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Rumen microbial diversity of Bonsmara cattle using amplicon sequencing during a 120-day growth trial

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Abstract

Improved understanding of the microbial populations during intensive feeding of feedlot cattle holds potential for optimizing production efficiency. Ionophores are used to increase the production and efficiency of ruminants and are commonly used in South African feedlots. Bonsmara bull calves (n=24) were subject to a four-phase feedlot diet in a growth trial commencing with backgrounding, followed by starter, grower and finisher diets. Animals were randomly divided into two groups: control and a group provided the in-feed ionophore monensin. Four animals from each group were randomly selected for rumen content collection using an oesophageal tube during the phases in the trial. Samples were analysed using 16S rRNA and internal transcribed spacers amplicon sequencing. Totals of 42 008 and 35 442 amplicon sequence variants were identified from 16S rRNA and internal transcribed spacers amplicon sequencing. The rumen microbiome composition and alpha diversity differed significantly between the phases, whereas no significant difference was observed between the control and monensin groups. Backgrounding had the highest bacterial richness, whereas the grower phase had the highest fungal richness. Bacteroidetes, Firmicutes and Proteobacteria were the most abundant phyla, with Bacteroidetes being most abundant in the backgrounding and starter phases, whereas Proteobacteria was the most abundant in the grower and finisher phases. Ascomycota, Basidiomycota and Neocallistigomycota were the most abundant fungal phyla. Improved knowledge of the shift in microbiome population during the growth period could assist in adapting feeding strategies to improve the efficiency of beef production.

Key words: feedlot, microbial shift, rumen microbiome, ruminant
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Introduction

Beef producers are presented with the challenge of increasing the supply of high-quality beef, while maintaining an economic and environmentally sustainable enterprise. A potential solution is to increase the feed efficiency (Capper, 2011). South African beef production is characterized by medium to large extensive commercial cow-calf operations with 65% to 70% of all cattle that are slaughtered having originated from feedlot systems (DAFF, 2019). Several factors play a role in economic beef production, such as the feeding regime, price of weaners, and general health and management of the animals. Overall, feed efficiency is the determining factor for sustainable feedlot production (Koenig *et al.*, 2020).

The feed efficiency is determined by various factors, including the rumen microbiome (Guan *et al.*, 2008; Myer *et al.*, 2015). Microbes (Firkins & Yu, 2015) are responsible for fermentation and degradation of the feed components into nutrients such as volatile fatty acids (VFAs) that provide approximately 70% of the energy available to the animal for its maintenance and production (Perea *et al.*, 2017). The composition and balance of the rumen microbiome determines the concentration of the VFAs and thus greatly influences the energy that is available for the animal to metabolise.

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In South Africa, the efficiency and production of feedlot animals are increased by adding ionophores, such as monensin, to the diet and thereby changing the rumen microbiome by inhibiting gram-positive bacteria and methanogens and shifting in VFA production in favour of propionate (Samuelson *et al.*, 2016). Propionate is glucogenic, thus providing more energy to the animal and thus increasing its efficiency.

Transitioning from a roughage-based diet to a concentrate-based diet has been reported to modify the rumen microbiome (Fernando *et al*., 2010; Stanton *et al*., 2020), with a sudden transition resulting in digestive disorders (Klieve *et al.*, 2003). A stepwise adaptation from a roughage diet to a high energy one is known to stabilize the rumen microbiome (Klieve *et al.*, 2003; Bevans *et al.*, 2005). These feeding regimes are characteristic of feedlot feeding and therefore necessitate improved understanding of the dynamics of the rumen microbiome (Mackie *et al.*, 1978; Tajima *et al.*, 2001). Improving feed efficiency in the livestock sector is crucial to sustainable animal production as it could improve nutrient utilization from feed, increase profitability, and reduce greenhouse gas emissions (Huws *et al.*, 2018).

Modern sequencing-based methods, such as 16S rRNA and internal transcribed spacer (ITS) amplicon sequencing, have improved the detection and quantification of microbes in the rumen. Previous methods such as culturing could not capture the full diversity of the rumen microbiome (Huws *et al.*, 2018; Gruninger *et al.*, 2019). These sequencing techniques can be used to conduct research on the total microbial diversity and microbial population function (Myer, 2019) and could lead to improved understanding of the interaction between the diet, the rumen microbiome, and efficiency of production (Pitta *et al.*, 2018).

In this study, it was hypothesized that the bacterial, archaeal and fungal populations in the rumen of Bonsmara cattle will differ across the phases in the feedlot period and in response to the feeding of monensin.

Materials and Methods

The Animal Ethics Committee of the University of Pretoria granted approval for the project (NAS445/2019). The trial was conducted on Sernick Group (Pty) LTD Farm, Edenvale, Free State, South Africa. Twenty-four Bonsmara bull calves (10–12 months old, 228 ± 22 kg) from a single Bonsmara breeder were backgrounded for 40 days on veldt grazing with lick supplementation before the start of the growth period. The animals were divided into two treatment groups: a group receiving a standard feedlot diet that included 30 mg/animal/day of monensin (n=12); and a control group that was fed the same diet without monensin (n=12). The diets were mixed at Sernick feed mill, marked and bagged for the trial.

The animals were allocated in a randomized block design according to weight, three to a pen, with eight pens in total for the growth study, which consisted of starter, grower, and finisher phases. The composition of the diets is presented in Table 1. All animals received a Revalor S (Intervet GesmbH, Austria) hormone implant at the beginning of the starter phase as per standard feedlot operations in South Africa.

Table 1 Composition of the diets (as fed in kg/day and % of diet) for the postweaning feeding of Bonsmara calves and their predicted daily feed intake

Ingredients	Starter		Grower		Finisher	
	kg/d	% of diet	kg/d	% of diet	kg/d	% of diet
Wheat straw (5% crude protein)	1.10	17.2	1.40	13.9	1.60	11.9
Yellow maize	1.80	28.1	3.12	31.0	4.36	32.3
Hominy chop	1.80	28.1	3.12	31.0	4.36	32.3
Salt	0.02	0.3	0.03	0.3	0.04	0.3
Urea	0.06	0.9	0.10	1.0	0.15	1.1
Limestone	0.10	1.6	0.11	1.1	0.14	1.0
Sunflower oilcake	0.26	4.1	0.15	1.5	0.00	0.0
Molasses	0.51	8.0	0.81	8.1	1.08	8.0
Wheat bran	0.70	11.0	1.01	10.0	1.35	10.0
Feedlot vitamin/mineral pre-mix	0.01	0.2	0.01	0.1	0.01	0.1
Megalac (rumen bypass fat)	0.03	0.5	0.20	2.0	0.41	3.0
Predicted feed intake (kg/d)	6.39		10.06		13.50	

Animals received the starter diet for 21 days, followed by the grower (fed for 80 days) and the finisher (fed for 14 days) diet. Three days was used for adaptation between phases by decreasing the percentage of the former diet and increasing the percentage of the new diet until the animals received only the diet of the new phase. Animals were given ad libitum access to feed and water.

Eight animals, one from each pen, were randomly selected at the start of the trial to collect rumen in the various phases (32 samples). The same animals were used for rumen collection throughout the trial. A trained veterinarian inserted a flexible plastic oesophageal tube into the rumen through the mouth to the ventral sac of the rumen to collect rumen. The microbial community composition of samples collected via oesophageal tube with fluid and solid particles was comparable with those collected via rumen fistula (Paz *et al.*, 2016). Care was taken to ensure fluid and solid particles were present in the samples. The first 50 ml of rumen content was discarded because of potential saliva contamination. A further 50 ml was collected in a sterilized 50 ml plastic container. Immediately after collection the pH was measured using a portable pH meter (EcoSense pH100A, YSI Environmental, USA) and the sample was frozen in liquid nitrogen and stored at -80 ºC until DNA extraction. Owing to technical problems with the portable pH meter, the pH readings of the backgrounding phase had to be discarded.

After thawing, the samples (300 mg) were homogenized using a BeadBug microtube homogenizer (Benchmark Scientific, USA) for approximately 12 mins at maximum speed (400 x 10 rpm) and the DNA was extracted with a QIAamp PowerFecal Pro DNA extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. After extraction, DNA concentration and purity (A260/A280) were quantified with a Qubit fluorometer v2 (Invitrogen, USA) and a Nanodrop ND-1000 spectrophotometer (Thermo Scientific, USA).

Thirty-two DNA samples were shipped to Novogene (NovogeneAIT, Singapore) for pair-ended (250 x 250 bp) sequencing with an Illumina NovaSeq 250 (Illumina, San Diego, California, USA). Sequencing targeted the V3-V4 hypervariable region of the 16S rRNA gene and the 5F region of ITS1. Data were received from Novogene Singapore with primers removed with an average number of reads per sample of 198 775 and 194 585 for 16S rRNA and ITS sequencing. Sequence data was deposited into the NCBI Sequence Read Archive under accession number PRJNA721531.

Microbiome analysis was performed with R software v z4.0.2 (R Core Team, 2013). Processing and analysis of reads were performed using DADA2 v1.16.0 (Callahan *et al.*, 2016) including read filtering, dereplications, sample inference, chimera removal, merging of paired-end reads, and taxonomic classification. Reads were trimmed at 220 base pairs for both the forward and reverse reads of the *16S rRNA* generated reads, resulting in 186 933 reads remaining after trimming. Taxonomy was assigned to genus level with RDP database (Cole *et al.*, 2014) for 16S rRNA and UNITE database (Nilsson *et al.*, 2019) for ITS. The ape package v5.4.1 (Paradis & Schliep, 2019) was used to construct a phylogenetic tree. The amplicon sequence variant (ASV) table, taxonomy table, phylogenetic tree and sample data were combined to construct a phyloseq object using phyloseq v1.32.0 (McMurdie & Holmes, 2013). The microbiome package v1.10.0 (Lahti *et al.*, 2017) was used to generate figures.

Low abundance ASVs (detected at least 10 times in 5% of the samples) were removed for downstream analysis. Reads were rarefied to minimum sampling depth for normalization. The numbers of remaining reads were reported as average and standard deviation. Weighed and unweighted UniFrac distances were used to perform a principle coordinate analysis (PCoA) for ordination analysis to visualize differences between the phases. Beta diversity was determined with Adonis, betadisper and permutest functions in vegan v2.5.6 (Oksanen *et al.*, 2020). This linear model was used to test for beta diversity with these functions: unifrac.dist ~ phase + animal + group. These same fixed effects (phase, animal, group) were tested for significant influence on alpha diversity and the relative abundance of the microbes with an analysis of variance. Animal and group were found not to have a significant effect and were not included in further tests. Three alpha diversity indices were calculated using phyloseq, namely the observed number of ASVs, Chao1 richness estimator, and Shannon diversity index. Kruskal-Wallis and Wilcoxon rank sum tests were used to determine statistical significance for the relative abundance of the taxa and alpha diversity. Analysis was corrected for multiple testing with Bonferroni correction. For all statistical tests, results were considered significant at $P < 0.05$ and trends were recognized at $P < 0.1$.

Results and Discussion

Following quality control, chimera detection and removal, the samples had an average read count of 116 943 ± 19 832 for the 16S rRNA and 149 447 ± 15 014 for the ITS sequencing. From the sequences, 42 008 and 35 442 amplicon sequence variants (ASVs) were detected for 16S rRNA and ITS sequencing.

There was no difference in the rumen microbiome composition between the control and monensin groups ($P > 0.05$), whereas a difference ($P = 0.001$) was observed between the four phases for the 16S rRNA and ITS rumen populations.

In Table 2, alpha diversity is shown based on the observed number of ASVs, Shannon and Chao1 indices. The diversity in the samples was higher for the backgrounding phase, with a consequent decrease up to the finisher phase for the bacterial and archaea population. Most ASVs for the fungi population were observed in the grower period, with the least in the finisher phase. All alpha diversity indices were significantly different across the four phases, with no significant difference between the groups or animals.

Table 2 Average and standard deviation of the alpha diversity indexes of rumen bacterial, archaeal, and fungal communities across phases in the feedlot period

Phase	Bacterial/Archaeal			Fungal		
	Observed	Shannon	Chao1	Observed	Shannon	Chao1
Backgrounding	1547±59[a]	6.51±0.11[a]	1554±58[a]	214±37[a]	3.76±0.33[a]	215±37[a]
Starter	1322±141[b]	5.89±0.30[b]	1331±142[b]	271±28[b]	3.58±0.27[b]	273±27[b]
Grower	923±125[c]	4.15±0.39[c]	935±126[c]	301±29[c]	3.93±0.51[c]	302±29[c]
Finisher	662±37[d]	3.34±0.47[d]	667±38[d]	162±18[d]	3.23±0.16[d]	162±18[d]

[*]Observed number of amplicon sequence variants, Shannon diversity index and Chao1 richness index
[a,b,c,d] Different superscripts within a column indicate significant difference at P <0.0

There was a difference in beta diversity between the phases (P = 0.001, R^2 = 0.601), but not between the animals or groups (P = 0.395, R^2 = 0.301). The backgrounding and starter phases clustered separately (Figure 1), whereas the grower and the finisher phases formed a larger dispersed cluster. The first principle coordinate explained 53.3% of the variation in the diversity of bacteria and archaea whereas the second principle coordinate only explained 7.1%.

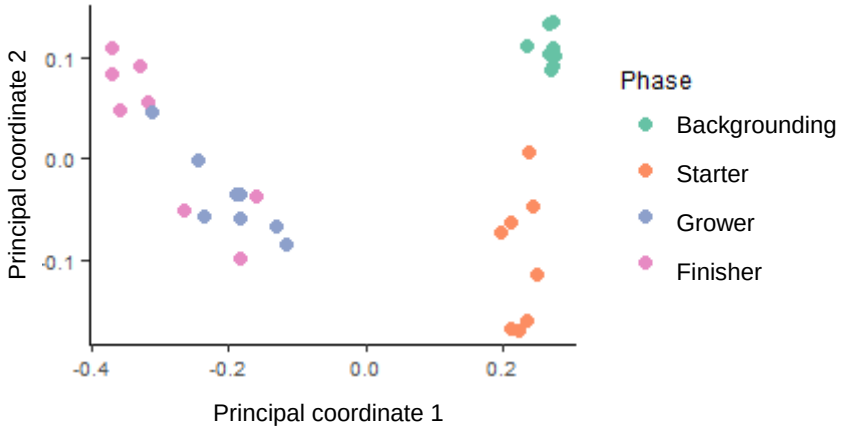

Figure 1 Principal coordinate analysis using weighed UniFrac distances to indicate beta diversity of phases in the feedlot period for bacteria and archaea

In Figure 2, a PCoA using weighed UniFrac distance of the fungal population of the phases can be observed to cluster separately. The groups (monensin and control) did not cluster separately within the phases. The first principle coordinate explained 32.6% of the variation in the rumen fungi whereas the second principle coordinate explained 20.1%.

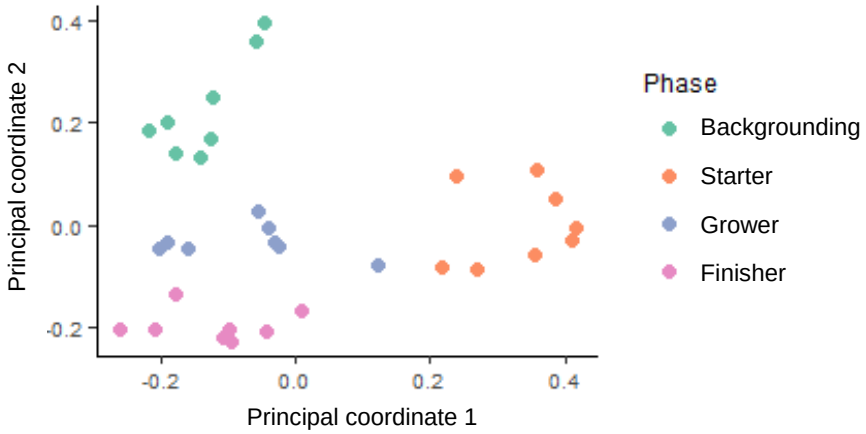

Figure 2 Principal coordinate analysis using weighed UniFrac distances to depict beta diversity between phases in the feedlot period for the rumen fungi

There was a difference in microbial composition between the phases (P =0.001, R^2 =0.568), but not between the animals or groups (P =0.221, R^2 =0.123) in the fungal population. There was also a difference in dispersion between the phases (P =0.002). The significance tests based on weighed UniFrac distances for the homogeneity of multivariate dispersions are reported in Table 3. There was a significant difference in the microbial composition between the backgrounding and starter phases in the 16S rRNA and between the starter and grower phases as shown in the ordination plot (Figure 1) and confirmed by the permutation test. As seen in Figure 2, the rumen fungi population present during the backgrounding phase clustered separately from the starter phase, showing a significance difference between the communities of these two phases (Table 3).

Table 3 Weighed UniFrac distance-based *P*-values for tests for homogeneity of multivariate dispersions for rumen microbial communities of the animals in the phases

Phases	Bacterial/Archaeal	Fungal
Backgrounding – starter	0.003	0.001
Starter – grower	0.046	0.946
Grower – finisher	0.902	0.763

The abundance of the phyla (in percentage) and significance of the various factors are depicted in Table 4. Most phyla did not differ (P >0.05) between the groups or between the animals. Overall, the most abundant phylum in the rumen microbiome was Bacteroidetes (56%), followed by Firmicutes (29%) and Proteobacteria (5%) (Figure 3). At phylum level, 2% of the microbes were not characterized. Of the ASVs identified in the rumen, 0.7% were archaea from the Euryarchaeota phylum. The relative abundance of most phyla did not differ (P >0.05) between the groups or between the animals.

Table 4 Relative abundance of bacterial, archaeal and fungal phyla (in percentage) and significance of differences among phases and for the contrasts of the backgrounding versus starter, starter versus grower and grower versus finisher phases

Phylum	Backgrounding	Starter	Grower	Finisher	*P*-values			
16S rRNA (bacterial & archaeal)					Phase	B vs S	S vs G	G vs F
Euryarchaeota	2.9	2.0	0.6	0.9	0.0060	0.7210	0.0030	0.9590
Actinobacteria	2.2	1.4	0.8	0.2	0.0004	0.1050	0.0280	0.0100
Bacteroidetes	62.4	62	26.2	26.6	<0.0001	0.0150	0.0002	0.7210
Elusimicrobia	0.3	0.1	0.0	0.1	0.0007	0.0030	0.0520	0.0010
Fibrobacteres	2.9	1.8	0.7	0.2	0.0006	0.1610	0.1560	0.1280
Firmicutes	27.3	26.8	26.7	14	0.0070	0.5050	1.0000	0.0030
Proteobacteria	2.3	5.3	0.44	57.8	<0.0001	0.0100	0.0002	0.0490
SR1	0.8	0.0	0.0	0.0	<0.0001	0.0009	0.0008	0.0760
Tenericutes	0.4	0.1	0.0	0.0	<0.00001	0.0020	0.0160	0.0020
ITS (Fungal)								
Ascomycota	37.2	74.5	89.4	49.8	0.0060	0.0150	0.7210	0.0210
Basidiomycota	0.6	5.7	1.3	0.9	0.0002	0.0002	0.0002	0.1950
Mucoromycota	0.7	0.4	2.2	1.2	0.4070	0.7980	0.0002	0.0380
Neocallimastigomycota	58.3	18.6	6.5	47.6	<0.0001	0.0002	0.0100	0.0003
Anthophyta	3.1	0.6	0.3	0.5	0.0590	0.0130	0.0150	0.0590

B vs S: backgrounding versus starter, S vs G: starter versus grower, G vs F: grower versus finisher

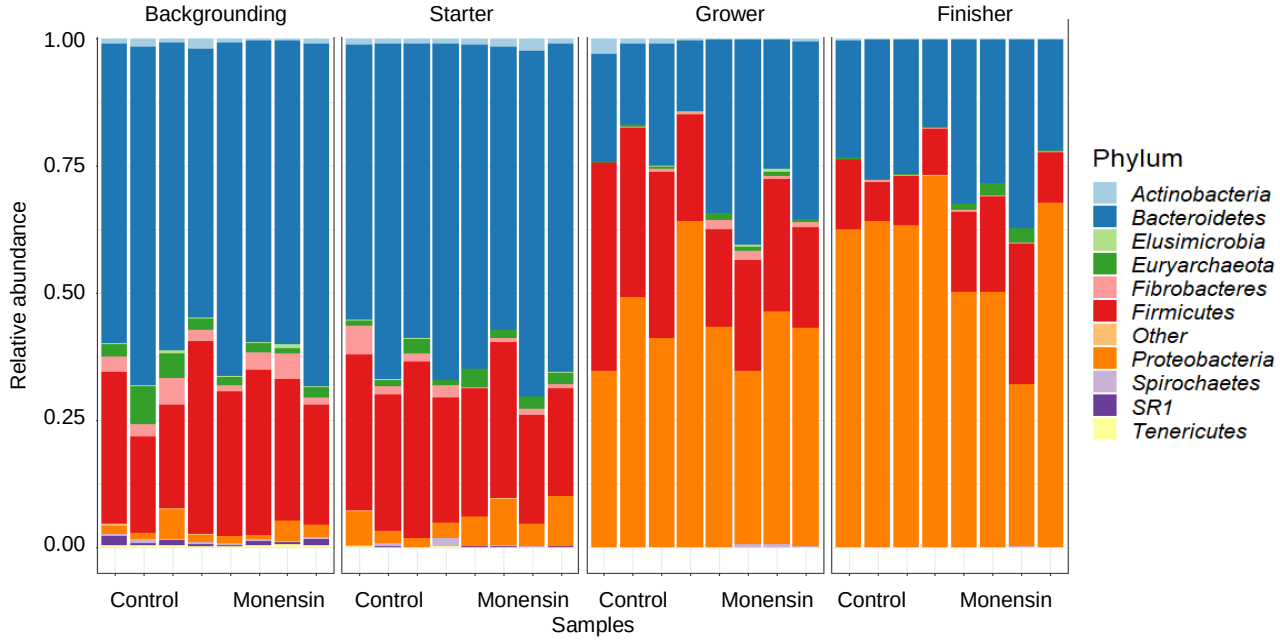

Figure 3 Relative abundance of phyla during the four phases of feeding the control and monensin groups

At family level, Prevotellaceae from the Bacteroidetes phylum and Ruminococcaceae from the Firmicutes phylum were the most abundant overall, with Prevotellaceae more abundant in the starter phase, whereas Ruminococcaceae was more abundant in the grower phase (Figure 4). Porphyromonadaceae, Lachnospiraceae, Sphingobacteriaceae, Fibrobacteraceae and Methanobacteriaceae were also present.

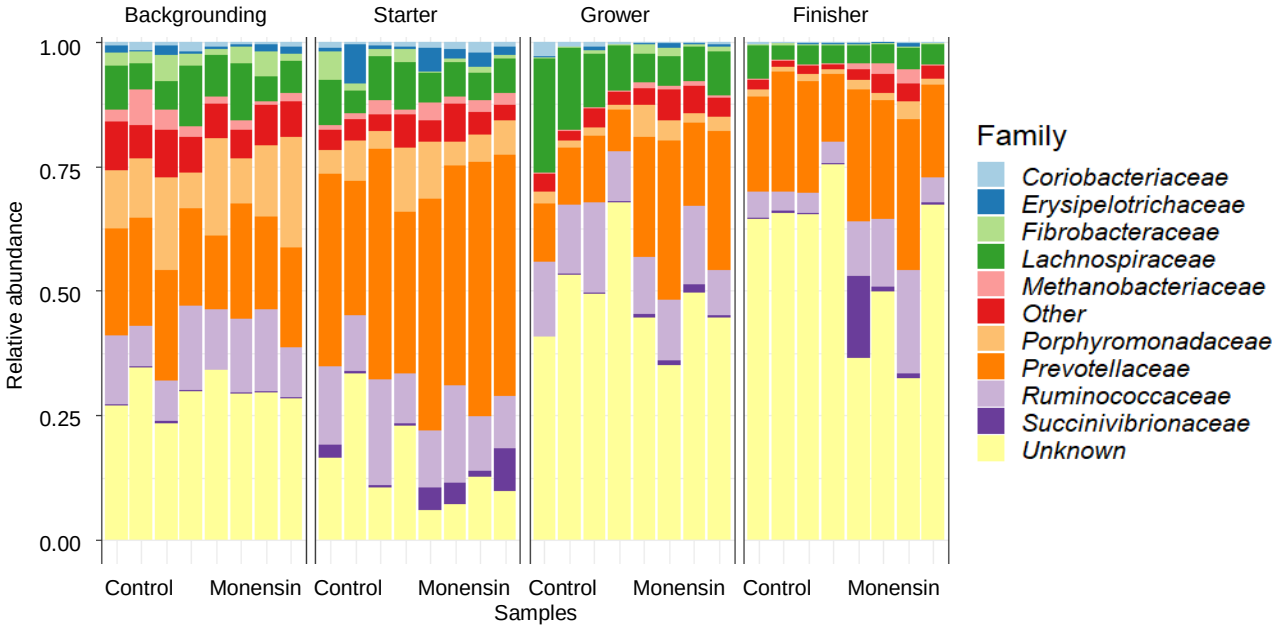

Figure 4 Relative abundance of bacterial families during the four phases of the feedlot in the control and the monensin groups

For the fungi population, Ascomycota, Neocallistigomycota and Basidiomycota were the most abundant (Figure 5, Table 4). Ascomycota was more abundant in the grower period, whereas Neocallimastigomycota was more abundant in the finisher phase.

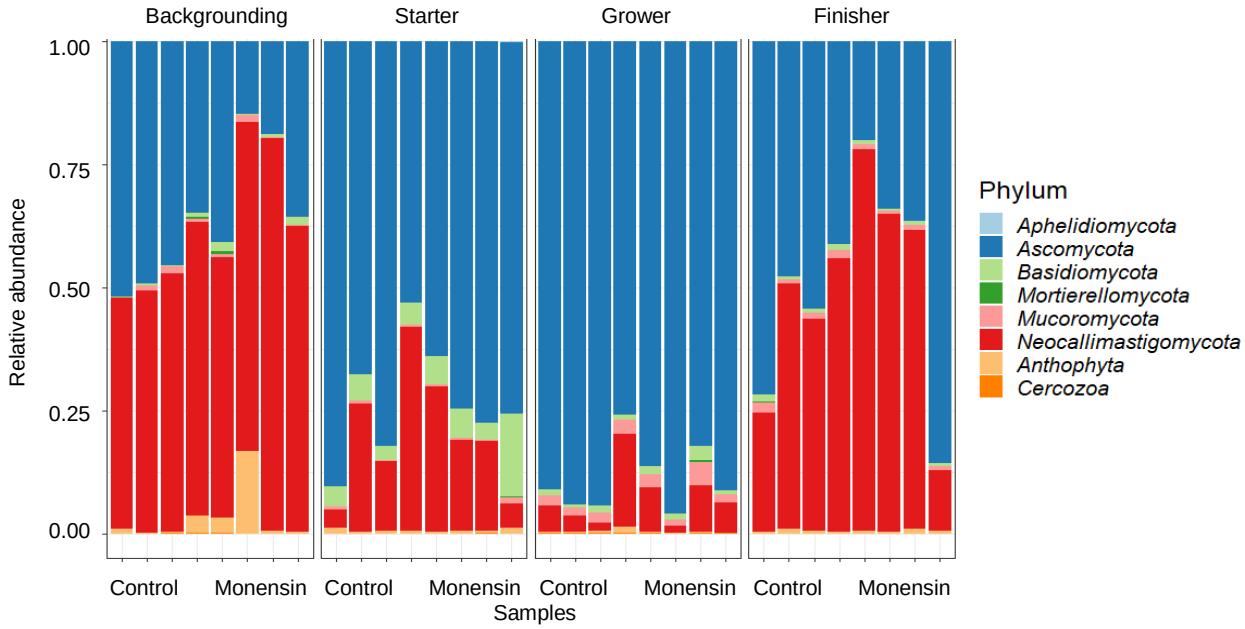

Figure 5 Relative abundance of fungal phyla in the feedlot phases of the control and monensin groups. The x-axis depicts the rumen samples per phase and the y-axis the relative abundance

At phylum level, 45% of the ASVs were non-characterized. Neocallimastigaceae from the Neocallimastigomycota phylum and Aspergillaceae from the Ascomycota phylum were the most abundant (Figure 6).

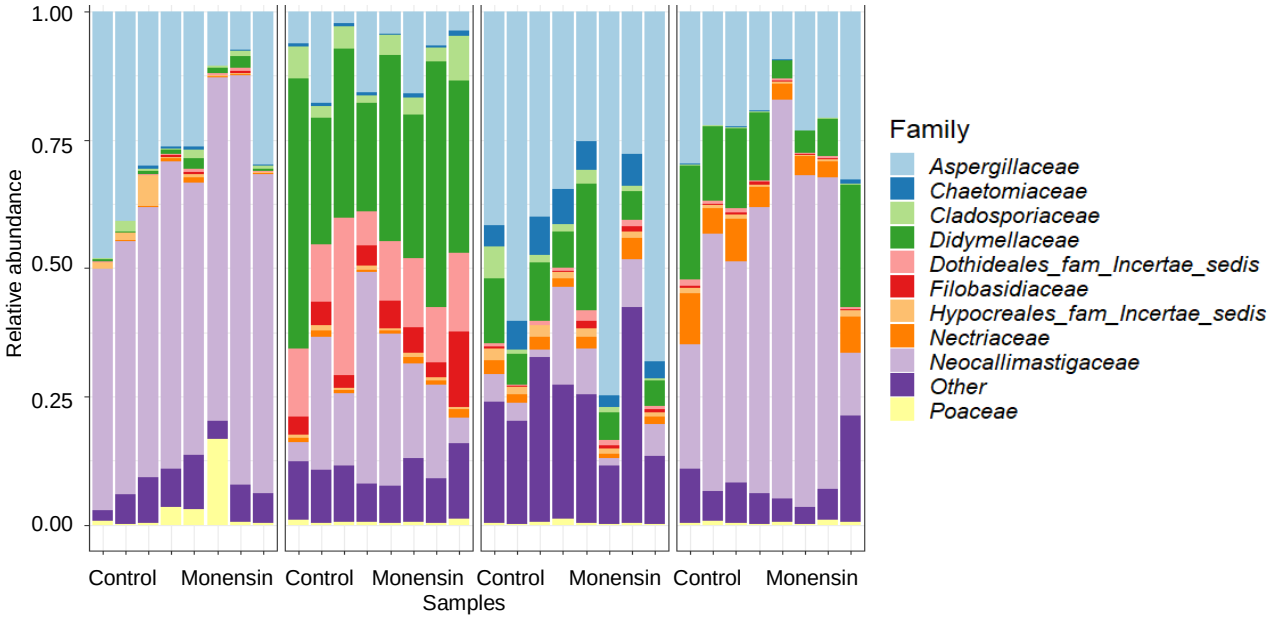

Figure 6 Relative abundance of fungal families in the feedlot phases of the control and monensin groups

There was no difference (P <0.5) in the pH measurement between the phases or between the control and monensin groups (Table 5).

Table 5 Mean, standard deviation and range of pH measurements for the monensin and control groups in the starter, grower and finisher phases

Group	Starter			Grower			Finisher		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Control	6.55	0.37	6.10-7.03	6.48	0.27	6.09-6.72	6.02	0.20	5.77-6.27
Monensin	6.09	0.27	5.76-6.43	6.29	0.25	6.06-6.58	6.24	0.48	5.52-6.56

Microbial diversity in the rumen microbiome determines the amount of energy, in the form of VFAs and other products, available to the animal for production (Guan *et al.*, 2008; Shabat *et al.*, 2016) with a higher propionate to acetate ratio resulting in more energy (Wolin, 1960). The weighed UniFrac distances differentiated bacterial and fungal communities between the phases significantly. This was expected as the composition of the diets differed, and diet is one of the most influential factors on microbial composition (Belanche *et al.*, 2012; Gruninger *et al.*, 2019; Stanton *et al.*, 2020).

Feed additives such as monensin are commonly used in feedlots in South Africa. Monensin is known to decrease the abundance of gram-positive bacteria and methanogens by limiting the nutrient supply, resulting in a decrease in the acetate to propionate ratio and in methane emissions (Boadi *et al.*, 2004; Thomas *et al.*, 2017). This decrease could result in a more energy efficient process. Feed additives have an effect on rumen microbiome composition (Schären *et al.*, 2017). In this study no significant difference was found in bacterial, archaeal and fungal populations for the group receiving monensin and the control. This finding warrants investigation as it may hold positive outcomes for countries or situations in which feed additives are not allowed in feedlot diets.

The most notable change in the rumen microbiome composition occurred during the transition from a forage-based to a concentrate-based diet as fermentation substrates switched from cellulolytic to amylolytic (Carberry *et al.*, 2012). This was observed in this study where the bacterial population of the backgrounding phase differed significantly from the starter phase, with alpha diversity indexes indicating a higher richness in the backgrounding phase. Roughage-based diets have a wider range of carbohydrate substrates such as cellulose and heteropolysaccharides that are fermented by microbes, resulting in a more diverse rumen microbiome (Belanche *et al.*, 2012). These diets have a less acidic rumen environment, which plays a role in rumen diversity because many microbes are sensitive to acidic conditions (Russell & Wilson, 1996). The pH measurements in this study (pH 6.0–7.0) indicated a less acidic environment for the starter phase.

Bacteroidetes phylum was the most abundant during backgrounding, as expected for animals on a roughage diet (Li *et al.*, 2012). *Prevotella* from the Bacteroidetes phylum was the most abundant genus in backgrounding compared with the starter phase. Several studies reported that *Prevotella* was the most abundant bacterial genus in the rumen microbiome, regardless of diet (Stevenson & Weimer, 2007; Jami & Mizrahi, 2012) because it is involved in the degradation of multiple substrates (Rosewarne *et al.*, 2014) and production of acetate, succinate and propionate (Carberry *et al.*, 2012; Chen *et al.*, 2017).

Ruminococcus, *Clostridium* and *Pseudobutyrivibrio* from the Firmicutes phylum are plant fibre degraders (Danielsson *et al.*, 2017) and were expected to be abundant in the backgrounding phase. *Pseudobutyrivibrio* had a significant effect on average daily feed intake (Paz *et al.*, 2018), and average daily gain (Myer *et al.*, 2015). *Methanobrevibacter* and *Pseudobutyrivibrio* were both more abundant in the backgrounding phase compared with the starter phase. These microbes were shown to be correlated because *Pseudobutyrivibrio* was identified as a potential biomarker for methane emissions (Auffret *et al.*, 2018) and *Methanobrevibacter* is a methanogen (Tapio *et al.*, 2017). Their lower abundance in the starter phase could be because of the higher energy content of the diet because propionate is favoured over acetate production. This results in more energy being available to the animal for production (Jeyanathan *et al.*, 2019).

Fungi play a role in degrading fibrous materials in the rumen (Gruninger *et al.*, 2014) and are therefore more abundant in roughage-based diets, such as during backgrounding. However, in this study the number of observed ASVs showed a higher abundance of fungi in the starter phase. Further research is needed. Most studies (Gruninger *et al.*, 2014; Zhang *et al.*, 2017, 2020; Belanche *et al.*, 2019) reported that Neocallimastigomycota was the most abundant fungal phylum in the rumen microbiome. In contrast, the Ascomycota phylum was the most abundant fungus in the current study, followed by Basidiomycota and Neocallimastigomycota. Few studies are available for Ascomycota and Basidiomycota as these phyla are aerobic fungi (Zhang *et al.*, 2020) and are rarely found in animals (Zhang *et al.*, 2017). Zhang *et al.* (2017) reported an increase in their abundance as the proportion of concentrates increased in dairy cattle, which was also observed in this study. It is unclear how these aerobic microbes survived in the anaerobic environment of the rumen. However, they might play a role in scavenging oxygen entering the rumen and have a beneficial effect on the anaerobic fermentation in the rumen (Zhang *et al.*, 2020).

Aspergillaceae family from the Ascomycota phylum have been used as feed additives in animal nutrition (Adegbeye *et al.*, 2020) because they decrease methane emissions by reducing the growth and activity of methanogenic bacteria (Wolin & Miller, 2006). The abundance of *Aspergillus* in the starter phase might indicate a decrease in methane emissions. The decrease in *Aspergillus*is from the backgrounding phase to the starter phase is in line with the earlier observations regarding *Methanobrevibacter* and *Pseudobutyrivibrio*.

There is a beneficial symbiotic relationship between anaerobic fungi from the Neocallimastigomycota phylum and methanogens, such as *Methanobrevibacter* (Cheng *et al.*, 2009). *Methanobrevibacter* and the Neocallistigaceae family were more abundant in the backgrounding phase. Roughage-based diets have a higher methane production per unit of feed compared with diets high in concentrates (Beauchemin & McGinn, 2006).

he most prominent shift in the rumen microbiome composition was between the starter and the grower phases. The increase in the proportion of carbohydrates in the diet shifts the rumen microbial composition from predominantly Firmicutes to Proteobacteria (Petri *et al.*, 2018). The proportion of carbohydrates in the diet has a significant effect on the rumen microbiome population (Raabis *et al.*, 2019) as an increase in easily digested carbohydrates results in more propionate-producing bacteria, lower fibre-degrading organisms, lower protein breakdown, and higher feed efficiency (Fernando *et al.*, 2010; Belanche *et al.*, 2012). Bacteroidetes was the most abundant phylum in the starter phase, whereas Proteobacteria was more abundant in the grower phase. Microbes from the Proteobacteria phylum have diverse metabolic functions and indicate an increase in the number of bacteria that are metabolically capable of handling easily fermentable carbohydrates (Fernando *et al.*, 2010). *Succinivibrio* was more abundant in the starter phase, whereas *Vampirovibrio* and *Ruminobacter* were more abundant in the grower phase. A higher abundance of propionate-producing bacteria such as *Succinivibrio* (Suen *et al.*, 2011) may divert hydrogen from methanogens, thus reducing enteric methane emissions and increasing available energy for metabolism (De Menezes *et al.*, 2011).

Eubacterium and *Ruminococcus* showed significantly higher abundance in the grower phase. *Eubacterium* is abundant in efficient steers and has a tolerance of low pH, whereas *Ruminococcus* has been associated with residual feed intake (Hernandez-Sanabria *et al.*, 2012). Because the grower diet had a higher proportion of easily fermentable carbohydrates, a lower pH could be expected in comparison with the starter phase. The pH measurements in this study did not show the expected decrease in pH from starter to grower. This might be explained by possible saliva contamination in the samples because of the method of rumen collection or selective feeding by the cattle before collection. In highly efficient steers, the acetate utilization characteristics of *Eubacterium* may interact with the acetate-producing capacity of *Succinivibrio* to utilize excessive hydrogen, which otherwise would be directed to methanogenesis (Chassard & Bernalier-Donadille, 2006).

Based on alpha diversity, more fungi were present in the grower phase compared with the starter. This was unexpected, as fungi are known to decrease in abundance as the proportion of carbohydrates in the diet increase. A low pH, which would be found in high concentrate diets such as the grower diet, can inhibit the growth of anaerobic fungi (Han *et al.*, 2019), leading to a decrease in their abundance. *Cyllamyces* and *Orpinomyces* were the genera more abundant in the starter phase. These genera from the Neocallistigomycota phylum are known to be present in the rumen (Gruninger *et al.*, 2014; Zhang *et al.*, 2017) and degrade cellulose and xylose (Kittelmann *et al.*, 2012). Genera from Ascomycota (*Neoascochyta*, *Selenophoma* and *Cecomyces*) were more abundant in the grower phase compared with the starter. However, few studies in the literature elucidated their abundance in the starter phase. Belanche *et al.* (2019) reported that fungi could be ingested with feed materials such as plant pathogens, saprotrophs, yeast and other species of unclassified fungi. The abundance of these genera might be because of external factors and their role in the rumen requires further research to confirm their origin and functions.

There was no significant difference in the rumen microbe population between the grower and finisher groups for either the bacteria and archaea or the fungi population. This might be because Proteobacteria had a high abundance in both the grower and finisher phases. Even though the high abundance of Proteobacteria could indicate possible dysbiosis in the rumen of the cattle (Auffret *et al.*, 2017), no physical effects of acidosis or metabolic disorders were observed in the animals during the grower and finisher phases. Many pathogenic bacteria belong to the Proteobacteria phylum and these pathogens are sensitive to dietary change (Baümler & Sperandio, 2016). Metabolic diseases in cattle such as bloat or acidosis have been associated with an unbalanced rumen microbiome (Khafipour *et al.*, 2009) and are known to occur in finishing diets. High concentrate diets, such as the finisher diet in this study, increase production of lactate and are associated with acid tolerant microbes such as Proteobacteria (Fernando *et al.*, 2010). Because the finisher period had the highest abundance of Proteobacteria, strategies could be formulated to decrease the abundance of pathogenic microbes while maintaining beneficial microbes.

The finisher phase had the lowest alpha diversity compared with the other phases. A lower alpha diversity has been associated with more efficient animals (Zhou *et al.*, 2009; Shabat *et al.*, 2016). It is therefore more desirable to have low diversity in the rumen microbial population to focus on promoting energy yield from the feed (Shabat *et al.*, 2016). However, a too low alpha diversity has been associated with an unbalanced and unhealthy rumen microbiome composition. It is therefore imperative to balance the microbiome composition to prevent dysbiosis.

The finisher phase exhibited the least abundance of fungi compared with the other phases based on the alpha diversity. A similar observation was reported by Kumar *et al.* (2015), but was in contrast to Zhang *et al.* (2017). This decrease in the richness and diversity of the fungi in the finisher might be because of the higher proportion of concentrates as fungi are mostly fibre degrading microbes. *Neocallismastix* from the Neocallistogomycota phylum was the most abundant in the finisher phase. This genus is able to utilize a wide range of substrates such as cellulose, xylose, glucose, starch, grass, and straw (Edwards *et al.*, 2017). However, its abundance decreased with increasing concentrates (Han *et al.*, 2019), which is in contrast with this study. Further studies on the function and prevalence of fungi in the rumen microbiome are needed.

There are other factors that must be mentioned when discussing the rumen microbiome. Although most of the differences discussed above can be explained by the influence of diet and the age of the animals (Jami *et al.*, 2013), host genetics can influence the rumen microbiome. Studies reported that animals fed the same diet could exhibit substantial differences in microbiome composition (Welkie *et al.*, 2010; Firkins & Yu, 2015) because of host genetics and the interaction between the host and the rumen microbiome (Hernandez-Sanabria *et al.*, 2013).

Conclusion

This is the first study to investigate the rumen microbiome of South African Bonsmara cattle under intensive feedlot conditions. Improvement of feed efficiency in feedlot cattle holds several advantages, including cost, a decrease in environmental impact and food safety. There was no significant difference in the overall rumen microbiome population between the monensin and the control groups, but differences within the phases require further investigation. This study allowed for an improved understanding of microbial shift in the feedlot period. This understanding could provide integrative information about rumen function and lead to improved ruminant production through changes in digestibility and feed efficiency.

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Authors' contributions

All authors participated in the planning of the project. DAL conducted the trial, performed the laboratory work, bioinformatics and statistical analysis and wrote the original paper. EvMK supervised the project, wrote, and edited the paper. CJLdT assisted in the nutritional aspects of the project. DS assisted in the bioinformatic and statistical analysis. MMS revised the paper. All authors have read and agreed to the published version of the manuscript.

Conflict of interest declaration

The authors declare there is no conflict of interest.

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