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

S.C. Fernando, H.T. Purvis II, G.W. Horn, B.G. Feiser and U. DeSilva

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 micoflora. Steers were randomly assigned to three treatments (n = 4/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 adlibitum 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

† DURING – During dosing of Monensin
‡AFTER - After cessation of Monensin dosing

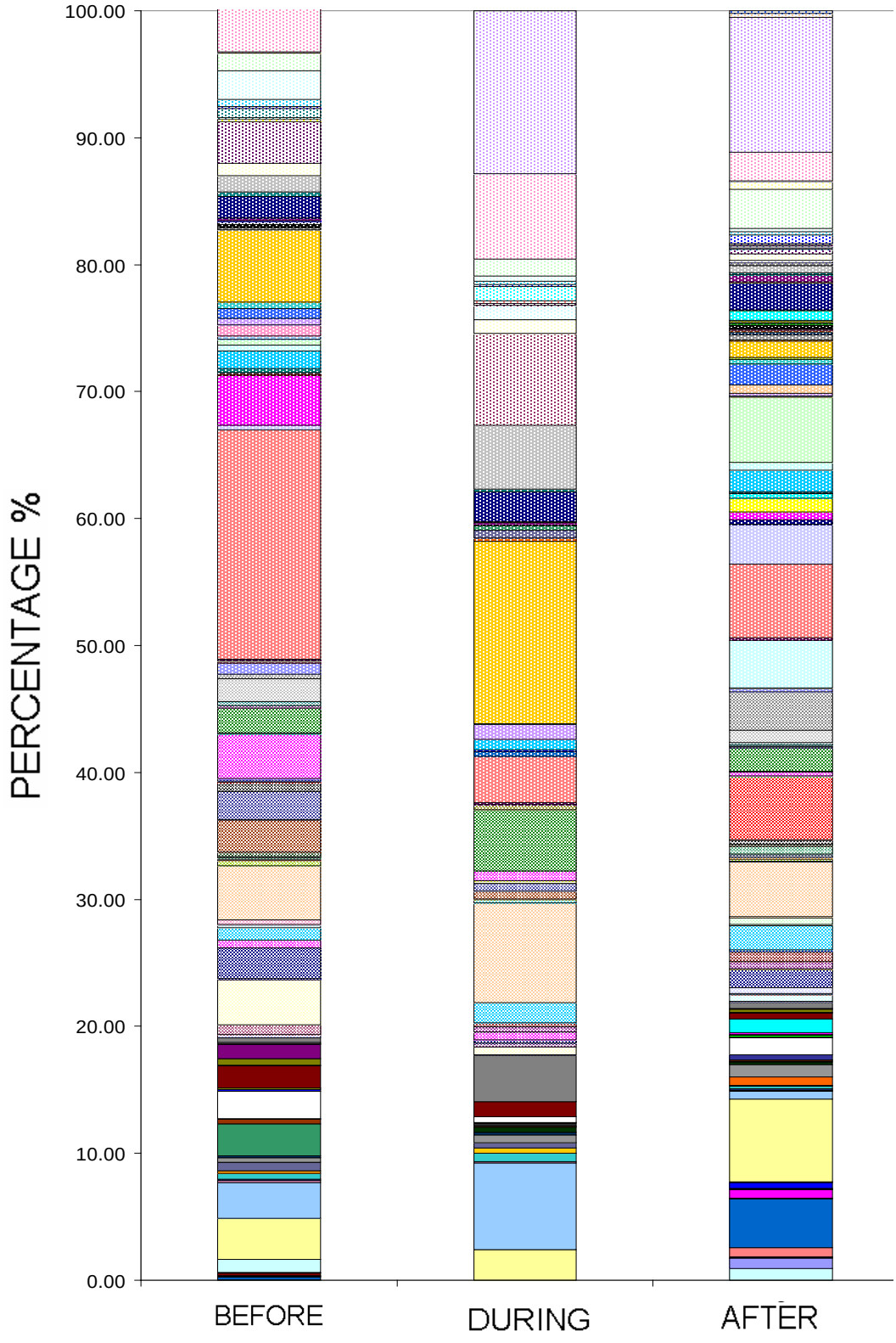
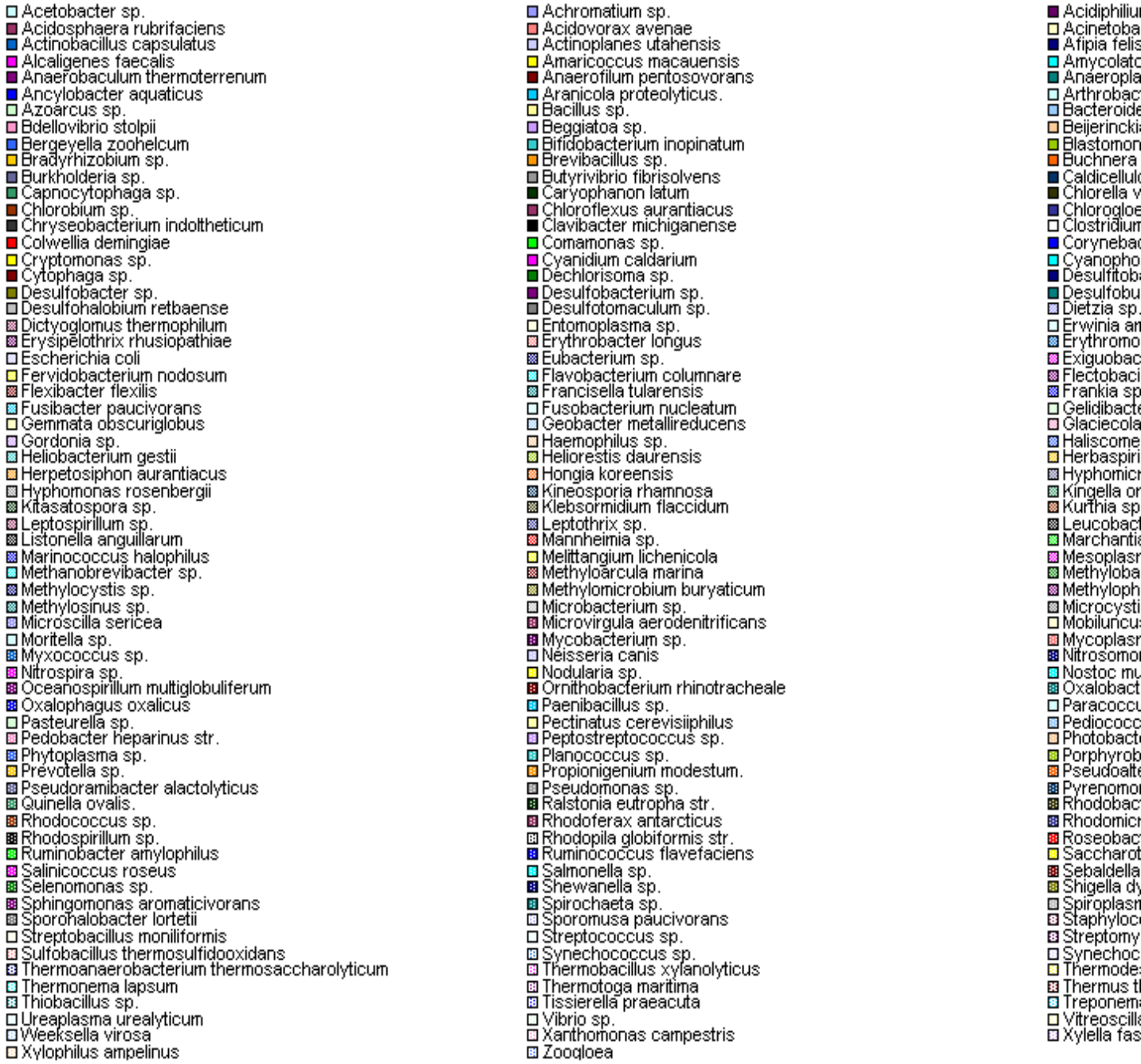


Figure 1. Bacterial fluctuations during monensin treatment. The legend corresponding to each color is given below. The percentages of each organism were calculated based on peak area.



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., *Clostredium* 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.).

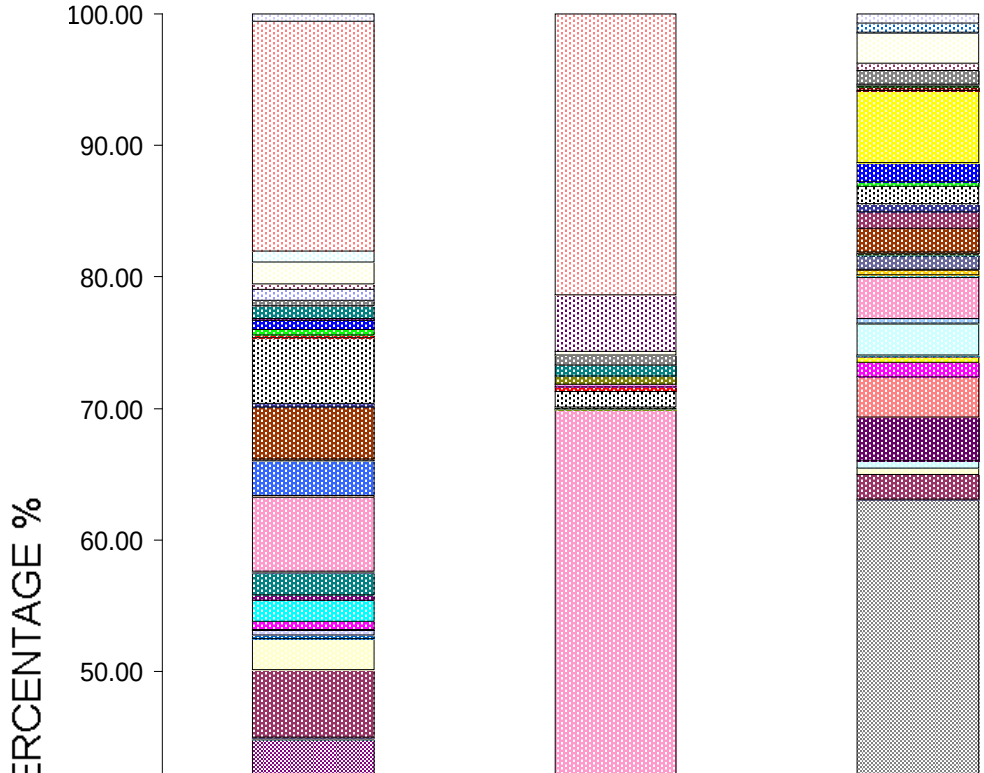
Table 2. Percentage of Gram + and Gram – Bacteria as Impacted by Lasalocid Dosed in the Rumen of Beef Steers Grazing Wheat Pasture.

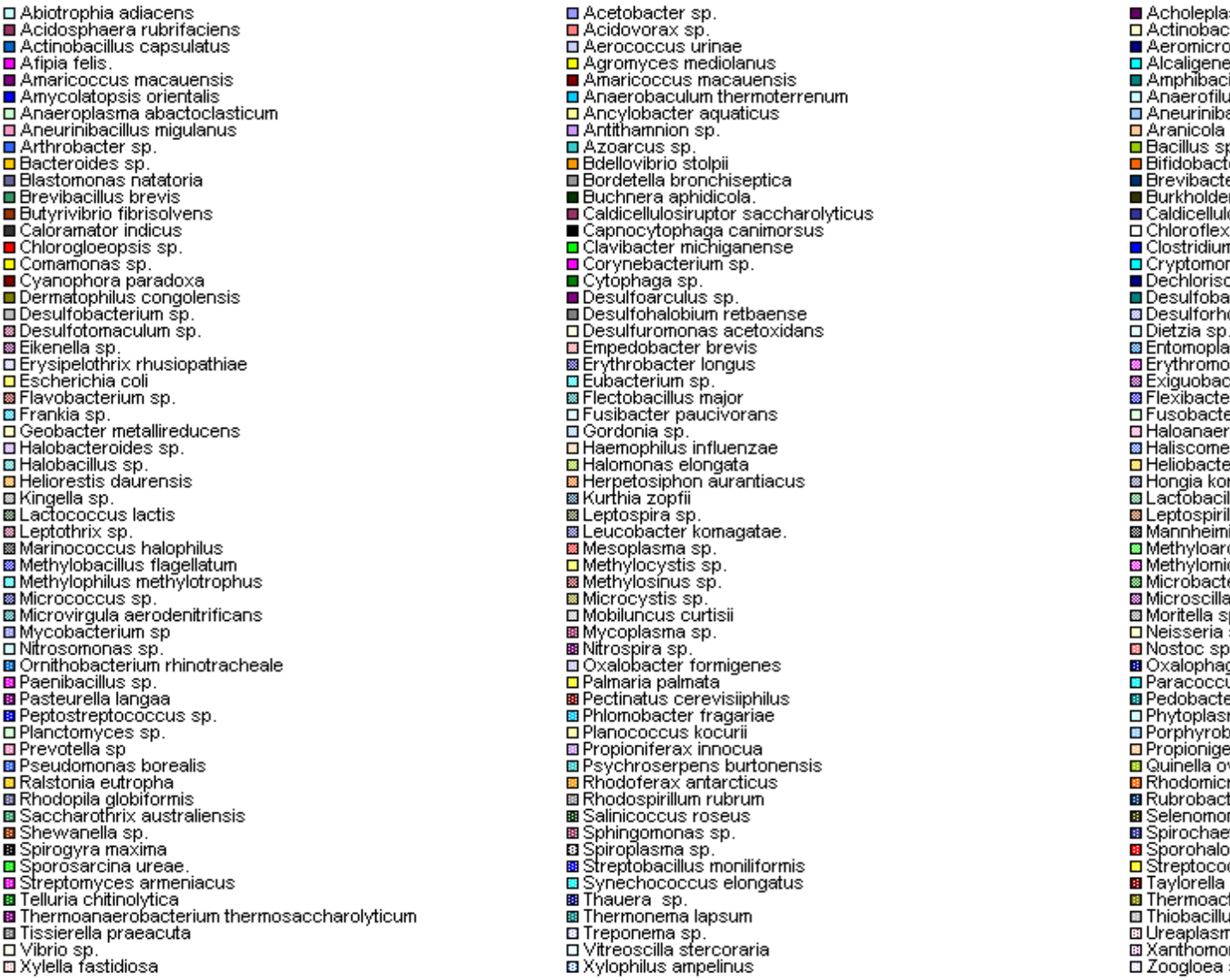
	BEFORE*	DURING†	AFTER‡
Bacterial Type	Percentage	Percentage	Percentage
Gram+	30.48	25.22	25.72
Gram-	69.52	74.78	74.28

* BEFORE – Before dosing with Lasalocid

† DURING – During dosing of Lasalocid

‡ AFTER - After cessation of Lasalocid dosing





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., *Clostredium* sp., *Mycoplasma* sp., *Prevotella* sp., and *Streptomyces* sp. fluctuated. See Figure 3.

Table 3. Percentage of Gram + and Gram – Bacteria for Non-Dosed Beef Steers Grazing Wheat Pasture.

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

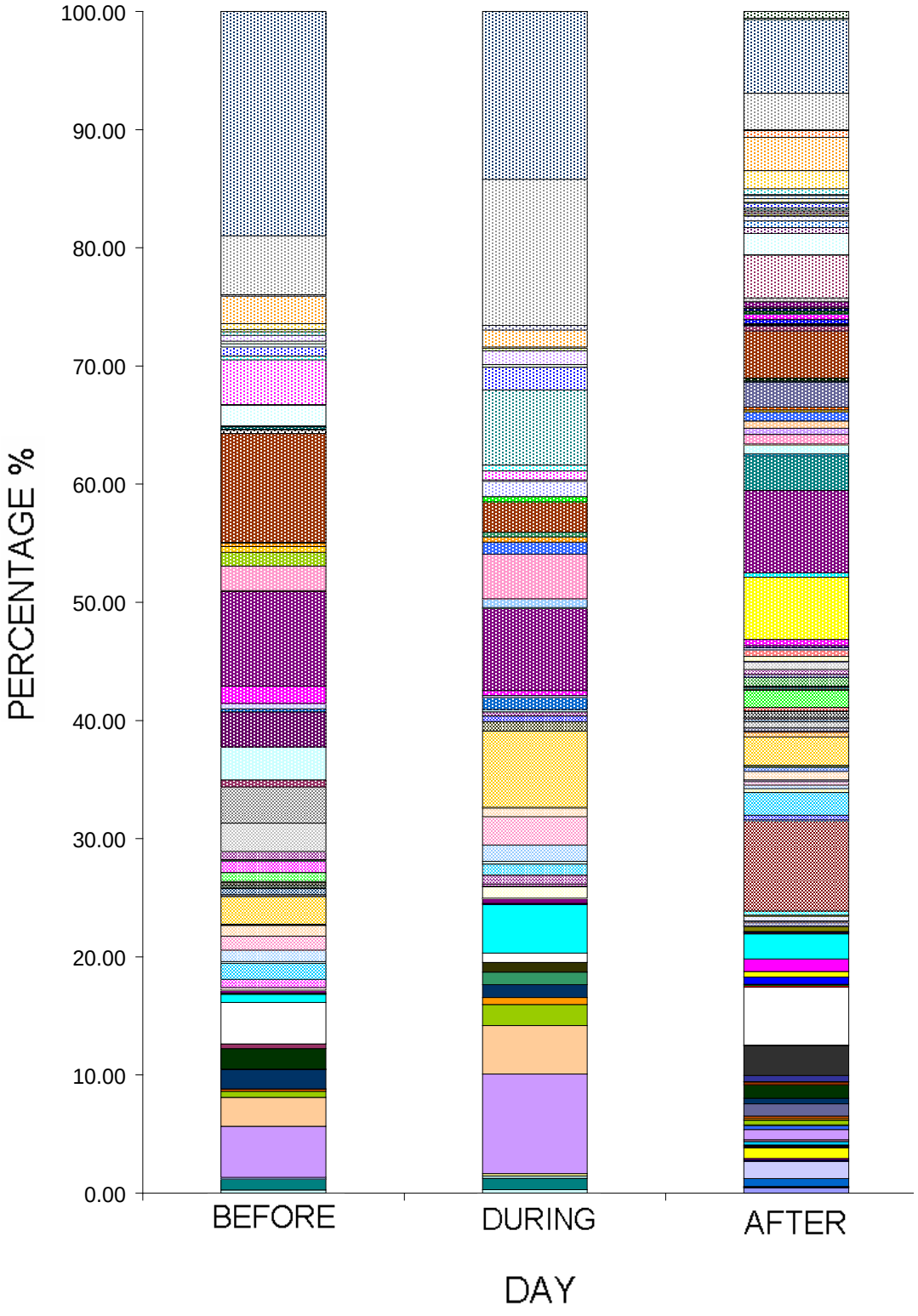


Figure 3. Bacterial fluctuations in control animals. The legend corresponding to each color is given below. The percentages of each organism were calculated based on peak area.

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.

Literature Cited

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