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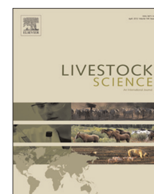

Production attributes of Merino sheep genetically divergent for wool growth are reflected in differing rumen microbiotas

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ABSTRACT

Divergent genetic selection for wool growth as a single trait has led to major changes in sheep physiology and metabolism, including variations in rumen microbial protein production and uptake of α-amino nitrogen in portal blood. This study was conducted to determine if sheep with different genetic merit for wool growth exhibit distinct rumen bacterial diversity. Eighteen Merino wethers were separated into groups of contrasting genetic merit for clean fleece weight (CFW; low: WG− and high: WG+) and fed a blend of oaten and lucerne chaff diet at two levels of intake (LOI; 1 or 1.5 times maintenance energy requirements) for two seven-week periods in a crossover design. Bacterial diversity in rumen fluid collected by esophageal intubation was characterized using 454 amplicon pyrosequencing of the V3/V4 regions of the 16S rRNA gene. Bacterial diversity estimated by Phylogenetic distance, Chao1 and observed species did not differ significantly with CFW or LOI; however, the Shannon diversity index differed ($P=0.04$) between WG+ (7.67) and WG− sheep (8.02). WG+ animals had a higher ($P=0.03$) proportion of Bacteroidetes (71.9% vs 66.5%) and a lower ($P=0.04$) proportion of Firmicutes (26.6% vs 31.6%) than WG− animals. Twenty-four specific operational taxonomic units (OTUs), belonging to the Firmicutes and Bacteroidetes phyla, were shared among all the samples, whereas specific OTUs varied significantly in presence/abundance ($P<0.05$) between wool genotypes and 50 varied ($P<0.05$) with LOI. It appears that genetic selection for fleece weight is associated with differences in rumen bacterial diversity that persist across different feeding levels. Moderate correlations between seven continuous traits, such as methane production or microbial protein production, and the presence and abundance of 17 OTUs were found, indicating scope for targeted modification of the microbiome to improve the energetic efficiency of rumen microbial synthesis and reduce the greenhouse gas footprint of ruminants.

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1. Introduction

Continuous divergent genetic selection for wool growth leads to major changes in the periphery of the animal at the skin and follicle level; (Williams and Winston, 1987;

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Nancarrow et al., 1998; Li et al., 2006) and in the digesta flow from the rumen and protein metabolism. Sheep with greater wool growth potential may exhibit shorter mean retention time of digesta (Smuts et al., 1995), greater efficiencies of microbial protein production in the rumen (Kahn, 1996), uptake of α-amino nitrogen in portal blood (Lush et al., 1991) and retention of ingested protein in wool and tissue (Li et al., 2008). However, De Barbieri et al. (2014, 2015) studying sheep with contrasting genetic merit for wool growth found no differences in rumen fermentation parameters or digesta kinetics. Kahn (1996) suggested that genotype differences in microbial protein production in the rumen may be related to differences in bacterial turnover and protozoal populations of the rumen. Differences in digesta flow rate can alter bacterial residence time and turnover in the rumen (Cottle, 2010). In parallel, it has been reported that variations in ruminal microbial communities may occur between cattle with different feed efficiencies (Guan et al., 2008; Carberry et al., 2012; Hernandez-Sanabria et al., 2012) and with differences in animal genetics (Hernandez-Sanabria et al., 2013) and these variations are moderated by the amount and type of feed.

We reasoned that if sheep divergently selected for wool growth exhibit variations in efficiency of microbial protein production and cattle with different genetics or feed efficiency displayed variations in microbial ecology, then sheep with contrasting estimated breeding values for fleece weight may harbor different bacterial communities in the rumen that may contribute to differences in ruminal and animal performance.

2. Materials and methods

All research work was conducted in accordance with the University of New England Animal Ethics Committee (AEC approval no.12/045).

2.1. Animals and treatments

Twenty 33 month-old Australian Merino wethers were selected from a group of 83 at Trangie Agricultural Research Center (Trangie, NSW, Australia). Selection was based on the following information: (a) estimated breeding values (EBV) for yearling clean fleece weight, yearling fiber diameter and yearling live weight (MerinoSelect ASBV, www.sheepgenetics.org.au); (b) phenotypic information on greasy fleece weight, live weight and fiber diameter at shearing from three previous annual shearings; and (c) pedigree information (sires and dams). From these sheep, two sub-groups with different average EBVs for clean fleece weight (wool genotype, CFW), and similar EBVs for live weight and fiber diameter were established. The average CFW EBV for low (WG−) and high (WG+) wool genotype groups was 6.2% and 28.1%, respectively. Across all sheep, average fiber diameter EBV and live weight EBV were −2.0 μm and 2.9 kg, respectively, with no differences in these traits between CFW groups. An incomplete cross-over design assessing the two factors (wool genotype and level of intake) was implemented. Two levels of feed intake were evaluated, being 1.0 and 1.5 times maintenance energy requirements (1.0 × M and 1.5 × M, respectively). The levels of intake (LOI) were calculated for wool-sheep housed indoors using the average live weight of the 20 animals and were fixed for the experimental period (SheepExplorer, 2003). The trial was divided into two periods of seven weeks each. In the first period half ($n=5$) of the animals of each wool genotype were chosen at random to be fed at 1.0 × M and the other half ($n=5$) were assigned to 1.5 × M. In the second period, the LOI of each animal was swapped. Each period was comprised of an initial four weeks for diet acclimatization and three subsequent weeks when measurements were performed. Animals were held in individual metabolism cages with fresh water always available and were fed once a day in the morning (10:00 h) with a blend of chaffed oaten and lucerne hay (Manuka Feeds Pty Ltd; CP, 14.5%; ME, 9.1 MJ/kg DM; DM, 89.5%; and DM digestibility, 61%). Information of animal production, rumen fermentation, gut anatomy and digesta kinetics has been reported (De Barbieri et al., 2014, 2015).

2.2. Rumen fluid collection, DNA extraction and quantification

Rumen fluid samples were collected using an esophageal tube with a perforated brass tip with openings of 1.25 mm, at the end of each measurement period four hours after feeding and stored at −80 °C. While this excluded large particles from the sample, it ensured microbes attached to small particles were sampled, with no filtering to remove these small-particle associated bacteria prior to DNA extraction. After the experimental period, rumen fluid samples were defrosted at room temperature, gently mixed and 1 mL was transferred to a new 1.5 mL micro-centrifuge tube. The sample was centrifuged at 17,000 g for 10 min and the supernatant was discarded. DNA extraction was performed following the repeated bead beating+column (RBB+C) method described by Yu and Forster (2005). To check the DNA quality, 5 μL aliquots were analyzed on 1% agarose gel in 1 × TAE (Tris-acetate, 0.04 mol/L; EDTA, 0.001 mol/L) containing 1 × gel red (Jomar Scientific, Australia) run at 95 V for 45 min with a 1 kb ladder (GeneRuler 1 kb DNA ladder Fermentas Life Sciences, Thermo Fisher Scientific) as a reference. DNA concentration and purity was evaluated using NanoDrop™ 8000 (UV–vis spectrophotometer, Thermoscientific®, Australia).

2.3. PCR and 454 – amplicon pyrosequencing

To characterize the bacterial population in rumen samples of each animal, purified DNA (10 ng/μL) was used as a template for the preparation of barcoded amplicon libraries for amplicon pyrosequencing by 454 GS-FLX Titanium (Macrogen Inc., Seoul, Korea). The V3 and V4 regions of the 16S rRNA gene were amplified by PCR using a Eppendorf Mastercycler® (Eppendorf, Australia) in a 50 μL reaction mixture containing; 2 μL DNA template (10 ng/μL), 0.5 μL Phusion™ DNA polymerase (2 UI/μL, Finnzymes, Australia), 1.25 μL primer A (341F MID-10 μM;

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