Comparative digestive ability and rumen microbial community of N'Dama and N'Dama x Jersey cattle fed different diets

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Abstract

Rumen fluid collected from three rumen fistulated N'Dama and three crossbred animals fed three different diets at medium supplementation level, was used to compare the plant cell wall degrading community of the two breeds of cattle. *In vivo* digestibility was also examined and compared using 12 animals (six of each breed) fed the same diets. The microbial community of the rumen was analyzed by 16S rRNA hybridisation, using phylogenetic probes of different levels: a universal probe, domain-specific probes for Bacteria, Eukarya and Archaea, and probes targeting cellulolytic organisms: *Chytridiomycetes*, *Fibrobacter* spp., *Ruminococcus albus* and *Ruminococcus flavefaciens*. *In vivo*, organic matter (OM) and neutral detergent fibre (NDF) digestibility were significantly higher in N'Dama compared to crossbred animals when they were fed either baby corn stover and concentrate, or groundnut hay and moringa. In contrast, when the animals were fed groundnut hay and concentrate there was no difference in OM and NDF digestibility between the two breeds. Results of the microbial community analysis showed that RNA concentration of total Bacteria and Archaea was not affected by the breeds of animal, but were significantly affected by diet. The eukaryotic RNA concentration was higher in crossbred animals compared to the N'Dama and was not affected by the diet. *Fibrobacter* and *R. flavefaciens* RNA concentrations (µg/mL) were significantly dependent on diet and breed. *Ruminococcus albus* and *Chytridiomycetes* RNA concentrations were neither affected by the breed, nor by the diet of the animals. This suggested that the differences between breeds observed in digestibility could be partially explained by the composition of the cell wall degrading community.

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Introduction

The rumen microbiome (bacteria, protozoa and fungi) plays a key role in the digestibility of feed which influences feed intake and animal performance. It can be expected that the proportions of various nutrients that are available for absorption by the animal change according to the population sizes and activities of individual microbial species. Although the dependence of the ruminant on its ruminal microbiota is well known, there is still a lack of information regarding the comparative composition of the rumen microbial community of different breeds of ruminants. The attachment of microbes to feed particles (Forsberg & Lam, 1977; Craig *et al*., 1987; Forsberg & Cheng 1992; Weimer *et al*., 1999) and the morphological and physiological similarities among related microbial species have confounded the quantification of these microbes based on classical microscopy or cultivation methods. Moreover, cultivation also selects for cultivable strains and neglects the vast majority of microbes that cannot be grown in pure cultures.

Over the last decade the development of oligonucleotide probes for species-specific segments of 16S rRNA has provided an unprecedented opportunity to characterize and quantify microbial communities in their natural environment without the need for cultivation (Fox *et al*., 1980; Stahl *et al*., 1985; Pace *et al*., 1986; Stahl *et al*., 1988; Shi *et al*., 2007; Goel *et al*., 2008). Oligonucleotide probe techniques have then been used to characterize rumen microbial communities of a single cow over time (Stahl *et al*., 1988), to investigate the competition among ruminal cellulolytic bacteria in a defined co-culture (Ondeyo *et al*., 1994; Shi & Weimer, 1997), to examine the relationship among ruminal bacteria *in vivo*, and with regards to diet,

feed digestion and animal production (Weimer *et al.*, 1999; Hooper *et al.*, 2002). However, these studies have not addressed the effects of the host animal's genetics on the rumen microbial communities in relation to differences in digestibility reported by several investigators (Givens & Moss, 1994; Ranilla *et al.*, 1998; Cruywagen *et al.*, 2001; Akinbamijo *et al.*, 2003).

Studies of microbial populations have raised many questions concerning the impact of host, environment and diet. Therefore the present study was undertaken to determine whether the plant cell wall degrading communities in the rumen vary between two cattle breeds (N'Dama and N'Dama x Jersey) kept under the same conditions and fed three different diets, in relation to *in vivo* digestibility.

Materials and Methods

Twelve bulls consisting of six N'Damas (152 ± 12 kg live weight, BW) and six Jersey x N'Dama crosses (295 ± 10 kg BW), aged between 4 - 6 years, were used for the study. Animal ethics approval was obtained from the International Trypanotolerance Centre (ITC) scientific committee in Gambia. The 12 bulls were housed individually in pens with concrete floors on an open-air platform. All animals had free access to a salt lick and access to water four times a day. Prior to the experiment, animals were dewormed with albendazole (Albenol®- 100, oral Inter Chemie, Holland) at the dose of 12 mL/kg BW and were sprayed against ticks with Decatix (Cooper® Zimbabwe Pvt. LTD). They were also tested for trypanosomiasis using the dark ground buffy coat technique, and infected animals were treated with diminazene aceturate (Berenil® Hœchst A.G., Frankfurt Am Main, Germany) at a dose of 3.5 mg/kg BW. They were fed three different diets: Baby corn stover and concentrate (BCS:Co); groundnut hay and concentrate (GNH:Co) and groundnut hay and moringa leaf (GNH:Mo). The supplements (concentrate or moringa) represented 20% of the diet.

Table 1 Chemical composition of test diets (g/kg dry matter)

	OM	CP	NDF	ADF
Baby corn stover & Concentrate	90.7	10.8	60.8	34.8
Groundnut hay & Concentrate	89.4	16.2	49.2	41.4
Groundnut hay & Moringa leaf	85.9	16.8	33.8	26.7

OM - organic matter; CP - crude protein; NDF - neutral detergent fibre; ADF - acid detergent fibre.

The feed was offered on a dry matter basis at 2.5% BW in two equal daily portions in the morning and in the afternoon to ensure constant availability. The roughage (basal diet) was only offered after an animal had completely consumed the supplement. During the adaptation period the average feed intake was measured individually and the bulls were offered this quantity plus 10 - 20% during the data collection period. Cleaning of the pens, removal and weighing of leftovers from the previous day were done daily before feeding. Representative samples of feed offered and of feed refused were taken daily for dry matter determination and bulked per breed for crude nutrients analysis.

After a 14-day adaptation period, faeces were manually collected immediately after being voided and sub-sampled, and weighed at 08:00 and 20:00. The dry matter was determined on a daily basis, and 5% of the representative faeces voided, were sampled from each animal, kept in a freezer and bulked on a weekly basis until required for further analysis. The collection period lasted 10 days.

Samples for microbial community analysis were collected from six rumen fistulated animals (three N'Damas and three N'Dama x Jersey crossbreds). In the morning before feeding rumen fluid and digesta were collected, strained and squeezed though a 100 µm pore size nylon bag in a prewarmed thermos flask. Laboratory handling of rumen fluid was done under continuous flushing with CO_2 to maintain anaerobic conditions. Approximately 300 µL rumen fluid were put into a 2 mL screwcap cup containing 600 µL phenol (pH 5), and stored at –25 °C. Every two months samples were sent to the University of Hohenheim where they were stored at –80 °C.

Animals were fed the same diets as used in the *in vivo* digestibility trial. Samples were collected before morning feeding from each fistulated animal. For each diet, samples were collected after a 14-day adaptation period for three consecutive days in triplicate per fistulated animal.

Total RNA was extracted using the modified (Muetzel & Becker, 2002) low pH hot phenol extraction procedure described by Stahl *et al.* (1988). To each sample containing phenol, 270 µL of a buffer (pH 5.1; 50 mM sodium acetate, 10 mM EDTA, pH 5.1), 30 µL SDS (20% w/v) and 1 g zircona silica beads (0.1 mm) were added. Cells were then lysed by beating the samples in a beat mill (Bartlesville, OK, USA) and shaken for 2 min. Samples were placed into a water-bath at 60 °C for 10 min and the beating was repeated. Approximately 300 µL chloroform was added to the samples which were shaken vigorously and then incubated at room temperature for 5 min. The incubation was repeated after another vigorous shaking. Aqueous and organic phases were separated by centrifugation (10 000 x g, 5 min, 4 °C), and the aqueous phase was transferred into a new vial containing 300 µL of 7.5 M ammonium acetate and 900 µL of isopropanol. Nucleic acids were precipitated at –20 °C overnight and recovered by centrifugation (16 000 x g, 10 min, 4 °C). Then the supernatant was discarded and the samples were washed in 80% ethanol. Nucleic acids were dissolved in 100 µL double distilled H_2O and samples were stored at –80 °C.

The quantification and the integrity of the RNA extracts were evaluated by agarose gel electrophoresis. Approximately 10 µL of nucleic acids were mixed with 10 µL of a sample loading buffer (40% saccharose and 0.05% bromophenol blue) and 4 µL of the mixture were loaded onto a 1.4% agarose gel. 16S Ribosomal RNAs (gels also showed the other rRNAs*)* were separated in a horizontal electrophoresis chamber containing a TBE buffer (0.1 M tris-base, 0.083 M boric-acid and 1 mM EDTA pH 8) at 200V for 2 h. After separation, the gels were stained for 30 min (1 µg/mL ethidium bromide), and destained for 30 min in the TBE buffer. Each gel contained six lanes of known concentrations of RNA (i.e. standards) that served as a calibration for an initial quantification of the RNA recovered for the calculation of the dilutions prepared for membrane hybridisation.

Membrane hybridizations were carried out as described in Stahl *et al.* (1988). The oligonucleotide probes used were custom synthesized by the Amersham Pharmacia Biotech, (Freiburg, Germany) and targeted *Fibrobacter* spp. (Stahl *et al.* 1988), *Ruminococcus albus* and *Ruminococcus flavefaciens* (Odenyo *et al.*, 1994), total bacteria (Amann *et al.*, 1990), the *Chytridiomycetes* (Dore *et al.*, 1993), total *Archaea* (Amann *et al.*, 1990) total eukaryotes (Hicks *et al.*, 1992) and total 16S rRNA (Zheng *et al.*, 1996) (Table 2).

Table 2 Oligonucleotide probe sequence with empirically determined thermal denaturation temperature (td) used in the experiment (Muetzel *et al.*, 2003).

Target	Sequence ('5-'3)	Wash temperature (^{o}C)
All organisms	GAC GGG CGG TGT GTA CAA	44
Bacteria	GCT GCC TCC CGT AGG AGT	54
Eukarya	TAC AAA GGG CAG GGA C	42
Archaea	GTG CTC CCC CGC CAA TTC CT	56
Chytridiomycetes	GTA CAC ACA ATG AAG TGC ATA AAG G	43
Fibrobacter	AAT CGG ACG CAA GCT CAT CCC	56
R. flavefaciens	AAC GGC AGT CCC TTT AG	46
R. albus	GTC AAC GGC AGT CCT GCT A	46

R. albus - Ruminococcus albus; *Ruminococcus flavefaciens - R. flavefaciens.*

The RNA samples were denatured with three volumes of glutaraldehyde (2% v/v) and diluted to approximately 2 ng/µL with dilution water (0.2 ng/mL bromophenol blue) 10 µg poly A. Then 50 µL of these dilutions were applied to a positively charged nylon membrane (Magna Charge, Micron Separation

Inc., Westboro, Ma) under slight vacuum using a Minifold II™ slot blotter (Schleicher and Schüll, Horb, Germany) in triplicate. For quantification purposes each membrane contained standards (i.e. a reference series) with known amounts of target RNA (Figure 1). Membranes were air-dried and RNA was fixed by baking at 80 °C for 1 h. Membranes were transferred to hybridization bottles and 3 mL of hybridization buffer were added per membrane. Membranes then were prehybridised at 40 °C for 1 h.
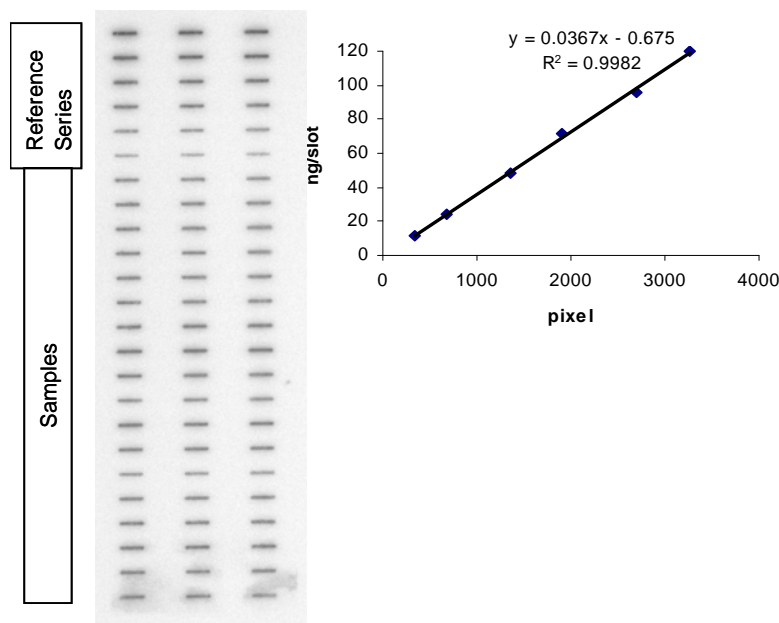

Figure 1 Membrane layout used for hybridisations. Each membrane contained 24 slots, the first six slots were hybridised with a universal probe using *Escherichia coli* as a reference strain, and the remaining 18 slots contained RNA from a member of the target group.

Probes were labelled with 32P-ATP (MP Biomedicals Inc., Eschwege, Germany) using T4 nuclease transferase (Amersham Pharmacia Biotech, Freiburg, Germany). The labelled probes were purified with spin columns (QIA Quick spin, Queen GmbH, Holden, Germany) and then added to the appropriate amount of hybridization buffer (3 mL per membrane). The pre-hybridization buffer was discarded and the hot buffer was added to the membranes. Hybridization was carried out overnight at 40 °C. Then the membranes were removed from the bottles and placed into a wash solution (SSC and 1% SDS), which was preheated to the probe specific wash temperature. After 15 min the membranes were transferred and kept for another 15 min in the fresh wash solution. There-after the membranes were air dried, covered with saran wrap and exposed to imaging plates (Type BAS-III, Fuji Photo Co., Ltd, Japan). The imaging plates were scanned in a phosphorus imager (BAS 1000, Raytest Isotopenmeßgeräte GmbH, Straubenhardt, Germany) and the resulting digital images were analyzed using the programme, Aida (Raytest Isotopenmeßgeräte GmbH, Straubenhardt, Germany).

Data were subjected to analysis of variance using the GLM procedure of SAS. The model used was:

$$Y_{ij} = \mu + B_i + D_j + E_{ij},$$

Where Y_{ij} represented the dependent variable for breed B_i (1 to 2), for diet D_j (1 to 3), μ the mean for the variable and E_{ij}, the error associated with the observation ij.

Results

The results of the *in vivo* organic matter digestibility (OMD) and neutral detergent fibre digestibility (NDFD) are shown in Table 3. When animals were fed BCS:Co or GNH:Mo, the OM and NDF digestibility were significantly higher in N'Dama cattle compared to the crossbreds. The difference was higher on the BCS:Co (7%) compared to the 4% on the GNH:Mo diet. However, when GNH:Co was fed to both the N'Dama and crossbred cattle, there was no significant difference in both OMD and NDFD between the two breeds.

Table 3 Mean (±s.d.) organic matter digestibility (OMD) and neutral detergent fibre digestibility (NDFD observed in two cattle breeds (N'Dama and N'Dama x Jersey crossbred) fed three different diets

Diet	OMD (%DM)			NDFD (%DM)		
	N'Dama	Crossbred	P value	N'Dama	Crossbred	P value
Baby corn stover & Concentrate (BCS:Co)	63.2 ± 1	55.9 ± 2	<0.01	58.0 ± 1	51.5 ± 2	<0.01
Groundnut hay & Concentrate (GNH: Co)	64.9 ± 1	66.6 ± 1	0.06	50.2 ± 2	52.0 ± 2	0.06
Groundnut hay & Moringa leaf (GNH: Mo)	66.8 ± 1	63.5 ± 1	<0.05	45.9 ± 2	41.7 ± 1	<0.05

The overall results for the microbial community analysis showed a huge variation among animals of the same breed. The bacterial RNA concentration (µg/mL of rumen fluid) was not significantly affected by the breed of animal. However, it was significantly (P <0.05) affected by diet (Table 4). The bacterial RNA concentration was higher in the groundnut based diets compared to the baby corn stover based diet. There was no significant breed x diet interaction.

The probe used to target all rumen eukaryotes did not differentiate between protozoa and anaerobic rumen fungi. When averaged for all the diets, the eukaryotic RNA concentrations (µg/mL of rumen fluid) were significantly (P <0.05) higher in crossbred animals compared to the N'Dama breed (7 *vs.* 4 µg/mL, respectively). However, diet had no effect on the microbial populations between the breeds (Table 4). When averaged for both breeds, there was no dietary effect, although it tended to be higher for GNH:Mo (4.63, 5.02 and 6.10 µg/mL for BCS:Co, GNH:Co and GNH:Mo, respectively). The breed x diet interaction was also non significant.

Table 4 Least-square means (± s.d.) RNA concentrations of Bacteria, *Eukarya* and *Archaea* (µg/mL) in rumen fluid of two cattle breeds fed different diets

Organisms	Breed	Diets			Breed	Diet	Day	Breed x Diet
		BCS:Co	GNH:Co	GNH:Mo				
Bacteria	Crossbred	62.52 ± 39.0	124.36 ± 63.0	128.85 ± 58.0	NS	<0.05	<0.05	NS
	N'Dama	97.69 ± 49.0	110.69 ± 43.0	98.89 ± 40.0				
Archaea	Crossbred	0.89 ± 0.9	2.08 ± 1.7	1.93 ± 0.7	NS	<0.01	NS	NS
	N'Dama	1.06 ± 0.8	1.75 ± 0.8	1.71 ± 07				
Eukaryotes	Crossbred	6.30 ± 5	6.62 ± 4	7.14 ± 6	<0.05	NS	NS	NS
	N'Dama	3.36 ± 3	3.56 ± 3	5.13 ± 4				

BCS:Co = Baby corn stover and concentrate; GNH:Co = Groundnut hay and concentrate; GNH:Mo = Groundnut hay and moringa; NS = non significant.

Contrary to the eukaryotic RNA concentration, the archaeal (i.e. methanogens) RNA concentration was significantly affected by the diet of the animals (P <0.01). The archaeal RNA concentration was lower in the baby corn stover diet compared to the groundnut hay diet (1 *vs.* 2 µg/mL, respectively) and was higher with GNH:Co compared to the GNH:Mo diet. However, the difference between the two breeds was not significant and no breed x diet interaction was observed.

Table 5 Least-square means (± s.d.) RNA concentrations of *Chytridiomycetes Fibrobacter* spp., *Ruminococcus albus* and *Ruminococcus flavefaciens* (µg/mL) in rumen fluid of two cattle breeds fed different diet

Organisms	Breed	Diets			Breed	Diet	Day	Breed x Diet
		BCS:Co	GNH:Co	GNH:Mo				
Chytridiomycetes	Crossbred	0.17 ± 0.1	0.21 ± 0.2	0.36 ± 0.2	NS	NS	NS	NS
	N'Dama	0.16 ± 0.2	0.14 ± 0.1	0.19 ± 0.2				
Fibrobacter spp.	Crossbred	0.68 ± 0.4	0.42 ± 0.2	0.39 ± 0.1	NS	<0.0001	NS	<0.05
	N'Dama	1.29 ± 0.9	0.33 ± 0.1	0.31 ± 0.1				
R. albus	Crossbred	0.40 ± 0.6	0.69 ± 0.4	0.61 ± 0.3	NS	NS	<0.01	NS
	N'Dama	0.68 ± 0.4	0.59 ± 0.2	0.47 ± 0.3				
R. flavefaciens	Crossbred	0.11 ± 0.1	0.10 ± 0.1	0.08 ± 0.1	NS	<0.05	NS	<0.05
	N'Dama	0.22 ± 0.2	0.05 ± 0.1	0.05 ± 0.1				

BCS:Co = Baby corn stover and concentrate; GNH:Co = Groundnut hay and concentrate; GNH:Mo = Groundnut hay and moringa; NS= non significant.

Four cell wall degrading organisms were also targeted *(Chytridiomycetes, Fibrobacter* spp., *R. albus* and *R. flavefaciens*). When averaged for all the diets, there was no significant difference between the two breeds in the RNA concentration of the four cell degrading organisms (Table 5). However, the RNA concentrations of *Fibrobacter* and *R. flavefaciens* were significantly affected by diet and a significant (P <0.05) breed x diet interaction was observed in the RNA concentration of these organisms. When averaged for all the breeds, the RNA concentrations of *Fibrobacter* and *R. flavefaciens* were respectively 3 and 2 times higher when animals were fed the baby corn diet than the groundnut hay based diets. Moreover, when the animals were fed BCS:Co, the N'Dama breed had higher *Fibrobacter* (P <0.05) and *R. flavefaciens* (P <0.05) RNA concentrations compared to the crossbred animals, whereas on groundnut based diets, there was no significant difference in *Fibrobacter* and *R. flavefaciens* RNA concentrations (µg/mL) between the two breeds.

The *R. albus* RNA concentration was neither influenced by the diet, nor by breed. Nevertheless, when animals were fed BCS:Co, the difference in *R. albus* RNA concentration tended to be in favour of the N'Dama breed (P = 0.06), whereas on the groundnut hay based diets, the crossbred animals tended to have a higher RNA concentration of *R. albus* (P = 0.2).

With the RNA concentration of *Chytridiomycetes* (Table 5), there was no significant affect of animal breed and diet. Moreover, no significant breed x diet interaction was observed. In general the *Chytridiomycetes* accounted for 3% to 5% of the total eukaryotic population 16S rRNA.

Discussion

Rumen microorganisms play a key role in the digestibility of feed, and this affects feed intake and animal performance. The effect of diet on composition, density and activity of the rumen microbiome has been investigated intensively (McAllister, 2000; Michalet-Doreau *et al*., 2002; Muetzel *et al*., 2003). In the present study these effects were also observed in *Fibrobacter*, *R. flavefaciens*, total bacterial and archaeal RNA concentrations. However, the effect of different breeds of cattle on the rumen microbial community is not well documented. In the present study there was no significant difference in RNA concentration of bacteria and Archaea between the two breeds and no breed x diet interaction was observed. The higher RNA concentration of eukaryotes observed in the crossbred cattle was most probably the result of a higher rumen protozoal RNA concentration since there was no difference between the two breeds in *Chytridiomycetes* RNA concentration. Protozoa have been reported to reduce intestinal protein supply (Muetzel *et al*., 2003). It could therefore be speculated that the low concentration of protozoa observed in rumen fluid of the native

N'Dama breed would increase the efficiency of protein utilisation and this could support a better conserving of nitrogen, and be related to the better digestibility observed in native cattle breeds compared to exotic breeds (Norton *et al.*, 1979).

The absolute total of cell wall degrading bacteria (consisting of the culturable species, *Fibrobacter* spp., *R. albus* and *R. flavefaciens*, but excluding uncultured species) was significantly higher in the N'Dama breed compared to the crossbred cattle. This difference corresponded to the difference (in favour of N'Dama) in *in vivo* OM and NDF digestibility when data were averaged for all the diets. However, there was a significant breed x diet interaction in *Fibrobacter* spp. and *R. flavefaciens* RNA concentrations. On the BCS:Co and GNH:Co diets, *in vivo* organic matter and NDF digestibility showed the same trend in *Fibrobacter* spp. and *R. flavefaciens* RNA concentrations. However, with moringa as supplement N'Dama cattle had in contrast the highest digestibility despite the absence of significant differences in RNA concentrations (μg/mL) of the targeted cell wall degrading microbial community between the two breeds. The highest digestibility observed in this case may be related to the lowered protozoa concentration and consequently to better nitrogen sparing, as reported by Norton *et al.* (1979). The two other cell wall degrading organisms targeted (i.e. *R. albus* and *Chytridiomycetes*), were not related to *in vivo* digestibility data. Stahl *et al.* (1988), Weimer *et al.* (1999) and Michalet-Doreau *et al.* (2002) did not find any relationship between digestion results and microbial ecosystem composition, determined using oligonucleotide probes to rRNA. The absence of this relationship may be due to the low identified bacterial proportion with the rRNA probes used (Michalet-Doreau *et al.*, 2002). Within the cell wall degrading community, the RNA concentration of *Chytridiomycetes* was not affected by the diet and breed of the animals. However, *Chytridiomycetes* was surprisingly high when animals were fed moringa as supplement; they represented 19% and 25% of the cellulolytic rRNA in N'Dama and crossbreds, respectively. The *Chytridiomycetes* are known for their high activity in cell wall breakdown (Williams & Withers, 1991; Lee *et al.*, 2000; Muetzel *et al.*, 2003; Mehmet *et al.*, 2005) and were expected to be higher on groundnut hay diets with a low NDF digestibility.

The RNA concentration of *Fibrobacter* and the ruminococci showed elevated amounts on the stover based diet, almost four times more than on the groundnut hay based diet. This was not surprising because these two organisms are recognized as the most active cell wall degrading species (Li & Heath, 1993; Malburg & Forberg, 1993). *Fibrobacter* is a widely examined rumen organism and the basic work with 16 rRNA targeted probes was done by Stahl *et al.* (1988) using a probe specific for this cell wall degrading organism. The proportion of *Fibrobacter* in the rumen fluid from our study showed a large variability ranging from 0.3 to 1.3% of the total bacterial RNA, with the lowest proportion in the groundnut hay based diet. These proportions are within the range reported by Muetzel *et al.* (2003) and much lower than those of Stahl *et al.* (1988). The difference between the data could be explained by the difference in the diet and more importantly the difference in the collection time. Our samples were collected before morning feeding (about 12 h after feeding), while those of Stahl *et al.* (1988) were collected 6 h after feeding. Species belonging to the genus *Fibrobacter* appeared to be the dominant cellulolytic bacteria targeted on stover based diets as they represented 50% to 55% of the cellulolytic rRNA, and the ruminococci (*R. albus* and *R. flavefaciens*) were the dominant (48 - 58%) bacteria on groundnut hay based diets. Weimer *et al.* (1999) and Martin *et al.* (2001) reported a higher proportion of the two ruminococci compared to *Fibrobacter* in the rumen microbial ecosystem of dairy cows, with *R. albus* as the dominant species. There was no difference in the proportion of the *Ruminococcus* and *Fibrobacter* relative to the total cell wall degrading community between the two breeds.

Conclusions

Differences in digestibility and most probably in productivity between breeds could be correlated to (and may be partially manifested through) a divergent community structure of rumen microbes. That, in turn, indicates that animals of different breeds might have a 'genetic background' that favours the establishment of a certain community, even if the animals are kept under identical conditions. This relationship should be investigated by more advanced molecular techniques.

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