Small Ruminant Research 136 (2016) 7–17

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Variability in microbial population and fermentation traits at various sites within the forestomach and along the digestive tract as assessed in goats fed either grass or browse

J.O. Zeitz[a,*,1], S. Ineichen[a], C.R. Soliva[a], F. Leiber[a], A. Tschuor[b], U. Braun[b], M. Kreuzer[a], M. Clauss[c]

[a] ETH Zurich, Institute of Agricultural Sciences, Universitätstrasse 2, 8092 Zurich, Switzerland
[b] University of Zurich, Vetsuisse Faculty, Department of Farm Animals, Winterthurerstrasse 260, 8057 Zurich, Switzerland
[c] University of Zurich, Vetsuisse Faculty, Clinic for Zoo Animals, Exotic Pets and Wildlife, Winterthurerstrasse 260, 8057 Zurich, Switzerland

ARTICLE INFO

Article history:
Received 25 September 2015
Received in revised form
16 December 2015
Accepted 17 December 2015
Available online 28 December 2015

Keywords:
Rumen
Hindgut
Microbial population
Rumen fermentation
Goat

ABSTRACT

In ruminants, feed is digested mainly in the reticulorumen but to some extent also in the hindgut, and the digestive processes are influenced by the diet. The aim of the study was to record changes in microbial population and fermentation along the digestive tract by considering defined rumen compartments, caecum and colon as assessed in goats fed two contrasting diets. Ten adult female Saanen goats were fed either on grass hay ('grass') or a 1:1:1 mixture of dried poplar, raspberry and chestnut leaves ('browse'). After at least 20 days on feed and 12 h of fasting, the goats were euthanised and frozen in the natural resting position to allow sampling of contents from exactly defined gastrointestinal locations. Lyophilised rumen and hindgut contents were used for isolation of genomic DNA and microbial quantification via real-time PCR. In filtered rumen and hindgut fluid, pH and concentrations of ammonia and short-chain fatty acids (SCFA) were measured. The experiment demonstrated that, within the rumen, the dry matter content decreased from dorsal and central (132 g/kg) to the ventral compartment (62 g/kg) as expected. The abundances of total and fibrolytic bacteria, protozoa and methanogens as well as SCFA concentrations were similar in the cranial and caudal dorsal and central rumen, but were lower in the ventral rumen compartment. Additionally, SCFA concentration and pH close to the rumen wall in the central rumen compartment resembled those in the ventral rumen more closely than those in the other central rumen samples. The pH was lower in the dorsal and central (6.4) than in the ventral (7.5) rumen compartment. In the caecum and colon, respectively, the copy numbers of bacteria (3.8 and 6.2×10^{10}/ml), protozoa (1.4 and 0.2×10^{7}/ml) and methanogens (4.0 and 3.5×10^{8}/ml) (means of all goats combined) were markedly lower than those found in the rumen even though SCFA concentrations were similar. For all variables measured, the differences within the rumen and along the digestive tract were more pronounced in the browse-fed compared to the grass-fed goats. Consistent with the higher nutritional quality of the browse, the concentration of fermentation end-products and nitrogen availability were higher in the rumen across all compartments in the browse-fed goats compared to the grass-fed goats (pH, 6.4 vs. 7.3; SCFA, 139 vs. 111 mmol/L; ammonia, 11.2 vs. 4.1 mmol/L; copy numbers of bacteria, 8.1 vs. 7.8×10^{10}/ml; protozoa, 1.7 vs. 0.3×10^{9}/ml; methanogens, 2.4 vs. 1.3×10^{9}/ml). Different from that, the hindgut pH was higher and ammonia concentration was lower in browse-fed goats compared to grass-fed goats.

* Corresponding author at: Heinrich-Buff-Ring 26-31, 35392 Gießen, Germany. Tel.: +49 641 9939234; fax: +49 641 9939239.
E-mail address: Johanna.O.Zeitz@ernaehrung.uni-giessen.de (J.O. Zeitz).
[1] Present address: Justus Liebig University Gießen, Institute of Animal Nutrition and Nutritional Physiology, Heinrich-Buff-Ring 26-31, 35392 Gießen, Germany.

http://dx.doi.org/10.1016/j.smallrumres.2015.12.029
0921-4488/

The results demonstrated that, when collecting rumen digesta to study the microbial population and rumen fermentation, it is important to distinguish between dorsal/central and ventral rumen sampling points and to consider forage type and quality. However, it is not decisive to distinguish between caudal and cranial rumen regions, provided the sampling is not done in rumen wall proximity and not in close proximity to the oesophagus.

1. Introduction

Studies in domestic ruminants revealed that there is a characteristic stratification of the rumen content which includes the liquid fraction in the ventral rumen, the fibre mat in the dorsal and central parts and the gas dome in the upper dorsal part of the rumen (Cheng and McAllister, 1997; Hummel et al., 2009). The stratification of the rumen content goes along with differences in the retention times between the fluid and particulate digesta phase (Clauss et al., 2010). The rumen microbial populations are generally divided into those adhering firmly or loosely to ruminal particulate matter (>80 % of the total microbial population), those which thrive in the ruminal liquid (<10 %), and those associated with the rumen epithelium (about 1 %) (Legay-Carmier and Bauchart, 1989; Cheng and McAllister, 1997). The community structure of these populations, as well as the functional abilities of the microbial species, differ between compartments (Cheng and McAllister, 1997; Kong et al., 2010). For example, fibre-degrading microbes like *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* are mainly found attached to the solid digesta and therefore in the dorsal rumen (Michalet-Doreau et al., 2001). Compartmental differences also exist in pH (dorsal < ventral rumen), SCFA concentration (dorsal > ventral) (Bryant, 1964; Shen et al., 2012) and abundance of bacteria and protozoa (dorsal > ventral) (Martin and Michalet-Doreau, 1995; Kong et al., 2010). Few studies have specifically investigated the difference between specific rumen sampling locations by collecting samples from fistulated animals or via oral stomach tubes (Li et al., 2009; Shen et al., 2012; Ramos-Morales et al., 2014). These authors described partly contrasting results concerning differences in fermentation traits between samples from dorsal and central, caudal and cranial, and the anterior sac. When studying diet effects on ruminal fermentation, rumen samples are typically collected from different locations and composited afterwards to one sample to avoid bias (e.g. Saro et al., 2012). However, this does not allow distinguishing between sample sites.

In the hindgut of ruminants, fermentation conditions, functional abilities of the microbes and the fermentation products generated are considered to be comparable to the rumen (Gressley et al., 2011). However, the actual fermentation intensity and the composition of the microbial population in the caecum, colon or faeces differ from those in the rumen content (Metzler-Zebeli et al., 2013; de Oliveira et al., 2013). It also depends on the substrate flow from the rumen, which is influenced by diet (Metzler-Zebeli et al., 2013). In studies where samples from different parts of the gastrointestinal tract have been collected, the animals were mostly fed on one diet only (Romero-Perez et al., 2011; de Oliveira et al., 2013) or concentrate- and forage-based diets were compared (Metzler-Zebeli et al., 2013). Studies investigating the differences in microbial population and fermentation traits in defined rumen compartments and along the lower part of the gastrointestinal tract (GIT) of ruminants comparing different forage types have not been performed so far.

Studies on the effect of the type of feed on fermentation and the microbial population in the rumen and in the hindgut have been mainly performed with mixed forage-concentrate diets (e.g. Saro et al., 2012; Anantasook et al., 2013). The quantitative influence of different forage types on the abundance of the microbial populations is less well known and has been mainly shown in mixed forage-based diets (Kong et al., 2010; Saro et al., 2012). Tree and shrub foliage are important supplements for (small) ruminants on low-quality grasslands or shrublands in many areas of Latin and Central America, Asia and Africa as well as in Southern Europe (Leng, 1997). However, studies comparing browse and grass diets in ruminants are restricted to few investigations of rumen fermentation and microbiota of tropical grass and browse (Osakwe and Steingass, 2006; Omoniyi et al., 2014). Forages differ both in chemical composition and physical structure, with the latter especially when comparing grass and browse (forbs, herbs, leaves and twigs of woody plants). Typically, the leaves of woody plants contain less fibre, hemicellulose and cellulose, and more lignin, pectin, crude protein and especially plant secondary compounds (PSC) than unfertilised (tropical) grass (Leng, 1997; Hummel et al., 2006). In addition, grass leaf degradation in the rumen results in rather long particles whereas degraded herbaceous forage leaves are more polygonally shaped which may be related to the different arrangement of vascular bundles (Clauss et al., 2011).

The present study aimed at investigating differences in ruminal stratification, and differences in fermentation traits and microbial population within different rumen compartments and along the digestive tract. A secondary objective was to evaluate if the effects measured depend on the forage type fed, and therefore, goats fed either grass or browse only were compared. Goats had been selected as experimental animals because they are classified as intermediate feeders (Hofmann, 1989), with the ability to subsist on both grass and browse diets (Pfister and Malechek, 1986). As a novel approach, euthanised animals were frozen completely in their natural resting position to allow precise sampling of digesta from specified rumen compartments.

2. Material and methods

2.1. Animals, diets and experimental design

This experiment was part of a study on abdominal anatomy of goats (Braun et al., 2011). Ten female adult non-lactating Saanen goats were kept in two groups in 5 × 5 m indoor enclosures on woodchips. They were fed exclusively on either grass hay ($n = 5$) or dried browse ($n = 5$). The latter consisted of a 1:1:1 mixture (as fed) of dried leaves of sweet chestnut (*Castanea sativa*), raspberry (*Rubus idaeus*) and poplar (*Populus tremula*) purchased from Alfred Galke GmbH (Gittelde, Germany). These diets were fed for at least 20 days, which was considered sufficiently long to adapt the entire gut microbial ecosystem to these diets. The nutrient composition of the forages is shown in Table 1. The browse hay had a lower fibre but higher lignin content and was richer in crude protein than the grass hay (Table 1). Feed was offered ad libitum with fresh portions given twice daily. Feed intake was not quantified. Water was available at all times. All animals were clinically healthy. The experiment was approved by the cantonal veterinary office (ZH 69/2008). After 20 days on the experimental feed, the goats were fasted for about