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Grain-rich diets differently alter ruminal and colonic abundance of microbial populations and lipopolysaccharide in goats

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ABSTRACT

High grain feeding has been associated with ruminal pH depression and microbial dysbiosis in cattle. Yet, the impact of high grain feeding on the caprine rumen and hindgut microbial community and lipopolysaccharide (LPS) release is largely unknown. Therefore, the objective was to investigate the effect of increasing dietary levels of barley grain on the microbial composition and LPS concentrations in the rumen and colon of goats. Effects were compared with respect to the responses of ruminal and colonic pH and short-chain fatty acid (SCFA) generation. Growing goats ($n = 5–6$) were fed diets containing 0, 30, or 60% coarsely ground barley grain for 6 weeks. Ruminal ciliate protozoa were counted with Bürker counting chamber, and quantitative PCR was used to compare bacterial populations. Increasing dietary grain level linearly increased ($P < 0.05$) ruminal numbers of entodiniomorphids. With the 60% grain diet, there was a reduction in ruminal abundance of the genus *Prevotella* and *Fibrobacter succinogenes*, whereas the ruminal abundance of *Lactobacillus* spp. increased compared to the 0 and 30% grain diets ($P < 0.05$). In the colon, abundance of the genus *Prevotella* and *F. succinogenes* increased ($P < 0.05$) in goats fed the 60% grain diet compared to those fed the other diets. Colonic abundance of *Clostridium* cluster I was related to the presence of grain in the diet. Ruminal LPS concentration decreased ($P < 0.05$) in response to the 60% grain diet, whereas its colonic concentration increased in response to the same diet ($P < 0.05$). Present results provide first insight on the adaptive response of rumen protozoa and rumen and colonic bacterial populations to increasing dietary levels of grain in goats. Although luminal pH largely affects microbial populations, fermentable substrate flow to the caprine hindgut may have played a greater role for colonic bacterial populations in the present study.

1. Introduction

The rumen is a complex and highly diverse microbial ecosystem consisting of bacteria, archaea, ciliate protozoa, fungi, and viruses [1]. Diet is one of the major factors influencing the rumen microbial composition due to substrate preferences of microbes and indirectly by modifying the rumen milieu due to fermentation of the ingested substrates [2,3]. Traditionally, meat goats were mainly fed roughage diets until slaughter [4]. Due to intensification of goat production, pasture alone does not provide sufficient energy and protein for fast growing goats. Consequently, goats are increasingly fed with concentrate-rich diets similar to intensive cattle and sheep production systems. Feeding of those diets does not only stimulate ruminal short-chain fatty acid (SCFA) generation and thus dietary energy intake but may also lead to acidotic conditions in the rumen being characterised by luminal pH depression and microbial dysbiosis [5]. Understanding how the microbial community adapts to

Abbreviations: BW, body weight; C_T, threshold cycle; LPS, lipopolysaccharide; qPCR, quantitative PCR; SCFA, short-chain fatty acids.

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different diets as being essential to formulate diets to ensure normal functioning of the rumen ecosystem is advancing for ruminants such as cattle [6,7]. Yet, comparatively little is known about the caprine rumen microbiota and their adaptation to dietary changes. The caprine rumen microbial composition appears to differ from that of cattle and sheep [8], making it difficult to simply predict the adaptive response of the caprine rumen microbiota to dietary changes from bovine and ovine data.

In cattle fed high-grain diets, molecular analysis of the rumen microbiome demonstrated predominant shifts in the populations of *Firmicutes*, *Bacteroidetes* and *Fibrobacteres* [5,6]. Also, an increased ruminal abundance of *Escherichia coli* was reported under low rumen pH conditions induced by high-grain diets [5,9]. During low rumen pH, gram-negative bacteria lyse more rapidly, increasing the ruminal concentration of lipopolysaccharides (LPS), also called endotoxin, a bioactive cell-wall component of all gram-negative bacteria [10]. Low rumen pH together with LPS may reduce barrier function of the rumen epithelium potentially allowing LPS to translocate into blood and stimulate a systemic inflammatory response [11].

Hindgut fermentation in ruminants becomes more important with high grain feeding due to increased flow of undigested substrate into the hindgut [12,13]. Up to now, fermentation processes in the hindgut of ruminants have received little research attention despite their importance for animal health and performance [12]. Therefore, adaptive shifts in microbial populations in the hindgut as response to dietary changes should be monitored to prevent that unfavourable dietary effects on animal health may be overlooked. We hypothesised that characteristic adaptations in the microbial community structure may not only occur in the rumen but also in the hindgut of goats in response to increasing dietary grain levels and subsequent changes in the luminal environment due to increased fermentation. It was further assumed that, similar to cattle [5,9,13], with increasing dietary grain levels, more LPS may be released in the rumen and hindgut of goats. Therefore, the present study aimed to characterise the effect of increasing dietary levels of easily fermentable barley grain on shifts in rumen ciliate protozoa and selected ruminal and colonic bacterial populations and LPS concentrations in growing goats.

2. Materials and methods

2.1. Animals, diets and experimental design

This study was carried out at the experimental station of the Institute for Organic Farming and Biodiversity in Wels, Austria, using 17 non-castrated male and 1 female growing goats (6 Boer breed, 6 White German Noble breed and 6 Toggenburg breed), aged 4 month at the commencement of the experiment. Upon arrival, all goats were treated for internal parasites with Hapadex 5% (1.5 mL/10 kg body weight (BW), netobimin; Intervet GmbH, Vienna, Austria). Prior to the experiment, goats consumed a high-forage diet of 1.5 kg chopped meadow hay (second cut) and 200 g coarsely ground barley grain per animal and day (as-fed basis). During the experiment, goats were housed in three pens (each 9.82 m^2). Each pen provided feeding, lying and tread areas and was equipped with an automatic drinking trough, a mineral lick stone (Alpenleckmasse für Rinder: Ca 12%; P, 6% Na, 5%; Mg, 2%, Zn, 6000 mg; Mn, 2000 mg; Cu, 1000 mg; I, 40 mg, Se, 40 mg; GARANT Tiernahrung, Pöchlarn, Austria) and a salt lick stone (Biosaxon Salzleckstein, sodium chloride, Na min. 39%; GARANT Tiernahrung, Pöchlarn, Austria). The feeding area was divided into 6 individual feeding places (1.10 m × 0.40 m). After an acclimation period of 2 weeks, goats were weighed and randomly allocated to one of the three experimental diets. Attention was paid to balance the groups for those factors that affect feed intake potential of animals (e.g., body weight and weight gain of animals), and consequently the availability of fermentable substrate in the rumen and colon, and hence the activity of respective microbiota. Because 3 different breeds were used, the 3 groups were balanced for breeds, so that two goats per breed received the same diet resulting in six observations per experimental diet. Goats fed the same diet were housed in the same pen. The experiment lasted for 6 weeks. One week before euthanasia, goats were transferred to the experimental barn of the Clinic for Ruminants at the University of Veterinary Medicine Vienna, Austria, and were kept under the same housing and feeding conditions as at the Institute for Organic Farming and Biodiversity.

Three experimental diets were formulated with increasing levels of coarsely ground barley grain (Lagerhaus, Wels, Austria) and decreasing levels of chopped meadow hay (second cut). Goats were fed either 100% hay (0% grain diet), 70% hay and 30% barley grain (30% grain diet), or 40% hay and 60% barley grain (60% grain diet) on dry matter basis (Table 1). Hay fed in this experiment originated from one batch. Barley grain was selected due to its rapid degradation in the rumen, and hence due to its high potential to cause an acidotic insult. Feed samples for chemical analysis were taken three times during the experiment (Table 1). Before the start of the experiment, goats were transitioned to the 30 and 60% grain diets by gradually increasing the dietary grain level by 2.5 and 5% per day, respectively. When the final dietary grain levels of 30 and 60% were reached, the experiment started. Barley grain was included at the expense of hay. Goats were fed restrictively with a daily feed allowance of about 650 and 1200 g per goat (as-fed basis) at the commencement and end of the experiment, respectively. Goats were weighed every week to adjust the feed allowance. The feed amount was calculated to meet energy and nutrient requirements for maintenance and to account for 250 g BW gain per week of goats fed the 0% grain diet [14]. Goats were fed equal portions of the experimental diets at 0630 and 1330 h. They were individually fed by closing the separate feeding places in each pen. The feed was provided for 45 min. Thereafter, feed residues were collected and individual feed intake per goat was recorded. On the last experimental day, goats were fed at 0630 h.

The animal protocol was discussed and approved by the institutional ethics committee of the University of Veterinary Medicine Vienna in accordance with Good Scientific Practice guidelines and national legislation (15/03/97/2011).

2.2. Digesta collection

Because goats were fed twice daily, variations in the microbial population structure during the course of the day could be expected. To ensure that an effect of high grain feeding on the rumen microbial populations may be visible, goats were euthanized two to 3 h after morning feeding on the last experimental day by an intravenous injection of Release (50 mg/kg BW, pentobarbital;

Table 1
Diet ingredients and analysed chemical composition of diet ingredients.

	% Grain		
	0	30	60
Chopped meadow hay (%, DM-basis)	100	70	40
Finely ground barley grain (%, DM-basis)	–	30	60
Analysed chemical composition (g/kg DM)	**Meadow hay**	**Barley grain**	
Dry matter	880	883	
Organic matter	796	852	
Crude protein	112	130	
Crude fibre	289	66	