

Can diet change the diversity of ruminal bacterial species in beef cattle?

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Abstract

Background The objective of this study was to assess the effects of diet on bacterial species in the solid fraction of the ruminal content using the gene sequences of the conserved 16S rDNA region steers fed one of the following diets: canola (C), cottonseed (A), sunflower (G), soybean (SO), corn silage (S) and control diet (PD). Canola, cottonseed, sunflower and soybean were fed as whole seeds. Six crossbred steers (Body weight = 416.33 ± 93.30 kg; mean \pm SD), castrated male, and fitted with ruminal cannula were used. The experimental design was a 6×6 Latin square design. **Results** Cellulolytic bacteria were predominant for all diets, with 47.75% of Operational Taxonomic Units (OTU) in animals fed the cottonseed diet. Amylolytic bacteria were identified for all diets, representing 62.51% OTU in animals consuming the sunflower diet. Proteolytic bacteria were identified for all diets, corresponding to 65.96% OTU in animals fed the sunflower diet. Lactic bacteria were identified for all diets. *Megasphaera elsdenii* bacterium was identified for all diets, with a greater diversity of this bacterium in steers fed the control diet. This bacterium may reduce the availability of hydrogen in the rumen due to propionate production and lactate utilization. **Conclusion** Oilseed in the diet showed a similarity of bacteria species with 47.5% of changing of the ruminal flora.

Background

Thousands of microorganisms belonging to the three domains [1] colonize the rumen environment: Bacteria, *Archaea*, and Eucarya (fungi and protozoa). Bacteria are highly diverse and abundant in the rumen representing approximately 95% of the total microbiota [2].

The main species of structural carbohydrate (i.e., cellulose and hemicellulose) bacteria, are *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* and the fermentation end-products are propionate, butyrate, succinate, formate, carbon dioxide, water, and especially acetate [3]. As *Ruminococcus* bacteria are hydrogen producers (via acetate production), their growth and consequently fibre degradation can be inhibited by hydrogen accumulation [4, 5].

The amylolytic bacteria *Treponema bryantii* and *Treponema saccharophilum* mainly ferment non-structural carbohydrates [6] using malt oligosaccharides [7]. *Ruminobacter amylophilus* is predominant in the rumen of starch-fed cattle and the range of substrate used by this bacterium is limited to starch, maltose and maltodextrins [8]. *Ruminobacter amylophilus* has also a significant proteolytic activity [9].

The proteolytic bacteria *Prevotella bryantii* and *Prevotella brevis* are capable of using starch and polysaccharides such as xylenes and plant cell wall pectin. However, they are not able to degrade cellulose [7, 10] and they have proteolytic activity. Lactic bacteria species are represented mainly by *Lactobacillus*. They are homofermentative bacteria and include *Lactobacillus amylovorus*, *Lactobacillus fermentum*, and *Lactobacillus pentosus* that play an important role as initiators of the ruminal fermentation [11, 12].

Eubacterium pyruvativorans is a rapidly growing bacterium that uses pyruvate [13] although pyruvate does not have is not present in high concentration in ruminal fluid [14]. *Megasphaera elsdenii* is considered as one of the main lactate fermenting bacteria converting lactate into propionate and. In the absence of lactate, *M. elsdenii* produces acetate and butyrate but does not ferment glucose into propionate [15, 16].

The higher abundance of bacterial populations involved in propionate production is associated with reduced methane emissions compared with acetate production because more hydrogen is used for propionate production, thus reducing the availability for methane production [4].

The use of oilseeds in ruminant nutrition is an alternative to improve feed efficiency, reducing the methane energy losses the use of hydrogen for biohydrogenation of unsaturated fatty acids. The metagenomic approach is a tool allowing the evaluation of bacterial diversity by studying the total microbial DNA extracted directly from the environment [17]. Using DNA sequencing technology, the ruminal microbiota can be quickly investigated [18]. In order to identify and quantify non-cultured microorganisms, the characterization of the population of prokaryote microorganisms is carried out through the sequencing of the 16S rDNA gene [19].

The objective of the present work was to use the metagenomic approach (i.e., gene sequences of the conserved region 16S rDNA) to determine the bacterial diversity in the solid fraction of ruminal content in steers fed corn silage and different oilseeds (canola, cottonseed, sunflower, soybean) rich with polyunsaturated fatty acids (i.e., omega-3 and omega-6 fatty acids).

Materials And Methods

Animals, experimental design

The experiment was carried out at the Dom Bosco Catholic University and at the Faculty of Veterinary Medicine and Animal Science of the Federal University of Mato Grosso do Sul, in Campo Grande, Mato Grosso do Sul, Brazil.

Six ruminally-cannulated male crossbred steers with and mean weight of 416 ± 93.3 kg at the beginning of the experiment were use in 6×6 Latin square design. Each experimental period consisted of 14 days of which 13 days for adaptation to the experimental diets and 1 day for ruminal sampling. The animals were vaccinated, dewormed and allocated in individual stalls (3×6 m, 18 m^2) with cover and having free access to water. Animals were fed (8:00 A.M.) ad libitum (5% refusals on as-fed basis) one of the 6 experimental diets: Canola (C); cottonseed (A); sunflower (G); soybean (SO); control diet (PD) and corn silage (CS).

Canola (187 kg/kg DM), cottonseed (350 g/kg DM), sunflower (132 kg/kg DM), and soybean (245 g/kg DM) were included in the diet (400:600 roughage:concentrate ratio; DM basis) as whole seeds. The

roughage:concentrate (DM basis) ratio of control diet was 400:600 (roughage:concentrate ratio; DM basis) and corn silage exclusive diet 950:50 roughage:mineral supplement ratio.

The oilseeds grains were included in the diets to achieve an ether extract (EE) concentration of 80 g/kg DM compared with an EE concentration of 50 and 30 g/kg DM, for control diet (PD) and corn silage exclusive diet, respectively.

The composition (chemical and composition) of the experimental treatments is shown in Table 1. The diets were formulated to meet nutrient requirements for an average daily gain of 1.25 kg/day [20].

Characterization of diets

Daily feed consumption was determined by weighing offered feed and refusals. Feed samples (offered and refused) were collected daily and kept frozen for later determination of dry matter (DM; method 930,15), organic matter (OM; method 942,05), crude protein (CP; method 976,05) and ether extract (EE; method 920,39) [21]. Determination of neutral detergent fiber (NDF) was assessed using α -amylase [22] (Termamyl[®]; Sigma Aldrich; add city and country please). Acid detergent fibre (ADF) [23].

Collection of ruminal content

Ruminal content was collected via the cannula on day 14 or each experimental period before feeding animals (i.e. 7:00 AM). Ten grams of the solid fraction of ruminal content were mixed with 10 ml of Tris-Borate-EDTA buffer (pH 7.0; Sigma Aldrich; add city and country please), shaken vigorously for 3 minutes (vortex; reference of the equipment) and filtered on 100-micron mesh tissue. The filtrate was centrifuged at 13,000 RCF for 13 minutes at 4°C. The supernatant was discarded, and the remaining pellet was suspended in 0.8 ml of TE Tris-EDTA buffer (10X, pH 8.0; Sigma Aldrich; add city and country please). The suspended content was centrifuged at 15,000 RCF for 11 min at 4°C, the supernatant was discarded, and the precipitate was stored at -20°C.

Extraction of genomic DNA

Obtaining the genomic DNA was performed on the suspended precipitate of the solid fraction of ruminal content. Thirty six samples were analyzed using the QIAamp[®] Fast DNA Stool Mini Kit extraction kit (Cat No. 51604, Qiagen, Hilden, Germany).

The total DNA extracted from the ruminal content was quantified using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific[®]; city, country), and the quality of the genetic material was assessed by 0.8% agarose gel electrophoresis. The extracted DNA samples were stored at -20°C for further amplification by the Polymerase Chain Reaction (PCR).

16S rDNA gene Sequencing

The samples were sequenced on a large-scale DNA for GenOne Solutions in Biotechnology. The amplification, as well as the sequencing of the V3-V4 (466 bp) regions of the 16S rDNA were performed by the Illumina HiSeq platform (<http://www.genone.com.br>) (V3 341F CCTAYGGGRBGCASCAG, V4 806R GGACTACNNGGGTATCTAAT). After amplification and sequencing a genomic library, data processing, species composition and abundance were obtained. The complexity and complexity difference for each sample, and clustering of species composition for each sample and between samples were made.

Analysis of 16S rDNA gene data

Subsequent data analysis was processed using the Quantitative Insights into Microbial Ecology (QIIME) program. The QIIME is a free program based on Python scripts that allows the classification of 16S rDNA sequences into Operational Taxonomic Units (OTU) and using them as a basis for building phylogenetic trees, plot taxonomic graphs, build interaction networks, alpha and beta diversity, among others [24]. Thus, OTU were defined by clustering at 97%, using as reference, the most recent OTUs database of Greengenes using the Uclust method [25].

The general structure of the bacterial community of phylum and genus were analysed using relative abundance plot. The alpha diversity analysed by a rarefaction curve and OTU observations. The beta-diversity analysis measured by the UniFrac distance matrix, which was used to demonstrate similarity or dissimilarity among the analyzed samples [26].

Statistical tests on the taxonomic differences between the samples were calculated using the STAMP software using Fisher's exact test with multiple Bonferroni correction ($P < 0.01$) (nominal coverage of 95%) [27].

Results

From the Venn diagram (Fig. 1), 2,495 Operating Taxonomic Units (OTU) were identified of which 1,188 OTUs were shared among all diets, corresponding to a similarity rate of 47.61%. The standard and canola diets shared 86 shared OUT while the standard and sunflower diets shared 42 OTU were. The standard and cottonseed diets shared 69 OTU whereas the canola and cottonseed diets shared 74 OTU. Canola and sunflower diets shared 62 OTU, and 41 OTU were shared between sunflower and cottonseed diets. The bacterial community changed slightly with the experimental diet fed to animals (Fig. 1).

The standard and canola diets exclusively shared 3.45% of the bacterial species; while 1.68% of the bacterial species were shared exclusively between the standard and sunflower diets shared. The control diets and cottonseed exclusively shared 2.76% of the bacterial species whereas the canola and cottonseed diets exclusively shared 2.96% of the bacterial species. Canola and sunflower diets exclusively shared 2.48% of the bacterial species and 1.64% of the bacterial species were exclusively shared between the sunflower and cottonseed diets. Thus, changing the experimental diet slightly affected bacterial species little changed due to the lipid level in the diets change more likely because of the chemical composition between the diets. i.e. lipid source from grains or fatty acid profile of them.

The Venn diagram (Fig. 2) revealed that there is an overlap of bacteria between the experimental diets. Corn silage and the control diets shared 1481 OTU, suggesting that the bacterial community present in this overlap probably does not change with the type of the diet fed to animals. Corn silage and control diets shared 66.71% of bacteria; suggesting that regardless of the type of diet, change in averaged 33.28% across all diets.

Comparing the control diet to corn silage diet, a total of 2220 OTU were observed, of which 20% and 13.28% were related to the corn silage diet and the control diet, respectively.

One hundred and fifty strains of bacteria, corresponding to 10.84% of OTU were identified while other bacteria (i.e., 89.16%) were not identified by the database. The identified bacteria, we classified according to the specificity activity (i.e., amylolytic, proteolytic and lactic acid) of each bacteria (Table 2).

The following cellulolytic bacteria were identified: *Prevotella ruminicola* (genus *Prevotella*); *Ruminococcus albus* (genus *Ruminococcus*); *Ruminococcus flavefaciens* (genus *Ruminococcus*); *Butyrivibrio fibrisolvens* (genus *Butyrivibrio*); *Butyrivibrio fibrisolvens* (genus *Pseudo Butyrivibrio*); *Rumen bacterium NK4B29* (genus *Ruminococcus*); *Clostridium papyrosolvans* (genus *Lachnospiraceae*); *Eubacterium uniforme* (genus *Lachnospiraceae*), and *Eubacterium* sp. F1 (genus *Rikenellaceae*). Cellulolytic bacteria were identified in the diets in the following proportions: 35.95% for the corn silage diet; 31.93% for the control diet; 47.75% for the cottonseed diet; 34.34% for the canola diet; 24.03% for the sunflower diet, and 24.02% for the soybean diet.

The following amylolytic bacteria were identified: *Treponema bryantii*, *Treponema saccharophilum* (genus *Treponema*); *Ruminobacter amylophilus* (genus *Ruminobacter*); and *Prevotella ruminicola* (genus *Prevotella*). Amylolytic bacteria were identified in the following proportions: 30.27% for the corn silage diet; 34.40% for the control diet; 27.24% for the cottonseed diet; 34.72% for the canola diet; 62.51% for the sunflower diet and 20.45% for the soybean diet.

The following proteolytic bacteria were identified: *Butyrivibrio fibrisolvens* (genus *Pseudo Butyrivibrio*); *Ruminobacter amylophilus* (genus *Ruminobacter*); *Prevotella albensis*; *Prevotella brevis*; *Prevotella bryantii*; *Prevotella nigrescens*; and *Prevotella ruminicola* (genus *Prevotella*). Proteolytic bacteria were identified in the following proportions: 24.40% for the diet corn silage; 25.63% for the control diet; 30.30% for the cottonseed diet; 35.97% for the canola diet; 65.96% for the sunflower diet; and 23.91% for the soybean diet.

The following lactic bacteria have been identified: *Lactobacillus agilis*; *Lactobacillus amylovorus*; *Lactobacillus aviaries*; *Lactobacillus fermentum*; *Lactobacillus mucosae*; *Lactobacillus murinus*; *Lactobacillus pentosus*; *Lactobacillus salivarius* (genus *Lactobacillus*); *Megasphaera elsdenii* (genus *Megasphaera*); *Eubacterium pyruvativorans* (genus *Eubacterium*); and *Pediococcus pentosaceus* (genus *Pediococcus*). Lactic bacteria were identified in the following proportions: 0.92% for corn silage diet; 0.29% for control diet; 0.22% for cottonseed diet; 0.14% for canola diet; 0.08% for sunflower diet, and 0.50% for soybean diet.

Other bacteria with specificities not described above were identified: *Synergites jonesii* (genus *Synergistes*; pyridinediole reduction); *Acetobacter pasteurianus* (genus *Acetobacter*, acetic acid production); *Selenomonas bovis* (genus *Selenomonas*; succinate production); *Clostridium amilophilum* (genus *Lachnoclostridium*; ammonia production); *Brevundimonas diminuta* (genus *Brevundimonas*; transform ethanol into acetic acid); *Sporomusa malonica* (genus *Sporomusa*; homoacetogenic); *Succiniclasticum ruminis* (genus *Succiniclasticum*; succinate production and use of sugar and urolithic); *Craurococcus roseus* (genus *Craurococcus*; nitrate reduction); and *Kandleria vitulina* (genus *Kandleria*; sugar production). The bacteria were identified in the following proportions: 17.12% for corn silage diet; 13.01% for control diet; 23.63% for cottonseed diet; 9.09% for canola diet; 11.06% for sunflower diet and 8.79% for soybean diet.

Some identified bacteria are involved in more than one activity, the latter is determined the specificity is the rumen environment and the type of diet fed to animals. These bacteria were: *Prevotella ruminicola* (genus *Prevotella*; cellulolytic, amylolytic and proteolytic); *Ruminobacter amylophilus* (genus *Ruminobacter*, proteolytic and amylolytic); *Butyrivibrio fibrisolvens* (genus *Pseudo Butyrivibrio*; cellulolytic and proteolytic); *Treponema saccharophilum* (genus *Treponema*; amylolytic and pectinolytic); *Treponema bryantii* (genus *Treponema*; amylolytic, saccharolytic, and interact with com cellulolytic); *Megasphaera elsdenii* (genus *Megasphaera*; production of acrylate, acetate, butyrate, succinate, lactic, and use of sugar and urolithic); *Lactobacillus fermentum* (genus *Lactobacillus*; use of sugar); and *Eubacterium pyruvativorans* (genus *Eubacterium*; pyruvate and lactic production).

In addition to these bacteria, others with unknown functions were reported so far in the literature. Other bacteria not belonging to the rumen environment were also found and the reason of their presence in the rumen environment is not yet known. Among them: pathogenic bacteria (*Propionibacterium granulosum*; *Ralstonia pickettii*; and *Serratia marcescens*); bacteria of human disease (*Rhodococcus hoagie*; *Aeromonas caviae*; *Klebsiella pneumonia*; *Gallibacterium salpingitidis*; and *Pseudomonas monteilli*); bacteria found in water (*Paladibaculum fermentans*; *Pseudoclavibacter caeni*; *Bacterium enrichment culture clone R4-41B*; and *Methylobacterium aquaticum*); bacteria found in plants (*Pseudoxanthomonas suwonensis* and *Sphingomonas melonis*); and bacteria of ruminant disease (*Dietzia maris*; *Micrococcus luteus*; *Prevotella heparinolytica*; and *Bibersteinia trehalosi*).

Discussion

Diversity indices showed that the ruminal bacterial microbiota is quite rich and diverse, reflecting the reliability of the metagenomic technique associated with 16S rDNA gene sequencing to describe the bacterial community, which would not have been revealed by traditional bacterial culture techniques in microbiology. Using the diversity analysis, it was possible to identify that 47.61% (on average) of changes in bacteria was due to the type of diet fed to animals and this diversity was related to cellulolytic bacteria.

Diet is probably the most important factor influencing the number and relative proportion of different species of ruminal microorganisms [28]. The change in population is a result of the change in ruminant

diet [29], and the change in diet imposes on the animal a transition period in the microbial rumen population, with changes in the proportions between different species to give a new balance and promote a better adaptation to the new diet [30]. The adaptation period used in the present study was 13 days as recommended by the literature [31].

The change in the diversity of rumen microorganisms also varies according to the phase of microbial growth and nutrient availability [32]. This variation also occurs according to the time at which sampling is performed [33]. Variations in the composition of ruminal bacteria can still be attributed to differences between isolation and bacterial composition determination techniques, and significant differences in the composition of isolated bacteria can occur in animals fed different diets [34]

For the cottonseed diet, 47.75% OTU of the were identified as cellulolytic bacteria and was probably due to NDF content of the cottonseed diet (Table 1). For the sunflower diet, 62.51% OTU were identified as amylolytic bacteria and this was more likely due to the starch content of the sunflower diet (Table 2). The bacterium *Ruminobacter amylophilus* was identified with 47.34% of total bacteria, this bacterium has proteolytic and amylolytic activities.

Proteolytic bacteria were identified in 65.96% in the sunflower diet and the bacteria *Ruminobacter amylophilus* was identified with 47.34% of total of bacteria in the sunflower diet and these bacteria possess proteolytic and amylolytic activities.

Lactic bacteria were identified in 0.92% with the silage diet. This proportion was three times higher than for the other diets, probably due to the lactate levels present in the diet, mainly in corn silage and the corn meal levels used in the experimental diets.

The bacteria *Megasphaera elsdenii* was identified in all of diets being 0.1% in the corn silage diet; 0.06% in control diet; 0.1% in cottonseed diet; 0.3% in canola diet; 0.02% in sunflower diet, and 0.03% in the soybean diet. These values suggest that the greater diversity of this bacterium in the ruminal fluid of animals may reduce the availability of rumen hydrogen due to propionate production and lactate utilization.

Cellulolytic bacteria were identified in high percentage in ruminal content of animals fed the cottonseed diet. Amylolytic and proteolytic bacteria were identified in great proportion in animals fed the sunflower diet. Lactic bacteria were identified in great proportion in ruminal content fed the corn silage diet.

Conclusions

Oilseed in the diet showed a similarity of bacteria species with 47.5% of changing of the ruminal flora.

Abbreviations

Canola (C); cottonseed (A); sunflower (G); soybean (SO); control diet (PD); corn silage (CS); dry matter (DM); ether extract (EE); organic matter (OM); crude protein (CP); neutral detergent fibre (NDF); acid detergent fibre (ADF); ethylenediamine tetraacetic acid (EDTA); deoxyribonucleic acid (DNA); polymerase chain reaction (PCR); operational taxonomic units (OTU)

Declarations

Ethics approval

This work was conducted in accordance with the ethical principles adopted by the National Council for the Control of Animal Experimentation (CONCEA) and approved by the Committee on Ethics in Animal Use/CEUA/UFMS (protocol number 654/2015).

Consent for publication

The authors declare that they agree for publication.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

Hilda Silva Araujo de Melo¹, acquisition, analysis, and interpretation of data; have drafted the work, read and approved the final manuscript;

Luis Carlos Vinhas Ítavo^{1*}, coordinator, conception and design of the work; analysis, and interpretation of data; and substantively revised the paper, read and approved the final manuscript;

Alinne Pereira de Castro², conception and design of the work and revised the paper, read and approved the final manuscript;

Camila Celeste Brandão Ferreira Ítavo¹, conception and design of the work and revised the paper, read and approved the final manuscript;

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Tables

Table 1. Ingredients and chemical composition of the experimental diets

	Diets (g/kg of DM)					
	Corn Silage	Control	Cottonseed	Canola	Sunflower	Soybean
Corn Silage	950	400	400	400	400	400
Cottonseed	-	-	350	-	-	-
Canola	-	-	-	187	-	-
Sunflower	-	-	-	-	132	-
Soybean	-	-	-	-	-	245
Corn meal	-	383	170	288	290	305
Soybean meal	-	167	30	75	128	-
Starea (200S)	-	20	20	20	20	20
Mineral nucleus	50	30	30	30	30	30
Chemical composition of diets						
DM (g/kg)	252	492	488	496	495	493
OM (g/kg of DM)	936	956	920	931	930	944
CP (g/kg of DM)	72	191	175	180	180	200
NDF (g/kg of DM)	630	389	497	422	434	375
ADF (g/kg of DM)	426	209	317	238	226	211
EE (g/kg of DM)	23	35	74	73	72	68

DM: dry matter; OM: organic matter; CP: crude protein; NDF: neutral detergent fibre; ADF: acid detergent fibre; EE: ethereal extract.

Table 2. Bacterial species identified by sequencing.

		Corn Silage	Control	Cottonseed	Canola	Sunflower	Soybean
Cellulolytic	<i>Prevotella ruminicola</i>	5.44%	14.37%	13.57%	12.62%	14.17%	9.46%
	<i>Ruminococcus albus</i>	3.88%	2.70%	11.16%	4.24%	3.27%	4.22%
	<i>Ruminococcus flavefaciens</i>	15.83%	6.45%	11.25%	7.15%	3.80%	5.13%
	<i>Butyrivibrio fibrisolvens</i>	0.20%	0.19%	0.66%	0.27%	0.08%	0.14%
	<i>Butyrivibrio fibrisolvens</i>	8.57%	7.54%	10.87%	9.55%	2.52%	4.67%
	<i>Rumen bacterium NK4B29</i>	0.04%	0.01%	0.03%	0.04%		0.01%
	<i>Clostridium papyrosolvens</i>	0.23%		0.01%	0.01%	0.01%	
	<i>Eubacterium uniforme</i>	0.71%	0.33%	0.16%	0.27%	0.14%	0.18%
	<i>Eubacterium sp. F1</i>	1.04%	0.36%	0.03%	0.20%	0.03%	0.21%
Amylolytic	<i>Treponema bryantii</i>	15.36%	17.18%	9.76%	8.88%	0.90%	3.93%
	<i>Prevotella ruminicola</i>	5.44%	14.37%	13.57%	12.62%	14.17%	9.46%
	<i>Treponema saccharophilum</i>		0.10%	0.03%	0.15%	0.10%	0.79%
	<i>Ruminobacter amylophilus</i>	9.47%	2.75%	3.88%	13.07%	47.34%	6.27%
Proteolytic	<i>Butyrivibrio fibrisolvens</i>	8.57%	7.54%	10.87%	9.55%	2.52%	4.67%
	<i>Prevotella ruminicola</i>	5.44%	14.37%	13.57%	12.62%	14.17%	9.46%
	<i>Ruminobacter amylophilus</i>	9.47%	2.75%	3.88%	13.07%	47.34%	6.27%
	<i>Prevotella bryantii</i>	0.52%	0.54%	0.76%	0.38%	1.23%	2.59%
	<i>Prevotella albensis</i>	0.16%	0.38%	1.20%	0.30%	0.68%	0.91%
	<i>Prevotella brevis</i>		0.05%	0.02%	0.05%	0.02%	0.01%
	<i>Prevotella nigrescens</i>	0.24%					
Lactic	<i>Lactobacillus amylovorus</i>	0.27%	0.13%	0.02%	0.02%	0.01%	0.04%
	<i>Lactobacillus fermentum</i>					0.01%	0.02%
	<i>Lactobacillus aviarius</i>	0.14%					
	<i>Lactobacillus mucosae</i>	0.43%	0.10%	0.09%	0.07%	0.03%	0.05%
	<i>Lactobacillus murinus</i>			0.02%			
	<i>Lactobacillus salivarius</i>	0.06%			0.01%	0.01%	0.01%
	<i>Lactobacillus pentosus</i>						0.31%
	<i>Megasphaera elsdenii</i>	0.01%	0.06%	0.10%	0.03%	0.02%	0.03%
	<i>Eubacterium pyruvativorans</i>	0.01%				0.02%	
	<i>Pediococcus pentosaceus</i>			0.01%		0.02%	

Figures

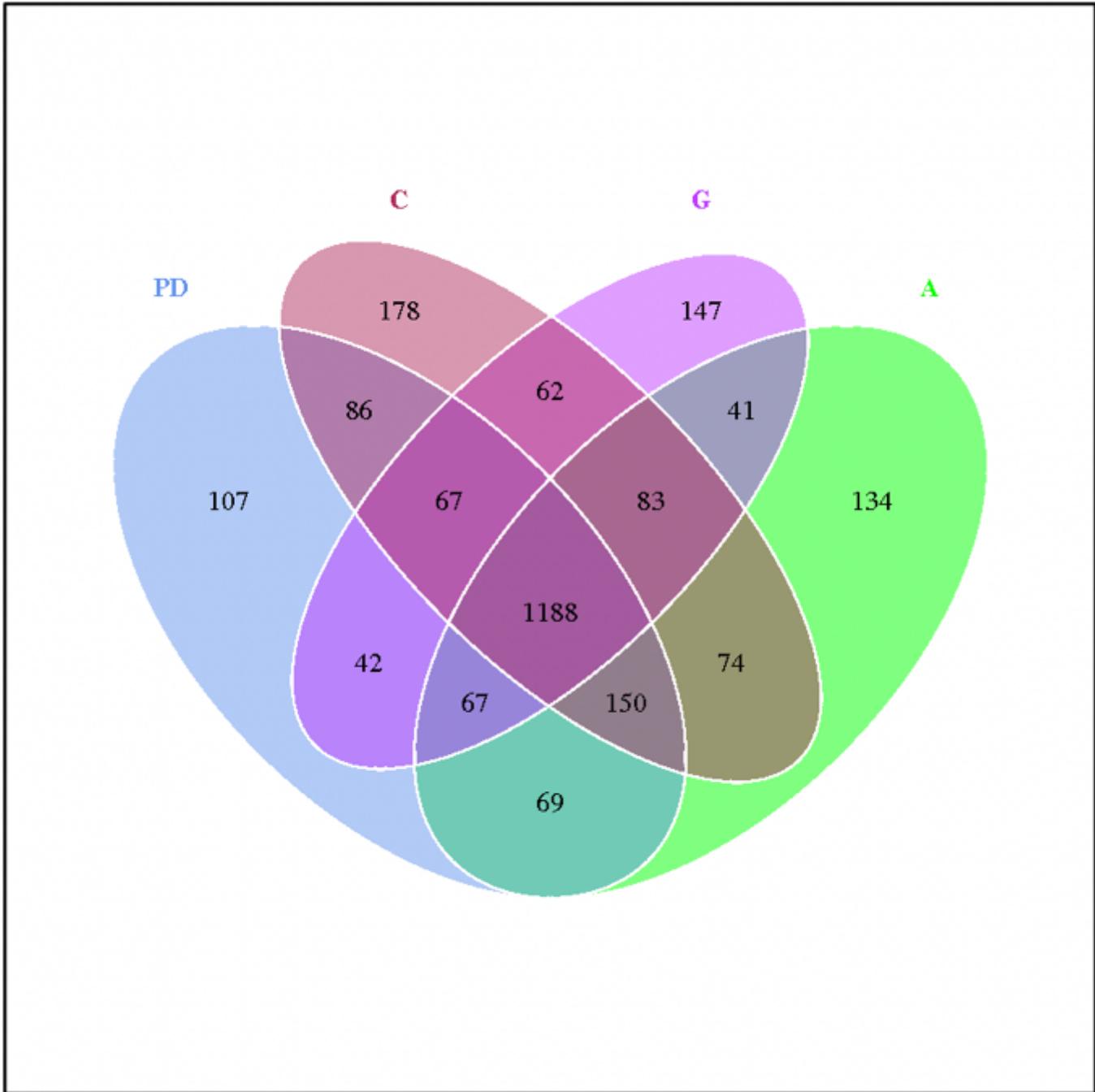


Figure 1

Venn diagram representing the OTU number of bacteria. The values represent the number of shared and non-shared OTUs between the analyzed diets regarding ruminal content. PD: control diet; A: cottonseed diet; C: canola diet; G: sunflower diet.

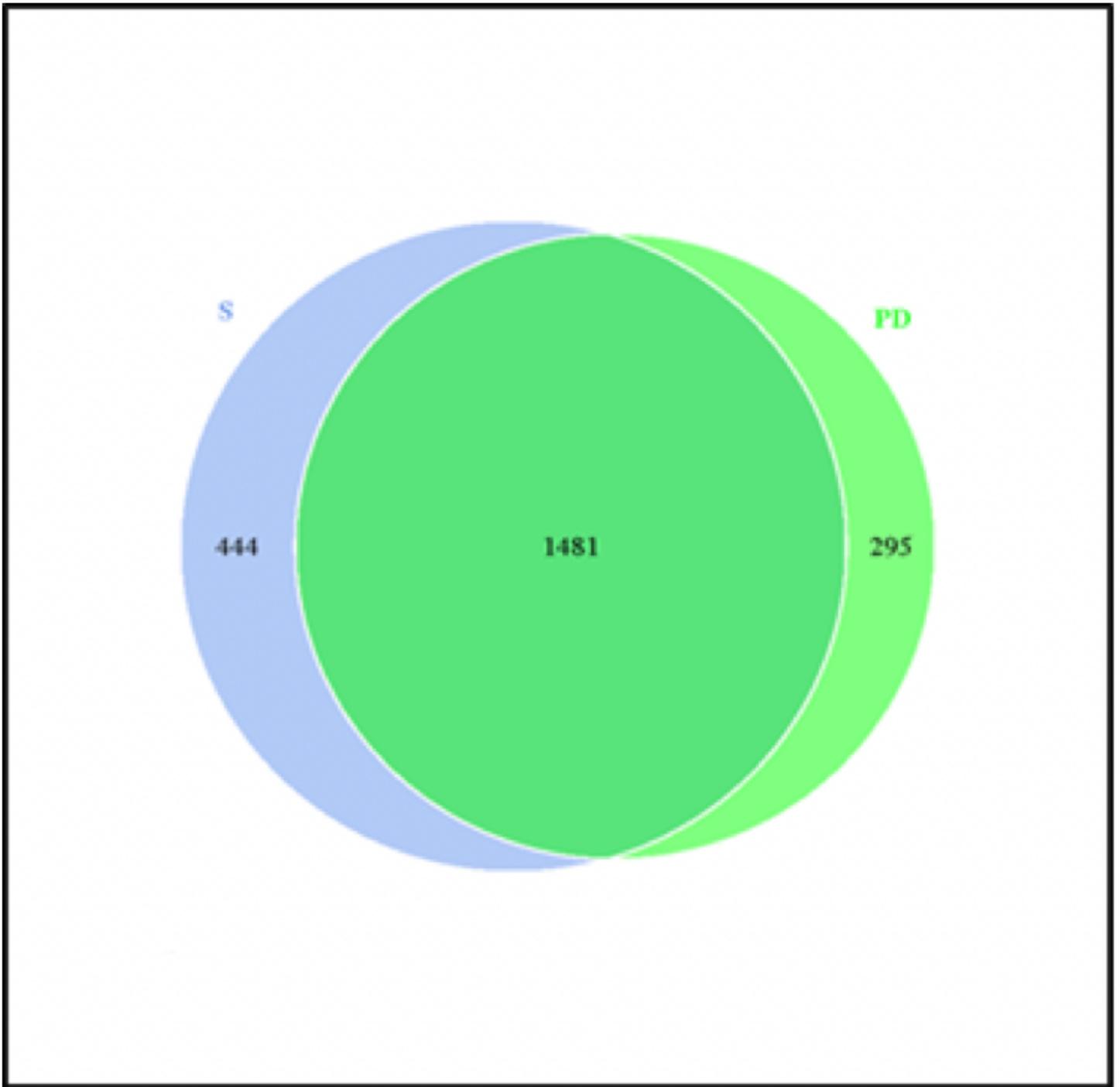


Figure 2

Venn diagram representing the OTU number of bacteria. The values represent the number of shared and non-shared OTUs between the rumen diets analyzed. PD: control diet; S: corn silage.