

Alterations in Ruminal Microflora of Steers Consuming Wheat Pasture Dosed with an Ionophore

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Story in Brief

Twelve ruminally cannulated steers (510 ± 20 kg) grazing wheat pasture (April 1 – April 20) were utilized to evaluate the impact of ionophores on alterations in ruminal microflora. Steers were randomly assigned to three treatments ($n = 4/\text{treatment}$); Control (no ionophore), Monensin (200 mg daily) or Lasalocid, (200 mg daily). Three sampling periods were used to determine the alterations due to ionophore dosing on microbial community; 1) before ionophore treatment (BEFORE); 2) following 8 days of ionophore treatment (DURING), and 3) after cessation of ionophore feeding (AFTER). Alteration in microbial diversity and microbial numbers were evaluated using Terminal Restriction Fragment Length Polymorphism (T-RFLP), and was analysed using Phylogenetic Analysis Tool (PAT). During dosing of monensin, Gram positive bacterial numbers decreased from 39% to 34% with respect to BEFORE and DURING bacterial numbers, and further declined from 34% to 29.5% with respect to AFTER bacterial numbers. Dosing with lasalocid decreased Gram positive bacterial numbers from 30.5% to 25% during the treatment period, but remained constant from the DURING to AFTER period. Overall, ionophore dosing altered the diversity of the microbial population in the rumen. Additionally, there was an apparent reduction in the Gram-positive bacteria in relation to total contribution to the total numbers. Additionally, lasalocid appeared to alter microbial diversity more than monensin.

Keywords: Beef Steers, Ionophore, Rumen Microflora, T-RFLP, 16S rDNA

Introduction

Ionophore supplementation is an efficient management tool that improves weight gains of grazing beef cattle. It is believed that ionophores alter ruminal microflora via changes in the microbial membrane permeability's to specific ions, thus altering various biological concentration gradients and selectively eliminating Gram-positive bacteria (Russell et al., 1987). These alterations in ruminal ecology impact fermented endproducts which are absorbed by beef cattle and ultimately impact animal metabolism (Bergen et al., 1984). The objective of this study was to evaluate the impact of ionophores on rumen microbial communities using T-RFLP and Phylongenetic Analysis.

Materials and Methods

Animals. Twelve ruminally cannulated steers (510 ± 20 kg) were maintained at the wheat pasture research facility located in Stillwater, OK. Steers had access to wheat pasture (April 1– April 20, 2004), and ad libitum water and salt during the entire duration of study.

Treatments/Sampling Periods. Steers were assigned to one of three treatments; Control (no ionophore, $n=4$); Monensin (200 mg daily, $n=4$); or Lasalocid (200 mg daily, $n=4$). The ionophore was delivered daily via ruminal dosing at 0800 daily. Ruminal sampling was done

before ionophore feeding (BEFORE, 4-1-2004), following 8 days of ionophore dosing (DURING, 4-9-2004), and 11 days after the cessation of ionophore dosing (AFTER, 4-20-2004). During each sampling time whole ruminal contents were removed at 0800 and 1300 for each steer. Samples (approximately 500 gm wet weight) were taken from the center of the ruminal mat and frozen immediately.

DNA Extraction. Frozen ruminal samples were thawed at room temperature, and were homogenized using a food processor. Half gram of the homogenized sample was used for genomic DNA extraction. DNA extraction was carried out using Qiagen[®] DNA stool Mini Kit according to manufactures protocols.

T-RFLP Analysis. The extracted DNA was quantified and was diluted to 50 ng/μl and was PCR amplified using fluorescently labeled universal 16S rDNA primers (BAC08F AGAGTTTGATCCTGGCTCAG and BAC 805R GGACTACCAGGGTATCTAATCC). Resulting PCR fragment was ethanol precipitated and was resuspended in 5 μl of sterile water. The PCR product was then digested with Hae III, Msp I and Rsa I individually. The restriction fragments generated were resolved using an ABI 377 DNA sequencer and analyzed using GENESCAN software.

The GENESCAN analysis data was further analyzed using PAT (Phylogenetic Analysis Tool) for assignment of species (<http://trflp.limnology.wisc.edu/index.jsp>); (Kent, et al. 2003).

Results and Discussion

Monensin. For steers dosed with monensin, Gram + bacterial numbers decreased from 39% to 34% with respect to BEFORE and DURING bacterial numbers (Table 1). *Desulfobacterium* sp., *Escherichia coli*, *Paracoccus* sp., and *Rhodobacter* sp. which were present in BEFORE were not detectable during Monensin dosing., but were, detectable again AFTER suggesting that these organisms may have been suppressed by monensin. *Prevotella* sp. (19%), *Bacillus* sp. (13%) and *Xylella fastidiosa* (8%) were predominant during supplementation of Monensin. *Xylella fastidiosa*, *Vibrio* sp. and *Xanthomonas campestris* levels increased from BEFORE to DURING and decreased by AFTER, suggesting that monensin may have induced the growth or removed other limiting factors that limited their contribution in terms of microbial numbers in a non-ionophore environment (Figure 1 and Appendix 1).

Table 1. Percentage of Gram + and Gram – Bacteria as Impacted by Monensin Dosed in the Rumen of Beef Steers Grazing Wheat Pasture

	BEFORE*	DURING [†]	AFTER [‡]
Bacterial Type	Percentage	Percentage	Percentage
Gram+	38.69	34.07	29.57
Gram-	61.31	65.93	70.43
* BEFORE – Before dosing with Monensin			

Figure 1. Bacterial fluctuations during monensin treatment. The legend corresponding to each color is given below. The percentages of each organism were calculated based on peak area.

- | | | |
|---|---|--|
| <input type="checkbox"/> <i>Acetobacter</i> sp. | <input type="checkbox"/> <i>Achromatium</i> sp. | <input type="checkbox"/> <i>Acidiphilium</i> |
| <input type="checkbox"/> <i>Acidosphaera rubrifaciens</i> | <input type="checkbox"/> <i>Acidovorax avenae</i> | <input type="checkbox"/> <i>Acinetobacter</i> |
| <input type="checkbox"/> <i>Actinobacillus capsulatus</i> | <input type="checkbox"/> <i>Actinoplanes utahensis</i> | <input type="checkbox"/> <i>Afipia felis</i> |
| <input type="checkbox"/> <i>Alcaligenes faecalis</i> | <input type="checkbox"/> <i>Amaricoccus macauensis</i> | <input type="checkbox"/> <i>Amycolata</i> |
| <input type="checkbox"/> <i>Anaerobaculum thermoerrenum</i> | <input type="checkbox"/> <i>Anaerofilum pentosovorans</i> | <input type="checkbox"/> <i>Anaeroplana</i> |
| <input type="checkbox"/> <i>Ancylobacter aquaticus</i> | <input type="checkbox"/> <i>Aranicola proteolyticus</i> | <input type="checkbox"/> <i>Arthrobacillus</i> |
| <input type="checkbox"/> <i>Azoarcus</i> sp. | <input type="checkbox"/> <i>Bacillus</i> sp. | <input type="checkbox"/> <i>Bacteroides</i> |
| <input type="checkbox"/> <i>Bdellovibrio stolpii</i> | <input type="checkbox"/> <i>Beggiatoa</i> sp. | <input type="checkbox"/> <i>Beijerinckia</i> |
| <input type="checkbox"/> <i>Bergeyella zoohelcum</i> | <input type="checkbox"/> <i>Bifidobacterium inopinatum</i> | <input type="checkbox"/> <i>Blastomonas</i> |
| <input type="checkbox"/> <i>Bradyrhizobium</i> sp. | <input type="checkbox"/> <i>Brevibacillus</i> sp. | <input type="checkbox"/> <i>Buchnera</i> |
| <input type="checkbox"/> <i>Burkholderia</i> sp. | <input type="checkbox"/> <i>Butyrivibrio fibrisolvens</i> | <input type="checkbox"/> <i>Caldicellulosibacter</i> |
| <input type="checkbox"/> <i>Capnocytophaga</i> sp. | <input type="checkbox"/> <i>Caryophanon latum</i> | <input type="checkbox"/> <i>Chlorella</i> v. |
| <input type="checkbox"/> <i>Chlorobium</i> sp. | <input type="checkbox"/> <i>Chloroflexus aurantiacus</i> | <input type="checkbox"/> <i>Chlorogloeum</i> |
| <input type="checkbox"/> <i>Chryseobacterium indoltheticum</i> | <input type="checkbox"/> <i>Clavibacter michiganense</i> | <input type="checkbox"/> <i>Clostridium</i> |
| <input type="checkbox"/> <i>Colwellia demingiae</i> | <input type="checkbox"/> <i>Comamonas</i> sp. | <input type="checkbox"/> <i>Corynebacterium</i> |
| <input type="checkbox"/> <i>Cryptomonas</i> sp. | <input type="checkbox"/> <i>Cyanidium caldarium</i> | <input type="checkbox"/> <i>Cyanophora</i> |
| <input type="checkbox"/> <i>Cytophaga</i> sp. | <input type="checkbox"/> <i>Dechlorisoma</i> sp. | <input type="checkbox"/> <i>Desulfatobacterium</i> |
| <input type="checkbox"/> <i>Desulfobacter</i> sp. | <input type="checkbox"/> <i>Desulfobacterium</i> sp. | <input type="checkbox"/> <i>Desulfobacterium</i> |
| <input type="checkbox"/> <i>Desulfotomaculum</i> sp. | <input type="checkbox"/> <i>Desulfotomaculum</i> sp. | <input type="checkbox"/> <i>Dietszia</i> sp. |
| <input type="checkbox"/> <i>Dictyoglomus thermophilum</i> | <input type="checkbox"/> <i>Entomoplasma</i> sp. | <input type="checkbox"/> <i>Erwinia</i> an. |
| <input type="checkbox"/> <i>Erysipelothrix rhusiopathiae</i> | <input type="checkbox"/> <i>Erythrobacter longus</i> | <input type="checkbox"/> <i>Erythromonas</i> |
| <input type="checkbox"/> <i>Escherichia coli</i> | <input type="checkbox"/> <i>Eubacterium</i> sp. | <input type="checkbox"/> <i>Exiguobacterium</i> |
| <input type="checkbox"/> <i>Fervidobacterium nodosum</i> | <input type="checkbox"/> <i>Flavobacterium columnare</i> | <input type="checkbox"/> <i>Flectobacterium</i> |
| <input type="checkbox"/> <i>Flexibacter flexilis</i> | <input type="checkbox"/> <i>Francisella tularensis</i> | <input type="checkbox"/> <i>Frankia</i> sp. |
| <input type="checkbox"/> <i>Fusibacter paucivorans</i> | <input type="checkbox"/> <i>Fusobacterium nucleatum</i> | <input type="checkbox"/> <i>Gelidibacter</i> |
| <input type="checkbox"/> <i>Gemmata obscuriglobus</i> | <input type="checkbox"/> <i>Geobacter metallireducens</i> | <input type="checkbox"/> <i>Glaciecola</i> |
| <input type="checkbox"/> <i>Gordonia</i> sp. | <input type="checkbox"/> <i>Haemophilus</i> sp. | <input type="checkbox"/> <i>Haliscometes</i> |
| <input type="checkbox"/> <i>Heliobacterium gestii</i> | <input type="checkbox"/> <i>Heliorestis daurensis</i> | <input type="checkbox"/> <i>Herbaspirillum</i> |
| <input type="checkbox"/> <i>Herpetosiphon aurantiacus</i> | <input type="checkbox"/> <i>Hongia koreensis</i> | <input type="checkbox"/> <i>Hyphomicrobium</i> |
| <input type="checkbox"/> <i>Hyphomonas rosenbergii</i> | <input type="checkbox"/> <i>Kineosporia rhamnosa</i> | <input type="checkbox"/> <i>Kingella</i> or. |
| <input type="checkbox"/> <i>Kitasatospora</i> sp. | <input type="checkbox"/> <i>Klebsormidium flaccidum</i> | <input type="checkbox"/> <i>Kurthia</i> sp. |
| <input type="checkbox"/> <i>Leptospirillum</i> sp. | <input type="checkbox"/> <i>Leptothrix</i> sp. | <input type="checkbox"/> <i>Leucobacterium</i> |
| <input type="checkbox"/> <i>Listonella anguillarum</i> | <input type="checkbox"/> <i>Mannheimia</i> sp. | <input type="checkbox"/> <i>Marchantia</i> |
| <input type="checkbox"/> <i>Marinococcus halophilus</i> | <input type="checkbox"/> <i>Melittangium lichenicola</i> | <input type="checkbox"/> <i>Mesoplasma</i> |
| <input type="checkbox"/> <i>Methanobrevibacter</i> sp. | <input type="checkbox"/> <i>Methyloarcula marina</i> | <input type="checkbox"/> <i>Methylobacterium</i> |
| <input type="checkbox"/> <i>Methylocystis</i> sp. | <input type="checkbox"/> <i>Methylomicrobium buryaticum</i> | <input type="checkbox"/> <i>Methylophilum</i> |
| <input type="checkbox"/> <i>Methylosinus</i> sp. | <input type="checkbox"/> <i>Microbacterium</i> sp. | <input type="checkbox"/> <i>Microcystis</i> |
| <input type="checkbox"/> <i>Microscilla sericea</i> | <input type="checkbox"/> <i>Microvirgula aerodenitrificans</i> | <input type="checkbox"/> <i>Mobiluncoccus</i> |
| <input type="checkbox"/> <i>Moritella</i> sp. | <input type="checkbox"/> <i>Mycobacterium</i> sp. | <input type="checkbox"/> <i>Mycoplasma</i> |
| <input type="checkbox"/> <i>Myxococcus</i> sp. | <input type="checkbox"/> <i>Neisseria canis</i> | <input type="checkbox"/> <i>Nitrosomonas</i> |
| <input type="checkbox"/> <i>Nitrospira</i> sp. | <input type="checkbox"/> <i>Nodularia</i> sp. | <input type="checkbox"/> <i>Nostoc</i> mu. |
| <input type="checkbox"/> <i>Oceanospirillum multiglobuliferum</i> | <input type="checkbox"/> <i>Ornithobacterium rhinotracheale</i> | <input type="checkbox"/> <i>Oxalobacterium</i> |
| <input type="checkbox"/> <i>Oxalophagus oxalicus</i> | <input type="checkbox"/> <i>Paenibacillus</i> sp. | <input type="checkbox"/> <i>Paracoccus</i> |
| <input type="checkbox"/> <i>Pasteurella</i> sp. | <input type="checkbox"/> <i>Pectinatus cerevisiiphilus</i> | <input type="checkbox"/> <i>Pediococcus</i> |
| <input type="checkbox"/> <i>Pedobacter heparinus</i> str. | <input type="checkbox"/> <i>Peptostreptococcus</i> sp. | <input type="checkbox"/> <i>Photobacterium</i> |
| <input type="checkbox"/> <i>Phytoplasma</i> sp. | <input type="checkbox"/> <i>Planococcus</i> sp. | <input type="checkbox"/> <i>Porphyrobacter</i> |
| <input type="checkbox"/> <i>Prevotella</i> sp. | <input type="checkbox"/> <i>Propionigenium modestum</i> | <input type="checkbox"/> <i>Pseudoalteromonas</i> |
| <input type="checkbox"/> <i>Pseudoramibacter alactolyticus</i> | <input type="checkbox"/> <i>Pseudomonas</i> sp. | <input type="checkbox"/> <i>Pyrenomonas</i> |
| <input type="checkbox"/> <i>Quinella ovalis</i> | <input type="checkbox"/> <i>Ralstonia eutropha</i> str. | <input type="checkbox"/> <i>Rhodobacter</i> |
| <input type="checkbox"/> <i>Rhodococcus</i> sp. | <input type="checkbox"/> <i>Rhodiferax antarcticus</i> | <input type="checkbox"/> <i>Rhodocyclonema</i> |
| <input type="checkbox"/> <i>Rhodospirillum</i> sp. | <input type="checkbox"/> <i>Rhodopila globiformis</i> str. | <input type="checkbox"/> <i>Roseobacterium</i> |
| <input type="checkbox"/> <i>Ruminobacter amylophilus</i> | <input type="checkbox"/> <i>Ruminococcus flavefaciens</i> | <input type="checkbox"/> <i>Saccharotoga</i> |
| <input type="checkbox"/> <i>Salinicoccus roseus</i> | <input type="checkbox"/> <i>Salmonella</i> sp. | <input type="checkbox"/> <i>Sebadella</i> |
| <input type="checkbox"/> <i>Selenomonas</i> sp. | <input type="checkbox"/> <i>Shewanella</i> sp. | <input type="checkbox"/> <i>Shigella</i> dy. |
| <input type="checkbox"/> <i>Sphingomonas aromaticivorans</i> | <input type="checkbox"/> <i>Spirochaeta</i> sp. | <input type="checkbox"/> <i>Spiroplasma</i> |
| <input type="checkbox"/> <i>Sporohalobacter lortetii</i> | <input type="checkbox"/> <i>Sporomusa paucivorans</i> | <input type="checkbox"/> <i>Staphylococcus</i> |
| <input type="checkbox"/> <i>Streptobacillus moniliformis</i> | <input type="checkbox"/> <i>Streptococcus</i> sp. | <input type="checkbox"/> <i>Streptomyces</i> |
| <input type="checkbox"/> <i>Sulfobacillus thermosulfidooxidans</i> | <input type="checkbox"/> <i>Synechococcus</i> sp. | <input type="checkbox"/> <i>Synechococcus</i> |
| <input type="checkbox"/> <i>Thermoanaerobacterium thermosaccharolyticum</i> | <input type="checkbox"/> <i>Thermobacillus xylanolyticus</i> | <input type="checkbox"/> <i>Thermodesulfobacterium</i> |
| <input type="checkbox"/> <i>Thermonema lapsum</i> | <input type="checkbox"/> <i>Thermotoga maritima</i> | <input type="checkbox"/> <i>Thermus</i> th. |
| <input type="checkbox"/> <i>Thiobacillus</i> sp. | <input type="checkbox"/> <i>Tissierella praeacuta</i> | <input type="checkbox"/> <i>Treponema</i> |
| <input type="checkbox"/> <i>Ureaplasma urealyticum</i> | <input type="checkbox"/> <i>Vibrio</i> sp. | <input type="checkbox"/> <i>Vitreoscilla</i> |
| <input type="checkbox"/> <i>Weeksella virosa</i> | <input type="checkbox"/> <i>Xanthomonas campestris</i> | <input type="checkbox"/> <i>Xylella</i> fas. |
| <input type="checkbox"/> <i>Xylophilus ampelinus</i> | <input type="checkbox"/> <i>Zooqloea</i> | |

Lasalocid. Treatment with lasalocid had the most drastic effect on the rumen microbial population diversity. During dosing with lasalocid Gram + bacterial numbers decreased from

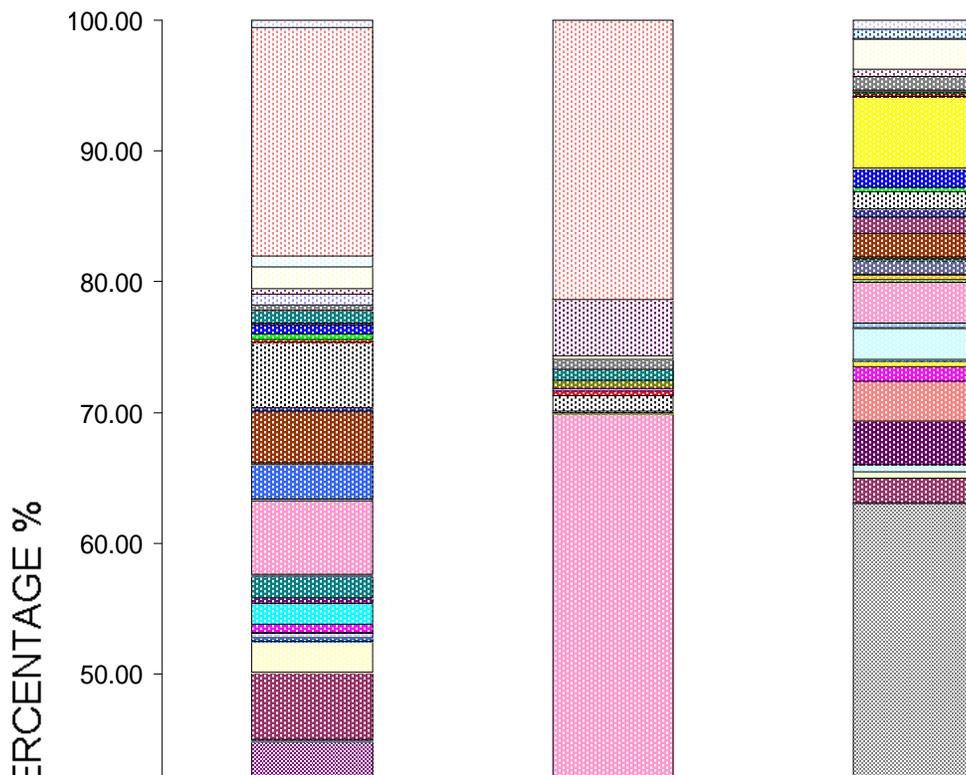
30.5% to 25% during the treatment period, but remained constant from the DURING to AFTER period (Table 2). Similar to monensin, *Prevotella* sp. was predominant during lasalocid treatment and accounted for 36% of the diversity. *Bacteroides* sp., *Clostridium* sp., *Flectobacillus major*, *Heliobacterium gestii*, and *Streptococcus* sp. were predominant during supplementation of lasalocid and increased in numbers from BEFORE to DURING and decreased by AFTER, suggesting that lasalocid may have induced their growth or removed other inhibitory factors. During treatment of lasalocid, *Desulfobacterium* sp., *Desulfobacter* sp., *Escherichia coli*, *Bradyrhizobium* sp., *Capnocytophaga* sp., *Gelidibacter algens*, *Methylosinus* sp., *Neisseria* sp., *Pseudomonas* sp., *Salmonella* sp., and *Ureaplasma urealyticum*, which were present BEFORE, were not detectable during lasalocid dosing. However they were detectable AFTER (Table 2) suggesting that these organisms may have been suppressed by lasalocid. Furthermore, microbial numbers of *Bacillus* sp., *Mycoplasma* sp., and *Nitrospira* sp. were suppressed during lasalocid dosing in comparison to BEFORE and AFTER, but were at detectable levels (Figure 2 and Appendix 2.).

	BEFORE*	DURING†	AFTER‡
Bacterial Type	Percentage	Percentage	Percentage
Gram+	30.48	25.22	25.72
Gram-	69.52	74.78	74.28

* BEFORE – Before dosing with Lasalocid

† DURING – During dosing of Lasalocid

‡ AFTER - After cessation of Lasalocid dosing



- Abiotrophia adiacens
- Acidosphaera rubrifaciens
- Actinobacillus capsulatus
- Atipia felis
- Amaricoccus macauensis
- Amycolatopsis orientalis
- Anaeroplasma abactoclasticum
- Aneurinibacillus migulanus
- Arthrobacter sp.
- Bacteroides sp.
- Blastomonas natatoria
- Brevibacillus brevis
- Butyrivibrio fibrisolvens
- Caloramator indicus
- Chlorogloeopsis sp.
- Comamonas sp.
- Cyanophora paradoxa
- Dermatophilus congolensis
- Desulfobacterium sp.
- Desulfotomaculum sp.
- Eikenella sp.
- Erysipelothrix rhusiopathiae
- Escherichia coli
- Flavobacterium sp.
- Frankia sp.
- Geobacter metallireducens
- Halobacteroides sp.
- Halobacillus sp.
- Heliorestis daurensis
- Kingella sp.
- Lactococcus lactis
- Leptothrix sp.
- Marinococcus halophilus
- Methylobacillus flagellatum
- Methylophilus methylotrophus
- Micrococcus sp.
- Microvirgula aerodenitrificans
- Mycobacterium sp.
- Nitrosomonas sp.
- Ornithobacterium rhinotracheale
- Paenibacillus sp.
- Pasteurella langaa
- Peptostreptococcus sp.
- Planctomyces sp.
- Prevotella sp.
- Pseudomonas borealis
- Ralstonia eutropha
- Rhodospila globiformis
- Saccharothrix australiensis
- Shewanella sp.
- Spirogyra maxima
- Sporosarcina ureae
- Streptomyces armeniacus
- Telluria chitinolytica
- Thermoanaerobacterium thermosaccharolyticum
- Tissierella praeacuta
- Vibrio sp.
- Xylella fastidiosa
- Acetobacter sp.
- Acidovorax sp.
- Aerococcus urinae
- Agromyces mediolanus
- Amaricoccus macauensis
- Anaerobaculum thermoterrenum
- Ancylobacter aquaticus
- Anthamnon sp.
- Azoarcus sp.
- Bdellovibrio stolpii
- Bordetella bronchiseptica
- Buchnera aphidicola
- Caldicellulosiruptor saccharolyticus
- Capnocytophaga canimorsus
- Clavibacter michiganense
- Corynebacterium sp.
- Cytophaga sp.
- Desulfoarculus sp.
- Desulfohalobium retbaense
- Desulfuromonas acetoxidans
- Empedobacter brevis
- Erythrobacter longus
- Eubacterium sp.
- Flectobacillus major
- Fusibacter paucivorans
- Gordonia sp.
- Haemophilus influenzae
- Halomonas elongata
- Herpetosiphon aurantiacus
- Kurthia zopfii
- Leptospira sp.
- Leucobacter komagatae
- Mesoplasma sp.
- Methylocystis sp.
- Methylosinus sp.
- Microcystis sp.
- Mobiluncus curtisii
- Mycoplasma sp.
- Nitrospira sp.
- Oxalobacter formigenes
- Palmaria palmata
- Pectinatus cerevisiiphilus
- Phlomobacter fragariae
- Planococcus kocurii
- Propioniferax innocua
- Psychroserpens burtonensis
- Rhodoferrax antarcticus
- Rhodospirillum rubrum
- Salinicoccus roseus
- Sphingomonas sp.
- Spiroplasma sp.
- Streptobacillus moniliformis
- Synechococcus elongatus
- Thauera sp.
- Thermonema lapsum
- Treponema sp.
- Vitreoscilla stercoraria
- Xylophilus ampelinus
- Acholeplasma
- Actinobaculum
- Aeromicrobium
- Alcaligenes
- Amphibacterium
- Anaerofilum
- Aneurinibaculum
- Aranicola
- Bacillus sp.
- Bifidobacterium
- Brevibacterium
- Burkholderia
- Caldicellulosiruptor
- Chloroflexus
- Clostridium
- Cryptomonas
- Dechloromonas
- Desulfobacterium
- Desulfotomaculum
- Dietzia sp.
- Entomoplasma
- Erythromonas
- Exiguobacterium
- Flexibacterium
- Fusobacterium
- Haloanaerobium
- Haliscometia
- Heliobacterium
- Hongia korarchaei
- Lactobacillus
- Leptospira
- Mannheimia
- Methylophilus
- Methylobaculum
- Microbacterium
- Microscilla
- Moritella
- Neisseria
- Nostoc sp.
- Oxalophagus
- Paracoccus
- Pedobacterium
- Phytoplasma
- Porphyrobacter
- Propionigenium
- Quinella
- Rhodococcus
- Rubrobacterium
- Selenomonas
- Spirochaeta
- Sporohalobium
- Streptococcus
- Taylorella
- Thermoactinomyces
- Thiobacillus
- Ureaplasma
- Xanthomonas
- Zoogloea

Control

The control group of animals who were not dosed with an ionophore also showed some fluctuation of microbial diversity (Table 3) where, bacterial numbers of *Bacillus* sp., *Clostridium* sp., *Mycoplasma* sp., *Prevotella* sp., and *Streptomyces* sp. fluctuated. See Figure 3.

	BEFORE	DURING	AFTER
Bacterial Type	Percentage	Percentage	Percentage
Gram+	22.54	28.15	36.43
Gram-	77.46	71.85	63.57

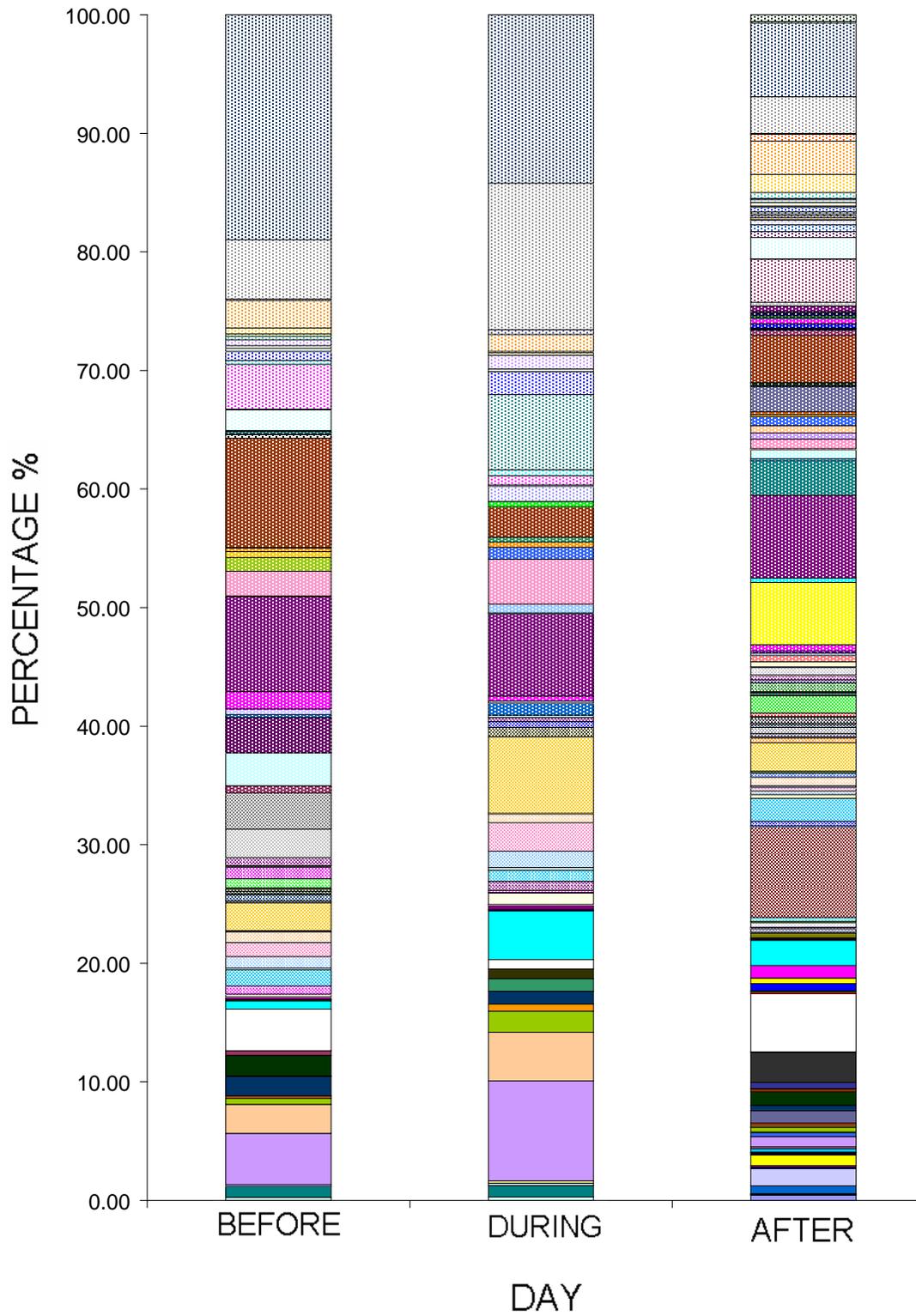


Figure 3. Bacterial fluctuations in control animals. The legend corresponding to each color is given below. The percentages of each organism were calculated based on peak area.

- Abiotrophia sp.
- Acidiphilium multivorum
- Afipia sp.
- Anaerobaculum thermoterrenum
- Ancylobacter aquaticus
- Arthrobacter sp.
- Beijerinckia indica
- Bradyrhizobium sp.
- Butyrivibrio sp.
- Chlorella sp.
- Closterium ehrenbergii
- Cryptomonas sp.
- Dechlorisoma suilla
- Desulfobacter sp.
- Desulfosarcina variabilis
- Desulfuromonas acetoxidans
- Enterococcus sp.
- Erysipelothrix rhusiopathiae
- Eubacterium sp.
- Flectobacillus sp.
- Fusobacterium sp.
- Hafnia alvei
- Halobacteroides sp.
- Herbaspirillum sp.
- Kitasatospora cystarginea
- Leptospirillum sp.
- Mannheimia granulomatosa
- Methyloarcula marina
- Methylococcus sp.
- Methylophilus methylotrophus
- Microscilla sp.
- Myxococcus sp.
- Nostoc sp.
- Paenibacillus sp.
- Pectinatus cerevisiiphilus
- Photobacterium sp.
- Planococcus sp.
- Propionigenium modestum
- Pseudomonas sp.
- Rhodobacter sp.
- Rhodospirillum sp.
- Ruminococcus flavifaciens
- Selenomonas sp.
- Sphingomonas sp.
- Sporobacter termitidis
- Staphylococcus sp.
- Sulfobacillus disulfidooxidans
- Thermoanaerobacterium
- Treponema sp.
- Weeksella virosa
- Zoogloea sp.
- Acetobacter sp.
- Acidosphaera rubrifaciens
- Agrobacterium vitis
- Anaerobranca horikoshii
- Angiococcus disciformis
- Azoarcus sp.
- Bergeyella zoohelcum
- Brevibacillus sp.
- Caldicellulosiruptor saccharolyticus
- Chlorobium sp.
- Clostridium sp.
- Cyanidium caldarium
- Dermatophilus sp.
- Desulfotulbus sp.
- Desulfotomaculum nigrificans
- Dietzia sp.
- Entomoplasma sp.
- Erythrobacter longus
- Exiguobacterium sp.
- Flexibacter sp.
- Geobacter sp.
- Haliscomenobacter hydrossis
- Halomonas sp.
- Herpetosiphon aurantiacus
- Klebsormidium flaccidum
- Leptothrix sp.
- Marchantia polymorpha
- Methylobacillus flagellatum
- Methylocystis sp.
- Methylosinus sp.
- Moritella sp.
- Neisseria sp.
- Oerskovia sp.
- Pantoea sp.
- Pedobacter heparinus
- Phytoplasma sp.
- Porphyrobacter sp.
- Proteus vulgaris
- Pseudoramibacter alactolyticus
- Rhodococcus sp.
- Rhodothermus marinus.
- Saccharothrix australiensis
- Shewanella sp.
- Spirochaeta sp.
- Sporohalobacter lortetii
- Streptobacillus sp.
- Telluria chitinolytica
- Thermus thermophilus
- Ureaplasma urealyticum
- Xanthomonas campestris
- Achleplasma laidlawii
- Acidovorax sp.
- Alcaligenes sp.
- Anaerofilum sp.
- Aranicola proteolyticus
- Bacillus sp.
- Bifidobacterium sp.
- Buchnera aphidicola.
- Capnocytophaga sp.
- Chlorogloeopsis sp.
- Comamonas sp.
- Cyanophora paradoxa
- Desulfotobacterium dehalogenans
- Desulfotulobium retbaense
- Desulfotomaculum sp.
- Eikenella sp.
- Epifagus virginiana
- Erythromonas ursincola
- Fibrobacter succinogenes
- Frankia sp.
- Glaciecola punicea
- Haloanaerobacter sp.
- Heliobacterium sp.
- Hongia koreensis
- Kurthia sp.
- Leucobacter komagatae.
- Melittangium lichenicola
- Methylobacter sp.
- Methylococcoides sp.
- Microbacterium sp.
- Mycobacterium sp.
- Nitrobacter winogradskyi
- Oxalobacter formigenes
- Paracoccus sp.
- Peptostreptococcus sp.
- Pirellula sp.
- Porphyromonas levii
- Pseudoalteromonas sp.
- Psychroserpens burtonensis
- Rhodomicrobium vannielii
- Roseobacter sp.
- Salinivibrio vallis mortis
- Shigella sp.
- Spirogyra maxima
- Sporomusa paucivorans
- Streptococcus sp.
- Thauera sp.
- Thiobacillus aquaesulis
- Vibrio sp.
- Xylella fastidiosa

Overall the use of terminal restriction fragment length polymorphism and pylogenetic analysis appears to be good tools to monitor shifts in microbial communities in the rumen. Ionophore supplementation alters rumen microbial diversity greatly and these changes impact animal performance. The two most prevalent bacteria in terms of contribution to total detectable microbes during ionophore feed was *Prevotella* sp. and *Bacteroides* sp. *Prevotella* sp. which are mostly obligatory anaerobic, non-spore-forming, nonmotile, gram-negative, rod-shaped bacteria have the ability to ferment carbohydrates and produce succinic and acetic acids (Moore, et al. 1994). *Bacteroides* sp. produce several exoenzymes such as collagenase, neuraminidase, and DNase. These anaerobic organisms are known to produce butyrate and acetate, and provide about 70% of the energy supply of the colonic enterocytes (<http://www.anaesthetist.com/icu/infect/bacteria/anaerobe/bfrag.htm>). They are also known to ferment carbohydrates, utilize nitrogenous substances, and biotransformation of bile acids and other steroids

(http://biology.kenyon.edu/Microbial_Biorealm/bacteria/bacteroidete_chlorob_group/bacteroides/bacteroides.htm). Additionally, it is interesting to note that while Gram + bacteria did decrease in numbers they still contributed to the total population during ionophore supplementation, suggesting that these particular Gram + bacteria are unaffected by these ionophores or have a mechanism(s) to adapt to the effects of the ionophore, in steers grazing wheat pasture.

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