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Programming rumen bacterial communities in newborn Merino lambs

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ABSTRACT

Establishment of the rumen microbiome can be affected by both early-life dietary measures and rumen microbial inoculation. This study used a 2 × 3 factorial design to evaluate the effects of inclusion of dietary fat type and the effects of rumen inoculum from different sources on ruminal bacterial communities present in early stages of the lambs' life. Two different diets were fed *ad libitum* to 36 pregnant ewes (and their lambs) from 1 month pre-lambing until weaning. Diets consisted of chaffed lucerne and cereal hay and 4% molasses, with either 4% distilled coconut oil (CO) provided as a source of rumen-active fat or 4% Megalac® provided as a source of rumen-protected fat (PF). One of three inoculums was introduced orally to all lambs, being either (1) rumen fluid from donor ewes fed the PF diet; (2) rumen fluid from donor ewes fed CO; or (3) a control treatment of MilliQ-water. After weaning at 3 months of age, each of the six lamb treatment groups were grazed in spatially separated paddocks. Rumen bacterial populations of ewes and lambs were characterised using 454 amplicon pyrosequencing of the V3/V4 regions of the 16S rRNA gene. Species richness and biodiversity of the bacterial communities were found to be affected by the diet in ewes and lambs and by inoculation treatment of the lambs. Principal coordinate analysis and analysis of similarity (ANOSIM) showed between diet differences in bacterial community groups existed in ewes and differential bacterial clusters occurred in lambs due to both diet and neonatal inoculation. Diet and rumen inoculation acted together to clearly differentiate the bacterial communities through to weaning, however the microbiome effects of these initial early life interventions diminished with time so that rumen bacterial communities showed greater similarity 2 months after weaning. These results demonstrate that ruminal bacterial communities of newborn lambs can be altered by modifying the diet of their mothers. Moreover, the rumen microbiome of lambs can be changed by diet while they are suckling or by inoculating their rumen, and resulting changes in the rumen bacterial microbiome can persist beyond weaning.

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1. Introduction

Manipulation of the rumen ecosystem can modify the relationship between the feed consumed, livestock performance and the overall environmental impact of livestock production (Calsamiglia et al., 2010). By developing strategies that target rumen nitrogen metabolism, fiber digestion, and methane production, the symbiotic relationship between the rumen microbiome and the ruminant host may be modified to improve ruminant host-beneficial fermentation processes and to minimize inefficient or non-beneficial fermentation processes (Nagaraja, 2012). Changing the symbiotic association to reduce specific energy and protein losses (methane and ammonia respectively; Yañez-Ruiz et al., 2010) may favorably affect the environment and livestock production efficiency.

The rumen at birth is not fully developed (Hobson, 1997) and its physical and metabolic development is favored by the intake of solid feed and the establishment of ruminal fermentation, which in turn results in a positive cause-consequence cycle which further stimulates the ingestion of solid feed (Baldwin et al., 2004; Bomba et al., 2005). The establishment of rumen fermentation depends on the inoculation and establishment of microbes into the rumen. Early investigations (Hobson, 1997; Dehority, 2003) established that the first inoculation of the rumen is with microbes from the mother of the newborn, other animals in the flock, and the surroundings. Other studies indicate (Fonty et al., 1987; Jami et al., 2013; Abecia et al., 2014a) that the rumen microbiome is established shortly after birth and before the rumen starts to be responsible for most of the feed digestion and fermentation. The rumen microbiome established early in ruminant life however, may change over time (Li et al., 2012; Jami et al., 2013) and can be affected by early-life microbial intervention (Yañez-Ruiz et al., 2010; Leahy et al., 2013; Abecia et al., 2014b). Based on previous research, inoculation of the rumen with complex microflora and the inclusion of non-protected fat in the diet of newborn lambs would be considered effective mechanisms to modify the rumen microbiome (Pounden and Hibbs, 1948; Machmüller, 2006; Patra, 2014; Zhong et al., 2014).

In the context of these previous findings, we reasoned that the rumen microbiome of lambs prior to weaning could be altered by post-natal intervention. This article evaluates how the inclusion of rumen-active fat in the diet of ewes during late pregnancy and of their lambs, together with artificial rumen inoculation of newborn lambs with fresh rumen fluid, affect the composition of ruminal bacterial communities over the first 5 months of the lamb's life.

2. Materials and methods

All research work was conducted in accordance with the University of New England Animal Ethics Committee (AEC No. 13/108) and CSIRO Animal Ethics Committee (No. 13/28).

2.1. Animals, treatments, and experimental design

Three hundred Australian Merino hogget ewes of the Elite commercial flock of FD McMaster Laboratory (CSIRO) were paddock-mated in May, shorn in mid-pregnancy, and scanned for pregnancy by ultrasound in August. From this group, 36 ewes with singleton pregnancies and eight non-pregnant ewes were selected. Their mean body weight was 39.1 ± 2.9 kg and mean condition score (Russel et al., 1969) was 2.9 ± 0.3 units. The average expected date for lambing based on ultrasound was 21st of October ±3 days. The experiment lasted 6 months, starting (17/09/2013) 1 month before the first date of lambing (16/10/2013), continued for 5 months after lambing (17/03/2014), and had 4 stages (Fig. 1). Stage one was from 1 month pre-lambing until lambing (involved only ewes), stage two was a 12-week period from birth until weaning of lambs (ewes and lambs; inoculations done during this period), stage three was for 2 weeks after weaning (lambs only; methane measurements), and then stage four lasted 7 weeks (lambs only). Stages one, two, and three were indoors (individual pens in stage one and three, and mother-lamb in combined pens in stage two), while stage four was conducted outdoors (grazing paddocks in treatment groups).

Table 1
Chemical composition of chaff-based diets with rumen protected fat (PF; Megalac®) or with coconut oil distillate (CO).

Parameter	Diet	
	PF	CO
Neutral detergent fiber (%)	50.3	50.3
Acid detergent fiber (%)	31.3	32.0
Crude protein (%)	13.0	13.1
Dry matter digestibility (%)	56.5	56.8
Organic matter (%)	90.5	90.8
Metabolisable energy (MJ/kgDM)	9.6	9.8
Dry matter (%)	89.6	89.2

The effect of two factors on lamb rumen bacteria communities was studied; (1) diet and (2) artificial inoculation of the lamb with rumen fluid. Two different diets were fed to both ewes and lambs from 1 month pre-lambing until 2 weeks post-weaning (Fig. 1). The diets consisted of 92% chopped and blended lucerne and cereal hay (Manuka Feeds Pty. Ltd., Quirindi, NSW, Australia), 4% Molasses and 4% of a fat source, all on dry matter (DM) basis. Fat was provided either as distilled coconut oil (CO) or as a rumen-protected fat (PF) (Megalac®, Church and Dwight Co., Inc., Exing, NJ, US)). PF was palm oil saponified with calcium. Formulation of the two mixed rations was based on creating iso-nitrogenous and iso-energetic diets. The most important variation between diets was the level of rumen protection of the lipids (rumen-active *versus* rumen-protected). Each diet was given *ad libitum* to half of the animals (half of pregnant ewes and subsequently their lambs) during all indoor stages from 1 month pre-lambing until 2 weeks post-weaning. Diets were prepared twice per week with subsamples dried to constant weight at 60 °C for dry matter (DM) calculations and nutritive value analysis. Analyses were performed by the Feed Quality Service (NSW DPI, Wagga Wagga, NSW, Australia) as described by Australian Fodder Industry Association (2014) including: CP – wet chemistry AOAC method 990.03 (AOAC, 1995); ADF and NDF – AFIA methods 1.8, 1.9; digestibility – pepsin-cellulase method AFIA method 1.7R; ME – estimated from digestibility AFIA method 2.2R (Table 1). Ewes were adjusted to the diets by incorporating a 1% point increase in coconut oil or Megalac® inclusion every 3 days from 0% until 4% inclusion was reached.

For the rumen fluid inoculation component of the experiment, non-pregnant ewes adjusted to each diet over a 35-day period were used as donors of fresh rumen fluid. Two-thirds of the lambs (24 individuals) were inoculated with fresh rumen fluid from these donor ewes by a structured inoculation program (Fig. 1). One third of lambs on each diet (12 individuals) was inoculated with rumen fluid from donor ewes consuming PF (group designated InPF), a second third of the lambs (12 individuals) were inoculated with rumen fluid from donor ewes eating the diet CO (InCO) and the remaining third (12 individuals) were inoculated with MilliQ-water (InWa). As a result of the factorial combination of two diets (CO and PF) and three different rumen inoculums (InPF, InCO, InWa) a total of six treatment groups were evaluated with six animals per treatment and these were managed in two blocks.

During the indoor stages of the experiment (Fig. 1), the animal house was subdivided into two blocks of 18 pens (each pen 3.75 m^2). Maternal ewes were penned individually on wood-chip bedding before lambing, gave birth in the pen, and remained in that pen until weaning. In order to avoid microbial cross-contamination between animals from different