ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 21 No. 9
October 2021
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Afr. J. Food Agric. Nutr. Dev. 2021; 21(9): 18676-18693 https://doi.org/10.18697/ajfand.104.19825

EFFECTS OF DIRECT-FED LACTIC ACID BACTERIA ON WEIGHT GAIN AND RUMINAL pH OF TWO SOUTH AFRICAN SHEEP BREEDS

Mani S[1, 2], Aiyegoro OA[1, 3]* and MA Adeleke[2]

Olayinka Aiyegoro

*Corresponding author email: ayoyinkaaiyegoro@gmail.com

[1]Agricultural Research Council- Animal Production Institute, GI Microbiology and Biotechnology Unit, P/Bag X02, Irene 0062, South Africa

[2]Discipline of Genetics, School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville, P/Bag X54001, Durban 4000, South Africa

[3]Research Unit for Environmental Sciences and Management North-West University, P/Bag X6001, Potchefstroom 2520, South Africa

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 21 No. 9 October 2021 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

ABSTRACT

A ruminant's digestion depends on microbial degradation of feed rather than endogenous enzyme degradation as in most monogastric animals. The study was conducted to assess the effects of Lactic Acid Bacteria (LAB) strains administered as direct-fed microbials on weight gain and ruminal pH of Damara and Meatmaster sheep breeds. Sixty-four Damara and Meatmaster sheep breeds [Damara males (36.6 ± 8.3 kg); Damara females (28.9 ± 6.9 kg); Meatmaster males (24.6 ± 3.4 kg); Meat master females (21.5 ± 3.1 kg)] were subjected to a 30-day trial and divided into five treatment groups as follows: pellets with no antibiotic and no probiotics administered (T1); pellets with no antibiotics, only *Lactobacillus rhamnosus* SCH administered (T2); pellets with no antibiotics, only *Lactobacillus rhamnosus* AF3G administered (T3); pellets with no antibiotics, with the combination of *Lactobacillus rhamnosus* SCH and *Lactobacillus rhamnosus* AF3G, administered (T4); pellets fortified with antibiotic and no probiotic administered (T5). The animals were fed on commercial pellets fortified with or without antibiotics. Animals were each weighed, and rumen fluids were collected using a stomach tube, and pH was read immediately, before and at the end of the trials. Data obtained were subjected to analysis of variance using SPSS version 4.0. The results showed that the effect of treatment, sex and some of their interactive effects were significant ($p < 0.001$) on the body weight of sheep irrespective of breed. The effect of treatments revealed that the animals in the combination of probiotics gained more weight than those in other groups. Damara breed had a heavier body weight than Meatmaster while males were 6 kg heavier relative to females ($p < 0.001$). Only breed was significant ($p < 0.05$) on weight gained. Treatment ($p < 0.05$), breed ($p < 0.001$) and their interactive effect ($p < 0.05$) were significant on ruminal pH. The highest pH value was 7.27 for the T5 group and 7.37 for the Damara breed. Results suggest that LAB may have beneficial effects on the growth performances of sheep and therefore may be suitable as future growth promoters in sheep production, as they don't have any harmful residues compared to antibiotics.

Key words: Sheep breeds, Lactic Acid Bacteria, Weight gain, Rumen fluid pH

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 21 No. 9 October 2021 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

INTRODUCTION

Sheep production is one of the most significant commercial livestock production activities in South Africa, especially in the arid, pastoral areas. The fat-tailed sheep are an important animal genetic resource in South Africa. They are very well adapted to harsh environmental conditions and can tolerate diseases and parasite-induced stress [1]. The Damara sheep breed is one of the indigenous fat-tailed sheep in South Africa. Meatmaster sheep breed is a South African developed breed from the combination of the well-adapted native Damara sheep and other locally developed sheep breeds such as Dorper and Van Rooy. The Meatmaster has the potential to adapt to optimal conditions [2].

Ruminants depend on the symbiotic interaction between the rumen microorganisms and the rumen environment of the host animal for the degradation of feed material ingested, with the microbial fermentation producing the nutrients required such as vitamins, protein and short-chain organic acids for the host [3]. The involvement of ruminal microbes in digesting and fermenting feed biomass is significant in mature ruminants, as they reflect dynamic characteristics of the rumen function and maintenance [4]. The rumen pH is one of the vital aspects of rumen function and it differs considerably from the rest of the gastrointestinal tract in ruminants as it is influenced by the type of diet ingested, feed additive supplementation, water intake or rumination. The pH stability in the rumen is maintained by the relationships amongst microbial populations, fermentation products and the saliva's buffering influence [5].

Probiotics have been explored as alternative feed additives to antibiotics to manipulate rumen fermentation to improve the health and productivity of animals; either lactic acid bacteria or yeast culture, probiotics have beneficial effects on higher nutrient utilization [6]. Probiotics consisting of lactic acid-producing bacteria (LAB) promote intestinal balance, resistance to pathogens [7] and they have been used as supplements in animal feed, which resulted in improved dry matter intake, weight gain and ruminants' health [8]. Lactic acid bacteria administration is said to aid rumen microbiota in adapting to lactic acid and prevent rumen lactate accumulation [9]. They also play a role in restoring mutualism between the gut bacteria, where the symbiotic relationship with the host has been disconcerted by either internal and external factors, where homoeostasis may be lost, leading to clinical conditions [10].

Probiotic supplementations are also acknowledged to modify molar proportions of volatile fatty acids [11] and regulate rumen pH [12]. Previous studies have reported the positive effect of probiotics supplementation as direct-fed microbials on nutrient intake, feed efficiency and higher growth associated with higher rumen fermentation and nutrient utilization in ruminants. Additionally, the level of Direct-Fed Microbials (DFMs) being expressed biologically in animals depend on certain factors like the type of strain, dose, feed, animal age [13–15]. Therefore, this study was designed to determine the effect of lactic acid bacteria strains used as direct-fed microbials, breed and sex on weight gain and ruminal pH of Damara and Meatmaster sheep breeds.

https://doi.org/10.18697/ajfand.104.19825

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 21 No. 9 October 2021 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

MATERIALS AND METHODS

Animals, treatments and sampling

All the procedures involving animals were permitted by the Agricultural Research Council- Animal Production Ethics committee (APIEC17/21). The trials were done at the Agricultural Research Council (ARC), GI Microbiology and Biotechnology unit and the Small Stocks Unit in Irene, Gauteng province, South Africa.

The lactic acid bacteria (putative probiotics) used in this study were isolated and characterized from fresh faecal samples of the Zulu sheep breed. The gene sequences obtained from the two bacteria compared to NCBI nucleotide blast (blastn) confirmed their identities as *Lactobacillus rhamnosus* SCH-MK246001.1 and *Lactobacillus rhamnosus* AF3G-MH478190.1. The putative probiotic bacteria were grown anaerobically in De Man Rogosa and Sharpe (MRS) broth (Oxoid, England) and preserved on 25% glycerol stored at -80^{0}C. The two candidate putative probiotics (*Lactobacillus rhamnosus* SCH and *Lactobacillus rhamnosus* AF3G) were later revived by inoculation in MRS broth. For suspension, MRS broth was inoculated with 1% (v/v) fresh culture and incubated anaerobically at 37 °C overnight to reach a cell density of approximately 2.0 × 10^9 CFU/ML prior to administering to the trial animals.

Sixty-four sheep (32 Damara sheep breed and 32 Meatmaster breed) were used for the trial and they were approximately 7 months old with 16 males and 16 females per breed, with the average initial weight as follows: Damara males (36.6 ± 8.3 kg); Damara females (28.9 ± 6.9 kg); Meatmaster males (24.6 ± 3.4 kg); Meatmaster females (21.5 ± 3.1 kg). The animals were housed per treatment with males and females separated in open barn trial pens with ± 4m^2 shelters. The sheep were randomly allocated to 5 treatment groups; (6-8 per treatment) based on the sex; the treatments were as follows: a) Diet with no antibiotics, no probiotics (negative control) (T1); b) Diet with no antibiotics, only *Lactobacillus rhamnosus* SCH (T2); c) Diet with no antibiotics, only *Lactobacillus rhamnosus* AF3G (T3); d) Diet with the combination of *Lactobacillus rhamnosus* SCH and *Lactobacillus rhamnosus* AF3G (T4); e) Diet with antibiotics, no probiotics (positive control) (T5). The animals were fed on commercial pellet feed fortified with or without probiotics or antibiotics and in case of the positive control, in-feed antibiotic rumensin was added according to the feed manufacturer's specification. Eragrostis hay and freshwater were supplied *ad libitum*. The experimental feed composition is presented in Table 1, and feed composition is analyzed as described by Mani *et al*. [41]. The animals in probiotic treatment groups were dosed once a week for the trial period using the dosing gun with 10 mL of the 24-hour old LAB culture suspensions of approximately 2 × 10^9 CFU/mL. On the first day of the experiment, after the two weeks adaptation period, ruminal pH and the weight of each experimental animal were measured to establish the basal levels. After the trial, ruminal pH and weight were recorded and difference in weights was used to calculate the weight gained during the trial. Rumen fluids were collected at 08:00 hours before feeding at each sampling time (before and after the trial).

Rumen fluid samples were collected using a stomach tube according to the procedures of Shen *et al.* [16]. About 40 mL of the collected rumen fluid contents were transferred

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 21 No. 9 October 2021 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

to 50 mL centrifuge tubes and the rumen pH was measured using a portable pH meter after calibration (Orion Star A121, Thermo Scientific, Singapore) immediately after sampling. Before each sample reading, the pH meter was rinsed with distilled water and blot dried, rinsed again after each sample reading. Rumen samples were kept on ice until they were transferred to the lab and they were stored at -80°C until further analysis.

DNA extraction and 16S rRNA amplification

According to the manufacturer's guidelines for pathogen detection, DNA from rumen samples (100 µl each) was extracted using QIAamp Fast DNA stool Mini Kit (Qiagen, Germany), and DNA concentration was evaluated with Nanodrop 2000 (Thermo Electron Corporation, USA). The total DNA obtained from each treatment group (male and female, separate) were combined, pooled DNA quality was assessed by electrophoresis on 1.5% agarose gels and visualized with UVP BioSpectrum 310 Imaging System (FisherScientific, UK). The pooled DNA samples were used as templates for amplifying a partial 16S rRNA sequence using the following primers, which include Illumina overhang adapter sequences:

Forward=5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG3'

Reverse=5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC3'

targeting the highly variable V3–V4 region of the prokaryotic 16S rRNA gene. PCR was done in 25 µl reaction mixture containing 5 µl template DNA, 1 µl of each primer, 5.5 µl nuclease-free water and 12.5 µl of 2× KAPA HiFi HotStart Ready Mix (KAPA Biosystems), with the following conditions: initial denaturation at 95°C for 3 min, 30 cycles of amplification at 95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec and final extension at 72°C for 5 min. Amplicons from PCR were visualized using agarose gel electrophoresis to verify the expected band size of 550 bp. PCR products were purified to remove any primer dimers using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Germany).

Sequencing and data analysis

Sequencing libraries were generated and index codes were added using Illumina MiSeq Nextera XT DNA Library Preparation Kit (Illumina, USA), according to manufacturer's guidelines. The library quality was evaluated using Qubit 2.0 Fluorometer (ThermoScientific, USA) and Agilent 2100 Bioanalyzer system. The library was sequenced on the Illumina MiSeq platform and 300bp paired-end reads were generated.

The raw data sequences generated by MiSeq illumine sequencer were trimmed using Trimmomatics version 0.36, where the low-quality sequence regions and Illumina universal adapter sequences were removed. Demultiplexed sequence files were imported to QIIME (version 2018.8) [17] for analysis. Using DADA2, the imported reads were denoised and trimmed. Representative reads were picked and operational

taxonomic units (OTU) feature table was generated; chimeric sequences were detected and removed from the representative OTU sequences. For taxonomic analysis, OTU representative sequences were aligned to the Greengenes database. All other statistical analyses were carried out using RStudio (version 3.5.3) with phyloseq package (version 1.24.2). Operational taxonomic units (OTUs) at a relative abundance ≥ 0.05% of the total reads in at least one sample were the ones retained and analyzed further in RStudio.

Statistical analysis

Data were subjected to two-way analysis of variance using SPSS (Statistical Package for the Social Sciences, version 25) with dietary treatment, breed and sex as the classification factors. Significant means were separated using Duncan's Multiple Range Test. The Kruskal-Wallis test was used to analyze microbial data. The degree of statistical significance was set as $P < 0.05$.

RESULTS AND DISCUSSION

In the current study, the results showed that the effect of treatment, breed, sex and their interaction effects were significant ($p < 0.001$) on the body weight of the sheep (Table 2). It has been reported that probiotic supplementation showed a positive effect on nutrient intake, weight gain and ruminal pH in ruminants [12,18,19]. In Table 3, the control group (T1) had the heaviest weight gain of 5.8 kg for Damara sheep and there was an increase in weight gain across the treatments except in the antibiotic group (T5), where there was a drop. However, in Meatmaster sheep, the lowest weight gain was 1.4 kg for the control group (T1), while weight gain for other treatments increased except the antibiotic group (T5) that recorded a decline. Body weight followed similar pattern. On average, body weight in other treatment groups was higher (about 4.85 kg) than the control group. The supplementation of probiotics influenced the weight improvement of the sheep as the negative control (T1) showed lower body weight in comparison to other treatment groups; *Lactobacillus rhamnosus* AF3G (T3) and combination of probiotics (T4) recorded the heaviest bodyweight, which implies that the probiotics may have an impact in the digestion of nutrients. Treatment with the combination of probiotics (T4) showed greater weight gain in comparison to the control and *Lactobacillus rhamnosus* SCH (T2), but there was no significant difference among *Lactobacillus rhamnosus* AF3G (T3), combination of probiotics (T4) and antibiotic group (T5) statistically. The observed comparative increase of body weight gain on microbial treated groups in this study cannot be explained by excess dry matter intake, as all experimental animals were given the same type and amount of feed. Rather, the body weight gain could be associated with the effectiveness of nutrient digestion stimulated by probiotics with microbiota interactions. A study by Roodposhti & Dabiri [20] reported that average weight gain was significantly greater for the synbiotic, prebiotic and probiotic treatments than the control. They observed that synbiotic-fed calves had greater weight gain than other treatments and no significant difference was found between prebiotic and probiotic treatments. Özsoy *et al.* [15] observed that final live weight of goats was not significantly affected. However, the weight gain was higher in the group fed with probiotics than the control group. Some studies have

discovered that the growth-stimulating effects of probiotics were equivalent or better than those of antibiotics in livestock [21,22].

Within the breed, Damara males were 8.9 kg heavier compared to their female counterparts, with Meatmaster males having 3.3 Kg higher than the females (Table 4). Between breed variation, Damara males were 15.1 kg heavier than Meatmaster males. The same trend was observed for the females except body weight was only about 62.91 % relative to that of males. While male Damara sheep gained 1.3 kg higher than the females, the Meat Master males only gained 0.5 kg higher compared to their female partners. The results also showed that Damara males gained 1.6 kg higher than Meatmaster sheep. Damara breed is a South African indigenous sheep breed, whereas Meatmaster is a developed breed (crossbred) with parental genetics of Damaras [2]. Damara sheep readily adapt to environmental conditions and are stress-tolerant under unfavourable natural environments [1]. This could be one of the main reasons they could obtain much greater weight gain than Meatmaster breed. This is in accordance with a study by Ptacek *et al*. [23], where the effect of breed on live weight was significantly greater on Suffolk purebred than the Suffolk × Merinolandschaf crossbreds. The ability of Damara sheep to obtain more nutrients from extensively different feed sources causes them to be an attractive alternate indigenous breed in sheep meat production [24].

The effect of breed on the body weight gain of the sheep was also reported by Wilkes *et al*. [24], where they recorded a difference in weight gain of the breeds with the Damara growing an average of 142 g/day and Merino at 80 g/day. However, no significant interaction between diet and breed was found, yet Damaras gained weight faster than Merinos in both diets. There have been reports on the difference between breeds regarding voluntary intake of low and high-quality diets and the digestibility difference between sheep and cattle breeds [25].

The sex of the animals exhibited a significant difference on body weight, where the rams were 6.5 kg heavier as compared to ewes. This sexual dimorphism between male and female sheep could be attributed to higher level of natural testosterone in males. However, there was no statistical difference in weight gained and in the sex interactions as shown in Table 5. De Souza Rodrigues *et al*. [26] reported similar results, where there was no significant effect of sexual class and feeding level interaction on weight gain. However, Kashani & Bahara [27] discovered that male lambs had enhanced performances compared to females in feed conversion ratio and average weight gain. Also, Siqueira *et al*. [28] reported a significant difference between non-castrated lambs, castrated males and female lambs. The non-castrated lambs had higher weight gain compared to castrated males and females. Such distinction was attributed to the physiological testosterone function, which is responsible for the high deposition of muscle mass and, subsequently, higher average weight gain and feed efficiency in males [29].

Ruminal pH is the principal guide signifying the internal balance in the rumen environment, hence it is vital to maintain a moderately stable pH to ensure efficiency in ruminal fermentation. Ruminants normally maintain ruminal pH in the physical range

between 5.5 - 7.0 [30]. In the present study treatment, breed and their interactions significantly affected ruminal pH ($p < 0.05$). Damara breed recorded a higher ruminal pH value than Meatmaster breed; this might be because Damaras can retain more fibrous constituents in the rumen for longer period, permitting the cellulolytic microorganisms to thoroughly degrade the feed's fibrous constituents. The ability of cellulolytic bacteria to digest fibres is maintained by optimal ruminal conditions, where pH between 6 and 9 is best [31].

The high ruminal pH recorded in the antibiotic-treated group (T5) in this study might be associated with a decrease of some lactic acid bacteria due to antibiotic supplementation, which in turn may have increased the pH. Some studies have reported that antibiotics such as monensin (rumensin) reduce the number of Gram-positive bacteria such as lactic acid bacteria leading to a higher ruminal pH, but alteration possibility could assist the microorganism in overcoming antibiotic effects in the rumen [32]. Probiotics stabilized ruminal pH, which may have enriched microbial ecology and led to increased nutrient absorption and lactate resulting in improved weight gain. Studies in literature have revealed that the important ruminal bacteria influencing ruminal fermentative aptitude are categorized as lactic acid producers and utilizers [33] and both bacterial groups have been used as probiotics. A study conducted by Nocek *et al*.[34] reported that bacterial probiotics consisting *of Enterococcus faecium* and *Lactobacillus plantarum* prompted alterations in the ruminal pH of high grain-fed cows. Yet, the mechanism of the effects of the bacterial probiotics on rumen fermentation was unclear.

At phylum level, 15 bacterial phyla and 1 archaeal phylum were detected across all treatments at 0.05 abundance. Bacteroidetes and Firmicutes were the most dominant across the treatments; with Proteobacteria and Euryarchaeota abundance ranging from 4.77% – 19.82% and 1.7% – 3.02%, respectively. Most abundances of the phyla were greater than 1% in particular treatments excluding Elusimicrobia, Planctomycetes, Actinobacteria and Synergistetes which had abundances less than 1% in all the treatments. Though Firmicutes were lower in T5 compared to other treatments and Eurychaeota higher in T4; there was no significant difference in microbial abundance across the treatments (Figure 1).

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 21 No. 9
October 2021
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

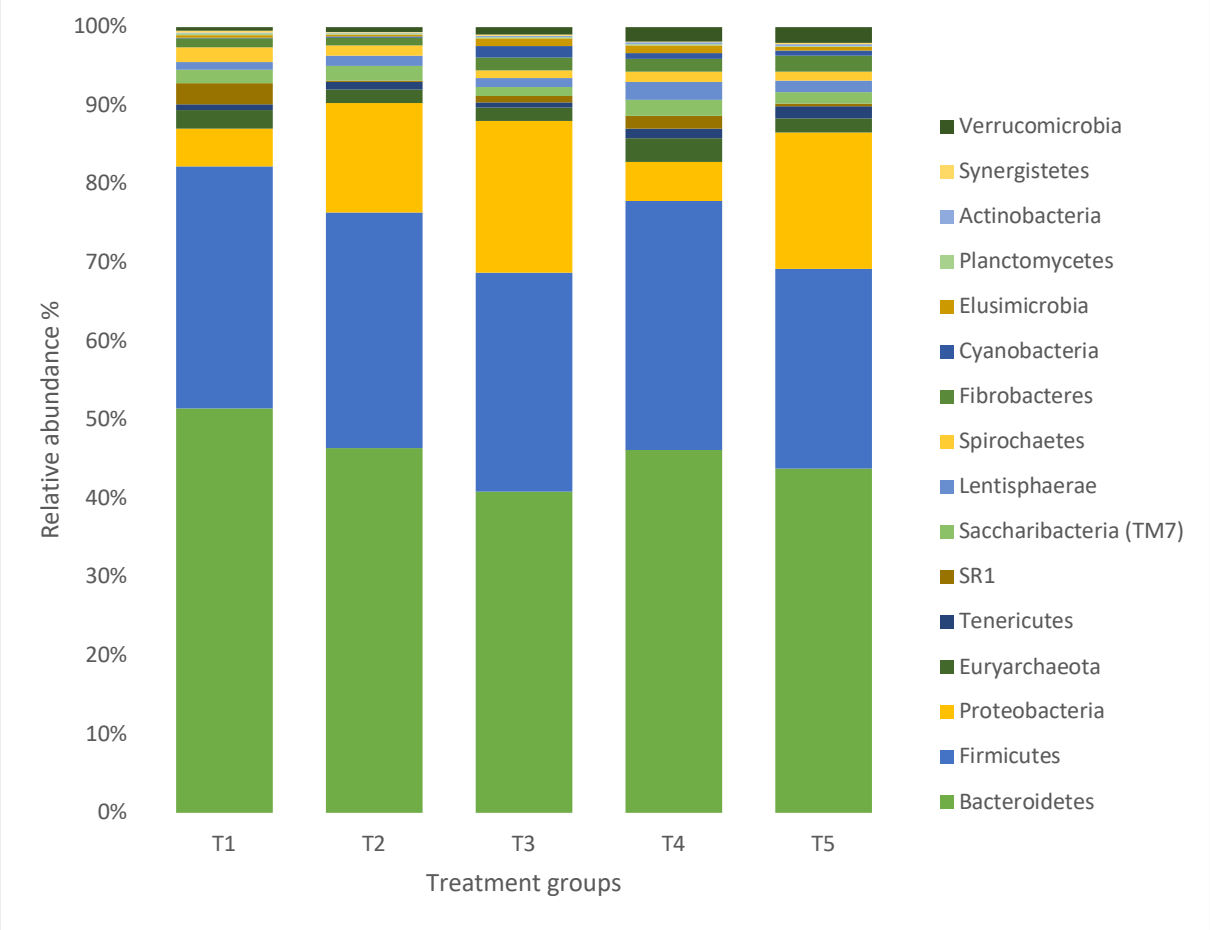

Figure 1: Relative abundance (%) of microbial phyla in rumen microbiome according to treatments

Treatments: T1- diet with no probiotic, no antibiotic (negative control); T2-diet with *Lactobacillus rhamnosus* SCH; T3- diet with *Lactobacillus rhamnosus* AF3G; T4- diet with a combination of *Lactobacillus rhamnosus* SCH and *Lactobacillus rhamnosus* AF3G; T5- diet with an antibiotic (positive control). Relative abundance = percent of the total microbial sequences, listed phyla were detected at greater than 0.05% relative abundance across all treatments

Common ruminal populations detected in our study were related to those reported in previous studies at phylum level [35,36], with Bacteroidetes and Firmicutes being the most abundant, then Proteobacteria, Euryarchaeota and other phyla. Treatments altered the relative abundance of the bacterial phyla differently. The observed decrease of Firmicutes in antibiotic group (T5) could be associated with monensin activity that inhibits the growth of pH-sensitive Gram-positive taxa of Firmicutes. Generally, Bacteroidetes produce propionate and acetate as fermentation end-products [37]. Higher quantities of Bacteroidetes might enhance feed utilization efficiency and body weight gain, though there is no definite correlation between the relative abundance of Bacteroidetes in combination of probiotics (T4) and the treatment's weight gain; also with weight gain of the negative control (T1) and its relative abundance of Bacteroidetes. Cooperatively, these microorganisms have essential roles in the cellulolytic and fermentative capacity of the rumen based on their presumed functions [38].

Bacterial probiotics appear to enhance ruminal bacteria's capability to metabolise lactic acid and regulate pH; also, the functionality and efficiency of bacterial probiotics have been assumed to be discovered based on the effects of dominant rumen microbiota [19]. Cao *et al*. [39] reported that lactic acid bacteria enhanced dry matter digestibility and reduced ruminal methane production. *Lactobacillus* has been widely used as feed enrichment in the dairy industry to improve feed conversion efficiency, intestinal health and milk production [30]. Earlier studies have shown that alterations in bacterial composition and diversity in the rumen, improved activities of lactate-utilizing bacteria and the greater lactate absorption affected the ruminal pH [40].

CONCLUSION

The study revealed that lactic acid bacteria administered as direct-fed microbials have beneficial effects on growth performances of sheep and they are suitable to be used as growth promoters, which may replace the use of antibiotics in the near future, as probiotics may not have any immediate public health and safety risks. The antibiotic-treated group displayed higher pH readings. It has been reported that high ruminal pH may lead to some ruminal essential bacteria to be inhibited. The combination of probiotic strains was more effective than the single probiotic strains, this may be due to the synergistic effect between strains. Genomic studies are needed to further investigate the interaction of probiotics with the rumen microbiota, to determine how their bio-availability resulted in better growth performance.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the material discussed in the manuscript.

ACKNOWLEDGEMENTS

Authors will like to acknowledge the Agricultural Research Council – Animal Production, Irene, South Africa; National Research Foundation (NRF) of South Africa and University of KwaZulu Natal, Discipline of Genetics for funds and provision of research facilities.

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 21 No. 9 October 2021 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

Table 1: Ingredient and chemical composition of experimental diet

Nutrients	Composition (g/kg)	Ingredient Composition (g/kg DM)
Dry Matter	893.3	Corn silage 600, Corn grain finely ground 250, Soybean meal 150, Urea:ammonium sulfate (9:1) 6.1
Moisture	106.8	
*Protein (N × 6.25)	152.8	
Fat (ether extraction)	120	
NDF (neutral detergent fiber)	370.9	
ADF (acid detergent fiber)	134.9	
ADL (acid detergent lignin)	55.9	
Ash	89.2	
Starch	327.1	
Fiber (crude)	123.8	
Calcium	13.9	
Phosphorus	3.2	
Potassium	13	
Magnesium	1.8	
Sodium	11	
Chloride	17	
Sulphur	3.5	

*For the conversion of nitrogen to protein content, the factor 6.25 was used. Table adopted from Mani *et. al.* [41]

https://doi.org/10.18697/ajfand.104.19825

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 21 No. 9 October 2021 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

Table 2: Interactive effect of treatment, breed and sex on body weight, weight gain and ruminal pH of sheep

			Parameters				
Treatment	**Breed**	**Sex**	**Initial BW (kg)**	**Final BW (kg)**	**Weight gained (kg)**	**Initial pH**	**Final pH**
	Damara	Female	19 .0 ± 0.0	24.0 ± 0.0	5.0 ± 0.0	6.9 ± 0.0	7.14 ± 0.0
T1		Male	38.5 ± 0.0	45.0 ± 0.0	6.5 ± 0.0	7.1 ± 0.0	7.3 ± 0.0
	Meatmaster	Female	21 ± 1.0	23 ± 2.0	2.0 ± 1.0	7.22 ± 0.03	7.22 ± 0.03
		Male	23.8 ± 1.8	24.67 ± 1.5	0.83 ± 0.3	7.12 ± 0.2	7.12 ± 0.2
	Damara	Female	32.5 ± 6.4	35.3 ± 6.0	2.75 ± 0.4	7.12 ± 0.1	7.16 ± 0.1
T2		Male	49.5 ± 0.0	52.0 ± 0.0	2.5 ± 0.0	7.68 ± 0.0	7.5 ± 0.0
	Meatmaster	Female	21.6 ± 3.5	23.3 ± 7.4	1.7 ± 4.04	6.9 ± 0.2	6.9 ± 0.2
		Male	24.8 ± 3.2	27.2 ± 3.3	2.3 ± 0.3	7.12 ± 0.15	7.12 ± 0.15
	Damara	Female	34.5 ± 0.0	37.0 ± 0.0	2.5 ± 0.0	7.5 ± 0.0	7.4 ± 0.0
T3		Male	43.0 ± 1.4	46.3 ± 1.1	3.25 ± 0.4	7.5 ± 0.4	7.3 ± 0.2
	Meatmaster	Female	21.3 ± 5.4	23.0 ± 4.4	1.7 ± 1.04	6.99 ± 0.13	6.9 ± 0.15
		Male	27.6± 1.6	31.3 ± 3.1	3.7 ± 2.9	6.9 ±0.3	6.89 ± 0.2
	Damara	Female	34 ± 1.4	37.5 ± 3.5	3.5 ± 2.12	7.26 ± 0.05	7.47 ± 0.2
T4		Male	41.8 ± 3.7	46.5 ± 2.4	4.75 ± 2.1	7.37 ± 0.4	7.43 ± 0.2
	Meatmaster	Female	19.63 ± 1.7	23.4 ± 2.8	3.75 ± 1.3	7.02 ± 0.2	7.02 ± 0.1
		Male	21.0 ± 3.1	25.4 ± 4.6	4.4 ± 1.8	6.7 ± 0.3	6.8 ± 0.2
	Damara	Female	29.8 ± 6.5	32.5 ± 6.5	2.75 ± 0.9	7.45 ± 0.23	7.28 ± 0.12
T5		Male	31.0 ± 7.1	35.5 ± 6.4	4.5 ± 1.08	7.55 ± 0.3	7.41 ± 0.14
	Meatmaster	Female	24.3 ± 2.02	26.2 ± 1.8	1.8 ± 0.3	7.05 ± 0.1	7.07 ± 0.1
		Male	26.7 ± 2.5	28.7 ± 2.6	2.0 ± 0.5	7.03 ± 0.2	7.05 ± 0.1

	Parameter	**Body Weight**	**Weight gained**	**pH**
P-value	Treatment	<0.001**	0.059	0.0053 *
	Breed	<0.001**	0.008*	<0.001**
	Sex	<0.001**	0.067 NS	0.24 NS
	Treatment × Breed	<0.001**	0.18 NS	0.004*
	Treatment ×Sex	0.01*	0.8 NS	0.31 NS
	Breed × Sex	0.000**	0.57 NS	0.30 NS
	Treatment × Breed × Sex	0.01*		0.7 NS

Treatments: T1- diet with no probiotic, no antibiotic (negative control); T2-diet with *Lactobacillus rhamnosus* SCH; T3- diet with *Lactobacillus rhamnosus* AF3G; T4- diet with a combination of *Lactobacillus rhamnosus* SCH and *Lactobacillus rhamnosus* AF3G; T5- diet with an antibiotic (positive control). BW- Body weight. ** denotes significance difference p< 0.001; * denotes significance difference p < 0.05; NS denotes no significance

ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 21 No. 9
October 2021
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Table 3: Interactive effect of treatment and breed on body weight, weight gain and ruminal pH of sheep

Parameter	T1		T2		T3		T4		T5	
	Damara sheep	Meat Master	Damara	Meat Master	Damara	Meat Master	Damara	Meat Master	Damara	Meat Master
n 1	4	12	6	12	6	12	12	16	16	12
Body weight (kg)	31.6 ± 12.2	23.1 ± 2.0	39.5 ± 9.7	24.3 ± 4.5	41.7 ± 4.9	25.8 ± 5.3	41.3 ± 5.4	22.3 ± 3.7	32.2 ± 6.3	26.5 ± 2.5
Ruminal pH	7.12 ± 0.18	7.17 ± 0.14	7.29 ± 0.25	7.03 ± 0.19	7.4 ± 0.19	6.9 ± 0.17	7.4 ± 0.25	6.9 ± 0.2	7.4 ± 0.2	7.05 ± 0.1
n 2	2	6	3	6	3	6	6	8	8	6
Weight gain (kg)	5.8 ± 1.1	1.4 ± 0.9	2.7 ± 0.3	2.0 ± 2.6	3.0 ± 0.5	2.7 ± 2.3	4.3 ± 2.0	4.1 ± 1.5	3.6 ± 1.3	1.9 ± 0.4

Treatments: T1- diet with no probiotic, no antibiotic (negative control); T2-diet with probiotic 1; T3- diet with probiotic 2; T4- diet with combination of probiotic 1 and 2; T5- diet with antibiotic (positive control). n. 1: number of observations for body weight; n 2: number of observations for the weight gained

ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 21 No. 9 October 2021 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

Table 4: Interactive effect of breed and gender on body weight, weight gain and ruminal pH of sheep

Parameter	Damara sheep		Meat Master sheep	
	Female	Male	Female	Male
n 1	20	24	32	32
Body weight (kg)	32.1 ± 6.2	41.0 ± 7.4	22.6 ± 3.5	25.9 ± 3.8
Ruminal pH	7.3 ± 0.2	7.42 ± 0.24	7.04 ± 0.16	6.98 ± 0.22
n 2	10	12	16	16
Weight gain (kg)	3.1 ± 1.2	4.4 ± 1.6	2.3 ± 1.9	2.8 ± 1.9

n 1: number of observations for body weight; n 2: number of observations for the weight gained

Table 5: Effect of sex on body weight, weight gain and ruminal pH of sheep

Parameter	Sex	
	Female	Male
n	26	28
Initial body weight (kg)	25.0 ± 6.3	30.6 ± 8.9
Final body weight (kg)	27.6^{b} ± 6.7	34.1^{a} ± 9.5
Weight gain (kg)	2.6^{b} ± 1.7	3.5^{a} ± 1.9
Initial ruminal pH	7.14 ± 0.23	7.17 ± 0.37
Final ruminal pH	7.13^{a} ± 0.2	7.17^{a} ± 0.26

n: number of observations; $^{a, b}$ Mean values in a row with different subscripts are significantly different $p < 0.05$

https://doi.org/10.18697/ajfand.104.19825

REFERENCES

1. **Almeida AM** The Damara in the context of Southern Africa fat-tailed sheep breeds. *Trop Anim Health Prod*. 2011;**43**:1427–1441.

2. **Peters FW, Kotze A, van der Bank FH, Soma P and JP Grobler** Genetic profile of the locally developed Meatmaster sheep breed in South Africa based on microsatellite analysis. *Small Rumin Res.* 2010;**90**:101–108.

3. **Pinloche E, McEwan N, Marden JP, Bayourthe C, Auclair E and CJ Newbold** The Effects of a Probiotic Yeast on the Bacterial Diversity and Population Structure in the Rumen of Cattle. *PLoS One*. 2013;**8**.

4. **Uyeno Y, Shigemori S and T Shimosato** Effect of Probiotics/Prebiotics on Cattle Health and Productivity. *Microbes Environ.* 2015;**30**:126–132.

5. **Reis LF, Minervino AHH, Araújo CASC, Sousa RS, Oliveira FLC, Rodrigues FAML, Meira-Júnior EBS, Barrêto-Júnior RA, Mori CS and EL Ortolani** Comparison of rumen fluid pH by continuous telemetry system and bench pH meter in sheep with different ranges of ruminal pH. *Sci World J.* 2014;**2014**:1–5.

6. **Kumar M, Dutta T, Singh G and I Chaturvedi** Effect of Lactobacilli culture on the performance of pre-weaned Barbari kids. *Indian Res J Genet Biotechnol*. 2013;**5**:278–286.

7. **Moarrab A, Ghoorchi T, Ramezanpour S, Ganji F and A Koochakzadeh** Effect of Synbiotic on Performance , Intestinal Morphology , Fecal Microbial Population and Blood Metabolites of Suckling Lambs. *Iran J Appl Anim Sci.* 2016;**6**:621–8.

8. **Elghandour MMY, Salem AZM, Castañeda JSM, Camacho LM, Kholif AE and JCV Chagoyán** Direct-fed microbes: A tool for improving the utilization of low quality roughages in ruminants. *J Integr Agric*. 2015;**14**:526–533.

9. **Goto H, Qadis AQ, Kim YH, Ikuta K, Ichijo T and S Sato** Effects of a bacterial probiotic on ruminal pH and volatile fatty acids during subacute ruminal acidosis (SARA) in cattle. *J Vet Med Sci*. 2016;**78**:1595–1600.

10. **Kumar S, Pattanaik AK, Sharma S, Jadhav SE, Dutta N and A Kumar** Probiotic Potential of a Lactobacillus Bacterium of Canine Faecal-Origin and Its Impact on Select Gut Health Indices and Immune Response of Dogs. *Probiotics Antimicrob Proteins*. 2017;**9**:262–277.

11. **Helal FIS and KA Abdel-Rahman** Productive performance of lactating ewes fed diets supplementing with dry yeast and/or bentonite as feed additives. *World J. Agric. Sci.* 2010. p. 489–498.

12. **Soren NM, Tripathi MK, Bhatt RS and SA Karim** Effect of yeast supplementation on the growth performance of Malpura lambs. *Trop Anim Health Prod.* 2012;**45**:547–554.

13. **Raeth-Knight ML, Linn JG and HG Jung** Effect of Direct-Fed Microbials on Performance, Diet Digestibility, and Rumen Characteristics of Holstein Dairy Cows. *J Dairy Sci*. 2007;**90**:1802–1809.

14. **Malik R and S Bandla** Effect of source and dose of probiotics and exogenous fibrolytic enzymes (EFE) on intake, feed efficiency, and growth of male buffalo (Bubalus bubalis) calves. *Trop Anim Health Prod*. 2010;**42**:1263–1269.

15. **Özsoy B, Yalçin S, Erdoğan Z, CantekİN Z and T Aksu** Effects of dietary live yeast culture on fattening performance on some blood and rumen fluid parameters in goats. *Rev Med Vet.* 2013;**164**:263–271.

16. **Shen JS, Chai Z, Song LJ, Liu JX and YM Wu** Insertion depth of oral stomach tubes may affect the fermentation parameters of ruminal fluid collected in dairy cows1. *J Dairy Sci.* 2012;**95**:5978–5984.

17. **Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang K Bin, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik A V, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R and JG Caporaso** Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol.* 2019;**37**:852–857.

18. **Fon FN and IV Nsahlai** Effect of direct-fed microbial consortia on ruminal fermentation of maize stover in sheep. *Small Rumin Res.* 2013;**111**:71–75.

19. **Qadis AQ, Goya S, Ikuta K, Yatsu M, Kimura A, Nakanishi S and S Sato** Effects of a Bacteria-Based Probiotic on Ruminal pH, Volatile Fatty Acids and Bacterial Flora of Holstein Calves. *J Vet Med Sci.* 2014;**76:**877–885.

20. **Roodposhti PM and N Dabiri** Effects of probiotic and prebiotic on average daily gain, fecal shedding of Escherichia coli, and immune system status in newborn female calves. *Asian-Australasian J Anim Sci.* 2012;**25**:1255–1261.

21. **Kumar M, Dutta TK and I Chaturvedi** Effect of probiotic supplementation with different roughage: concentrate rations on in vitro rumen fermentation metabolites. *J Sci.* 2016;**2**:30–39.

22. **Dlamini Z, Langa S and OA Aiyegoro** Effects of probiotics on growth performance , blood parameters , and antibody stimulation in piglets. *South African J Anim Sci.* 2017;**47**:766–75.

23. **Ptáček M, Ducháček J, Stádník L, Beran J and D Němečková** Influence of selected factors on growth performance of Suffolk lambs and their crossbreds. *J Cent Eur Agric*. 2015;**16**:188–96.

24. **Wilkes MJ, Hynd PI and WS Pitchford** Damara sheep have higher digestible energy intake than Merino sheep when fed low-quality or high-quality feed. *Anim Prod Sci.* 2012;**52**:30–4.

25. **Silva AMA, Sobrinho AGS, Trindade IACM, Resende KT and OA Bakke** Food intake and digestive efficiency in temperate wool and tropic semi-arid hair lambs fed different concentrate : forage ratio diets. *Small Rumin Res.* 2004;**55**:107–15.

26. **de Souza-Rodrigues RT, Chizzotti ML, Martins SR, Queiroz MAÁ and KC Busato** Digestibility , ingestive behaviour and performance of non-descript breed hair lambs of different sexual classes subjected to feed restriction. *J Anim Feed Sci.* 2014;**23**:117–23.

27. **Kashani SM and M Bahari** The Effect of Sex and Weaning Age on Growth Performance of First Generation Lambs Derived from Crossing ½Romanov and Zel. *J Anim Res Nutr.* 2017;**2**:3–8.

28. **Siqueira ER de, Simões CD and S Fernandes** Efeito do sexo e do peso ao abate sobre a produção de carne de cordeiro. I. Velocidade de crescimento, caracteres quantitativos da carcaça, pH da carne e resultado econômico. *Rev Bras Zootec*. 2001;**30**:844–8.

29. **Joo ST, Kim GD, Hwang YH and YC Ryu** Control of fresh meat quality through manipulation of muscle fiber characteristics. *Meat Sci.* 2013;**95**:828–36.

30. **Chen L, Ren A, Zhou C and Z Tan** Effects of Lactobacillus acidophilus supplementation for improving in vitro rumen fermentation characteristics of cereal straws. *Ital J Anim Sci.* 2017;**16**:52–60.

31. **Castillo-Gonzalez A, Burrola-Barraza M, Dominguez-Viveros J and A Chavez-Martinez** Rumen microorganisms and fermentation. *Arch Med Vet.* 2014;**46:**349–61.

32. **Kišidayová S, Lauková A and D Jalč** Comparison of nisin and monensin effects on ciliate and selected bacterial populations in artificial rumen. *Folia Microbiol*. 2009;**54**:527–32.

33. **Belanche A, Doreau M, Edwards JE, Moorby JM, Pinloche E and CJ Newbold** Shifts in the Rumen Microbiota Due to the Type of Carbohydrate and Level of Protein Ingested by Dairy Cattle Are Associated with Changes in Rumen Fermentation. *J Nutr.* 2012;**142**:1684–92.

34. **Nocek JE, Kautz WP, Leedle JAZ and JG Allman** Ruminal supplementation of direct-fed microbials on diurnal pH variation and in situ digestion in dairy cattle. *J Dairy Sci*. 2002;**85**:429–33.

35. **Jewell KA, Mccormick CA, Odt CL, Weimer PJ and G Suen** Ruminal Bacterial Community Composition in Dairy Cows Is Dynamic over the Course of Two Lactations and Correlates with Feed Efficiency. *Appl Environ Microbiol*. 2015;**81**:4697–710.

36. **Mao S, Zhang M, Liu J and W Zhu** Characterising the bacterial microbiota across the gastrointestinal tracts of dairy cattle: Membership and potential function. *Sci Rep.* 2015;**5**:1–14.

37. **Macfarlane S and GT Macfarlane** Regulation of short-chain fatty acid production. *Proc Nutr Soc.* 2003;**62**:67–72.

38. **Myer PR** Bovine Genome-Microbiome Interactions: Metagenomic Frontier for the Selection of Efficient Productivity in Cattle Systems. *mSystems.* 2019;**4**:1–4.

39. **Cao Y, Cai Y, Takahashi T, Yoshida N, Tohno M, Uegaki R, Nonaka K and F Terada** Effect of lactic acid bacteria inoculant and beet pulp addition on fermentation characteristics and in vitro ruminal digestion of vegetable residue silage. *J Dairy Sci.* 2011;**94**:3902–12.

40. **Khafipour E, Li S, Plaizier JC and DO Krause** Rumen microbiome composition determined using two nutritional models of subacute ruminal acidosis. *Appl Environ Microbiol.* 2009;**75**:7115–24.

41. **Mani S, Aiyegoro OA and MA Adeleke** Characterization of Rumen Microbiota of two Sheep Breeds Supplemented With Direct-Fed Lactic Acid Bacteria. *Front Vet Sci.* 2021;**7**:570074. https://doi.org/10.3389/fvets.2020.570074