to water. The daily feeding rate was adjusted to 9.8 and 8.7 kg of DM per animal for the low- and high-fiber diets respectively to avoid sorting and orts. The animals fed at 90% of their feed intake capacity according to Jarrige (1989). For both diets the net energy and protein recommended allowances of the animals were covered by the dietary supplies (Table 2, Jarrige, 1989). The diets were offered twice daily in equal portions and were kept at the heifer's disposal until the subsequent feeding. The dry matter and chemical composition of all feedstuffs were determined from samples withdrawn each week throughout the study (Table 3).

2.2. Measurements and sampling

Body weights were recorded with both weighing two consecutive days in weeks 1, 7, 10 and 15.

The individual orts were recorded twice daily, *i.e.* just before the subsequent feeding. After a 7-week period of adaptation to the diet, measurements were made over 15 consecutive weeks. Physico-chemical and fermentation parameters of rumen contents were measured once a week. Redox potential and pH were measured as described by Marden et al. (2005). The *ex-vivo* device was quickly (less than 30 s) inserted into the rumen to limit oxygen ingress 4 h before sampling and measurements. The device allowed a continuous sampling of ruminal fluid in anaerobic conditions while maintaining the ruminal temperature and mixing. A lead weight in the sampling device ensured that the sampling site was always located on the ventral side of the rumen. The more redox potential is negative the more the ruminal environment is reductive and anaerobic (Marounek et al., 1982). Each hour from the morning (T0) to the evening

Table 3
Percentage of dry matter and chemical composition of the different feedstuffs on a dry matter basis (%).

	Dry matter (%)	Organic matter (% of DM[a])	Starch	NDF	CP	Ashes
Corn silage	33.5 ± 2.1[b]	95.3 ± 0.3	31.3 ± 2.4	44.6 ± 2.2	7.2 ± 1.0	4.7 ± 0.3
Dehydrated alfalfa	87.4 ± 1.9	88.9 ± 0.3	ND[c]	44.1 ± 0.3	21.6 ± 0.7	11.1 ± 0.3
Ground corn	87.5 ± 1.9	98.5 ± 0.1	73.1 ± 0.7	15.0 ± 2.5	8.4 ± 0.2	1.5 ± 0.1
Soybean meal	88.3 ± 1.4	91.9 ± 1.3	2.1 ± 0.8	15.1 ± 1.1	51.9 ± 0.8	8.1 ± 1.3
Wheat straw	90.6 ± 2.5	95.0 ± 0.2	ND	86.0 ± 1.3	5.3 ± 1.6	5.0 ± 0.2

[a] DM = dry matter.
[b] Mean of the 15 values from the weekly sampling throughout the study ± standard error of the mean.
[c] ND = not determined.

feeding (T8), pH and redox potential values were recorded with a digital pH-meter (model 713, Metrohm, Herisau, Switzerland), a glass pH electrode (combined with Ag–AgCl reference, Metrohm, Herisau, Switzerland), a redox potential platinum electrode (Ag/AgCl as reference, Metrohm, Herisau, Switzerland) and a thermoelectrode (Pt100, Metrohm, Herisau, Switzerland). On the day of the pH and redox potential measurements, 0.5 L of ruminal content was collected from each heifer on the ventral side just before the morning feed and 2, 3, 4, 6 and 8 h after feeding. The sample was filtered with a metal sieve (250-μm mesh) to isolate the liquid phase and 3 aliquots of 10-mL of liquid phase were preserved with the addition of 1 mL of mercuric chloride (2% wt/vol) and stored at −18 °C. The volatile fatty acids and ammonia concentrations were determined from one of the aliquots.

To study the bacterial community and *in vitro* fiber degradation, an additional sample of the ventral ruminal content (0.5 L) was collected on the same day, 3 h after feeding. The content of these samples had to be homogenized by removing the large feed particles to be representative of the total ruminal content with both bacterial fractions, *i.e.* liquid-linked bacteria and solid-attached bacteria. A filtration was done through a 1.6-mm metal sieve to obtain a filtrate with small and medium particles. Filtration through a 1.6 mm sieve was previously used to study the bacterial community structure (Michelland et al., 2009b) and bacterial activity (Privé et al., 2010) in rumen contents. The samples were preserved at −80 °C before DNA extraction and treatment.

2.3. Bacterial community

Total DNA was extracted with QIAamp® DNA Stool Mini kit (Qiagen Ltd, West Sussex, England) from 0.2 g of filtered sample. The V3 region of the 16S rRNA genes of bacterial species was used as a diversity marker by performing PCR as previously described by Michelland et al. (2009b). PCR products were checked for appropriate size by 1% agarose gel electrophoresis. The community structure was studied by Capillary Electrophoresis-Single-Strand Conformation Polymorphism (CE-SSCP), a capillary electrophoretic method based on heterogeneity of single-stranded secondary structure which can provide different electrophoretic mobility through a gel as previously described by Michelland et al. (2009b). Alignment and normalization guaranteed reliable comparison between samples. CE-SSCP profiles processing was computed with the StatFingerprints program version 1.2 (Michelland et al., 2009a) working under R version 2.9.2 (R Development Core Team, 2009). The community structures were compared by calculating the Euclidian distance between the two profiles throughout the scans. The matrix of Euclidian distances thus obtained was analyzed statistically.

2.4. In vitro fiber degradation

Ruminal contents were incubated in a water bath rotary shaker (Aquatron, Infors AG, Bottmingen, Germany). The 1.6-mm strained ruminal fluid sampled 3 h after feeding was kept in anaerobic conditions at 39 °C until transfer to the laboratory. The wheat bran was used as substrate for its crude proteins, cellulose and starch contents allowing microorganism survey and growth for the incubation and was ground through a 1.5-mm sieve (SK 100, Retsch GmbH & Co. KG, Haan, Germany). Eighty milliliters of strained ruminal fluid was incubated in a 250-mL Erlenmeyer flask containing 3 g of wheat bran, and 80 mL of a phosphate–bicarbonate buffer solution. The composition of the buffer solution and the *in vitro* incubation process were described by Privé et al. (2010). Two replicates of the same sample were incubated for 3 h (Time 3), one blank incubated 3 h and one replicate without incubation were used as control. At the end of the incubation, fermentations were stopped by placing the flasks into iced water. The contents of the flasks were then immediately frozen. Samples were freeze-dried (Virtis Freezemobile 25, Virtis, Gardiner, NY), weighed, ground and homogenized in a ball mill (Dangoumau, Prolabo, Nogent-sur-Marne, France) for 3 min.

2.5. Chemical analysis

The dry matter content was determined by oven drying at 105 °C for 24 h, and the organic matter content by ashing at 550 °C for 6 h. Chemical compositions of feedstuffs were determined by the official methods: NF V18-100-1 for crude protein (AFNOR-NF V18-121, 2005), NF V18-121 for starch (AFNOR-NF V18-100-1, 2005) and NF V18-122 for NDF (AFNOR-NF V18-122, 1997).

VFA concentrations were determined by gas chromatography using an adaptation of the method of Playne (1985). Briefly, the liquid phase was separated from the sample by centrifugation (20 min at 4000 *g*). Then the proteins were removed with metaphosphoric acid (200 μL of 25% metaphosphoric acid for 1 mL of liquid phase) and centrifugation (15 min at 20000 *g*). An internal standard (200 μL of 4-methylvaleric acid 1% vol/vol) was added to 1 mL of supernatant, and 1 μL of this mixture was injected into a gas chromatograph (Model 5890 Series II equipped with a flame-ionization detector, Hewlett-Packard, Avondale, PA). The determination of ammonia concentration was based on the modified Berthelot reaction with the Skalar Method followed by a colorimetric test. According to the manufacturer's advice the ammonia was chlorinated to monochloramine which reacted with salicylate. After oxidation and oxidative coupling a green complex was formed. Its absorption was measured by spectrophotometry at 660 nm.

2.6. Calculation

The covering rate of the net energy and the protein corresponds to the supplies: recommended allowances ratio. The French system of ruminant nutrition (Jarrige, 1989) was used for the calculation. The nutrient supplies (net energy and protein) were determined for each diet by the daily intake of the diets and their nutritive compositions (Jarrige, 1989). The net energy and protein recommended allowances of the growing heifers were determined according to the race, the digestive efficiency, the body weight and the average daily gain between each weighing time (Jarrige, 1989).

The redox potential corresponds to a potential difference between a platinum electrode and a hydrogen reference electrode. In this study, the reference electrode used was an Ag–AgCl electrode. Hence a correction must be made to the redox potential measurements corresponding to the potential difference between the Ag–AgCl electrode used and the standard hydrogen electrode, *i.e.* +199 mV at 39 °C.

The *in vitro* fiber degradation was evaluated by the percentage of NDF which disappeared during the 3 h incubations.

2.7. Statistical analyses

All data were analyzed using the R software (R Development Core Team, 2009). The means by cow and by week were calculated for all the physico-chemical and fermentation data (*i.e.* 90 observations for each parameter) and included in the data file to run the statistical analyses. The data were reported as mean values with their standard errors. Ruminal physico-chemical and fermentation parameters, *in vitro* fiber degradation rates and body weight were analyzed using a model with repeated-measurements that included the effect of diet as fixed effect, and the effects of week and heifer as random. The week was a class parameter in the model and the linear effect of week was tested. The statistical model was:

$$Y_{ij} = \mu + D_i + W_j + (DxW)_{ij} + \varepsilon_{ij}$$

where Y is the dependent variable, μ the overall mean, D_i the diet effect, W_j the week effect, $(D \times W)_{ij}$ the interaction between diet and week and ε_{ij} the residual error.

Differences between weeks were assessed by pairwise comparisons (Tukey's test) and differences were declared significant at $P<0.05$.

The analysis of the community structure was based on the calculation of pairwise Euclidean distance between the CE-SSCP profiles to obtain a distance matrix as previously described by Michelland et al. (2009b). Analysis of similarity (ANOSIM) was calculated on the distance matrix using 10 000 Monte Carlo permutations. The fixed effects of diet, week and heifer were tested. Effects were declared not significant at $P>0.05$ whatever the value of ANOSIM-R. If $P<0.05$ the value of ANOSIM-R indicated the degree of similarity between the groups: $R>0.75$: well separated groups, $0.50<R<0.75$: separated but overlapping groups, $0.25<R<0.50$: separated but strongly overlapping groups, $R<0.25$: unseparated groups (Ramette, 2007).

3. Results

3.1. Body weight and covering rates of animal recommended allowances

The body weight evolved throughout the trial with a significant diet × week interaction ($P=0.017$). The growth of

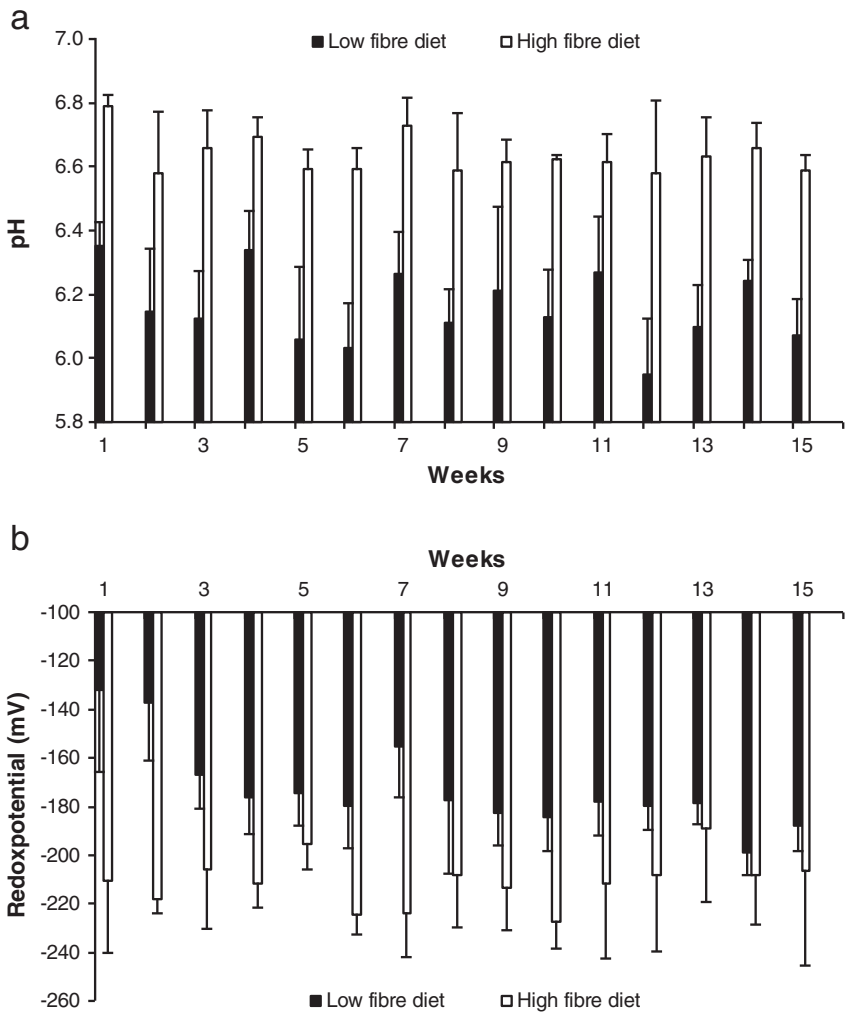

Fig. 1. Variation in rumen pH (a) and redox potential (b) during the 15-week period and according to the diet (low fiber diet: ■, or high fiber diet: □). The bars correspond to the standard error of the mean.

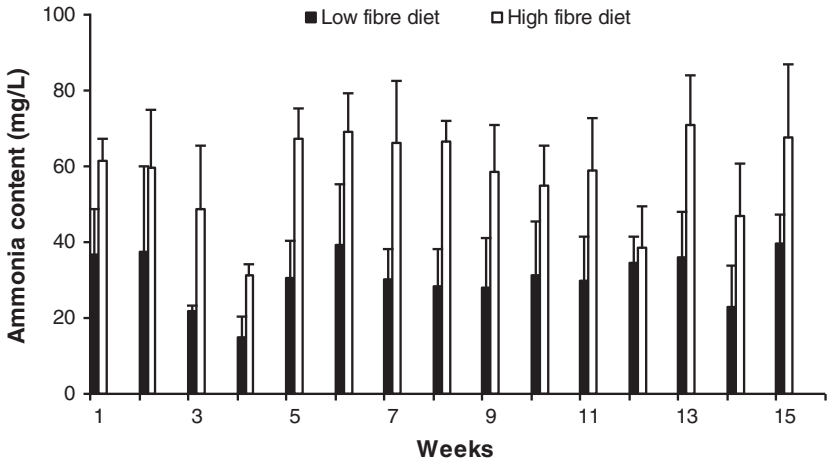

Fig. 2. Ruminal ammonia content during the 15-week period and according to the diet (low fiber diet: ■, or high fiber diet: □). The bars correspond to the standard error of the mean.

heifers fed the low-fiber diet was higher (+99 kg vs +58 kg between weeks 1 and 15 for the low- and high-fiber diets respectively). Throughout the experiment the net energy and protein recommended allowances of heifers were covered by both diets (Table 2).

3.2. Ruminal physico-chemical and fermentation parameters

The interaction between diet and week was not significant for the physico-chemical and fermentation parameters, except for the redox potential. The ruminal pH was lower for the low-fiber diet than for the high-fiber one (6.16 and 6.64 respectively, $P<0.01$). The pH changed significantly over time for both diets without any clear trend ($P<0.01$, Fig. 1a). The ruminal pH values obtained with the low-fiber diet were more variable between two consecutive weeks and throughout the study than those obtained with the high-fiber diet. For the redox potential, the interaction between diet and week was significant ($P<0.05$). The redox potential differences between the two diets were clearest at the beginning of the study, with a maximum for the 1st week of measurement, (−78 mV, Fig. 1b). The redox potential was not significantly different between the diets from week 13 to the end of the study. For the low-fiber diet the week effect was significant ($P<0.01$) and the redox potential decreased rapidly from -132 ± 6 mV for week 1 to -188 ± 3 mV for week 15. For the high-fiber diet the week effect was not significant ($P=0.37$). The ammonia concentration (Fig. 2) was about twice as high ($P<0.01$) for the high-fiber diet (64.9 ± 2.7 mg/L, on average) as for the low-fiber diet (33.4 ± 2.1 mg/L, on average). The ammonia concentrations varied over time ($P<0.01$), sometimes with very big changes between two consecutive weeks, especially for the high-fiber diet between weeks 4 and 5, and between weeks 12 and 13 for example. The low-fiber diet led to a significant ($P<0.05$) higher total VFA content (86.5 ± 1.0 mmol/L on average) than the high-fiber diet (75.1 ± 0.8 mmol/L on average) (Fig. 3a). For both diets the total VFA content varied randomly throughout the study ($P<0.01$). The acetate content was the same in the low- and high-fiber diets (53.3 and 53.4 mmol/L on average, respectively, Fig. 3b) and varied with time during the study ($P<0.01$) with random changes. The propionate content was significantly higher for the low- than for the high-fiber diet (18.5 and 11.5 mmol/L on average, respectively, $P<0.01$, Fig. 3c). No week effect was observed but larger random changes were observed for the low-fiber diet. This diet led also to a higher butyrate content (10.5 and 6.9 mmol/L on average for the low- and high-fiber diets respectively, $P<0.01$, Fig. 3d). This parameter had time-related changes throughout the study for both diets ($P<0.05$). The amplitudes of these changes were larger for the low-fiber diet.

3.3. Bacterial community structure

The effects of diet and week were tested on the structure of the bacterial community. Both were significant ($P<0.01$) but the associated ANOSIM-*R* values were very weak and showed a large overlap between groups (Table 4). Thus the bacterial community structures were similar for both diets throughout the study.

3.4. In vitro fiber degradation

The interaction between diet and week was not significant for the *in vitro* fiber degradation. The percentage of fiber degraded was not significantly different between the two diets (21.7 ± 9.90 and 18.8 ± 6.41% on average for the low- and high-fiber diets respectively, $P=0.18$, Fig. 4). Nevertheless, a week effect was observed with numerous random changes over time ($P<0.05$). The greatest variability between two consecutive weeks was observed for the low-fiber diet, especially between weeks 2 and 3.

Fig. 3. Ruminal VFA content (a: total VFA, b: acetate, c: propionate, d: butyrate) throughout the 15-week period and according to the diet (low fiber diet: ■, or high fiber diet: □). The bars correspond to the standard error of the mean.

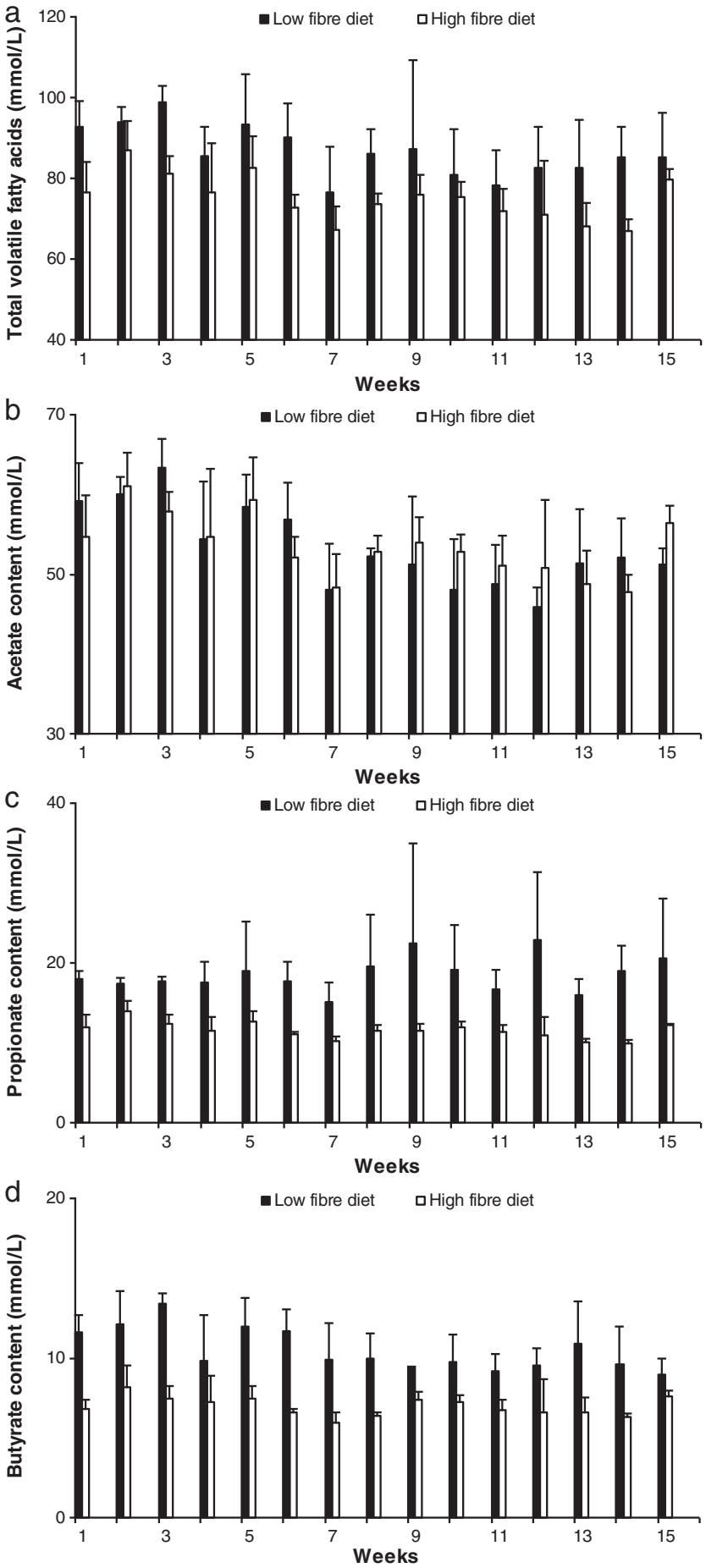
a
Low fibre diet
High fibre diet
Total volatile fatty acids (mmol/L)
120
100
80
60
40
1
3
5
7
9
11
13
15
Weeks
b
Low fibre diet
High fibre diet
Acetate content (mmol/L)
70
50
30
1
3
5
7
9
11
13
15
Weeks
c
Low fibre diet
High fibre diet
Propionate content (mmol/L)
40
20
0
1
3
5
7
9
11
13
15
Weeks
d
Low fibre diet
High fibre diet
Butyrate content (mmol/L)
20
10
0
1
3
5
7
9
11
13
15
Weeks

Table 4
Effect of diet and week on the structure of bacterial communities using ANOSIM.[a]

CE-SSCP profile groups	Number of observations per group	Degree of proximity: ANOSIM-R[b]	*P*
Diet	45	0.04	<0.01
Week	6	0.15	<0.01
Animal	15	0.08	<0.01

[a] The community structure was studied with a SSCP-profiles comparison from samples withdrawn each week (15 weeks) for each animal (6 heifers) 3 h after feeding.
[b] ANOSIM-R value between 0 and 1 (Ramette, 2007).

4. Discussion

This study demonstrates for the first time the time-related changes of the physico-chemical and fermentation parameters of the rumen over a long period (15 weeks) without any applied disturbance. The effect of the diet on the fermentation pattern is well known (Bannink et al., 2006) and the values obtained in the present study with low- and high-fiber diets were in agreement with those reported in the literature (Julien et al., 2010; Marden et al., 2008; Michelland et al., 2009b). However, the long-term evolution of the physico-chemical and fermentation parameters is less well-known. In this current study the changes were more frequent and larger for the low- than for the high-fiber diet. The daily intake and dietary composition were constant throughout the study and could not explain the variability of fermentation between the weeks. Moreover Wertz et al. (2001) showed that an intake restriction to growing beef heifers can achieve a moderate rate of gain without compromising feed efficiency. Nevertheless the daily intake kinetics may have differed as water intake or water intake kinetics. Particular precautions were taken to reduce any difference in daily kinetics of intake or water consumption with the measurements of physico-chemical and fermentation parameters using post-prandial kinetics with at least 6 time points. Numerous external parameters (temperature, day length…) may be also affecting the ruminal ecosystem during this study. This potential effect was intensified by the long-term study. Throughout the experiment the body weight of heifers continuously increased. This data have an effect on the animal recommended allowances which were taken account. In spite of the growth the dietary supplies of net energy and protein allowed the covering of the recommended allowances from the beginning to the end of the experiment.

It appears that the pH values for the low-fiber diet fluctuated around a mean value. For the high-fiber diet, the pH values did not vary much. These data do not support the observation of no time effect reported by Michelland et al. (2009b) over a shorter period. Zosel et al. (2010) explained the temporal change of ruminal pH by single sampling carried out in studies. In our study the temporal changes observed could not be attributed to a small number of sampling. The redox potential values at the beginning of the study for both diets were in accordance with data from short-term studies (Julien et al., 2010; Marden et al., 2008). For the redox potential the interaction between diet and week was significant. For the high-fiber diet the redox potential did not vary with time. For the low-fiber diet time-related changes were observed with the redox potential which became more and more reductive. The redox potentials were not statistically different between both diets from week 13, suggesting that the value obtained with both diets would be similar, but takes longer to be reached with the low-fiber diet. The similarity of redox potential between diets after a long time was only observed in the current study and so needs to be confirmed by additional data.

Propionate and butyrate contents presented similar trends, with very variable values for the low-fiber diet and less variable values and a lower mean for the high-fiber diet. For propionate content the changes were not significant due to their large standard error. Lower production of propionate and butyrate with a high-fiber diet compared to high-concentrates diet has previously been reported (Calsamiglia et al., 2008; Penner et al. 2009). The total VFA and acetate contents showed strong time-related changes with similar variations for both diets. The equivalent capacity of such diets to produce acetate has already been observed (Calsamiglia et al., 2008). In the present study, for these parameters the amplitudes of the changes were similar for both diets. Michelland et al. (2009b) did not find time effect in the physico-chemical parameters and VFA production in their shorter study (3 weeks), probably because the time span of the study was too short. The trend for total VFA content was parallel to that of acetate content because acetate was the principal VFA.

The ammonia content was the only fermentation parameter which showed greater variability for the high-fiber diet. The CP contents were close for both diets but the high-fiber diet contained a higher part of rumen degradable proteins. The types of proteins, the presence of bonds and the slower passage through the rumen with the high-fiber diet could explain a difference in ruminal degradation of proteins as reported by Bach et al. (2005). Nevertheless the authors have no explanation for the higher variability for the high-fiber diet.

In spite of the significant time effect on the environmental parameters, most of the observed differences were slight and had quite likely no physiological sense and no consequences.

The community structure of an ecosystem is based on the species present and their relative abundance in the community (Begon et al., 1996). The fingerprint technique allows a broad approach to the bacterial community and provides information on its structure. Events of co-migration of ribotypes belonging to different bacterial species may occur during capillary electrophoresis, resulting in a single peak on the CE-SSCP profile (Zinger et al., 2007). Thus we preferred to assume that a single peak corresponds to an operational taxonomic unit (OTU) assembly rather than to a single ribotype or bacterial species. Moreover the dominant OTUs are more easily detected with this technique. In spite of these disadvantages, the CE-SSCP technique has advantages and remains useful to determine the microbial fingerprint profiles. Since the migration of ribotypes within the CE-SSCP capillary has been shown highly reproducible (Zinger et al., 2007), the comparison of the peak sizes for each scan of the profile shows which OTUs appear, disappear or change in abundance. Consequently, in the current work, the study of the structure of bacterial communities refers to the fine

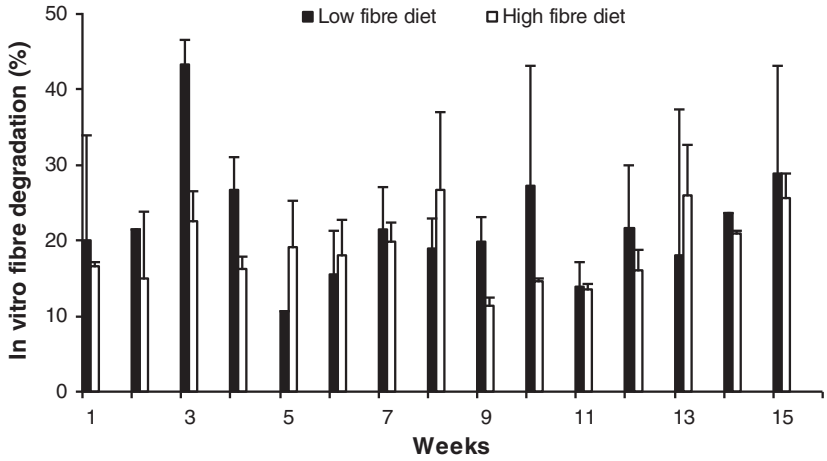

Fig. 4. *In vitro* fiber degradation measured after 3 h of incubation during the 15-week period and according to the diet (low fiber diet: ■, or high fiber diet: □). The bars correspond to the standard error of the mean.

analysis of the size of the various peaks throughout the profiles. In our study the bacterial community structure did not differ between the diets or from week to week. This data was not in agreement with the diet effect observed by Sadet et al. (2007) in the ruminal bacterial community of lambs fed forage and high concentrate diets. Phylogenetic analysis showed changes in the rumen bacterial community according to the dietary composition (Tajima et al., 2000). Substantial differences in bacterial community composition were observed within and across the feeding cycles of lactating cows fed diets with equal proportions of fiber and starch (Welkie et al., 2009). Nevertheless the two diets probably resulted in a different bacterial community composition but the global bacterial community structure was not changed. An extremely dynamic community can sustain a functionally stable ecosystem (Fernandez et al., 1999). The values of *in vitro* fiber degradation, around 20% for both diets, were in accordance with the literature (Eun and Beauchemin, 2007; Lila et al., 2006). However some changes observed between consecutive weeks were not biologically valid. The short time of the *in vitro* incubation could induce variations even if the wheat bran used as substrate was mainly fermented in this period. The equal capacity to digest NDF could explain the equal acetate content observed in the rumen with both diets. Similarly to the physico-chemical and fermentation parameters, the *in vitro* fiber degradation data displayed high variability around a mean value throughout the study.

All the physico-chemical and fermentation parameter data suggested a strong link between the random changes of the ruminal parameters and the level of the fermentative activity. The low-fiber diet induced a higher level of the fermentative activity which was confirmed by lower pH and higher VFA content, and higher amplitudes of variations of the physico-chemical and fermentation parameters.

In spite of the precautions taken for the sampling, an effect on the data of the repeated samplings with brief breaks in the anaerobic conditions in the rumen caused by oxygen ingress cannot be totally ruled out. Finally in the studies carried out with breeding conditions unruly parameters (temperature, humidity…) appear during the long term course and can affect the ruminal functioning. More specifically in this study the growing of the heifers can also affect the ruminal functioning in spite of the similar intake over time.

5. Conclusion

With the adaptation period applied in this study, most of the parameters of the ruminal ecosystem had time-related changes with random fluctuations around a mean value except for redox potential. This study showed the lack of a strict steady state without time changes. Nevertheless an unstable equilibrium was observed for most of the parameters of the ruminal ecosystem. The magnitudes of changes were higher for the low-fiber diet. Further studies with more animals will be necessary to confirm these data. Moreover longer-term studies and a deeper analysis of the bacterial community structure will allow completing and supporting our data.

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