

Chapter One

1 Introduction

1.1 General introduction

Improving the efficiency of feed conversion into animal products and reducing the environmental impact of livestock productions are increasingly important challenges in animal nutrition (Hegarty, 2012; Herrero and Thornton, 2013; Morgavi et al., 2013). Changing the yield of animal product (i.e. milk, wool and meat) arising from feed consumed can be achieved by dietary change (Leng, 1990) or by selecting animals for either lower residual feed intake (Waghorn and Hegarty, 2011) or higher animal growth efficiency (Oddy, 1999) or by manipulation of rumen fermentation (Calsamiglia et al, 2010; Poppi and McLennan, 2010).

Nagaraja (2012) indicated that the objectives of rumen manipulation are to improve host-beneficial rumen fermentation processes and minimize or alter inefficient or non-beneficial fermentation processes. Specifically, this author identified that modifications of nitrogen metabolism, microbial fiber-degradation and methane production processes would be relevant targets in manipulating rumen fermentation. Nagaraja (2012) noted that modification of rumen microbial fermentation process and thus animal performance and efficiency of production can be performed at three levels; being animal, feed and microbiota. These three levels of intervention are in agreement with the three components that form the rumen ecosystem defined by Mackie et al. (2002), being: i) factors related with the host, ii) factors related with the diet and iii) the microbiome.

In the case of nitrogen metabolism in the rumen, Calsamiglia et al. (2010) in their study of strategies to modify nitrogen use by ruminants noted that efficiency of nitrogen utilization is low and variable in comparison with other livestock animals. They also indicated that key factors to optimize the efficiency of nitrogen utilization in the rumen are the manipulation of the rumen microbiome and the efficiency of nitrogen (N) captured in the rumen by bacteria. Most of the metabolisable protein for ruminants comes from the microbial protein produced in the rumen (Poppi and McLennan, 2010). Poppi and McLennan (2010) indicated that improvements in the knowledge of rumen microbiome will help in improving the efficiency of microbial protein production and will be of benefit to the livestock industries. Successful

examples on how modification of rumen microbial communities can alter nitrogen metabolism in the rumen exist (Miller et al., 1995; 1996).

In the case of methane production, Beauchemin et al. (2008) highlighted that methane is an important loss of dietary energy. Consequently reducing methane production may potentially improve energy capture and feed-use efficiency (Trei et al., 1972). Morgavi et al. (2010) in their study of microbial ecosystems and methanogenesis provided evidence that altering the rumen microbiome can affect methane production. They observed that the protozoa population, which is much affected by diet, explained 47% of the variations in methane emissions.

In accordance with microbial fiber digestion process enumerated by Nagaraja (2012), recent work of Hegarty (2012) has concluded that the success of ruminant livestock production in the future depends on technologies to maximize animal utilization of cellulosic plant by-products. Precedents of an altered rumen microbiome leading to a modified rumen fermentation exist, for instance by inclusion of dietary fat it is possible to affect dry matter (DM) and neutral detergent fiber (NDF) digestibility and this effect has been partially explained by the effect of fat on protozoa and bacterial communities (Patra, 2014).

From the above examples, it is apparent that the three rumen fermentation processes (nitrogen metabolism, methane production and microbial fiber-degradation) noted by Nagaraja (2012) are all able to be affected favourably by altering the rumen microbiome and the rumen microbiome itself is affected by both the diet and the host. The study of the association between rumen microbiome and productivity of Merino sheep and strategies for manipulating this association form the substance of this thesis.

1.2 Literature review

In accordance with the approach of Mackie et al. (2002) and Nagaraja (2012) this section of the thesis consists of two subsections, the first one is focused on the development of the rumen ecosystem and its relation with the three components forming points of intervention: microbiota, feed and host. The second section discusses in more detail the implications of different host genetics on animal physiology, metabolism and performance in Merino sheep.

1.2.1 Association of the rumen ecosystem with host, feed and microbiota

1.2.1.1 Development of the rumen environment

The rumen of ruminants at birth is not fully developed, either physically (keratinization, relative size, papillary growth, musculature) or metabolically (absorption and metabolism of nutrients). Its development is favoured by the intake of solid feed and establishment of the ruminal fermentation, which in a positive cause-consequence cycle will stimulate the ingestion of solid feed (Hobson, 1997; Baldwin et al., 2004; Bomba et al., 2005). The presence of solid feed in the rumen is responsible for increases in rumen weight and muscularity, while ruminal fermentation is mostly responsible for papillary density and height development (Baldwin et al., 2004). The establishment of rumen fermentation depends on the inoculation or establishment of microbes into the rumen. Hobson, (1997) and Dehority (2003) noted that the first inoculation of the rumen is with microbes from the mother of the newborn, other animals in the flock and surroundings (e.g. saliva, faeces, pasture).

The rumen of a grazing lamb is totally developed by 8 weeks of age (Wardrop and Coombe, 1961). The development process (function and physical) has 3 stages: non-ruminant (0-3 weeks of age), transition (3-8 weeks of age) and adult (8 weeks of age). The non-ruminant stage is almost fixed but could be extended by avoiding forage feed in the lamb diet and during the last development stage the rumen works as in an adult animal, therefore, they indicated that it is the transition phase that is the most variable. The type of diet and the rumen microbial inoculation from adults have been reported to be the most important factors affecting this stage. The volume of the rumen contents, rumen volatile fatty acid (VFA) concentrations, ammonia and roughage DM intake, relative to live weight, in that study were constant from 8 weeks of age onwards. It has been also mentioned (Cottle, 2010), that the rumen of lambs on pasture is functional as early as 3 weeks of age but while the adult proportions are reached at 4-6 months, the rumen is in general fully functional at 6-8 weeks of age.

The host provides a stable environment and feed for growth and development of a microbial population in the reticulum and rumen. The later provide a pre-gastric digestion service to the host, in a symbiotic relationship between the ruminant and the microbiome (Dehority, 2003). The relationship is also called mutualism, where the microorganisms digest

and ferment the feed provided by the host, forming short chain fatty acids, carbon dioxide and methane and providing proteins, vitamins and other essential nutrients (Mackie et al., 2002), while the host removes the fatty acids by absorption and the gases by eructation.

1.2.1.2 Development of the rumen microbiome

The microbes that are common residents in the rumen belong to three domains of life Eubacteria (bacteria), Archaea (methanogens), Eukarya (protozoa and fungi) (Mackie et al., 2000; Mackie et al., 2002; McSweeney and Mackie, 2012; Nagaraja, 2012). In addition, bacteriophages (bacterial virus) are also present in the rumen. It has been indicated that bacteria are the largest member of the rumen microbiome, being 10^{11} cells per ml; and then ciliate protozoa, $10^4 - 10^6$ cells per ml; flagellate protozoa, $<10^3$ cells per ml; fungi 10^3-10^5 zoospores per ml (or 10% of microbial mass); bacteriophage particles, $10^7 - 10^{11}$ particles per ml; archaea, 0.6% of total rRNA in the rumen (Lin et al., 1997; Mackie et al., 2000; Nagaraja, 2012; Krause et al., 2013).

Ruminal bacteria are the largest population in the rumen microbiome and are able to convert feed into nutritionally valuable products (mostly short-chain fatty acids and microbial proteins) for the host (Hobson, 1997; Mackie et al., 2002). This conversion is based on the hydrolysis of feed polymers to short oligomers and monomers which are transported into the microorganisms and fermented, having as a consequence the synthesis of bacterial cells and production of end products (VFA, CO_2 and H_2) (McSweeney and Mackie, 2012). Kim et al. (2011) have found that 19 bacterial phyla may be present in the rumen, with Firmicutes, Bacteroidetes, and Proteobacteria the most prevalent; representing 57.8%, 26.7%, and 6.9% of the total sequences in their work respectively. These authors calculated that the rumen may harbour 7400 species of bacteria. Class Clostridia represented 90.6% of the total Firmicutes, while Bacilli, Erysipelotrichi and Unclassified Firmicutes accounted for the rest of the sequences. Inside the class Clostridia, the families Lachnospiraceae (23.8%), Ruminococcaceae (25.8%) and Veillonellaceae (7.7%) were the most frequent. On the other hand, the largest class within the phylum Bacteroidetes was Bacteroidia, followed by Sphingobacteria and Unclassified Bacteroidetes. The most prevalent Bacteroidetes genus was

Prevotella, accounting for almost 42% of this phylum sequences and 11.1% of the total sequences in this study.

Methanogenic archaea, which can occur associated with the rumen liquid, solid digesta, attached to protozoa and on rumen epithelium, use the final products of the fermentation of other microorganisms within the rumen as substrates (Morgavi et al., 2010). Methanogens are partially responsible firstly for the regulation of the partial pressure of hydrogen in the rumen and secondly for methane production by using hydrogen and mainly CO₂ as the carbon source (although other compounds such as acetate and ethanol can be used). The phylum Euryarchaeota accounted for 99.6% of total sequences reported by Kim et al. (2011), while the phylum Crenarchaeota was represented for only 11 sequences of 3516. This study estimated that at species level the rumen may contain 1400 species of archaea. Within the phylum Euryarchaeota, Methanobacteria, Methanomicrobia, and Thermoplasmata were the most prevalent classes. While at genera level the most frequent are Methanobrevibacter, Methanomicrobium, and RCC.

As described by Krause et al. (2013), fungi were not recognized as part of the rumen microbiome until approximately 35 years ago. Fungi can account for about 10% of the microbial mass (Nagaraja, 2012) divided in 6 genera (Anaeromyces, Caecomyces, Cyllamyces, Neocallimastix, Orpinomyces, and Pyromyces; McSweeney and Mackie, 2012). Their role in ruminal digestion is not yet totally understood. However, ruminal fungi include fibrolytic and proteolytic activities in their function, and zoosporic fungi help plant bacterial colonization and degradation by penetrating and disrupting plant fragments (Krause et al., 2013; McSweeney and Mackie, 2012).

McAllister and Newbold (2008) and Mackie et al. (2002) indicated that bacteriophages (microbial viruses) are obligate pathogens and they can lyse (lytic and temperate types) the host or integrate their DNA into the bacterial chromosome (temperate types). While it has been suggested that they are involved in the homeostasis of microbial populations (Nagaraja, 2012) or in the efficiency of feed utilization (Mackie et al., 2002), further evidence to fully understand their role in the rumen ecosystem is still required.

Protozoa are one of the members of the rumen microbiome that have been extensively studied over the last 50 years. As mentioned, ciliate and flagellated protozoa are present in the rumen, being the former more abundant (Dehority, 2003). Rumen ciliate protozoa belongs to the phylum Ciliophora and to two orders (Vestibuliferida and Entodiniomorpha), with more than 25 genera present. Veira (1986) specified that rumen protozoa are responsible of stable rumen fermentation and can alter ammonia concentration, bacteria population, protein energy ratio, liquid volume and turnover rate of ruminal contents. Bird and Leng (1984) and Hegarty et al. (2008) have indicated that total elimination of rumen protozoa can positively affect animal performance, for instance they obtained increases in live-weight gain and importantly increases in wool growth. Moreover, Morgavi et al. (2010) in their analysis of the relationship between the rumen microbial ecosystem and methanogenesis have found that the number of protozoa in the rumen explained 47% of the variability in methane emissions.

Fonty et al. (1984) working with newborn lambs showed that strict anaerobic bacteria are established in the rumen and dominate as early as 2 days after birth. Cellulolytic bacteria appear in the fourth day of life and protozoa establish at 2 weeks of age, however their populations are not stable until four months. Ciliate protozoa have been found at 9 days after birth, being frequently detected at 2 weeks and were present in all animals by 21 days in the work of Eadie (1962). In addition, the rumen of the newborn is also characterized by the presence of populations of methanogens and anaerobic fungi during the first week of life (Fonty et al., 1987). Facultative anaerobic bacteria are the dominant microbes in the first two days, however after the first 48 h they gradually decreased and strictly anaerobic bacteria establish within the first 72 h. They also found that methanogens could be present 30 h after birth and their population is close to that in an adult at two weeks and their establishment occurs prior to the establishment of cellulolytic bacteria. Although, methanogens have been reported as early as 17 h after birth in the rumen of lambs (Gagen et al., 2012).

This process has been summarized by McSweeney and Mackie (2012) in their review, they have noted that the rumen ecology of the growing ruminant is subjected to strong changes during the first weeks of life (from birth to 7 weeks), where the microbial colonization will have the following pattern after birth: methanogens establishing in the first 17 hours, facultative

and strict anaerobic bacteria in the first two days, fibrolytic bacteria during the first week, fungi during the first 7-10 days and finally protozoa between week 3 and 7. These authors have indicated two key points of this process that should be taken into account in studies of microbial ecology in ruminants: a) methanogens colonize the rumen early in life and remain stable during rumen development and life, b) stabilization of bacterial populations is an intricate process and depends on age, physiological state, host genetics and feed composition (Guan et al., 2008; Hernandez-Sanabria et al., 2012; 2013; Jami et al., 2013)

It has been apparent (Fonty et al., 1987; Jami et al., 2013) that the rumen microbiome is established soon after birth and before the rumen starts to be fully functional. However the rumen microbiome established early in ruminant life could change over time. Studying pre-ruminant calves at different age (14 versus 42 days after birth) Li et al. (2012) indicated that animals at different age presented differences at genus level in their ruminal bacterial composition, although they presented a stable community function and metabolic potentials. Furthermore, Jami et al. (2013) studied the ruminal bacteria of five age groups of cattle (from 1 day to 2 years-old), and have shown that each group has its own characteristic microbiota.

Zilber-Rosenberg and Rosenberg (2008) have suggested in their theory of evolution that the host and its microbiota (the holobiont) might be considered as a unit of selection in evolution, that acts in consortium with all the genetic information of the microbes and the host. This particular association between an animal and its microbiota acting in a symbiotic way, has also been called a 'superorganism' (Morgavi, et al., 2013). Adaptation and evolution of the holobiont can be positively affected by the rapid variations that the microbiota can provide to both entities. Zilber-Rosenberg and Rosenberg (2008) acknowledged that genetic variation plays a key role in evolution, and in holobionts genetic variation can be provided by the genome of the host or of the microbiota and by changes in the genome of both entities. The genome of the microorganisms can be affected (Zilber-Rosenberg and Rosenberg, 2008) during: a) reproduction, chromosome rearrangements, mutation, and recombination (conjugation, transduction and DNA transformation, and gene transfer); b) microbial amplification; and c) microbial acquisition.

1.2.1.3 Dietary manipulation of the rumen microbiota

Three dietary factors are the most important in affecting or interacting with the microbial ecology, being diet composition or nutritive value, quantity or level of feed intake and supplements/additives in the feed (e.g. ionophore antibiotics, feed enzymes, live microbial feed supplements, buffering agents, and methane inhibitors) (Mackie et al., 2002).

The following paragraphs are focused on three specific examples of dietary related factors that are relevant for the thesis reported here, being: i) timing of microbial intervention, ii) inclusion of live microbial feed supplements in the rumen, and iii) inclusion of coconut oil in the diet. In relation to the timing of intervention, Leahy et al. (2013) indicated that an intervention early in the life of the ruminant would be ideal to generate a lifetime effect on the rumen microbiome and thus on the host in terms of animal performance and environmental impact. Early-life intervention strategies have been studied with promising results.

Yañez-Ruiz et al. (2010) found changing the ratio of forage to concentrate in the diet of lambs around weaning affected the bacterial and methanogen populations in the rumen in the short-term, and effects on the structure of the bacterial community persisted in the medium-term (four months). Furthermore a recent study from the same group (Abecia et al., 2014) working with a methanogenic inhibitor (bromochloromethane, BCM) in goats (kids and does) indicates scope to modify the archaeal community of the host during the development of the rumen. By weaning and one month post-weaning (end of BCM treatment), the methanogen community could be affected, in this case by the use of BCM. Does and kids were treated or untreated with BCM (from birth to 1 month post-weaning) resulting in four treatments (2 categories * with/without BCM). The interaction found in the results led these authors to conclude that microbial intervention of the offspring could be achieved by treating the mothers even before parturition, identifying the key role of the dam microbiota on rumen colonization of the offspring.

In relation to the second factor (inclusion of live microbial feed supplements in the rumen), it has been suggested that inoculation of the rumen can improve or accelerate rumen function, rumen development and the complexity of microbial ecology (Pounden and Hibbs, 1948; Fonty et al., 1983; Miller et al., 1996; Krause et al., 2003; Bomba et al., 2005), animal

health and performance (Muscato et al., 2002) and feed conversion efficiency (Zhong et al., 2014).

Pounden and Hibbs (1948) inoculated calves from day 5 to 20 (every 5 days) with fresh cuds from cows. They found that inoculation assisted the establishment of protozoa and particular groups of bacteria which they reported were associated with hay intake. Additionally, they noted that the results in the establishment of different microorganisms were associated with the diet (proportion of hay and grain) of the young calves. An example of the effect of rumen fluid inoculation on animal performance and rumen function has been reported by Miller et al. (1995; 1996). They found in a series of studies that the inoculation of sheep and domestic goats with fresh rumen fluid or even with an *in vitro* fermented inoculum (based on rumen fluid) from feral goats could improve feed intake, nitrogen retention, nitrogen digestibility and reduce live-weight loss when animals were eating a mulga diet (rich in condensed tannins).

In a different approach, Muscato et al. (2002) inoculated rumen fluid into newborn calves with the objective of evaluating the consequences of the inoculation on calf performance and health. The inoculation of the rumen of calves with rumen fluid had a positive effect on live-weight gain and on occurrence of scours (fewer scours) before weaning at 6 weeks of age. Different types of inoculums (rumen fluid cells, just rumen fluid, rumen fluid supernatant after centrifugation, autoclaved rumen fluid) and period of inoculations (5 or 42 days) were tested and indicated that rumen fluid was not acting as a “probiotic” (positive responses were found even after oxygen exposing or autoclaving) and positive responses can be achieved with short early periods of inoculation (first week of life). Specifically, fresh rumen fluid, centrifuged rumen fluid (solid and liquid) and autoclaved rumen fluid had similar effects; they indicated that the benefit of rumen fluid inoculation in this case was due by the administration of bacterial polysaccharides. Muscato et al. (2002) hypothesized that based on the antigenic properties of bacterial polysaccharides and because they were present in all the inoculums, these compounds positive influenced the immune system of the calves.

Krause et al. (2003) in their work about the opportunities to improve fiber degradation in the rumen have reviewed the research about the potential effects of rumen inoculation on improving the digestion of fibre. They concluded that rumen function is benefited by

inoculation, indicating that rumen inoculation positively affects the transition of animals between diets (forage to grain), helps an early adaptation of calves to a roughage diet, improves the health and animal performance on newborn calves, and could increase forage utilization (e.g. *Leucaena leucocephala*),

In a recent study, Zhong et al. (2014) evaluated the inoculation with fresh or lyophilized rumen fluid on lambs weaned at 28 days of age. The lambs were inoculated for 7 days and animal performance, intake, digestibility and rumen function were evaluated until 56 days after weaning. Fresh rumen fluid had a positive effect on DM and NDF digestibility, live-weight gain and feed conversion ratio while lyophilized rumen fluid affected feed conversion rate and fat digestibility. These authors indicated that differences found in live-weight gain and feed conversion ratio are caused by differences in presence/abundance of microorganisms and their activity between both rumen fluids. They also suggested a relationship between the type of rumen fluid and carbohydrate fermentation and fiber digestion, based on the potential activity of the microorganisms and rumen papillae development after inoculation with fresh rumen fluid, and although they specify that further research is needed to understand the effect of the oral administration of fresh rumen fluid.

The following concept of Stewart et al. (1988) should be taken into account when modification of the rumen ecosystem through inoculations is considered: “The further manipulation of functions that have already been subject to intense natural selective pressure is likely to be particularly difficult. However manipulation to control disorders, utilise exotic substrates or to detoxify toxic compounds produced by plants may be more promising”.

In relation to the third dietary factor (supplements/additives in the feed), the inclusion of coconut oil in the diet of ruminants induces major changes on the fermentation and digestion of the feed, on rumen microbial ecology and on methane production (Sutton et al., 1983; Machmüller, 2006; Liu et al., 2011; Patra and Yu, 2013; Patra, 2014).

Sutton et al. (1983) studied the effect of inclusion of rumen-active or rumen-protected linseed or coconut oils in the diet (30:70, hay:concentrate) of wethers. In a first experiment, they found that the intake of DM was not affected until 4% of rumen-active oil in the diet, when the concentration was 5% animals started to refuse feed. After 5 weeks of

supplementation, they also detected that oil had an effect on the profile of VFA and protozoa numbers in the rumen. Acetic and butyric acids were present in lower proportions while propionic was higher in animals fed the diet with 4% oil, in parallel, protozoa numbers were lower in this diet. In a second experiment, they found that oil affected intake, DM and organic matter digestion, the site of the digestion of the feed, VFA profile, number of protozoa, N leaving the rumen and efficiency of microbial protein synthesis; and those effects were reversible once the oil was removed from the diets. These authors indicated that the direct (defaunation, inhibition of cellulolytic bacteria) and indirect (coating) effects of oil on the rumen microbiome explain the major effects of oils on rumen metabolism.

In vitro evaluations of the inclusion of coconut oil (Machmüller et al., 1998) have shown that coconut oil may eliminate ciliate protozoa and suppress methane production by 43% (at 3% oil inclusion). These results were confirmed in vivo (Machmüller et al., 2000), and together with results on energy balance, indicated that medium chain fatty acids are the most beneficial supplementary form of dietary fat to reduce methane production. Subsequent, *in vivo* experiments including up to 7% of coconut oil have shown the beneficial effects of the fat inclusion without some of the adverse effects reported by Sutton et al. (1983). In addition, a specific effect of medium chain fatty acid has been found (Machmüller et al., 2003) on methanogenic communities in the rumen.

The effect of coconut oil on protozoa and methanogen populations has been further reported by Liu et al. (2011). The inclusion of 2.5% coconut oil in the diet of 16 week-old sheep did not affect live-weight gain, DM intake or feed conversion ratio, however methane production and methane yield were decreased by the inclusion of coconut oil in the diet. A decrease of methanogens and protozoa populations after inclusion of fat in the diet was explained by the toxicity of the unsaturated fatty acids on those microbes. Finally, 2.5% fat affected the population of *Fibrobacter succinogenes*, without inducing changes in fungi, *Ruminococcus flavefaciens* or *Ruminococcus albus* populations. Recently, Patra and Yu (2013) have shown that coconut oil affects cellulolytic bacteria, protozoa, and archaeal communities, in *in vitro* work. In addition to the antimethanogenic effect of fat on rumen microbiome (methanogens, fiber digesting bacteria and protozoa), Liu et al. (2011) hypothesized that the

unsaturated fatty acids of the oil are potential electron acceptors for hydrogen instead of CO₂, thereby contributing to the methane suppressing effect of coconut oil.

Finally, from his meta-analysis about the effect of dietary fat in sheep, Patra (2014) concluded that the inclusion of fat in the diet of sheep: i) reduces methane production, ii) reduces numbers and activity of methanogens and protozoa, iii) may reduce digestibility of DM and NDF and rumen fermentation, iv) may alter fibrolytic bacteria populations, v) increases propionate percentage in VFA profile, and vi) decreases butyrate percentage and ammonia concentration.

1.2.1.4 Host animal influence on gut microbial ecology

Characteristics of the host are the third component (of three components or points of intervention specified in the general introduction) intimately related with dietary factors and the microbiota in the rumen ecosystem (Mackie et al., 2002). Rawls et al. (2006) evaluated whether the gut microbiome of mice and zebrafish is primarily affected by legacy effects (microbiota available for colonization) or by gut habitat effects (host genome-encoded variations which affect the environment the microbiota occupy). They cross inoculated gut microbiome from either mice or zebrafish in germ-free mice or zebrafish and found that the gut habitat of the host differentially shaped the final bacterial community. The final community structure of each host was predictable and partially resembles its original community, however the relative abundance of each phyla in the community ends being similar than the normal microbiota of the host. Furthermore, Ley et al. (2008) found that bacterial diversity of the host is influenced by the diet and the host genotype, indicating that in mammals the gut microbiota has co-evolved with the host. In cattle it has been suggested that the breed of the sire can affect the frequency of particular microbial phylotypes (Hernandez-Sanabria et al., 2013). Furthermore, McSweeney and Mackie (2012) mentioned (unpublished data, Kailang Liu) that species of cattle from Asia have a different rumen bacterial community than Holstein cattle. These results support the concept that the genetics of the host have a role in shaping the rumen microbiome if other potential sources of variation were controlled (for instance, environment, diet).

The relation between cattle feed efficiency and rumen microbial ecology has been studied. Guan et al. (2008) compared bacterial profiles from cattle divergent on residual feed intake (RFI) and found that bacterial profiles from low-RFI animals clustered together and separated from high-RFI animals. Moreover, bacterial components in the rumen of low-RFI were more similar than those in the rumen of high-RFI. These authors also found that bacterial profiles appear to cluster together within a certain breed, concluding that host genetics and phenotypes may play an important role in shaping rumen bacterial profiles.

Carberry et al. (2012) and Hernandez-Sanabria et al. (2012) confirmed that differences in the rumen microbiome contribute to differences in cattle feed efficiency phenotype, and noted that those differences are influenced by the quality or type of diet. In parallel, Carberry et al. (2012) reported for the first time a relationship between host RFI phenotype and the abundance of particular microorganism in the rumen; they found an association between RFI phenotype and the abundance of rumen *Prevotella* sp., being more abundant on high-RFI cattle and this association was irrespective of the diet. These results have been further supported, McCann et al. (2014) studying Brahman bulls with different residual feed intake under grazing conditions at two levels of forage allowance found differences on the rumen microbiome between divergent animals for RFI and also detected that the relative abundance of *Prevotella* was greater in inefficient animals.

Hernandez-Sanabria et al. (2012) found three bacterial phylotypes (*Succinivibrio* sp., *Eubacterium* sp. and *Robinsoniella* sp.) that were differentially associated with cattle feed efficiency phenotype. In relation to another member of the rumen ecosystem, methanogens, Zhou et al. (2010) reported that cattle with different feed efficiency harbor methanogen populations of similar size (cells/mL), but the structure (at strain or genotype level) of that population will vary with RFI in cattle.

Waghorn and Hegarty (2011) hypothesized that individual animals have their own specific microbiota. In their work, they have indicated that nutrient availability, methanogenesis and rate of ruminal feed degradation among animals could be affected by selecting animals according to their efficiency of digestion. In addition, Smuts et al. (1995) have reported differences in digesta kinetics between sheep with different wool growth.

Differences in digesta kinetics may affect animal productivity by changing the number and diversity of rumen microorganisms, the volatile fatty acid pattern, composition and maintenance energy requirement of the microbes, the energetic efficiency and yield of microbial growth, and proteolysis and deamination of amino acids (Hegarty, 2004; Waghorn and Hegarty, 2011). Finally, Kittelmann et al. (2014) have linked different bacterial communities with high- and low-methane emitting sheep. They indicated that while the factors that caused those differences are not yet clear, genetic, epigenetic or environmental factors could be the drivers to lead to different methane production between those sheep phenotypes.

1.2.2 Influence of host (wool) genetics on animal physiology

1.2.2.1 Wool biology

The number of cells produced per unit of time in the wool follicle, the volume of the cells after keratinization, proportion of cells that enter the fibre and the volume of the intercellular cement determine the total volume of fibre produced (Schinckel, 1961; Wilson and Short, 1979; Williams and Winston, 1987; Hynd and Masters, 2002). Hynd (1989) indicated that phenotypic differences in fibre production were strongly related to differences in the rate of bulb cell division and to a smaller degree with the proportion of bulb cells entering the fibre cortex, while cortical cell volume has a weak association with fibre production.

Wilson and Short (1979) established that only 9-18% of the germinal cells were destined for the fibre cuticle and cortex, while the other cells enter the inner root sheath. Short et al. (1965; cited by Hynd and Masters, 2002), established that the proportion of the cells that could enter the wool fibre is between 10 and 20%. These results showed that the formation of a wool fibre is not a very efficient process. Nutrition (Hynd and Masters, 2002), genotype (Wilson and Short, 1979), and season (Holle et al., 1994) among others such as health, could affect the cell distribution between wool fibre and inner root sheath. The effect of nutrition on wool production is through altering the size of the follicle bulb; the size and number of cells in the germinative region of the bulb; the rate division of bulb cell division; and the ultimate size of cortical cells. With respect to nutrition, Kempton (1979) suggested that energy intake has only minor effect on wool growth in comparison with protein availability. The amount and

array of amino acids available to the wool follicle is the major limitation for wool growth. The results for several studies indicated that for any given digestible energy or protein intake, there is an optimum balance of amino acids to energy required for maximum wool growth. An average of about 12 for CP/ME ratio (g/MJ) is required in the diet for maximum wool growth, depending on physiological state and health of the animal, composition of amino acids, degradability of the diet, others (Kempton, 1979).

1.2.2.2 Association of wool growth with nutrient metabolism and skin characteristics

Li et al. (2006) indicated that skin characteristics such as skin thickness, follicle density, secondary to primary follicle (S/P) ratio, skin weight, dermis weight and true protein and collagen content are affected by genetic selection for wool growth rate, fibre length and fibre diameter and nutritional variation. The authors suggested that the greater wool growth achieved by sheep selected for high estimated breeding values (EBVs) in fleece weight are a consequence of the higher S/P ratios, higher skin and protein masses, more responsive dermises and higher rate of methionine usage in the skin of sheep with high EBV than sheep with low EBV. In concordance, Nancarrow et al. (1998), who worked with animals genetically selected for high or low wool production, obtained significant associations of follicle density, S/P ratio, follicle branching and follicle shutdown with clean fleece weight.

In the experiment of Williams and Winston (1987), animals selected for high wool production had a greater density of wool follicles and produced more wool per unit area of skin. Also the wool produced by those animals selected for high wool production had less sulphur content than animals selected for low production. Despite the diameter of the follicle bulbs being equal for both flocks, the follicles from high wool production sheep were deeper in the skin and they also had a greater area of mitotically active tissue. Finally, high production animals showed a higher rate of incorporation of cortical cells into fibre, without differences on the average volume of cortical cells isolated from the fibres.

Hales and Fawcett (1993) reported that the blood flow rate per unit area of wool-bearing skin is greater in animals with high fleece production than in animals with low fleece production. Other tissues that exhibit significant blood flow differences between the two

genetic groups differing in wool production include adrenal glands and fat, being greater in animals who produced more wool, and thyroid glands, reticulum, rumen, and extremity skin (no wool-bearing), which are also greater in animals who produced more wool.

Differences in plasma metabolites could reflect differences in utilization of nutrients related to genetic capacity for wool production. Hough et al. (1988) found that high wool production ewes have higher levels of plasma α -amino-N, blood acetate and lower concentrations of plasma cysteine and thyroxin, blood lactate and wool sulphur. No differences in plasma glucose, plasma NEFA, blood beta-hydroxybutyrate, plasma urea, growth hormone or plasma insulin were apparent. Williams et al. (1990) evaluated the activity of the thyroid glands in relation with genetic control of wool production and did not find differences between the wool breeding lines studied. However, Adams et al. (2006) found that the plasma concentration of glucose, insulin (also Adams et al., 2004) and IGF-1 were lower in high fleece weight sheep; plasma concentration of growth hormone was higher in those animals, while plasma glucagon was not different between both groups of animals. Those researchers hypothesized that selection for clean fleece weight resulted in leaner sheep with lower metabolic energy reserves, greater feed intake, and greater lean tissue mass.

Plasma urea concentration could be an indicator of the incorporation of N into protein under controlled feeding conditions, with a hypothesis that animals with low plasma urea concentration might have higher efficiency of nitrogen retention, and so low plasma urea indicates more protein available in the animal and thus more protein available for wool production (McCutcheon et al., 1987). Animals selected for high fleece weight had lower plasma urea concentration than non-selected animals supporting this hypothesis (McCutcheon et al., 1987; Thomson et al., 1989). McCutcheon et al. (1987) further linked the result of higher plasma urea concentration of non-selected animals with an increased deamination of amino acids and thus a lower ability to retain dietary nitrogen.

Piper and Dolling (1969) with their series of experiments concluded that the better efficiency of conversion of food into wool in animals selected for wool production was not explained by differences in apparent digestive ability. Furthermore, they indicated that the

efficiency of feed conversion of high wool producers could be related with a better nutrient metabolism after absorption from the alimentary tract.

Masters et al. (2000) showed that protein synthesis rate and total synthesis in the skin and wool were higher in animals selected for high clean fleece weight than animals with low clean fleece weight. In this work the results are explained by differences in the proportion of follicular tissue in the skin, differences in the proportion of active follicles and differences in the efficiency of follicles between both groups. In this sense Adams et al. (2004), also found that high fleece weight sheep had a high turnover rate of body protein (wool and non-wool tissues), and they suggested that the low concentrations of insulin in high fleece weight sheep could be partly responsible for increased whole-body protein turnover.

Li et al. (2007) showed that fractional synthesis rates of protein in the epidermis, dermis, whole skin and muscle are affected by genotype, being greater in high wool production sheep (independent of the level of intake). These results indicate that selection for wool growth affects the rate of protein turnover in components of the skin and skeletal muscle. Li et al. (2007) did not observe increased ruminal microbial protein supply, suggesting that the changes in protein metabolism between skin and muscle of high wool production sheep came about despite an unaltered supply of amino acids from the digestive tract. Li et al. (2007) also suggested that genetic selection for high clean fleece weight increases the utilisation efficiency of amino acids available to the body for wool production. A complementary study (Li et al., 2008), indicates that sheep with high-fleece EBVs synthesised and degraded more body protein and retained more ingested protein in wool and body tissue than did sheep with low-fleece EBVs. However, these differences disappeared when whole-body protein flux, synthesis and degradation were expressed in relation to metabolic weight. From the energy point of view, energy expenditure was higher in high wool producers than in low wool sheep. Finally, high wool producers retained more energy in wool and wool-free body tissue than did low wool producers. Li et al. (2008) concluded that it is possible to increase efficiency of dietary protein and energy use for wool production and body-tissue growth by genetic selection for wool growth.

1.2.2.3 Wool genetics influence on fermentation, digestion and utilization of the feed

Lush et al. (1991) and Kahn (1996) have presented evidence of increased portal amino acid uptake and increased ruminal microbial protein supply in genetic lines of sheep selected for increased wool growth. Animals selected for high wool growth had, at the same feed intake, an uptake of α -amino nitrogen in portal blood 30% higher than animals selected for low wool growth (Lush et al., 1991). Kahn (1996) explained the differences in the yield of microbial nitrogen from the rumen by the variation in the yield of microbial nitrogen per unit of DM intake (it explains 35% of the variation), and the variation in DM intake (it explains 65% of the variation) between lines of sheep. Digestibility of feed does not differ between these lines of sheep, but microbial protein production per unit of feed is higher in sheep from lines selected for high wool growth (Kahn, 1996). Furthermore, this author using models suggested that the variation on yield of microbial nitrogen per unit of DM intake explains 8% and DM intake explains 11% of the variation in wool growth between lines selected for different wool growth. Based on these results, among others, Herd and Arthur (2009) indicated that differences in the processes of digestion and in substrate availability exist, and this is a possible mechanism to explain variation in efficiency of feed utilization. Li (2007), concluded that it appears to be possible to breed lines of sheep with higher microbial yield per unit of feed intake and higher efficiency of use of absorbed amino acids by both skin and muscle tissues.

Kahn (1996) reported that voluntary feed intake (adjusted for live weight) was different between lines selected for wool growth, without differences in apparent DM digestibility. Thomson et al. (1989) found differences in voluntary feed intake or apparent DM digestibility between genetic lines for wool production on a high plane of nutrition however no differences were detected when fed medium-quality diet. Animals selected for wool production had higher voluntary feed intake than non-selected animals and a lower apparent DM digestibility. Changes in apparent digestibility and voluntary feed intake could be associated with a change in the rumen pool size, degradation rate, passage rate, or a combination of these effects. If genetic selection produced no increase in rumen pool size per unit of metabolic body size, they suggested that faster particulate matter passage rate is the principal reason why voluntary feed intake was higher and apparent digestibility lower in animals selected for wool production on

high plane of nutrition. The increase in water consumption detected in selected animals and the possibility of the increase in fluid passage rate associated with particulate matter passage rate could indicate a greater rumen liquid passage rate. An increase in the rumen fluid passage rate is associated with an increase in the amount of microbial protein that leaves the rumen and reaches the lower digestive tract (Harrison et al. 1975; 1976; cited by Thomson et al., 1989). In addition, undegraded protein outflow also increases with passage rate, thus the quantity of protein arriving at the duodenum at greater passage rates could have been greater due to both more dietary and microbial protein flow in selected animals (Thomson et al., 1989).

Later work by Faichney et al. (1998), does not find differences in voluntary feed consumption, feed digestibility, urea synthesis and excretion, or renal function between lines selected or non-selected for wool production, however they found that sheep selected for high wool production drink more water. Also, Lee et al. (2002) established that genetic selection for wool growth will have little effect on intake. Finally, Adams et al. (2006) established that selection for clean fleece weight EBVs resulted in leaner sheep with increased phenotypic feed intake (when fed medium-quality diets).

Kahn (1996) found that animals selected for high wool production ate faster than low wool production sheep. Voluntary feed intake and mean retention time are related to eating rate (Forbes, 1972). High eating rate implies low mean retention time and high voluntary feed intake. Smuts et al. (1995) indicated that the probability of having low wool production with short retention time of digesta in the rumen is close to zero. These researchers also mentioned that high wool producers may have faster metabolic rates; high producers also may possess more active gastrointestinal contractions and exhibit characteristics in the skin such as shorter generation times in bulb cells, or more active follicle bulb cell division. They suggested that more investigations are needed using feeds of varying qualities to assess: the effects of passage rate differences on amino acid supply to the lower digestive tract; the influence of variation in digesta retention time between animals; and the heritability of this parameter. Retention time of digesta in the rumen is a repeatable characteristic, which contributes in part to differences in wool growth rate. Because digestibility, as influenced by retention time, did not affect wool

growth rate, the effect of retention time on wool production is accounted for the effect of retention time on level of intake (Smuts et al., 1995).

Oddy (1999) suggested, that nutrients available for absorption and metabolism could be affected by genetic selection, even if the trait is on the outside of the host (wool growth). The mechanism could be by altering the environment in the rumen, allowing the gut bacterial population to produce a greater quantity of performance-limiting nutrients. In accord with this, Kahn (1996) suggested that differences in yield of microbial nitrogen per unit of DM intake induced by selecting for wool growth could be explained by differences in the rate of turnover of bacterial protein and of rumen protozoa.

1.3 Conclusions from the literature and development of the hypothesis of this thesis

1.3.1 Scope for modifying the rumen microbiome of newborn Merino lambs

The relationship between the microbes in the rumen and the host is a product of symbiotic evolution (Ley et al., 2008; Rosenberg et al., 2010; Morgavi et al., 2013). Research has reported that this relationship between rumen microbial ecology and the host is related to multiple factors including: host genetics (Hernandez-Sanabria et al., 2013), inoculation (Fonty, et al., 1983; Dehority, 2003), age of the host (Li et al., 2012; Jami et al., 2013), diet (Liu et al., 2011), early-life intervention (Leahy et al., 2013) and feed conversion efficiency (Guan et al., 2008), Leahy et al. (2013) indicated that a microbial intervention early in the life of the ruminant would be ideal to generate a lifetime effect on the host in terms of animal performance and environmental impact. Early-life intervention strategies have been studied with promising results (Yañez-Ruiz et al., 2010 and Abecia et al., 2014).

It is hypothesized that the rumen microbiome of lambs could be altered post-natally by an early microbial intervention and as a consequence, rumen fermentation, development and animal performance of newborn lambs until weaning and beyond may be modified. From the reviewed literature, two treatments appear suitable as models for modifying the early-life rumen microbial composition were selected as appropriate to test this hypothesis: (I) inclusion of coconut oil as a non-protected fat in the diet of the ewes during late pregnancy plus the lambs during lactation; and (II) inoculation of the newborn lambs during the development of the

rumen ecosystem with fresh rumen fluid from sheep on an alternate diet. In the case of treatment (I) (inclusion of coconut oil), it is expected that this non-protected fat will affect the rumen microbiota by particularly modifying the presence/abundance of cellulolytic bacteria, protozoa and methanogens in the rumen. In parallel, to test this hypothesis it will be important to consider a control group fed the same amount of fat and that fat should be fully protected in order to not alter rumen function.

1.3.2 Role of the host animal in regulating the ruminal microbiome

Research has demonstrated that continuous divergent genetic selection for wool growth leads to major changes on the periphery of the animal (skin and follicle level) (Williams and Winston, 1987; Nancarrow et al., 1998; Li et al., 2006) and on digesta flow from the rumen and protein metabolism. Gut parameters in which differences have been observed between animals with different potential for wool growth include: mean retention time of digesta (Smuts et al., 1995), efficiency 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 for wool and tissue (Li et al., 2008). Kahn (1996) suggested that the changes in microbial protein production in the rumen may be related to differences in bacterial turnover and protozoa populations of the rumen and Hegarty (2004) hypothesized that animals with shorter mean retention time may exhibit differences in microbial ecology to deliver reduced CH₄ emissions. In parallel, it has been suggested that variations in ruminal microbial communities may occur between cattle with different feed efficiency (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 modulated by the feed.

It is hypothesized that if sheep with different potential for wool growth present variations in digesta retention times and efficiency of microbial protein production, then sheep with contrasting estimated breeding values for fleece weight may harbour a different microbiome in the rumen, in association with differences in gut morphology, physiology, microbial protein flow from the rumen and digesta retention times, regardless of the level of intake.

1.4 Research plan

The research plan of this thesis was designed to build on existing knowledge by testing the hypotheses mentioned above (sectors 1.3.1 and 1.3.2). In this context, two major animal studies were performed and their outcomes are presented in the following five chapters of the thesis.

The first experimental chapter (chapter 2) studies the effect of an early-life microbial intervention on the rumen microbiome of newborn lambs and investigates the consequences of natural or artificial microbial inoculations on the rumen ecosystem. Modification of the natural inoculum (dam's rumen microbiome) received by the lambs was achieved by the inclusion of non-protected fat in the ewe's diet, while variable artificial inoculation was accomplished by the inoculation of the newborn lamb with specific rumen fluids.

Chapter three investigates and reports the consequences of the treatments implemented in chapter two as early-life microbial interventions on the development and fermentation of the rumen and on the performance of lambs at weaning and at 5 months of life.

The fourth chapter reports a study that was conducted to assess whether differences in the rumen bacterial communities were associated with differences in sheep genetic potential for wool growth and whether these differences persisted across intake levels.

Chapters five and six are focused on studies of the differences in wool production in animals with different genetic potential for wool growth and similar genetic merit for live weight and fibre diameter to ascertain associations with differences in gut anatomy, rumen function, and digesta retention times regardless of the level of intake.

These three studies (chapter 4 to 6) present evidence on differences in the rumen microbiome (bacteria and protozoa) of animals with different genetic potential merit for fleece weight, and provide information on the consequences of wool genotype on gut metabolism and morphology.

Finally chapter seven integrates results and conclusions from the five experimental chapters and gives a general discussion in relation to the conclusions from the literature and hypotheses expressed earlier in this chapter.

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Chapter Two

Modulating rumen microbiota in newborn Merino lambs¹

Chapter contents

De Barbieri, I., Hegarty, R., Silveira, C., Gulino, L.M., Oddy, V., Gilbert, R.A., Klieve, A.V.,
Ouwkerk, D. (2015). Programming rumen bacterial communities in newborn Merino lambs.
Small Ruminant Research, doi: <http://dx.doi.org/10.1016/j.smallrumres.2015.05.015>

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Chapter Three

Positive consequences of maternal diet and post-natal rumen inoculation on rumen function and animal performance of Merino lambs²

Chapter contents

De Barbieri, I., Hegarty, R., Silveira, C., & Oddy, V. (2015). Positive consequences of maternal diet and post-natal rumen inoculation on rumen function and animal performance of Merino lambs. *Small Ruminant Research*, doi: <http://dx.doi.org/10.1016/j.smallrumres.2015.05.017>

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Chapter Four

Production attributes of Merino sheep genetically divergent for wool growth are reflected in differing rumen microbiotas³

Chapter contents

De Barbieri, I., Gulino, L., Hegarty, R., Oddy, V., Maguire, A., Li, L., Klieve, A.V., Ouwerkerk, D. (2015). Production attributes of Merino sheep genetically divergent for wool growth are reflected in differing rumen microbiotas. *Livestock Science*, doi: <http://dx.doi.org/10.1016/j.livsci.2015.05.023>

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Chapter Five

Association of wool growth with gut metabolism and anatomy in sheep⁴

Chapter contents

De Barbieri, I., Hegarty, R. S., Li, L., & Oddy, V. H. (2015). Association of wool growth with gut metabolism and anatomy in sheep. *Livestock Science* 173, 38-47.

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Chapter Six

Sheep of divergent genetic merit for wool growth do not differ in digesta kinetics while on restricted intakes⁵

Chapter contents

De Barbieri, I., Hegarty, R. S., Oddy, V. H., Barnett, M. C., Li, L., & Nolan, J. V. (2014). Sheep of divergent genetic merit for wool growth do not differ in digesta kinetics while on restricted intakes. *Animal Production Science*, 54(9), 1243–1247.

Wool information presented here (Chapter Six) has also been submitted for publication (Chapter Five).

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Chapter Seven

7 General discussion

7.1 General introduction

Manipulation of the rumen ecosystem can modify the relationship between feed consumed, livestock performance and environmental impact (Calsamiglia et al., 2010). Manipulations can target different rumen fermentation processes, including nitrogen metabolism, fiber digestion and methane production (Nagaraja, 2012), and as a consequence will affect the symbiotic relationship between the rumen microbiome and the host ruminant. The rumen has specific energy and protein losses (methane and ammonia respectively; Yañez-Ruiz et al., 2010), that can affect the environment, livestock production and efficiency. The quantity and array of products supplied from the rumen are themselves products of the interaction of three components (Mackie et al., 2002). These components are the rumen microbiome, diet and aspects of the host. A modification of any of these three components will generate consequences and potentially a new equilibrium (not necessarily long-lasting) in the rumen ecosystem. Knowledge of the interrelation among the three components and consequences of any kind of manipulation is necessary in order to be effective in targeting specific modifications in the rumen ecosystem for desired outcomes.

This program sought to explore the plasticity of the rumen microbiome to manipulation by changing the microbial inoculum or diet, and to further explore the role of the host animal in rumen microbiome regulation. Further, we sought to assess the contribution of the rumen microbiota to long-term animal productivity differences between animal genotypes.

7.2 Major outcomes

7.2.1 *Scope for modifying the rumen microbiome of newborn Merino lambs*

A microbial intervention early in the life of the ruminant has been postulated to have the potential to generate a long-lasting effect on animal performance and environmental impact (Leahy et al., 2013); a proposal supported by the promising results of early-life microbial interventions of the research of Yañez-Ruiz et al. (2010) and Abecia et al. (2014a). It was hypothesized that an early-life microbial intervention could alter the rumen microbiome of

newborn Merino lambs while suckling and as a consequence could alter rumen fermentation, development and animal performance of lambs until weaning and beyond. Contrasting rumen-protected (PF) and rumen-active (CO) lipids were used to modify the microbes of the natural maternal inoculum and the rumen microbiome of the lambs while suckling, as was with cross inoculation of the lamb with fresh rumen fluid from donor ewes on the alternate or same diet.

The key findings of the study reported in Chapter 2 are that ruminal bacterial communities of newborn lambs can be altered by modifying the diet of the ewes from late pregnancy and of the lambs while suckling, or by inoculating the rumen of the lambs during the first weeks of their life with specific fresh rumen fluids. Those modifications can have an effect at least over the mid-term (21 weeks). Specifically, inclusion of CO in the diet of pregnant or non-pregnant ewes can lead to different rumen bacterial communities which can present different species richness and biodiversity. When OTUs are considered at equal abundance they cluster as different populations. Furthermore, the form of lipid in the diet of ewes affected the presence and abundance of specific ruminal microbes. The coating effect of the feed particles by the lipids that physically disrupts the association between microbes and digesta (Wanapat et al., 2011) and the inhibitory effect of coconut oil on protozoa, methanogens and bacterial populations (Sutton et al., 1983; Machmüller, 2006; Rasmussen and Harrison, 2011) are two of the factors (Nagaraja et al., 1997) that can explain the observed effects of the inclusion of rumen active lipid on bacterial communities of the rumen.

Modifications in species richness, biodiversity and taxonomy of ruminal bacterial communities of lambs while suckling and beyond weaning can be achieved by the inclusion of coconut oil in the diet of the lamb and its mother. These results are supported by the findings of Mao et al. (2010) and Yañez-Ruiz et al. (2010) who altered the rumen microbiome by changes in the diet of the lambs. However, the findings of our study are not simply attributable to differences in diet consumed by the lambs; because in our case the mothers of the lambs shared the same diet than the lambs from one month pre-lambing, and in accordance with Hobson (1997); Dehority (2003) and Abecia et al. (2014a), the mothers microbiota may have been the most significant source of inoculum to lambs.

Characteristics of the ruminal bacterial communities of lambs were also modified by inoculating the rumen with alternate complex microbial communities (rumen fluid) from other adult animals. Previous studies have reported that the inoculation of the rumen of young animals can improve the complexity of the final microbial ecology on that rumen (Pounden and Hibbs, 1948; Fonty et al., 1983; Krause et al., 2003; Bomba et al., 2005). In our study a difference in the diet appeared to modify the rumen microbiome more than did the inoculation. This indicates that the natural inoculation and fostering of microbiota in the rumen of the lamb associated with the diet of the ewe and lamb could more effectively shape rumen microbiota than could systematic early inoculations with specific rumen fluid. One possible explanation is that the uninterrupted maternal inoculation for three months plus the constant diet were continuously impacting at the time the developing rumen was most sensitive to microbial intervention, while the infrequent though systematic weekly inoculation with an exogenous microbiota may have been less able to modify the resident microbiome.

The effect of both diet and inoculation on ruminal bacteria diminished after treatments were suspended. This in accordance with Rawls et al. (2006) and Ley et al. (2008) and can be explained by the existing relationship between the host and the gut microbiota, a relationship that can influence the final bacterial community profile in each host. Furthermore, this also shows that the extent to which microbial intervention can be restrained by the genotype of the host, and this opens new questions on the potential for early-life microbial interventions in relation to different host genetics and plasticity of possible rumen microbiotas. The diminished effect once treatments were suspended also can be associated with an effect of the post-weaning diet rather than a host effect.

In summary, ruminal bacterial communities of newborn lambs can be altered by modifying the diet of the ewes and lambs during lactation or by inoculating the rumen of the lambs during the first weeks of their life. While both diet and inoculation can affect species richness, biodiversity and composition of those communities, diet appeared to generate a greater and more lasting effect on the groups of bacteria than did inoculation.

Regarding consequences of modifying the diet of ewes and their lambs through to weaning results presented in Chapter 3 indicate the inclusion of non-protected (rumen-active)

fat in the diet affected DM intake, rumen fermentation, rumen size, methane production and protozoa numbers in the rumen of lambs by the time of weaning, but animal performance evaluated through live weight, condition score and wool growth was not affected. The major digestive changes could be a consequence of two factors acting separately or together: i) the effect of the diet on the gut microbiology of the ewes and in consequence the divergent natural inoculation to which lambs between treatments were subjected, and ii) the direct effect of the fat in the diet on the rumen physiology and microbiome of the lambs, which is consistent and supported by the findings reported in Chapter 2.

Inoculation with rumen fluid also modulated some aspects of the rumen fermentation and microbiome, indicating that detected changes in the rumen microbiome (Chapter 2 and 3) can be responsible for modifications in rumen function. Results from both Chapters in regards to rumen fluid inoculation, consistently indicate that the effects of inoculation on rumen function and ecology were greater in early lactation and tended to be less important when the animals were older. Finally, even though inoculation early in the life of the animal created some differences in the rumen ecosystem; changes were not large enough to generate detectable modifications in live weight, condition score or wool production and quality on the lambs in the short- or medium-term up to five months of age. In parallel, it is important to consider that a shift in the rumen microbiome will not necessary be associated with different biochemistry or fermentation outcomes of the microorganisms (Li et al., 2012). Abecia et al. (2014b) suggested that the first two weeks of life would be the best opportunity for an early microbial intervention, in this context, lambs studied here were inoculated two times during that window. This could indicate that further research would be of interest to determine the potential impact of different protocols of inoculation, specifically investigating the time-window of intervention to achieve greater and longer-lasting modifications not only in rumen function and ecology but also in animal performance or adaptation.

7.2.2 Role of the host animal in regulating the ruminal microbiome

Based on sheep with different potential for wool growth exhibiting variations in digesta flow (Smuts et al., 1995) and protein metabolism (Lush et al., 1991; Kahn, 1996; Li et al., 2008) and

these variations potentially being related to distinct rumen microbiomes (Kahn, 1996; Hegarty, 2004), it was hypothesized that sheep with contrasting estimated breeding values for fleece weight may harbour different microbiomes in the rumen in association with differences in gut morphology, physiology, microbial protein flow from the rumen and digesta retention times.

The study of ruminal bacteria of two groups of Merino sheep with divergent EBVs for clean fleece weight and similar EBVs for live weight and fibre diameter reported in Chapter 4 indicates that differences in bacterial community diversity exist between sheep with different genetic merit for fleece weight. Additionally and reported in Chapter 5, small differences in the population of Large Isotrichidae protozoa were found between groups of Merino sheep. These findings support the idea that selection for wool growth may affect the rumen ecosystem (Oddy, 1999) and also support the concept of considering the host and its microbiota as one entity (Zilber-Rosenberg and Rosenberg, 2008; Morgavi, et al., 2013).

Even though, differences between bacterial communities in the rumen of sheep with distinct EBVs for wool growth were found, they were not large enough to cluster groups separately in a Principal coordinate analysis. In parallel and in accordance with the hypothesis of the host's biology controlling the gut microbiome, rumen fermentation, gut anatomy and digesta kinetics in the gut of divergent sheep groups for wool production were evaluated (Chapters 5 and 6). Results indicated that differences in wool growth between sheep divergently selected for fleece weight were apparently not induced by differences in gut metabolism and anatomy or digesta kinetics (mean retention time of particulate and solute fractions in the fore- and hind-gut and whole tract). This is consistent with the assessments of Piper and Dolling (1969) that found a better nutrient metabolic utilization after nutrients were absorbed from the tract could explain an improved efficiency of conversion of feed into wool between animals selected for wool production.

Despite the fact that rumen parameters were not different and bacterial communities did not cluster separately between wool genotypes, associations between the presence/abundance in the rumen of seventeen OTUs and at least one of seven continuous traits (pH, protozoal counts, concentration of VFA, microbial protein leaving the rumen, microbial protein yield, methane production and methane yield) were found (Chapter 4). Even though the

rumen is an extremely complex ecosystem, these simple associations between specific OTUs and products supplied from the rumen as VFA, methane or microbial protein indicate a scope to improve rumen fermentation processes by targeted modifications of the rumen microbiome.

Together, the outcomes from Chapters 4, 5 and 6 indicate that Merino sheep with distinct EBVs for fleece weight evaluated here had: i) small differences between their ruminal bacterial communities and protozoa populations, and ii) no differences in their gut anatomy, rumen fermentation, and digesta kinetics. Consideration of these results in respect to previous research suggests at least two factors that can explain discrepancies between studies reported here and studies previously published. Firstly, single-trait genetic selection for wool growth for many generations was used to generate the sheep of some previous studies, while multi-trait genetic selection (wool growth, fibre diameter, live weight) was used to produce genetic diversity among the sheep of this study. Different genetic selection approaches for wool growth could be associated with different genetic progress for wool production (and other traits such as live weight or feed intake) and may have different consequences on the rumen ecosystem. Fleece weight has a positive genetic correlation with live weight (Safari et al., 2007a) and live weight is positively correlated with dry matter intake (Fogarty et al., 2009). Consequently in a single trait selection process, animals selected for wool growth could exhibit a greater intake than sheep selected against wool growth, and this higher intake, live weight and wool growth together may be responsible for differences found in the rumen fermentation processes between divergent lines for wool growth by Kahn (1996) but not by us.

Secondly, it would be expected that the contribution of differences in the rumen ecosystem to explaining variations in wool growth may be small in comparison with other metabolic processes such as those occurring in the wool follicle. In this context, the differences in wool growth achieved by genetically divergent sheep groups in this thesis were less substantial than differences registered in previous research (Smuts et al., 1995; Kahn, 1996), being potentially too small to generate differences of measurable significance in the evaluated traits.

The study of the association between sheep with contrasting estimated breeding values for fleece weight and rumen microbiome, gut metabolism, gut anatomy, digesta retention times

in the gut and animal performance was performed at two levels of intake. No interaction between level of intake and wool genotype was detected in any of the traits evaluated and reported in Chapters 4, 5 and 6. Studies of Li et al. (2008) evaluating protein metabolism and energy expenditure in sheep with different EBVs for fleece weight have only reported interaction in live-weight change, when levels of intake under (0.8) and above (1.8) energy maintenance requirements were studied. In parallel, while Lee et al. (2002) noted that there was no genetic correlation of feed intake with wool growth, Adams et al. (2006) found that different types of feed (straw versus mixed) can lead to a phenotypic interaction between wool genotype and feed intake. Based on previous studies (Lee et al., 2002; Adams et al., 2006; Li et al., 2008) and results reported in this thesis, further studies including different type of diets and more divergent levels of intake may add knowledge on the association between wool genotype and the rumen ecosystem through providing increased diversity.

Analysis of the results reported in this thesis confirms that level of intake could be utilized as a tool for modifying rumen function and animal performance. Level of intake induced major changes in gut morphology, rumen digestion and fermentation, digesta kinetics and animal performance, indicating that managing the level of intake towards *ad libitum* on animals of either genetic merit for wool production could deliver desirable outcomes as increased animal performance and reduced methane yield.

7.2.3 *Non-invasive methods to evaluate gut parameters*

Computed tomography (CT) scanning as a novel non-invasive method to estimate rumen characteristics was evaluated during the course of the thesis. As mentioned in Chapter 5, two outcomes are important from that investigation. Firstly, the repeatability between several measurements of the weight and volume of reticulum and rumen with the CT scanning were in the range of 0.84-0.88. Secondly, when the weight of the reticulo-rumen was evaluated pre-mortem by CT scanning and was then weighed immediately post-mortem, results indicate that both measures were highly correlated ($r=0.96$). Relationships between physical rumen characteristics and rumen ecosystem have been reported (Purser and Moir, 1966a; b; Goopy et

al., 2014), therefore future understanding of rumen function may be improved by the complementary utilization of CT scanning and traditional techniques.

7.3 Future research

Results reported here indicate that the rumen microbiome may be associated with genetic characteristics of the host and potentially correlate with animal productivity traits. In this context, if a variable such as fleece weight which is under moderate genetic control ($h^2 \approx 0.29$, Ciappesoni et al., 2013; Safari et al., 2007b) and is phenotypically expressed in the surface of the animal is related with the rumen microbiota of the host, it is reasonable to expect that traits closely related with rumen function, anatomy and physiology including rumen microbiota would also be to some extent under genetic control. Recently, Kittelmann et al. (2014) have reported a link between sheep phenotypically divergent for methane emissions and bacterial communities in the rumen. Furthermore, Shi et al. (2014) noted that while methanogen populations and methanogenic genes between high- and low-methane sheep were similar, the transcription of the genes associated with methane production were different between groups. Therefore, study of sheep with different genetics and phenomics for: feed conversion efficiency, methane production and performance traits for the purpose of relating these traits to their rumen microbiome, metagenomics, transcriptomics, proteomics and metabolomics would be relevant to improve our knowledge of the processes involved during sheep genetic selection and how we can utilize these processes to create beneficial lifelong modifications in the rumen.

Effects of different diets on the rumen microbiome have been previously reported. Outcomes from this thesis add new knowledge to understanding implications of the manipulation of the diet in late pregnancy of the dams and early lactation of the lambs. Specifically these relate to opportunities to manipulate rumen function and animal performance through inducing simple changes in the rumen microbiome without the necessity of complex long term supplementary feeding practices in the sheep industry.

In relation to inoculation with rumen fluid, it is recommended that future research considers genetic and phenotypic characteristics of the host, as the Merino study indicated the

host's role in influencing the microbiome. Knowing those animal characteristics affecting the rumen suitability to receive an external inoculum and to achieve long-lasting rumen modification can be important in successfully manipulating the rumen microbiota. In this context, exploring different protocols of inoculation and specific aspects of the inoculum so that lambs achieve improved lifetime productivity would be worthy of consideration in future projects.

7.4 References

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Appendix 2 - Supplementary information of Chapter 2.

Table S1. Basic statistics of the number of sequences and OTUs per sample in ewes and lambs at three different stages during the analysis with QIIME

		Stage 1	Stage 2	Stage 3
Ewes	Sequences/sample			
	Minimum	5418	5334	5299
	Maximum	41798	40164	39453
	Median	18431	17992	17808
	Mean	19276	18856	18695
	Standard deviation	9937	9555	9394
	Total number of sequences	790297	773080	766474
	OTUs/sample			
	Minimum	841	796	758
	Maximum	4617	3325	2518
	Median	1232	116	1102
	Mean	1601	1412	1251
	Standard deviation	995	683	459
	Total number of OTUs	18407	13196	6590
Lambs	Sequences/sample			
	Minimum	8304	7869	7756
	Maximum	23928	23804	23771
	Median	13394	12539	12339
	Mean	13466	12715	12533
	Standard deviation	2821	2733	2720
	Total number of sequences	1252301	1182520	1165523
	OTUs/sample			
	Minimum	1063	935	854
	Maximum	3735	2732	2182
	Median	2037	1551	1349
	Mean	2019	1536	1353
	Standard deviation	492	323	255
	Total number of OTUs	55708	32888	15891

Stage 1 = after reassigning taxonomy using the Greengenes database. Stage 2 = after removing chimeras. Stage 3 = after removing singletons.

Table S2. Effect of diet (PF, CO) and inoculation (InPF, InCO, InWa) on number of sequences per sample (after reassigning taxonomy, removing chimeras and removing singletons) of ruminal bacteria of maternal ewes at week 8 and of lambs at three ages (week 8, 12 or 21) (mean±s.e.)

Group	Week	Diet		Inoculation		
		PF	CO	InPF	InCO	InWa
Ewes	8	14566±1597	17297±112	na	na	na
Lambs	8	12444±653	12867±725	12787±815	12901±859	12279±859
Lambs	12	12573±1564	11168±1755	11217±1735	11117±1876	13277±1591
Lambs	21	14117±1515	13314±1667	14194±1640	13249±1823	13704±1482

^(a,b) Means within rows and factor with differing letters are significantly different (P<0.05). No interaction was detected between Diet and Inoculation (P>0.05). (PF) protected fat. (CO) coconut oil. (InPF) lambs inoculated with rumen fluid from donor ewes consuming PF. (InCO) lambs inoculated with rumen fluid from donor ewes consuming CO. (InWa) lambs inoculated with MilliQ-water. (na) non applicable.

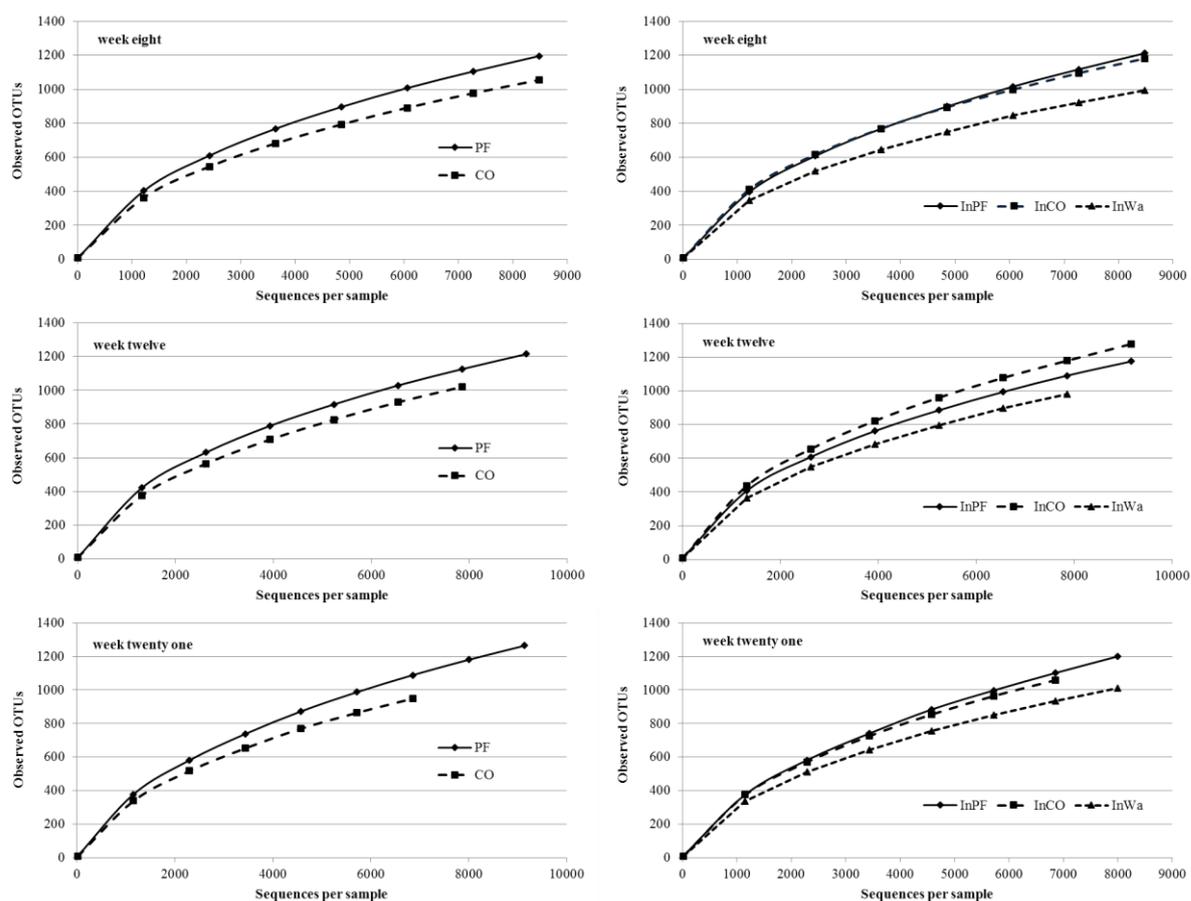


Figure S1. Graphical results of the alpha rarefaction (on the basis of Observed OTUs) analysis of lambs in accordance with the diet (PF and CO, on the left) or with the inoculation (InPF, InCO, InWa, on the right) at three ages (week 8, 12 or 21).

(PF) protected fat. (CO) coconut oil. (InPF) lambs inoculated with rumen fluid from donor ewes consuming PF. (InCO) lambs inoculated with rumen fluid from donor ewes consuming CO. (InWa) lambs inoculated with MilliQ-water.

Appendix

Appendix 3 - Supplementary tables of Chapter 4.

Table S1. Bacteria OTUs that present differences in presence/abundance ($P < 0.05$) between wool genotypes and classified by using Blast Algorithm and compare with the Greengenes database.

OTU ID	Taxonomic Classification	Accession number	Closest named relative	Max id	Host	Reference
11379	Unclassified Prevotellaceae	AB501155.1	Prevotella sp. BP1-56 gene for 16S rRNA, complete sequence	99.55	Sheep rumen	Unpublished
11926	Unclassified Prevotellaceae	AB501152.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: BP1-34	95.68	Sheep rumen	Unpublished
14006	Unclassified Clostridiales	Z49863.1	T. acetius gene for 16S ribosomal RNA	91.02	Termite gut	Int. J. Syst. Bacteriol. 46, 512-518 (1996)
14139	Unclassified Clostridiales	HM004597.1	Caloramator sp. TC17 16S ribosomal RNA gene, partial sequence	85.58	---	Unpublished
14468	Unclassified Prevotellaceae	AB501166.1	Prevotella sp. BP1-148 gene for 16S rRNA, complete sequence	94.09	Sheep rumen	Unpublished
15550	Unclassified Clostridiales	AY312403.2	Alkalibacter saccharofermentans strain Z-79820 16S ribosomal RNA gene, partial sequence	87.47	---	Extremophiles 8 (4), 309-316 (2004)
16377	Unclassified Prevotellaceae	AB501155.1	Prevotella sp. BP1-56 gene for 16S rRNA, complete sequence	90.68	Sheep rumen	Unpublished
18192	Unclassified Prevotellaceae	AB501174.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: AC5-20	94.77	Sheep rumen	Unpublished
20200	Unclassified Prevotellaceae	AF218619.1	Prevotella ruminicola strain TC2-28 16S ribosomal RNA gene, partial sequence	92.74	Rumen	FEMS Microbiol. Ecol. 33 (1), 69-79 (2000)
21479	Unclassified Bacteroidales	AB267809.1	Barnesiella viscericola gene for 16S ribosomal RNA, partial sequence	83.18	Chicken caecum	Int. J. Syst. Evol. Microbiol. 57 (PT 2), 342-346 (2007)
23366	Unclassified Clostridiales	AF202264.1	Syntrophococcus sucromutans 16S ribosomal RNA gene, partial sequence	97.86	Rumen	Unpublished
24094	Unclassified Lachnospiraceae	Y12289.1	Clostridium sp. 16S rRNA gene	97.38	Deer rumen	Syst. Appl. Microbiol. 21 (1), 135-143 (1998)
27650	Unclassified Bacteroidia	AB501173.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: AC5-13	88.86	Sheep rumen	Unpublished
28267	Unclassified Clostridiales	X71853.1	C. populeti gene for 16S ribosomal RNA	95.45	---	FEMS Microbiol. Lett. 113 (2), 125-128 (1993)
29010	Unclassified Clostridiales	AJ133124.1	Blautia luti 16S rRNA gene, strain bln9	96.91	Human faeces	Syst. Appl. Microbiol. 25 (2), 189-193 (2002)
29599	Unclassified Prevotellaceae	AB501163.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: BP1-90	96.59	Sheep rumen	Unpublished
31336	Unclassified Bacteroidales	EF151805.1	Parapedobacter soli strain DCY14 16S ribosomal RNA gene, partial sequence	83.79	---	Int. J. Syst. Evol. Microbiol. 58 (PT 2), 337-340 (2008)
32992	Unclassified Ruminococcaceae	EU815224.1	Clostridium sp. NML 04A032 16S ribosomal RNA gene, partial sequence	89.83	---	Unpublished
36659	Unclassified Ruminococcaceae	EU815224.1	Clostridium sp. NML 04A032 16S ribosomal RNA gene, partial sequence	93.85	---	Unpublished

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38584	Unclassified Prevotellaceae	AB501157.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: BP1-60	95.57	Sheep rumen	Unpublished
41898	Unclassified Prevotellaceae	AJ009933.1	Prevotella aff. ruminicola Tc2-24 16S rRNA gene	93.89	Rumen	Int. J. Syst. Bacteriol. 44 (2), 246-255 (1994)
44226	Unclassified Prevotellaceae	DQ278861.1	Prevotella sp. 152R-1a 16S ribosomal RNA gene, partial sequence	87.27	Rumen	J. Appl. Microbiol. 100 (6), 1261-1271 (2006)
47923	Unclassified Prevotellaceae	AB501151.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: BP1-32	98.18	Sheep rumen	Unpublished
50066	Unclassified Prevotellaceae	AJ009933.1	Prevotella aff. ruminicola Tc2-24 16S rRNA gene	94.77	Rumen	Int. J. Syst. Bacteriol. 44 (2), 246-255 (1994)
5219	Unclassified Lachnospiraceae	EF564277.1	Clostridium lavalense strain CCRI-9842 16S ribosomal RNA gene, partial sequence	98.10	Human faeces	Int. J. Syst. Evol. Microbiol. 59 (PT 3), 498-503 (2009)
53298	Unclassified Prevotellaceae	AB501163.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: BP1-90	97.50	Sheep rumen	Unpublished
5719	Unclassified Bacteroidia	AB501173.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: AC5-13	92.05	Sheep rumen	Unpublished
57388	Unclassified Prevotellaceae	AJ009933.1	Prevotella aff. ruminicola Tc2-24 16S rRNA gene	92.95	Rumen	Int. J. Syst. Bacteriol. 44 (2), 246-255 (1994)
58648	Unclassified Clostridiales	AY949857.1	Clostridium sp. strain P6 16S ribosomal RNA gene, complete sequence	92.73	---	Int. J. Syst. Evol. Microbiol. 60 (12), 2735-2738 (2010)
61776	Unclassified Lachnospiraceae	AF202264.1	Syntrophococcus sucromutans 16S ribosomal RNA gene, partial sequence	96.9	Rumen	Unpublished
63183	Unclassified Prevotellaceae	AY158021.1	Prevotella sp. RS2 16S ribosomal RNA gene, partial sequence	90.91	Pig colon	J. Bacteriol. 182 (11), 3002-3007 (2000)
6403	Unclassified Prevotellaceae	AJ009933.1	Prevotella aff. ruminicola Tc2-24 16S rRNA gene	95.00	Rumen	Int. J. Syst. Bacteriol. 44 (2), 246-255 (1994)
65608	Unclassified Clostridia	NR_025289.1	Eubacterium sulci strain ATCC 35585 16S ribosomal RNA, partial sequence	91.71	---	Int. J. Syst. Bacteriol. 49 (PT 4), 1375-1379 (1999)
66850	Unclassified Clostridia	AB547675.1	Prevotella buccae gene for 16S ribosomal RNA, partial sequence, strain: JCM 12245	85.68	---	J. Med. Microbiol. 59 (PT 11), 1293-1302 (2010)
69725	Unclassified Lachnospiraceae	FM954977.1	Roseburia sp. 11SE39 partial 16S rRNA gene, strain 11SE39	94.54	Human gut	FEMS Microbiol. Ecol. 74 (1), 205-213 (2010)
70689	Unclassified Bacteroidales	AY239469.1	Gram-negative bacterium cL10-2b-4 16S ribosomal RNA gene, complete sequence	86.36	Rat faecal flora	Can. J. Microbiol. 49 (10), 589-601 (2003)
8786	Unclassified Lachnospiraceae	HM596276.1	Eubacterium infirmum strain F0142 16S ribosomal RNA gene, partial sequence	91.47	---	Unpublished

Table S2. Bacteria OTUs that present differences in presence/abundance ($P < 0.05$) between wool genotypes and classified by using Blast Algorithm and compare with the NCBI Genbank database.

OUT ID	Taxonomic Classification	Accession number	Closest match	Max id	Host	Reference
11379	Unclassified Prevotellaceae	AB612385	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-A-43	100	Bovine rumen	unpublished

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		JN857628	Uncultured bacterium clone 186139-18	100	Ovine lung	Emerging Infect. Dis. 18 (3), 406-414 (2012)
		AB009238	Unidentified rumen bacterium RFN91	99	Bovine rumen	Published Only in Database (1997)
11926	Unclassified Prevotellaceae	GQ327165	Uncultured rumen bacterium clone CAPT2SD07	97	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		EU850489	Uncultured rumen bacterium clone BF255	97	Buffalo rumen	unpublished
		AB616282	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-A-CA157	97	Bovine rumen	unpublished
14006		Unclassified Clostridiales	AB824596	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: T3_3_23	99	Bovine rumen
	GQ327705		Uncultured rumen bacterium clone CTPT2SF06	99	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
	FJ172854		Uncultured bacterium clone RB-3G3	99	rumen	unpublished
14139	Unclassified Clostridiales	EU843854	Uncultured bacterium clone 1103206140824	99	Bovine rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
		GQ327884	Uncultured rumen bacterium clone CTPT2B04	99	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		EU259470	Uncultured rumen bacterium clone YRC94	99	Yak rumen	Mol. Biol. Rep. 37 (1), 553-562 (2010)
14468	Unclassified Prevotellaceae	EU871370	Uncultured rumen bacterium clone CF183	96	Bovine rumen	unpublished
		AB614917	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-B-180	95	Bovine rumen	unpublished
		GQ402127	Uncultured bacterium clone ALP22	95	Alpaca forestomach	Anaerobe 16 (4), 426-432 (2010)
15550	Unclassified Clostridiales	JF797361	Uncultured rumen bacterium clone UG-B0_024	100	Bovine rumen	FEMS Microbiol. Ecol. 78 (2), 275-284 (2011)
		AB821810	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: A26_4_17	99	Bovine rumen	unpublished
		GQ326922	Uncultured rumen bacterium clone CAL1B05	99	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
16377	Unclassified Prevotellaceae	AB821635	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: I14_2_12	94	Bovine rumen	unpublished
		EU850565	Uncultured rumen bacterium clone BF375	93	Buffalo rumen	unpublished
		AB612635	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-B-CA27	93	Bovine rumen	unpublished
18192	Unclassified Prevotellaceae	AB555482	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-C-CA68	96	Bovine rumen	unpublished
20200	Unclassified Prevotellaceae	AB821609	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: Y14_2_32	99	Bovine rumen	unpublished
		AB616462	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-B-CA163	99	Bovine rumen	unpublished
		GU304013	Uncultured rumen bacterium clone L406RC7-A09	99	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
21479	Unclassified Bacteroidales	KC290782	Uncultured bacterium clone LGM56	99	goat rumen	direct submission
		GU303922	Uncultured rumen bacterium clone L406RC-5-G09	98	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)

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		AB614982	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-B-CA158	94	Bovine rumen	unpublished
23366	Unclassified Clostridiales	HM105338	Uncultured Firmicutes bacterium clone L2d23UD	99	Bovine rumen	Appl. Environ. Microbiol. 76 (22), 7482-7490 (2010)
		FJ684320	Uncultured bacterium clone 6-4H15	99	Bovine faeces	Appl. Environ. Microbiol. 76 (14), 4858-4862 (2010)
		EU842532	Uncultured bacterium clone 1103200825082	98	Bovine rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
24094	Unclassified Lachnospiraceae	AB821777	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: l26_4_15	100	Bovine rumen	unpublished
		HM104724	Uncultured Firmicutes bacterium clone p1c01cow63	99	Bovine rumen	Appl. Environ. Microbiol. 76 (22), 7482-7490 (2010)
		GQ327836	Uncultured rumen bacterium clone CTRS2E03	99	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
27650	Unclassified Bacteroidia	GU303831	Uncultured rumen bacterium clone L406RC-4-G10	94	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		EU774555	Uncultured bacterium clone DL_1aaa03d02	92	Dooc langur feces	Science 320 (5883), 1647-1651 (2008)
		AB824365	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: B1_4_19	91	Bovine rumen	unpublished
28267	Unclassified Clostridiales	JX218699	Uncultured rumen bacterium clone MXML_C-O11	100	Ovibus moschatus rumen	unpublished
		JF797552	Uncultured rumen bacterium clone UG-B6_037	100	Bovine rumen	FEMS Microbiol. Ecol. 78 (2), 275-284 (2011)
		GQ327362	Uncultured rumen bacterium clone CARS2H02	100	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
29010	Unclassified Clostridiales	EU462921	Uncultured bacterium clone arma_aaj55a07	98	armadillo feces	Science 320 (5883), 1647-1651 (2008)
		KC290765	Uncultured bacterium clone LGM39	98	goat rumen	direct submission
		KC632940	Uncultured bacterium clone SG.166.98.2_325108	97	kangaroo foregut	ISME J (2014) In press
29599	Unclassified Prevotellaceae	GQ327165	Uncultured rumen bacterium clone CAPT2SD07	98	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		KC290860	Uncultured bacterium clone LGM134	97	goat rumen	direct submission
		AF018508	Unidentified rumen bacterium 30-11	97	rumen	Anaerobe 4 (3), 153-163 (1998)
31336	Unclassified Bacteroidales	GQ327881	Uncultured rumen bacterium clone CTPT2A12	99	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		AY244965	Uncultured rumen bacterium clone BS11	99	Bovine rumen	unpublished
		HM104929	Uncultured Bacteroidetes bacterium clone p1i09pool	98	Bovine rumen	Appl. Environ. Microbiol. 76 (22), 7482-7490 (2010)
32992	Unclassified Ruminococcaceae	AB821806	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: A26_4_9	98	Bovine rumen	unpublished
		JX095359	Uncultured bacterium clone Hda1-58	98	Bovine hindgut	Gut Pathog 5 (1), 8 (2013)
		EU794098	Uncultured Ruminococcaceae bacterium clone EMP_A26	97	Bovine faeces	Vet. Microbiol. 136 (3-4), 285-292 (2009)

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36659	Unclassified Ruminococcaceae	GU304166	Uncultured rumen bacterium clone L406RT-2-A03	100	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		EU381431	Uncultured rumen bacterium clone L3A_E01	100	Bovine rumen	unpublished
		EU844382	Uncultured bacterium clone 1103200831444	99	Bovine rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
38584	Unclassified Prevotellaceae	AB821633	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: I14_2_10	98	Bovine rumen	unpublished
		HQ399919	Uncultured rumen bacterium clone L102RC-6-B01	98	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		AB034100	Uncultured rumen bacterium 6C3d-16	98	rumen	Anaerobe 6, 273-284 (2000)
41898	Unclassified Prevotellaceae	AB821831	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: A26_4_42	96	Bovine rumen	Unpublished
		GQ402134	Uncultured bacterium clone SH17	96	Ovine rumen	Anaerobe 16 (4), 426-432 (2010)
		KJ159259	Uncultured bacterium clone LlaJ65	96	Llama forestomach	unpublished
44226	Unclassified Prevotellaceae	FJ172869	Uncultured bacterium clone RB-3D1	97	Yak rumen	unpublished
		EU259398	Uncultured rumen bacterium clone YRC22	96	Yak rumen	Mol. Biol. Rep. 37 (1), 553-562 (2010)
		AB615059	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-C-132	95	Bovine rumen	unpublished
47923	Unclassified Prevotellaceae	AB821652	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: I14_2_51	100	Bovine rumen	unpublished
		KC290806	Uncultured bacterium clone LGM80	100	goat rumen	unpublished
		GU303519	Uncultured rumen bacterium clone L406RC-1-A01	100	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
50066	Unclassified Prevotellaceae	AB746666	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: M14_1_40	99	Bovine rumen	unpublished
		AY244934	Uncultured rumen bacterium clone BE23	99	Bovine rumen	unpublished
		EU381970	Uncultured rumen bacterium clone P5_H20	98	Bovine rumen	unpublished
5219	Unclassified Lachnospiraceae	AB746664	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: M14_1_37	100	Bovine rumen	unpublished
		GU124467	Rumen bacterium NK4A66	99	Ovine rumen	Appl. Environ. Microbiol. 76 (7), 2058-2066 (2010)
		AB494866	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: N27	99	Ovine rumen	Published Only in Database (2010)
53298	Unclassified Prevotellaceae	AB009237	Unidentified rumen bacterium RFN90	98	Bovine rumen	Published Only in Database (1997)
		JX003961	Uncultured bacterium clone v09b89pf	98	Buffalo rumen	unpublished
		HM008812	Uncultured bacterium clone Camel_AAR_122	98	Camel rumen	Environ. Microbiol. 13 (11), 3024-3035 (2011)
5719	Unclassified Bacteroidia	EU850489	Uncultured rumen bacterium clone BF255	94	Buffalo rumen	unpublished
		AF018508	Unidentified rumen bacterium 30-11	94	Bovine rumen	Anaerobe 4 (3), 153-163 (1998)
		GQ327155	Uncultured rumen bacterium clone CAPT1SC08	93	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
57388	Unclassified Prevotellaceae	GU302957	Uncultured rumen bacterium clone L206RC-6-D08	98	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		AF018501	Uncultured rumen bacterium clone L102RC-5-H11	96	Bovine rumen	Anaerobe 4 (3), 153-163 (1998)
		KJ159259	Uncultured bacterium clone LlaJ65	96	Llama forestomach	unpublished
58648	Unclassified Clostridiales	GU324387	Rumen bacterium NK4A76	100	Ovine rumen	J. Microbiol. Methods 84 (1), 52-60 (2011)

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		GQ327737	Uncultured rumen bacterium clone CTRS1B05	100	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		EU845442	Uncultured bacterium clone 1103200828836	100	Bovine rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
61776	Unclassified Lachnospiraceae	EU842355	Uncultured bacterium clone 1103200821219	100	Bovine rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
		GQ327344	Uncultured rumen bacterium clone CARS2F06	99	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		AY854287	Uncultured bacterium clone CATTLE_25	99	Bovine rumen	Environ. Microbiol. 5 (11), 1212-1220 (2003)
63183	Unclassified Prevotellaceae	EU843625	Uncultured bacterium clone 1103200842910	100	Bovine rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
		AB612655	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-B-CA47	99	Bovine rumen	unpublished
		GQ327327	Uncultured rumen bacterium clone CARS2E01	99	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
6403	Unclassified Prevotellaceae	GQ402134	Uncultured bacterium clone SH17	99	ovine rumen	Anaerobe 16 (4), 426-432 (2010)
		AB821831	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: A26_4_42	99	Bovine rumen	unpublished
		HM104800	Uncultured Bacteroidetes bacterium clone p1k01cow63	97	Bovine rumen	Appl. Environ. Microbiol. 76 (22), 7482-7490 (2010)
65608	Unclassified Clostridia	GU303794	Uncultured rumen bacterium clone L406RC-4-D08	100	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		EU842135	Uncultured bacterium clone 1101352028130	100	Bovine rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
		DQ673511	Uncultured rumen bacterium clone GRC46	99	Gayals rumen	Mol. Biol. Rep. 37 (4), 2063-2073 (2010)
66850	Unclassified Clostridia	HQ399802	Uncultured rumen bacterium clone L102RC-4-F02	100	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		HM105077	Uncultured Bacteroidetes bacterium clone L1f03UD	89	Bovine rumen	Appl. Environ. Microbiol. 76 (22), 7482-7490 (2010)
		GQ358317	Uncultured Prevotellaceae bacterium clone SGYA529	89	Wallaby foregut	Proc. Natl. Acad. Sci. U.S.A. 107 (33), 14793-14798 (2010)
69725	Unclassified Lachnospiraceae	AB824628	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: T3_4_8	99	Bovine rumen	unpublished
		EF686521	Uncultured rumen bacterium clone YNRC10	99	Bovine rumen	Mol. Biol. Rep. 39 (2), 1181-1192 (2012)
		HM008777	Uncultured bacterium clone Camel_AAR_068	99	Camel rumen	Environ. Microbiol. 13 (11), 3024-3035 (2011)
70689	Unclassified Bacteroidales	AB821668	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: A14_2_22	100	Bovine rumen	unpublished
		AM884127	Uncultured rumen bacterium partial 16S rRNA gene, clone 46-P5	97	Ovine rumen	Vet. Microbiol. 146 (1-2), 98-104 (2010)
		HQ400225	Uncultured rumen bacterium clone L102RT-4-E01	97	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
8786	Unclassified Lachnospiraceae	JF797412	Uncultured rumen bacterium clone UG-B0_106	100	Bovine rumen	FEMS Microbiol. Ecol. 78 (2), 275-284 (2011)
		AB034147	Uncultured rumen bacterium 4C28d-20	99	Bovine rumen	Anaerobe 6, 273-284 (2000)
		GU303804	Uncultured rumen bacterium clone L406RC-4-E06	99	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)

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Table S3. Bacteria OTUs that present differences in presence/abundance ($P < 0.05$) between levels of intake and classified by using Blast Algorithm and compare with the Greengenes database.

OTU ID	Taxonomic Classification	Accession number	Closest named relative	Max id	Host	Reference
11727	Unclassified Bacteroidales	AY239469.1	Gram-negative bacterium cL10-2b-4 16S ribosomal RNA gene, complete sequence	86.14	Rat faecal flora	Can. J. Microbiol. 49 (10), 589-601 (2003)
11926	Unclassified Prevotellaceae	AB501152.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: BP1-34	95.68	Sheep rumen	Unpublished
12210	Unclassified Lachnospiraceae	AJ229251.1	Clostridium from anoxic bulk soil 16S rRNA gene (strain FCB90-3)	87.44	Soil	Appl. Environ. Microbiol. 65 (11), 5042-5049 (1999)
12341	Unclassified Lachnospiraceae	AY442822.1	Ruminococcus sp. YE58 16S ribosomal RNA gene, partial sequence	94.52	Kangaroo forestomach	Lett. Appl. Microbiol. 41 (4), 327-333 (2005)
14095	Unclassified Clostridiales	AY554413.1	Alkalibacterium sp. 12A2 16S ribosomal RNA gene, partial sequence	88.15	landfill leachate bioreactor	Unpublished
16856	Unclassified Prevotellaceae	AB501161.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: BP1-76	98.86	Sheep rumen	Unpublished
19128	Unclassified Ruminococcaceae	DQ882649.1	Ruminococcus bromii strain YE282 16S ribosomal RNA gene, partial sequence	96.91	Cattle rumen	J. Appl. Microbiol. 103 (6), 2065-2073 (2007)
23190	Unclassified Bacteroidia	AB547651.1	Paraprevotella clara gene for 16S ribosomal RNA, partial sequence, strain: JCM 14859	87.73	---	J. Med. Microbiol. 59 (PT 11), 1293-1302 (2010)
23836	Unclassified Anaeroplasmataceae	M25050.1	A.abactoclasticum 16S ribosomal RNA small subunit	99.77	---	J. Bacteriol. 171 (12), 6455-6467 (1989)
23845	Unclassified Prevotellaceae	AF414826.1	Prevotella intermedia strain 36-PIN 16S ribosomal RNA gene, partial sequence	88.44	---	Int. J. Syst. Evol. Microbiol. 52 (PT 4), 1391-1395 (2002)
24126	Unclassified Bacteroidales	DQ421387.1	Olivibacter sitiensis strain AW-6 16S ribosomal RNA gene, partial sequence	84.13	---	Int. J. Syst. Evol. Microbiol. 57 (PT 2), 398-404 (2007)
25190	Unclassified Leconostocaceae	AB494723.1	Weissella confusa gene for 16S ribosomal RNA, partial sequence, strain: MJJ8	100.0	Fermented juice	Unpublished
25534	Unclassified Prevotellaceae	AB501173.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: AC5-13	94.75	Sheep rumen	Unpublished
25962	Unclassified Bacteroidales	AB078046.1	Flexibacter canadensis gene for 16S rRNA, strain: IFO 15130	85.39	---	J. Gen. Appl. Microbiol. 48 (3), 155-165 (2002)
25986	Unclassified Bacteroidales	DQ012324.2	Capnocytophaga genosp. AHN8471 strain AHN9528 16S ribosomal RNA gene, partial sequence	82.95	---	Int. J. Syst. Evol. Microbiol. 58 (PT 2), 324-336 (2008)
26251	Unclassified Prevotellaceae	AB501151.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: BP1-32	92.73	Sheep rumen	Unpublished
27901	Unclassified Lachnospiraceae	AB372004.1	Eubacterium cellulosolvens gene for 16S ribosomal RNA, partial sequence	91.19	---	FEMS Microbiol. Lett. 287 (1), 34-40 (2008)
28910	Unclassified Clostridiales	AY574991.1	Catabacter hongkongensis 16S ribosomal RNA gene, partial sequence	87.47	---	J. Clin. Microbiol. 45 (2), 395-401 (2007)
29809	Unclassified Ruminococcaceae	X85099.1	Ruminococcus bromii 16S rRNA gene	95.48	---	FEMS Microbiol. Lett. 129 (1), 69-73 (1995)

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30034	Unclassified Bacteroidales	FJ754320	Parapedobacter luteus strain 4M29 16S ribosomal RNA gene, partial sequence.	84.47	cotton waste compost	Int. J. Syst. Evol. Microbiol. 60 (PT 8), 1849-1853 (2010)
3044	Unclassified Prevotellaceae	AB501163.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: BP1-90.	95.45	sheep rumen	unpublished
31615	Unclassified Prevotellaceae	AB501151.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: BP1-32.	94.32	sheep rumen	unpublished
34568	Unclassified Bacteroidales	AY278613.1	Capnocytophaga genomosp. C1 16S ribosomal RNA gene, partial sequence.	84.55	dental caries	J. Clin. Microbiol. 42 (7), 3023-3029 (2004)
37079	Unclassified Bacteroidales	AY643076.1	Capnocytophaga cynodegmi strain CIP 103937 16S ribosomal RNA gene, partial sequence.	82.95	---	unpublished
37154	Unclassified Prevotellaceae	AB501152.1	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain: BP1-34.	95.23	sheep rumen	unpublished
37920	Unclassified Ruminococcaceae	X85099.1	R.bromii 16S rRNA gene	94.06	---	FEMS Microbiol. Lett. 129 (1), 69-73 (1995)
38632	Unclassified Prevotellaceae	EU728757.1	Prevotella sp. DJF_RP53 16S ribosomal RNA gene, partial sequence.	93.86	pigs	unpublished
39025	Unclassified Prevotellaceae	AJ009933.1	Prevotella aff. ruminicola Tc2-24	92.95	rumen	Int. J. Syst. Bacteriol. 44 (2), 246-255 (1994)
40161	Unclassified Prevotellaceae	AB501152.1	Prevotella ruminicola str. BP1-34	96.36	sheep rumen	unpublished
41635	Unclassified Bacteroidales	AY643076.1	Capnocytophaga cynodegmi str. CIP 103937	85.45	---	unpublished
46534	Unclassified Lachnospiraceae	ACIQ02000009.1	Oribacterium sp. oral taxon 078 str. F0262	92.38	---	unpublished
4658	Unclassified Bacteroidales	EU281854.1	Eubacterium sp. str. F1	87.19	cattle rumen	unpublished
47244	Unclassified Clostridia	X76161.1	Clostridium aminobutyricum str. DSM 2634	93.81	---	Int. J. Syst. Bacteriol. 44 (4), 812-826 (1994)
50622	Unclassified Prevotellaceae	AJ009933.1	Prevotella aff. ruminicola Tc2-24	95.23	rumen	Int. J. Syst. Bacteriol. 44 (2), 246-255 (1994)
53298	Unclassified Prevotellaceae	AB501174.1	Prevotella ruminicola str. AC5-20	97.50	sheep rumen	unpublished
53542	Unclassified Prevotellaceae	AB547651.1	Paraprevotella clara str. JCM 14859	89.07	---	J. Med. Microbiol. 59 (PT 11), 1293-1302 (2010)
56548	Unclassified Clostridiales	FJ848369.1	Clostridium sp. str. P1-2-2	89.05	---	unpublished
60104	Unclassified Bacteroidia	AB547685.1	Prevotella histicola str. JCM 15637	88.18	---	J. Med. Microbiol. 59 (PT 11), 1293-1302 (2010)
60120	Unclassified Prevotellaceae	L16465.1	Prevotella corporis	91.82	---	J. Bacteriol. 176 (3), 725-732 (1994)
63019	Unclassified Lachnospiraceae	X76748.1	Clostridium colinum str. DSM 6011	95.27	---	Int. J. Syst. Bacteriol. 44 (4), 812-826 (1994)
64400	Unclassified Clostridiales	ACFX02000046.1	Clostridium sp. str. M62/1	90.71	humans	unpublished
64787	Unclassified Prevotellaceae	AB547651.1	Paraprevotella clara str. JCM 14859	89.07	---	J. Med. Microbiol. 59 (PT 11), 1293-1302 (2010)
66074	Unclassified Clostridiales	GU124472.1	Blautia sp. str. Ser8	95.71	rumen	Appl. Environ. Microbiol. 76 (7), 2058-2066 (2010)
6627	Unclassified Prevotellaceae	FJ717335.2	Prevotella sp. oral taxon 472 str. F0295	89.77	humans	J. Bacteriol. 192 (19), 5002-5017 (2010)
66823	Unclassified Prevotellaceae	AB501157.1	Prevotella ruminicola str. BP1-60	98.18	sheep rumen	unpublished
68265	Unclassified Bacteroidales	DQ680836.1	Pedobacter koreensis str. Jip14	85.84	---	Int. J. Syst. Evol. Microbiol. 57 (PT 6), 1336-1341 (2007)
70747	Unclassified Clostridiales	AY534872.1	Clostridium sp. str. IMSNU 40011	96.19	forest soil	Int. J. Syst. Evol. Microbiol. 57 (PT 8), 1784-1787 (2007)
71886	Unclassified Prevotellaceae	AB501158.1	Prevotella ruminicola str. BP1-64	91.82	sheep rumen	unpublished

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7794	Unclassified Prevotellaceae	AB501169.1	Prevotella ruminicola str. BP5-13	95.92	sheep rumen	unpublished
797	Unclassified Clostridiales	FJ848364.1	Clostridium sp. str. P4-6	88.33	---	unpublished

Table S4. Bacteria OTUs that present differences in presence/abundance ($P < 0.05$) between levels of intake and classified by using Blast Algorithm and compare with the NCBI Genbank database.

OUT ID	Taxonomic Classification	Accession number	Closest match	Max Id	Host	Reference
11727	Unclassified Bacteroidales	JX218491	Uncultured rumen bacterium clone MXAP1bG7	98	Ovibos moschatus	unpublished
		AB612541.1	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-B-22	98	Cattle rumen	unpublished
11926	Unclassified Prevotellaceae	GQ327165.1	Uncultured rumen bacterium clone CAPT2SD07	97	Cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		EU850489.1	Uncultured rumen bacterium clone BF255	97	swamp buffalo rumen	unpublished
		AB616282.1	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-A-CA157	97	Cattle rumen	unpublished
12210	Unclassified Lachnospiraceae	AB185649.1	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: F24-H05	99	Cattle rumen	Biosci. Biotechnol. Biochem. 69 (3), 499-506 (2005)
		AY858395.3	Uncultured bacterium clone GRANT29	99	gastrointestinal tract of gazelle	Environ. Microbiol. 5 (11), 1212-1220 (2003)
		AB506500.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: ST-C-A10	99	sheep rumen	Published Only in Database (2013)
12341	Unclassified Lachnospiraceae	EU777964.1	Uncultured bacterium clone RHSD_aaa04h11_1	99	Procavia capensis	Science 320 (5883), 1647-1651 (2008)
		EU771852.1	Uncultured bacterium clone AFEL3_aao13g04	97	Loxodonta africana	Science 320 (5883), 1647-1651 (2008)
		AB507497.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: CONT-51	97	sheep rumen	Published Only in Database (2013)
14095	Unclassified Clostridiales	JF797385	Uncultured rumen bacterium clone UG-B0_053	100	cattle rumen	FEMS Microbiol. Ecol. 78 (2), 275-284 (2011)
		GQ327565	Uncultured rumen bacterium clone CTL2SD11	100	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		GU302589	Uncultured rumen bacterium clone L206RC-1-G06	100	cattle rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
16856	Unclassified Prevotellaceae	AB501161	Prevotella ruminicola gene for 16S rRNA, complete sequence, strain:BP1-76.	100	sheep rumen	unpublished
		EU777991	Uncultured bacterium clone RHSD_aaa01g04_1	100	Procavia capensis	Science 320 (5883), 1647-1651 (2008)
		KJ159325	Uncultured bacterium clone LlaBA88	100	Lama glama	unpublished
19128	Unclassified Ruminococcaceae	AB506551	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: ST-C-F08	100	sheep rumen	Published Only in Database (2013)

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		HM105434	Uncultured Firmicutes bacterium clone L2110UD	100	cattle rumen	Appl. Environ. Microbiol. 76 (22), 7482-7490 (2010)
		FJ679516	Uncultured bacterium clone 4-2B18	100	cattle faeces	Appl. Environ. Microbiol. 76 (14), 4858-4862 (2010)
23190	Unclassified Bacteroidia	AB615039	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-C-112.	99	cattle rumen	unpublished
		AB612840	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-C-CA56	99	cattle rumen	unpublished
		AB555141	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-A-CA76	99	cattle rumen	unpublished
23836	Unclassified Anaeroplasmataceae	GQ327377	Uncultured rumen bacterium clone CAPT2C01	99	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		AB614663	Uncultured rumen bacterium 16S rRNA, partial sequence, clone:R-A-113	99	cattle rumen	unpublished
		HQ616131	Uncultured rumen bacterium clone KF34-140	99	cattle rumen	unpublished
23845	Unclassified Prevotellaceae	GU302822	Uncultured rumen bacterium clone L206RC-4-E06	100	cattle rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		EU843073	Uncultured bacterium clone 1103200821500	100	cattle rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
		GQ402136	Uncultured bacterium clone SH14 16S	99	sheep rumen	Anaerobe 16 (4), 426-432 (2010)
24126	Unclassified Bacteroidales	JX941442	Uncultured rumen bacterium clone G34	99	sheep rumen	unpublished
		EU290034	Uncultured bacterium clone 06SM90_11H	99	sheep lungs	J. Clin. Microbiol. 46 (2), 423-430 (2008)
		JX218536	Uncultured rumen bacterium clone MXAP1bB1	98	Ovibos moschatus rumen	unpublished
25190	Unclassified Leconostocaceae	KJ095645	Weissella cibaria strain SD3S3L2	100	soidon	unpublished
		KF598945	Weissella confusa strain 3133O2	100	mesquite flower	PLoS ONE 8 (12), E83125 (2013)
		AB911503	Weissella cibaria gene for 16S rRNA, partial sequence, strain: JCM 7777	100	culture collection	Unpublished
25534	Unclassified Prevotellaceae	AB746641	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: M14_1_9.	98	cattle rumen	unpublished
		GQ327350	Uncultured rumen bacterium clone CARS2F12	98	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		EU382025	Uncultured rumen bacterium clone P5_115	98	cattle rumen	Unpublished
25962	Unclassified Bacteroidales	EU259414	Uncultured rumen bacterium clone YRC38	99	yak rumen	Mol. Biol. Rep. 37 (1), 553-562 (2010)
		AB824357	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: B1_4_6.	99	swamp buffalo rumen	unpublished
		DQ394590	Uncultured rumen bacterium clone NP18	99	reindeer rumen	Microb. Ecol. 54 (3), 424-438 (2007)
25986	Unclassified Bacteroidales	JX218498	Uncultured rumen bacterium clone MXAP1bC12	99	Ovibus moschatus rumen	unpublished
		HM008775	Uncultured bacterium clone Camel_AAR_066	98	camel foregut	Environ. Microbiol. 13 (11), 3024-3035 (2011)
		KC163041	Uncultured rumen bacterium clone YRMCB21	97	yak rumen	unpublished

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26251	Unclassified Prevotellaceae	GQ327247	Uncultured rumen bacterium clone CARS1D11	96	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		AB821652	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: I14_2_51.	95	cattle rumen	unpublished
		KC290806	Uncultured bacterium clone LGM80	95	goat rumen	unpublished
27901	Unclassified Lachnospiraceae	AB507565	Uncultured bacterium gene for 16S rRNA, partial sequence, clone:CONT-119.	100	sheep rumen	Published Only in Database (2013)
		JN857795	Uncultured bacterium clone ZB SBN B-31	100	sheep lungs	Emerging Infect. Dis. 18 (3), 406-414 (2012)
		HQ399879	Uncultured rumen bacterium clone L102RC-5-E05	100	cattle rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
28910	Unclassified Clostridiales	GQ327290	Uncultured rumen bacterium clone CARS2A07	99	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		EU843999	Uncultured bacterium clone 1101352050153	98	cattle rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
		KJ159282	Uncultured bacterium clone LlaBA11	98	Lama forestomach	unpublished
29809	Unclassified Ruminococcaceae	AB506540	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: ST-C-E08.	98	sheep rumen	Published Only in Database (2013)
		DQ673585	Uncultured rumen bacterium clone GRC120	98	Bos frontalis rumen	Mol. Biol. Rep. 37 (4), 2063-2073 (2010)
		JF797511	Uncultured rumen bacterium clone UG-B3_103	98	cattle rumen	FEMS Microbiol. Ecol. 78 (2), 275-284 (2011)
30034	Unclassified Bacteroidales	AM884129	Uncultured rumen bacterium partial 16S rRNA gene, clone 48-P5.	99	sheep rumen	Vet. Microbiol. 146 (1-2), 98-104 (2010)
		GQ327050	Uncultured rumen bacterium clone CAL1SD05	97	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		EU259446	Uncultured rumen bacterium clone YRC70	97	yak rumen	Mol. Biol. Rep. 37 (1), 553-562 (2010)
3044	Unclassified Prevotellaceae	AF018508	Unidentified rumen bacterium 30-11	98	rumen	Anaerobe 4 (3), 153-163 (1998)
		GQ327155	Uncultured rumen bacterium clone CAPT1SC08	98	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		AY244942	Uncultured rumen bacterium clone BE28	98	cattle rumen	unpublished
31615	Unclassified Prevotellaceae	AB616572	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-C-187.	98	cattle rumen	unpublished
		GQ327918	Uncultured rumen bacterium clone CTPT2G09	98	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		JX889341	Uncultured bacterium clone SDCS44 16S	98	Cervus nippon rumen	BMC Microbiol. 13, 151 (2013)
34568	Unclassified Bacteroidales	AB506616	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: ST-D09.	100	sheep rumen	Published Only in Database (2013)
		EU844637	Uncultured bacterium clone 1103200949791	100	cattle rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
		GU302714	Uncultured rumen bacterium clone L206RC-3-C09	99	cattle rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
37079	Unclassified Bacteroidales	EU382048	Uncultured rumen bacterium clone P5_D12	99	cattle rumen	unpublished
		AB824562	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: T1_5_38.	99	Thai native cattle rumen	unpublished

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		AB746544	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: Y14_1_33.	99	cattle rumen	unpublished
37154	Unclassified Prevotellaceae	AB612385	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-A-43.	96	cattle rumen	unpublished
		JN857628	Uncultured bacterium clone 186139-18	96	sheep lung	Emerging Infect. Dis. 18 (3), 406-414 (2012)
		GU324406	Rumen bacterium NK4A62	96	sheep rumen	J. Microbiol. Methods 84 (1), 52-60 (2011)
37920	Unclassified Ruminococcaceae	EU842638	Uncultured bacterium clone 1103200826910	99	cattle rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
		JX941411	Uncultured rumen bacterium clone G3	99	sheep rumen	unpublished
		AB506559	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: ST-C-G05.	98	sheep rumen	Published Only in Database (2013)
38632	Unclassified Prevotellaceae	AB185797	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: U29-F10.	99	cattle rumen	Biosci. Biotechnol. Biochem. 69 (3), 499-506 (2005)
		HQ399751	Uncultured rumen bacterium clone L102RC-4-A02 16S ribosomal RNA gene, partial sequence	99	cattle rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		FJ681782	Uncultured bacterium clone 5-8G3	99	cattle faeces	Appl. Environ. Microbiol. 76 (14), 4858-4862 (2010)
39025	Unclassified Prevotellaceae	FJ028784	Uncultured rumen bacterium clone TWBRB69	99	buffalo rumen	unpublished
		HM008796	Uncultured bacterium clone Camel_AAR_092	96	camel rumen	Environ. Microbiol. 13 (11), 3024-3035 (2011)
		EU773702	Uncultured bacterium clone CAP_aah97c05	95	Capybara faeces	Science 320 (5883), 1647-1651 (2008)
40161	Unclassified Prevotellaceae	AB746642	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: M14_1_11.	97	cattle rumen	unpublished
		GQ327507	Uncultured rumen bacterium clone CTL2C09	97	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		AB821682	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: A14_2_42	97	cattle rumen	unpublished
41635	Unclassified Bacteroidales	JN857609	Uncultured bacterium clone 186195-30	100	sheep lungs	Emerging Infect. Dis. 18 (3), 406-414 (2012)
		JF797427	Uncultured rumen bacterium clone UG-B0_128	100	cattle rumen	FEMS Microbiol. Ecol. 78 (2), 275-284 (2011)
		JX218503	Uncultured rumen bacterium clone MXAP1bG11	99	Ovibos moschatus rumen	unpublished
46534	Unclassified Lachnospiraceae	AB614763	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-A-CA116.	100	cattle rumen	unpublished
		HM105290	Uncultured Firmicutes bacterium clone L1p21UD	99	cattle rumen	Appl. Environ. Microbiol. 76 (22), 7482-7490 (2010)
		EF436369	Uncultured rumen bacterium clone BRC83	99	Bubalus bubalis rumen	Mol. Biol. Rep. 37 (4), 2063-2073 (2010)
4658	Unclassified Bacteroidales	EU843838	Uncultured bacterium clone 1103206092360	99	cattle rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
		GU302859	Uncultured rumen bacterium clone L206RC-5-A12	99	cattle rumen	Vet. Microbiol. 155 (1), 72-80 (2012)

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		AB746793	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: A26_3_10.	99	cattle rumen	unpublished
47244	Unclassified Clostridia	GQ327762	Uncultured rumen bacterium clone CTRS1E02	100	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		EU842499	Uncultured bacterium clone 1103200824956	99	cattle rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
		FJ833992	Uncultured bacterium clone P2Q202_96H12	98	crop, hoatzin	ISME J 4 (5), 611-620 (2010)
50622	Unclassified Prevotellaceae	KC290864	ncultured bacterium clone LGM138	99	goat rumen	unpublished
		EU290069	Uncultured bacterium clone 06WA06_8A	99	sheep lungs	J. Clin. Microbiol. 46 (2), 423-430 (2008)
		GQ327587	Uncultured rumen bacterium clone CTL2SE11A	97	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
53298	Unclassified Prevotellaceae	AB009237	Unidentified rumen bacterium RFN90 gene	98	cattle rumen	unpublished
		JX003961	Uncultured bacterium clone v09b89pf	98	buffaloes rumen	unpublished
		HM008812	Uncultured bacterium clone Camel_AAR_122	98	Camel foregut	Environ. Microbiol. 13 (11), 3024-3035 (2011)
53542	Unclassified Prevotellaceae	AB615193	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-C-CA177.	100	cattle rumen	unpublished
		GU303845	Uncultured rumen bacterium clone L406RC-5-A01	99	cattle rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		EU459537	Uncultured bacterium clone CAP_aah99d09	97	capybara feces	Science 320 (5883), 1647-1651 (2008)
56548	Unclassified Clostridiales	KJ159249	Uncultured bacterium clone LlaJ51	99	Llama forestomach	unpublished
		AB494742	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: A8.	99	sheep rumen	Published Only in Database (2010)
		EU844442	Uncultured bacterium clone 1103200831750	99	cattle rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
60104	Unclassified Bacteroidia	EU459518	Uncultured bacterium clone CAP_aah99a04	99	capybara feces	Science 320 (5883), 1647-1651 (2008)
		EU381764	Uncultured rumen bacterium clone P5_H23	99	cattle rumen	unpublished
		GQ327275	Uncultured rumen bacterium clone CARS1G10	98	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
60120	Unclassified Prevotellaceae	AB615110	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-C-183.	100	cattle rumen	unpublished
		EU719293	Uncultured rumen bacterium clone 814383	100	cattle rumen	unpublished
		AB555249	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-B-CA9	100	cattle rumen	unpublished
63019	Unclassified Lachnospiraceae	JX218652	Uncultured rumen bacterium clone MXML_C-J8	100	Ovibos moschatus rumen	unpublished
		EU850609	Uncultured rumen bacterium clone BF444	100	Swamp nuffalo rumen	unpublished
		GQ327045	Uncultured rumen bacterium clone CAL2SC07	99	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
64400	Unclassified Clostridiales	AB612842	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-C-CA58.	100	cattle rumen	unpublished

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		AB185567	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: F23-H04	100	cattle rumen	Biosci. Biotechnol. Biochem. 69 (3), 499-506 (2005)
64787	Unclassified Prevotellaceae	EU773602	Uncultured bacterium clone CAP_ aai00g02	99	Capybara feces	Science 320 (5883), 1647-1651 (2008)
		AB555057	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-A-76.	97	cattle rumen	unpublished
66074	Unclassified Clostridiales	KC163111	Uncultured rumen bacterium clone YRMCB126	98	Yak rumen	unpublished
		GQ358233	Uncultured Lachnospiraceae bacterium clone SGYA396	98	Wallaby foregut	Proc. Natl. Acad. Sci. U.S.A. 107 (33), 14793-14798 (2010)
		EU344424	Uncultured bacterium clone hoa2_44g08	98	hoatzin	Appl. Environ. Microbiol. 74 (19), 5905-5912 (2008)
6627	Unclassified Prevotellaceae	AM884031	Uncultured rumen bacterium partial 16S rRNA gene, clone 23-P1.	99	sheep rumen	Vet. Microbiol. 146 (1-2), 98-104 (2010)
		AB612864	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-C-CA80	92	cattle rumen	unpublished
		EU844208	Uncultured bacterium clone 1103200823762	92	cattle rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
66823	Unclassified Prevotellaceae	AB009237	Unidentified rumen bacterium RFN90 gene	99	cattle rumen	Published Only in Database (1997)
		GQ327385	Uncultured rumen bacterium clone CAPT2D06	98	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		HM008812	Uncultured bacterium clone Camel_AAR_122	98	camel rumen	Environ. Microbiol. 13 (11), 3024-3035 (2011)
68265	Unclassified Bacteroidales	GQ327795	Uncultured rumen bacterium clone CTRS1H05	99	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		EU471807	Uncultured bacterium clone AE2_ aaa03b03	91	Elephant faeces	Science 320 (5883), 1647-1651 (2008)
70747	Unclassified Clostridiales	AB612604	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-B-85.	100	cattle rumen	unpublished
		AF018547	Unidentified rumen bacterium JW11	99	rumen	Anaerobe 4 (3), 153-163 (1998)
		JX218429	Uncultured rumen bacterium clone MXAP1aG2	99	Ovibos moschatus rumen	unpublished
71886	Unclassified Prevotellaceae	AF018441	Unidentified rumen bacterium JW9	96	rumen	Anaerobe 4 (3), 153-163 (1998)
		GQ327582	Uncultured rumen bacterium clone CTL1SC12A	95	cattle rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		EU842487	Uncultured bacterium clone 1103200823366	95	cattle rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
7794	Unclassified Prevotellaceae	KJ159325	Uncultured bacterium clone LlaBA88	96	Llama forestomach	unpublished
		AB616236	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-A-CA111.	96	cattle rumen	unpublished
		AF018450	Unidentified rumen bacterium JW31	96	rumen	Anaerobe 4 (3), 153-163 (1998)
797	Unclassified Clostridiales	EU381686	Uncultured rumen bacterium clone L7B_D10	100	cattle rumen	unpublished
		EF686530	Uncultured rumen bacterium clone YNRC19	100	cattle rumen	Mol. Biol. Rep. 39 (2), 1181-1192 (2012)
		AB270093	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: T33H60F18	100	cattle rumen	Anaerobe 13 (2), 57-64 (2007)

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Table S5. Bacteria OTUs that were significant ($P < 0.001$) correlated with continuous variables and classified by using Blast Algorithm and compare with the Greengenes database.

OTU ID	Taxonomic Classification	Accession number	Closest named relative	Max id	Host	Reference
1903	Unclassified Ruminococcus	ACFX02000046.1	Clostridium sp. str. M62/1	96.43	human colon	direct submission
10263	Unclassified Lachnospiraceae	FP929051.1	Ruminococcus bromii str. L2-63	95.01	---	direct submission
23270	Unclassified Clostridiales	AB537979.1	Garciella sp. GK3 gene for 16S rRNA, partial sequence	87.71	anaerobic digester	Unpublished
25190	Unclassified Weissella	AB494723.1	Weissella confusa str. MJJ8	100.0	fermented foods	unpublished
26305	Unclassified Prevotella	AB501152.1	Prevotella ruminicola str. BP1-34	95.68	sheep rumen	unpublished
26763	Unclassified Clostridiales	FJ848364.1	Clostridium sp. str. P4-6	88.57	peat	direct submission
36936	Unclassified Butryvibrio	DQ278862.1	Clostridium aminophilum 152R-1b	95.95	cattle rumen	J. Appl. Microbiol. 100 (6), 1261-1271 (2006)
40161	Unclassified Prevotella	AB501152.1	Prevotella ruminicola str. BP1-34	96.36	sheep rumen	unpublished
41506	Unclassified Sphaerochaeta	DQ833400.1	Sphaerochaeta sp. str. TQ1	87.42	pure culture	direct submission
41635	Unclassified Bacteroidales	AY643076.1	Capnocytophaga cynodegmi str. CIP 103937	85.45	---	direct submission
44836	Unclassified Bacteroidales	AJ431253.1	Cytophaga sp. str. Dex80-37	83.64	---	direct submission
47696	Unclassified Lachnospiraceae	FJ957875.1	Clostridium saccharolyticum str. JPL_23	97.14	---	Appl. Environ. Microbiol. 76 (9), 2837-2845 (2010)
58734	Unclassified Prevotellaceae	AB331896.1	Paraprevotella clara gene for 16S ribosomal RNA, partial sequence	90.21	Human faeces	Int. J. Syst. Evol. Microbiol. 59 (PT 8), 1895-1900 (2009)
64787	Unclassified Prevotellaceae	AB547651.1	Paraprevotella clara str. JCM 14859	89.07	---	direct submission
66823	Unclassified Prevotella	AB501174.1	Prevotella ruminicola str. AC5-20	98.18	sheep rumen	unpublished
71604	Unclassified Prevotella	AB501155.1	Prevotella sp. str. BP1-56	91.14	sheep rumen	unpublished
71886	Unclassified Prevotella	AB501163.1	Prevotella ruminicola str. BP1-90	91.82	sheep rumen	unpublished

Table S6. Bacteria OTUs that were significant ($P < 0.001$) correlated with continuous variables and classified by using Blast Algorithm and compare with the NCBI Genbank database.

OUT ID	Taxonomic Classification	Accession number	Closest match	Max id	Host	Reference
1903	Unclassified Ruminococcus	HQ400396	Uncultured rumen bacterium clone L102RT-6-G11	99	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		AB185810	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: U29-G12	98	Bovine rumen	Biosci. Biotechnol. Biochem. 69 (3), 499-506 (2005)
		AB821639	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: 114_2_16	98	Bovine rumen	unpublished
10263	Unclassified Lachnospiraceae	AB730785	Lachnospiraceae bacterium G11 gene	97	Bovine rumen	Lett. Appl. Microbiol. 56 (1), 63-70 (2013)
		FJ834079	Uncultured bacterium clone P2Q401_16H02	97	Hoatzin	ISME J 4 (5), 611-620 (2010)

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		GQ449028	Uncultured bacterium clone calf783_6wks_grp1_H10	97	Calves faeces	unpublished
23270	Unclassified Clostridiales	AB507483	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: CONT-37.	100	Ovine rumen	Published Only in Database (2013)
		GU303900	Uncultured rumen bacterium clone L406RC-5-E11	100	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		GQ402103	Uncultured bacterium clone SH4	100	Ovine rumen	Anaerobe 16 (4), 426-432 (2010)
25190	Unclassified Weissella	HF562954	Weissella confusa partial 16S rRNA gene, strain qz-488-2	100	Oat	unpublished
		KF842967	uncultured bacterium clone SG_B030	100	Human faeces	unpublished
		KF330573	Uncultured bacterium clone GXTJ5A301A5ZJQ	100	Prawn intestine	PLoS ONE 9 (3), E91853 (2014)
26305	Unclassified Prevotella	AB555482	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-C-CA68.	99	Bovine rumen	unpublished
		EU850489	Uncultured rumen bacterium clone BF255	97	Buffalo rumen	unpublished
		AB244160	Uncultured rumen bacterium gene for 16S ribosomal RNA, partial sequence, clone:T20H80G03.	97	Bovine rumen	Anaerobe 13 (2), 57-64 (2007)
26763	Unclassified Clostridiales	HQ716656	Uncultured bacterium clone T2WK15B11	97	Pig faeces	J. Appl. Microbiol. 110 (6), 1414-1425 (2011)
		EU844787	Uncultured bacterium clone 1101352057827	97	Bovine rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
		GU303684	Uncultured rumen bacterium clone L406RC-3-B06	97	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
36936	Unclassified Butyrivibrio	GU303967	Uncultured rumen bacterium clone L406RC-6-D10	100	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		KC632867	Uncultured bacterium clone SG.166.98.2_323968	100	Kangaroo foregut	ISME J (2014) In press
		EU844734	Uncultured bacterium clone 1103206141160	99	Bovine rumen	Proc. Natl. Acad. Sci. U.S.A. 106 (6), 1948-1953 (2009)
40161	Unclassified Prevotella	GQ327507	Uncultured rumen bacterium clone CTL2C09	97	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		AB746642	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: M14_1_11	97	Bovine rumen	unpublished
		AB821682	Uncultured bacterium gene for 16S rRNA, partial sequence, clone A14_2_42.	97	Bovine rumen	unpublished
41506	Unclassified Sphaerochaeta	HM049828	Uncultured Spirochaeta sp. clone RF104	100	Ovine rumen	unpublished
		AB537633	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: ALTC68	99	Ovine rumen	FEMS Microbiol. Lett. 316 (1), 51-60 (2011)
		HM104815	Uncultured Firmicutes bacterium clone p1112cow63	95	Bovine rumen	Appl. Environ. Microbiol. 76 (22), 7482-7490 (2010)
41635	Unclassified Bacteroidales	JN857609	Uncultured bacterium clone 186195-30	100	Sheep lungs	Emerging Infect. Dis. 18 (3), 406-414 (2012)
		JF797427	Uncultured rumen bacterium clone UG-B0_128	99	Bovine rumen	FEMS Microbiol. Ecol. 78 (2), 275-284 (2011)

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		JX218503	Uncultured rumen bacterium clone MXAP1bG11	99	Ovibos moschatus rumen	unpublished
44836	Unclassified Bacteroidales	AB616340	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: D-B-128	100	Bovine rumen	unpublished
		EU773671	Uncultured bacterium clone CAP_aah99e07	100	Capybara feces	Science 320 (5883), 1647-1651 (2008)
		EU381867	Uncultured rumen bacterium clone P5_G16	100	Bovine rumen	unpublished
47696	Unclassified Lachnospiraceae	HQ399713	Uncultured rumen bacterium clone L102RC-1-F12	100	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		AB555075	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-A-CA10	99	Bovine rumen	unpublished
		EU779220	Uncultured bacterium clone TU1_aaa02c09	99	Ovis vignei faeces	Science 320 (5883), 1647-1651 (2008)
58734	Unclassified Prevotellaceae	AB821632	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: I14_2_9	99	Bovine rumen	unpublished
		GU302574	Uncultured rumen bacterium clone L206RC-1-E12	99	Bovine rumen	Vet. Microbiol. 155 (1), 72-80 (2012)
		EU459456	Uncultured bacterium clone CAP_aah97e06	99	capybara feces	Science 320 (5883), 1647-1651 (2008)
64787	Unclassified Prevotellaceae	EU773602	Uncultured bacterium clone CAP_aai00g02	99	capybara feces	Science 320 (5883), 1647-1651 (2008)
		AB555057	Uncultured rumen bacterium 16S rRNA, partial sequence, clone: R-A-76.	97	Bovine rumen	unpublished
		GQ327696	Uncultured rumen bacterium clone CTPT1SE06	96	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
66823	Unclassified Prevotella	AB009237	Unidentified rumen bacterium RFN90	99	Bovine rumen	Published Only in Database (1997)
		GQ327385	Uncultured rumen bacterium clone CAPT2D06	99	Bovine rumen	FEMS Microbiol. Ecol. 74 (3), 612-622 (2010)
		HM008812	Uncultured bacterium clone Camel_AAR_122	98	Camel rumen	Environ. Microbiol. 13 (11), 3024-3035 (2011)
71604	Unclassified Prevotella	AM884081	Uncultured rumen bacterium partial 16S rRNA gene, clone 21-P3	96	Ovine rumen	Vet. Microbiol. 146 (1-2), 98-104 (2010)
		JX218862	Uncultured rumen bacterium clone MXMP-M2	95	Ovibos moschatus rumen	unpublished
		EU719289	Uncultured rumen bacterium clone 823013	94	Bovine rumen	unpublished
71886	Unclassified Prevotella	AF018441	Unidentified rumen bacterium JW9	96	rumen	Anaerobe 4 (3), 153-163 (1998)
		EU850530	Uncultured rumen bacterium clone BF318	95	Swamp buffalo rumen	unpublished
		AB270110	Uncultured rumen bacterium gene for 16S rRNA, partial sequence, clone: T33H60F35	95	Bovine rumen	Anaerobe 13 (2), 57-64 (2007)