Small Ruminant Research 159 (2018) 38–44

Contents lists available at ScienceDirect

Small Ruminant Research

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

Grain grinding size of cereals in complete pelleted diets for growing lambs: Effects on ruminal microbiota and fermentation

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ARTICLE INFO

Keywords:
Cereal processing
Digestion
Lamb
Rumen health
Ruminal fatty acids
Ruminal microbiota

ABSTRACT

Lambs from intensive dairy sheep production systems are sold out either after weaning as milk-fed lambs or after a fattening characterized by high intakes of concentrate feeds (mainly cereals) which can lead to ruminal disorders. Therefore, it is essential to explore different feeding alternatives in order to reach a balance between economic or productive benefits and animal welfare. Twenty weaned male lambs (6–8 weeks age, 14.8 ± 0.16 kg body weight) were allocated randomly to one of two different groups (n = 10 per group) to study the effect of feeding a complete pelleted diet (CPD) in which cereal grains were ground at two different sieve sizes (either 2-mm with 13.5% dry matter (DM) of the particles > 1.7 mm or 6-mm with 47.1% DM of particles > 1.7 mm) on DM intake, nutrient digestibility, ruminal microbiota and fermentation. Lambs were fed the corresponding CPD *ad libitum* and slaughtered when they reached 27 kg. DM intake was greater in the lambs fed 2-mm grains than in those fed 6-mm grains ($P < .05$). Feeding 2-mm grains in comparison to 6-mm grains resulted in darker colour of ruminal mucosa (100 *vs*. 127, from a scale where 0 is black and 256 white; SED = 9.8; $P = .017$) and thicker stratum corneum (45.3 *vs*. 30.9 µm; SED = 3.67; $P = .001$), suggesting more severe acidotic conditions in the rumen with the smaller cereal particle size. Analysis of microbial communities revealed that the presence of ruminal bacteria such as *Prevotella* and *Quinella* was increased in the digesta of animals fed the 2-mm diet. These data were corroborated by the odd- and branched-chain fatty acid profile of ruminal contents, which would indicate a shift from cellulolytic to amylolytic bacteria in the rumen of lambs fed the 2-mm diet. In conclusion, feeding 6-mm in comparison to 2-mm ground cereal grains included in CPD formulated for growing lambs is an alternative strategy that may reduce ruminal acidosis through changes in the microbiota.w

1. Introduction

In the Mediterranean area, dairy sheep are primarily reared to produce milk for high-quality cheese and also meat lambs (Milán et al., 2011). In dairy sheep farms, lambs are separated from the mothers soon after birth, and then fed with milk replacers. Most lambs are sold after 3–4 weeks as milk-fed lambs, a valuable product of dairy sheep production systems due to their organoleptic properties, and its demand is strongly tied to local traditions and consumer preference (De Rancourt et al., 2006). Due to seasonal variations in lamb production and demand, its price strongly fluctuates throughout the year (Milán et al., 2011; European Commission, 2017), with periods where market value does not cover production costs. An alternative strategy to break the seasonal variation and get a better return is to produce fattening lambs efficiently to expand the market and promote exports of lamb to other countries (Rodríguez et al., 2011). These lambs are fed high concentrate diets from weaning to slaughter weight. Complete pelleted diets (CPD) simplify feedlot feeding management of fattening lambs when compared to the traditional *ad libitum* supply of concentrates and cereal straw in separate feeding troughs (Manso et al., 1998). It has been

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https://doi.org/10.1016/j.smallrumres.2017.12.009
Received 14 March 2017; Received in revised form 13 November 2017; Accepted 19 December 2017
Available online 22 December 2017
0921-4488/

shown that feed efficiency attained with CPD is similar to that with the traditional feeding system (Blanco et al., 2015). However, CPD developed so far for fattening lambs do not mitigate ruminal acidosis when compared to feeding concentrates and cereal straw in separate troughs (Blanco et al., 2014).

A possible approach to reduce the fermentation rate of starch in the rumen would be to modify the cereal particle size (grinding size) included within the pellet, as it is well known that lambs can use the whole grain cereal efficiently (Ørskov and Fraser, 1975). Feed intake, rumen ecosystem and fermentation parameters can be affected by cereal particle size (Pérez-Torres et al., 2011; Gimeno et al., 2015a; Kazemi-Bonchenari et al., 2017), but to the authors' knowledge there are no studies integrating all this information in weaned lambs intensively fattened during short periods of time (less than 50 days) with CPD. Changing grinding size of grain may cause changes in the composition of ruminal microbiota, affecting the rate of fermentation and the formation of end-products, and then the composition of ruminal contents and digesta reaching the abomasum. Consequently, the effect of different grinding sizes of cereal must be properly evaluated from a holistic perspective to get the best balance between physiological, welfare and economic criteria. The objectives of the present study were to investigate the effects of increasing the grinding size of cereal grain included in CPD on dry matter intake (DMI), nutrient digestibility, ruminal mucosa and fermentation, and composition of ruminal microbiota in growing lambs.

2. Materials and methods

All handling practices followed the recommendations of the Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes and the IGM-CSIC Animal Experimentation Committee (protocol number 2010-E80).

2.1. Animals and diets

Twenty intact male Merino lambs (age 47 ± 0.78 days, all lambs were between 6 and 8 weeks old) were used in this experiment. The lambs were allocated randomly to one of two different groups (n = 10 per dietary treatment) balanced by body weight (14.8 ± 0.16 kg). Both groups were fed the same CPD (43% barley, 15% maize, 24% soybean, 15% barley straw, and 3% vitamin-mineral premix). Soybean meal and the premix were in a finely milled form, and the barley straw used in both diets had been ground using a 2-mm screen size (average particle size 550 µm, with 97, 82 and 15% of the particles with a size < 1700, < 1180 and < 300 µm, respectively). The only difference between diets was the grinding size of the cereals included in the CPD before pelleting. The cereals included in the CPD of one group were ground using a sieve with a screen diameter of 2-mm (2-mm diet or grains) while for the other group the screen diameter was 6-mm (6-mm diet or grains). A 100-horsepower hammermill (VRE, Rosal, Spain) equipped with screens of either 2 or 6-mm openings was used to grind cereal grains (barley and maize). Particle size distribution of both CPD was measured by dry sieving analysis (ADSA, 1970; Waldo et al., 1971), and shown in Table 1. After mixing the ground cereals with the other ingredients, both diets were steam-conditioned at 70 °C and then extruded through a 6 mm (hole diameter) × 44 mm (thickness) die using a 150-horsepower pellet mill (PVR, MABRIK, Spain). The die thickness to hole diameter ratio was 7.3. Physical characteristics of the pellets for both diets are shown in Table 1. Length and diameter of each pellet were measured using a digital caliper (0.01 mm resolution). It was confirmed that in all cases pellet diameter was 6 mm. Bulk density was determined by weighing the amount of pelleted feed required to fill a 155 (height) × 120 (diameter) glass cylinder. Pellet hardness was determined using a Kahl tester (Amandus Kahl GmbH & Co. KG, Reinbek, Germany). Durability index was determined with 100 g samples (in triplicate for each diet) using a Holmen tester equipped with 2.5-mm diameter hole mesh (NHP100, Holmen, UK).

Table 1
Particle size distribution (cumulative% w/w of feed retained in each sieve size) of diets before pelleting, physical characteristics of the pellets and chemical composition (g/kg dry matter unless otherwise stated) of the complete pelleted diets formulated with cereals ground at 2-mm or 6-mm.

	2-mm	6-mm
Particle size distribution		
> 2360 µm	2.3	20.4
> 1700 µm	13.5	47.1
> 1180 µm	36.1	65.4
> 300 µm	82.0	91.8
> 105 µm	99.6	99.9
Mean particle size of mash diets before pelleting (µm)	640	1270
Pellet characteristics [mean (standard deviation)]		
Pellet length (mm)[a]	1.08 (0.106)	0.96 (0.112)
Bulk density (kg/m^3)[b]	651 (6.55)	674 (5.94)
Kahl-hardness (kg)[a]	7.6 (1.06)	6.8 (1.61)
Pellet durability index (%)[b]	96.7 (2.34)	95.1 (3.06)
Chemical composition		
Dry matter, g/kg feed	898	895
Crude protein	174	175
Neutral detergent fibre	224	218
Acid detergent fibre	112	109
Physically effective fibre	81	143
Starch	345	346
Total fat	27	28
Ash	76	72

[a] Measured in 15 pellets randomly selected from each diet (n = 15).
[b] Measured in triplicate (n = 3).

Data on dry matter (ISO 6496:1999), ash (ISO 5984:2002), crude protein (ISO 5983:2009), neutral detergent fibre (NDF), acid detergent fibre (ADF) and total fat composition of both CPD are summarized in Table 1. NDF and ADF were determined by the automated fibre analysis methods for feeds and forages using an ANKOM 200 Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA), and total fat by the acid hydrolysis filter bag technique using the Ankom^{HCl} Hydrolysis System. Physically effective fibre for each diet was calculated by multiplying the proportion of particles retained on the 1.18-mm sieve by the NDF content of the feed (Grant and Cotanch, 2005).

Animals were housed in individual concrete-floor pens (1.45 m width × 1.40 m length × 1.30 m height) with sawdust bedding and equipped with individual feeding troughs and automatic drinkers. After seven days of adaptation, lambs received the experimental diets *ad libitum* at 09:00 h daily. The amount of feed offered to each lamb was adjusted daily according to the previous day intake to allow refusals of approximately 200 g/kg of feed offered. Samples of both the feed and refusals were collected daily for subsequent analyses after pooling all the subsamples refused by each animal after one week. The total duration of the experiment was 48 days including the adaptation period (7 days), until lambs reached a weight of 27 kg.

2.2. Digestibility

Four male Merino lambs per treatment (22.8 ± 2.45 kg) were used for a digestibility trial. The number of animals used was the minimum considered necessary according to both statistical and welfare criteria. After being fed with the experimental diets for 21 days, these lambs were confined to individual metabolic cages (0.65 m width × 1.10 m length × 0.90 m height) with individual feeding troughs and drinkers, and fitted with specific devices to collect faeces and urine separately. During the digestibility trial, DMI was adjusted to 35 g/kg body weight to avoid differences in the rate of passage due to possible variations in DMI of both CPD. After two days of adaptation to the pens, faeces were collected for five days. The faeces of each animal were collected daily, weighed, mixed thoroughly and sampled (10%). Aliquots from each