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Evaluation of various barley processing methods on rumen microbial population, histomorphometry, and fermentation characteristics in fattening lambs

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Abstract: Barley deserves a top place in the farm for feeding livestock. It is irreplaceable by any other grain in sheep diets for producing capacious rumen microbial yields. The present study was conducted to investigate the effect of different methods of barley processing on a number of microbes, fermentation parameters, and the rumen tissue characteristics of fattening lambs. For this purpose, 20 fattening male lambs with a mean weight of 25 ± 1.1 kg were tested for 80 days based on a completely randomized design with four treatments (GRB: ground barley, SRB: steam-rolled barley, GEB: germinated barley, SOB: soaked barley) and five replicates. Three lambs from each treatment were slaughtered and sampling was done on the rumen liquid and tissue to determine their pH and volatile fatty acid (VFA), amylolytic bacteria, proteolytic, cellulolytic, heterophilic, and protozoa contents. The findings showed that the number of cellulolytic bacteria in ground barley treatment was lower than that of soaked barley treatment. In addition, there existed fewer heterophilic bacteria in germinated barley groups compared with other treatments. The lactic acid level in the GRB was increased compared to that in SRB and GEB treatments ($P < 0.05$). The GRB further reduced the thickness of the rumen wall in comparison with SRB. In general, replacing GRB with SOB improved certain thickness of the epithelial tissue and cellulolytic bacteria of the rumen.

Key words: Bacteria, protozoa, volatile fatty acids, histomorphometry, fattening lambs

1. Introduction

Microbes (bacteria, protozoa, and fungi) digest a high amount of feed and generate energy in animals (1,2). Starch consumption, rumen fermentation and the production of volatile fatty acids (VFAs) in animals may induce the growth and development of the rumen tissue structure (3). One approach to increasing the utilization and efficiency of edible products is to increase their digestibility. Moreover, feed processing via various physical and chemical methods can increase nutrient efficiency and the digestive ratio. Although ruminants are highly able to consume whole grains such as barley and maize, processing cereal grains via different ways such as grinding improves their nutritional value and increases their efficiency and digestibility (4). Barley processing involves grounding, rolling, steam- rolling, soaking, tempered rolling and germinating. Germinated barley is produced through soaking barley in water at a properly maintained temperature and humidity and taking the sprouts out after they reach a length of approximately 0.5 centimeters (5, 6). The primary role played by the mechanical processing of barley is cracking and softening the outer covering of grain and increasing the availability of microbes to stored starch, thereby augmenting the total degradability of starch in the rumen and the entire digestive tract. Through processing cereal grains, particularly barley, a rapid growth and development of the rumen papillae is achieved. In addition, such processing results in high starch degradability and the provision of the energy required for the growth of fattening animals (5). Rough concentrate diets or appropriately ground diets enhance the rumen capacity and its muscular tissue more than flour or pelletized concentrates. Therefore, the processing size or the concentrate particle size might be effective in its ability to stimulate the rumen capacity and its muscular tissue (7). Processed diets containing coarser particles may be more favorable for the overall development of the rumen, probably owing to their ability to stimulate the evolution of the rumen's epithelial tissue, capacity, and muscular tissue (8,9). Barley processed via the soaking method generates amylose and amylopectin in starch granules leading to semicrystalline and gelatinous components; this gelatination may increase

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the bioavailability of ruminal bacterial derived enzymes and consequently could promote diet digestibility, a matter that could affect sheep tract absorption efficiency (10).

Accordingly, a method of barley grain processing capable of increasing digestion-resistant starch in the rumen can preclude gastrointestinal disorders in fattening lambs on a barley-based diet. It seems that barley processing methods have different results in terms of the number of the main bacteria–protozoa and the characterized histomorphometry and volatile fatty acids (VFA) of the rumen (5,11). The purpose of the present study is to compare the effects of various barley grain processing methods such as soaking and steam flaking on the microbial mass, VFAs, and the rumen histomorphometry of fattening lambs.

2. Materials and methods

2.1. Lambs and treatments

This research was conducted in the Department of Animal Sciences, Faculty of Agriculture Sciences, Yasouj University, Yasouj, Iran. In a completely randomized design, 20 male lambs of Lory breed with an average age of 90 days and an average weight of 25 ± 1.1 kg were used in four treatments and five replicates for 80 days. Prior to the experiment, the animals were given 1 month of adaptation. During this period, nutrition and livestock management conditions were equal, and the lambs were vaccinated and immunized against enterotoxemia and treated with antiparasitic drugs (Albendazole: Razi Co.; Iran). The sheep were cared for and managed in accordance with the ethical considerations for handling animals, and prior to its commencement, the experiment was critiqued by the staff of the Department for Animal Science, University of Yasouj. Experimental treatments included: I) soaked barley for 24 h (SOB), II) germinated barley (GEB), III) steam-rolled barley (SRB), and IV) ground barley (GRB). In order to make the GRB-based treatment, barley seeds were ground with hammer rollers (4 mm pore diameter, Pardazan, Iran). In GEB-based treatment, the seeds were soaked in water during the germination process for 24 h, stored for 3 days in moist conditions and prepared for the following experiments. In the SOB treatment, the barley was weighed and soaked in fresh water that covered the surface of the grains. After 24 h, the water was removed and the grains were added to the diet. In the steam-rolling method, the seeds were passed through a chamber and exposed to hot water for half an hour. Next, the seeds were compressed, passed through rollers, and dried with a special cooler. The diets were formulated using National Research Council publications (1985) (Table 1). The lambs were fed a total mixed ration three times a day. After the end of the experiment and 5 h of starvation, three lambs from each treatment were slaughtered (5).

2.2. Feed sampling and analyses

Feed samples of each treatment were frozen at −20 °C for later analysis. At the end of the experiment, all samples were oven dried to a constant weight at 55 °C, ground to pass a 1 mm screen, and prepared for analysis for organic matter by ashing in a muffle furnace at 600 °C overnight, and CP was measured using the standard Kjeldahl procedure. NDF and ADF were determined with the ANKOM fiber system in a hot water rinse using heat stable α-amylase and Na_2SO_3 for NDF (12).

2.3. Microbial experiment

After mixing the entire contents of the rumen, 1 kg of the content was taken and mixed in anaerobic conditions (with the help of carbon dioxide, Sanyo, Japan). After that, the rumen fluid was purified, filtered, and stored at 4 °C. Under sterile conditions, 10-fold serial dilutions of rumen samples were prepared in sterilized saline solution (0.85% NaCl [Merck, Germany] diluted in deionized water). To isolate and count cellulolytic, amylolytic, proteolytic, and heterophilic bacteria, 100 µL of each dilution was cultured on Carboxymethyl cellulose (CMC) agar medium, Starch agar (SA), peptides gelatin agar (PGA), and tryptone soy Agar (TSA), respectively. The CMC medium (Merck, Germany) was incubated (Binder, Germany) for 24 h at 37 °C. At the end of incubation, 2 mL of Congo red dye was added to the medium which was washed with phosphate buffer saline after 15 min. The SA medium (Merck, Germany) was incubated under anaerobic conditions (Gas pack A) for 48 h at 37 °C; 1 mL of Lugol was then added to the medium which was subsequently washed with phosphate buffer saline. The PGA (Merck, Germany) and TSA (Merck, Germany) were incubated under anaerobic conditions for 48 h at 37 °C. Finally, colonies were counted using the colony counter (Tebsaran, Iran) and the number of bacteria per gram of rumen suspensions were calculated and analyzed based on Log10 CFU/g formula (13).

2.4. Protozoan

To count the number of protozoa, equal volumes of MFS solution (0.6 g of methyl green, 8 g NaCl, and 100 mL formaldehyde were mixed with 25 mL of phosphate buffer saline) and rumen fluid were mixed in Eppendorf microtubes and stored at room temperature for 20 min. Subsequently, suspensions were poured on a Hemocytometer slide and counted with an optical microscope (Olympus, Japan; magnification 100×) (11,13).

2.5. Fatty acids, lactate, and pH assay

To measure the fatty acids, lactate, and pH, certain rumen contents were mixed (Sanyo, Japan) and the pH was measured. The concentration of VFAs of the rumen fluid was measured (acetate, propionate, butyrate, valerate and iso-valerate) using gas chromatography (GC) (PU 4410 PHILIPS, Netherlands). Furthermore, for the GC analysis, the samples were prepared in the following

Table 1. Ingredients and chemical composition of experimental diets (DM basis). SOB: soaked barley for 24 h; GEB: germinated barley; SRB: steam-rolled barley; GRB: ground barley. *Protected fat supplement (Behparvaran Co.; Iran) **Contained 500K IU/kg vitamin A, 100K IU/kg vitamin D3, 100 IU/kg vitamin E, and 400 ppm Antioxidant; ***Contained 195 g/kg Ca, 90 g/kg P, 2000 mg/kg Mn, 3000 mg/kg Zn, 3000 mg/kg Fe, 280 mg/kg Cu, 100 mg/kg I, 100 mg/kg Co, and 20 mg/kg Mg.

	Percentage of ingredients and chemical composition of diet			
	SOB	GEB	SRB	GRB
Chopped alfalfa	30	30	30	30
Wheat straw	10	10	10	10
Barley grain	25	25	25	25
Soybean meal	7.5	7.45	7.45	7.45
Wheat bran	21.35	21.35	21.35	21.35
Royal fat*	4.15	4.15	4.15	4.15
Vitamin supplement**	1	1	1	1
Mineral supplement***	1	1	1	1
Total	100	100	100	100
The ratio of the forage in the diet (percent)	40	40	40	40
The ratio of the concentrate in the diet (percent)	60	60	60	60
Nutrients composition (DM basis)				
Metabolizable energy (Mcal/kg)	2.5	2.5	2.5	2.5
Crude protein (%)	14.7	14.7	14.7	14.7
NDF %))	39.93	39.93	39.93	39.93
NFC (%)	33	33	33	33
Crude fiber (%)	16.3	16.3	16.3	16.3
Ether extract (%)	6	6	6	6
Organic matter (%)	93.1	93.1	93.1	93.1
Ash (%)	6.9	6.9	6.9	6.9
Ca (%)	0.81	0.81	0.81	0.81
P (%)	0.51	0.51	0.51	0.51
K (%)	1.25	1.25	1.25	1.25
Cl (%)	0.2	0.2	0.2	0.2
Na (%)	0.07	0.07	0.07	0.07

way: 200 uL of metaphosphoric acid (Merck, Germany) (25%) and formic acid (3:1) mixture was added to 1 mL of rumen liquid. After 30 min of centrifugation, the clear supernatant was diluted 10-fold in water and injected into the chromatograph. Also, Lactic acid was measured by spectrophotometry (Colorimetric) methods (UV-VIS, Japan). Lactic acid concentration was calculated with the following formula: lactic acid concentration (mg/dL) = (amount of absorption × 50)/0.774 (14).

2.6. Histological tests

After preparing the paraffin sections (with a thickness of 5 µm), tissue sampling was prepared in 10% buffered formalin (Merck, Germany) from the dorsocaudal part of the rumen. After staining with Hematoxylin and Eosin (Qlab, Spain), the desired indicators were measured. In each group, four samples from each sample, four tissue sections, and in every tissue section, at least four microscopic fields were surveyed and measured in different magnifications through the use of a digital microscope (Dino-Lite), digital lens (Dino-Lite), and software (DinoCapture). Micrometry investigations included the length and thickness of papillae, the thickness of epithelial and muscular tissue layers, and the thickness of the entire wall (3,14).

2.7. Statistical analysis

The data analysis of microbial counts, tissue histomorphometry, and VFAs was performed using the

GLM procedure, SAS software (version 9.1). The mean comparisons were performed using Duncan method at a 5% significance level. The following fixed effect model was fitted to the data:

$Y_{ij} = \mu + T_i + E_{ij}$

where Y_{ij} is the value of each observation, μ is the population mean, T_i is the treatment effect, and E_{ij} is the residual error.

3.Results

3.1. Measurements of fatty acids, lactate, and pH

The amount of acetic and propionic acid decreased in the ground barley (GRB) treatment (37.10 mg/mL and 13.76 mg/mL, respectively) compared with those of other treatments ($P < 0.05$). Additionally, the amount of lactic acid in the GRB was increased compared with steam-rolled (SRB) and germinated barley (GEB) treatments ($P < 0.05$). Fattening lambs fed with ground barley grains had reduced rumen pH fluid compared with SRB, GEB, and SOB treatments (Table 2).

3.2. Microbial population of the rumen

The results of this study showed that there were significantly different ($P < 0.05$) numbers of cellulolytic, amylolytic, proteolytic, and heterophilic bacteria. GRB treatment, compared with SOB treatment, had a lower cellulolytic bacterial count ($P < 0.05$). There were statistically significant differences among the treatments in the number of amylolytic bacteria. The highest (9.16 log CFU/g) and least (6.96 log CFU/g) count of amylolytic bacteria were respectively observed in lambs receiving ground barley and germinated barley ($P < 0.05$). The highest number of proteolytic bacteria was related to the lambs receiving ground barley (Table 3). There was a significant difference among the treatments in the number of heterophilic bacteria, where the lowest number was observed in germinated barley (9.30 log CFU/g) ($P < 0.05$).

3.3. Histological findings

The only statistically significant difference among the treatments was observed in the mean thickness of the rumen epithelial tissue (Table 4). As seen in Figure, the

Table 2. The effects of barley grain processing on rumen fermentation characteristics (mg/L). (a, b, c): Values with different letters differ significantly ($P < 0.05$). The lactic acid mean was based on milligrams per deciliter; GRB: ground barley, SRB: steam-rolled barley, GEB: germinated barley, SOB: soaked barley.

	Experimental treatments					
Item	GRB	SRB	GEB	SOB	SEM	P-value
Acetic	37.10^{b}	39.81^{a}	42.70^{a}	40.93^{a}	2.30	0.427
Propionic	21.10	23.26	23.80	22.13	2.59	0.838
Butyric	13.76^{b}	18.26^{a}	17.93^{a}	17.393^{a}	0.97	0.034
Valeric	1.96	2.13	2.20	2.03	0.13	0.625
Isovaleric	2.13	2.13	1.70	1.90	0.22	0.498
Lactic	2.41^{a}	0.62^{b}	0.80^{b}	1.47^{ab}	0.42	0.062
pH	5.16^{b}	6.04^{a}	5.75^{a}	5.88^{a}	0.10	0.001

Table 3. The effects of barley grain processing on the count of rumen bacterial population (log CFU/g) and protozoa (log the number of protozoa counted/g). SOB: soaked barley for 24 h; GEB: germinated barley; SRB: steam-rolled barley; GRB: ground barley.

	Experimental treatments					
Treatments	GRB	SRB	GEB	SOB	SEM	P-value
Celluloytic	6.75^{b}	6.96^{ab}	7.04^{ab}	7.34^{a}	0.015	0.0150
Amylolytic	9.16^{a}	8.58^{b}	6.96^{c}	8.34^{b}	0.12	0.0001
Proteolytic	9.01^{a}	7.09^{b}	7.47^{b}	7.16^{b}	0.23	0.001
Heterophylic	11.04^{a}	9.94^{b}	9.30^{c}	10.86^{a}	0.15	0.0002
Protozoa	6.19	6.18	6.24	6.34	0.06	0.353

Table 4. The effects of barley grain processing on the rumen histomorphometry (in μm). *Values with different letters differ significantly (P < 0.05); GRB: ground barley, SRB: steam-rolled barley, GEB: germinated barley, SOB: soaked barley.

	Experimental treatments					
Treatments	GRB	SRB	GEB	SOB	SEM	P-value
Papillae length	2859.2	3163	2024.4	3028.8	337.27	0.075
Papillae thickness	293.71	317.84	331.42	333.8	15.93	0.359
Thickness of the epithelial tissue	94.59^{b}	104.96^{ab}	104.52^{ab}	121.32^{a}	5.08	0.041
Thickness of the muscular layer	1313.5	1201.1	1416.3	1316.5	128.34	0.764
Thickness of the wall	1835.8	2198.1	2211. 4	1920.1	227.58	0.611

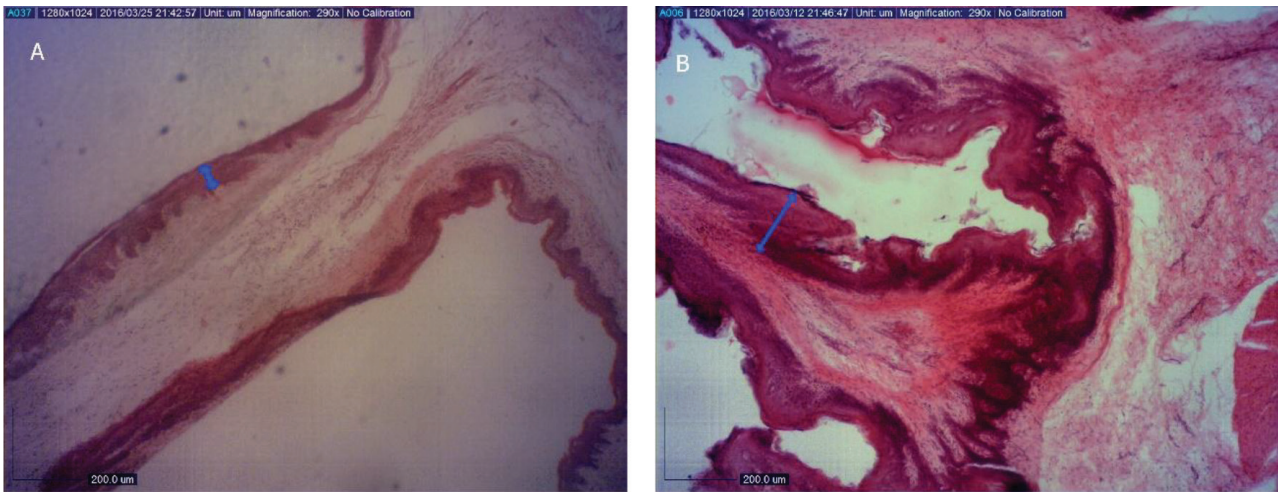

Figure. Photomicrograph of a rumen section at 4.50 cm CRL (crown-rump length) in lambs fed with ground barley (A) and soaked barley (B). The thickness of the epithelial tissue was considerable (blue arrow) (H & E, 250×).

thickness of the rumen epithelial tissue of lambs receiving ground barley was reduced (94.59 μm) compared with soaked barley (P < 0.05); the greatest thickness of epithelial tissue was found in the SOB treatment (94.59 μm). There were no significant differences in papillae length, papillae thickness, thickness of the epithelial tissue, thickness of the wall, or thickness of the muscular layer between SRB and GRB groups.

4. Discussion

4.1. Microbial population of the rumen

The use of ground and soaked barley increased the number of heterophilic bacteria compared with steam-rolled and germinated barley. In one study, grinding maize grain reduced the starch resistance of grain compared with steaming and soaking, and increased its degradability in the rumen (5). Also, the dairy cattle on starch diets had reduced acetate ratio compared to propionate (15). The researchers attributed the results to the reduction in the activity of fiber degrading bacteria, stating that the number of fiber degrading bacteria decreased by reducing the pH of the rumen fluid. In another study that investigated ruminal microbial population densities due to changes of different type of forages, the results showed that the total number of protozoa was higher in alfalfa hay fed sheep compared with sheep on a grass hay diet. The sheep fed with lower forage quality showed a higher abundance of certain cellulolytic bacteria and fungi, a matter that could be fairly and likely interpreted as the adaptation of rumen populations to be able to digest more fibrous and complex forage feedstuffs (16). Ying and Allen (17) used two types of maize grain processing, namely grinding and maize with high moisture, in the Holstein dairy cattle diet; they reported that the maize grain with high moisture increased starch degradability and the count of amylolytic and proteolytic microbes in the rumen, which is in accordance with the findings of the present research.

4.2. Fermentation characteristics

Results of the present study showed that feeding fattening lambs with ground barley reduced the rumen pH compared with steam-rolled, germinated, and soaked barley. Murphy et al. (18) reported that fattening cattle fed with dried steam-rolled maize had decreased acetic acid and augmented rumen propionic and butyric acids compared with whole grain. Nagaraja and Titgemeyer (19) reported that the severity of acidosis, generally depending on the amount, frequency, and duration of grain feeding, varied from acute acidosis caused by lactic acid accumulation to subacute acidosis due to the accumulation of VFAs in the rumen. Ruminal microbial changes associated with acidosis are indicative of the increased availability of fermentable substrates and subsequent accumulation of organic acids. In another study, researchers used two kinds of processing cereal grains, including finely steam-rolled grains (thin roller) and coarsely steam-rolled grains (wide roller) in the diet of dairy cattle. They reported that processing grain, based on its core size, strongly affected the rumen fermentation. Differences in the fermentation parameters among the diets indicate a difference in the chemical composition and degradability of the rumen (20). Crocker et al. (21) used two types of maize processing, including steam-rolled and dried steam-rolled, to feed dairy cattle, reporting that the type of processing did not significantly affect the amount of different VFAs in the rumen. It seems that diets high in fast-fermenting starch and a sudden increase in VFAs result in reduced rumen pH, and are therefore able to decrease the absorption capacity of VFAs through damaging the rumen tissue.

4.3. Rumen histomorphometry

The thickness of the rumen epithelial tissue of lambs receiving ground barley decreased compared with that of the soaked barley. Such an increase could be due to the slower release of carbohydrates in soaked barley. Another possible reason for the greater thickness of epithelial tissue in lambs fed with soaked barley may be the higher abrasion ability of soaked barley. Furthermore, germinated barley can facilitate the absorption of fatty acids. The increase in the "thickness of the rumen epithelial tissue" by replacing the ground-processing treatments with proper methods can affect absorption efficiency. Engstrom et al. (22) reported that the degradability of steam-rolled barley was lower compared to that of the dried steam-rolled and ground barley with regards to the rumen of fattening calves. The increase in the length, width, and count of papillae enhanced the absorption surface and prevented both the accumulation of VFAs in the rumen and acute and subacute acidosis in the animals (3). It has been reported that dried steam-rolled, steam-roasted, and steam-rolled corns significantly affect the ruminal papillae height in the calves; however, the maximum height of papillae was observed in steam-rolled corn treatment while the shortest was found in animals treated with dried steam-rolled corn, and papillae thickness remained unaffected. It has also been reported that the use of large-drilled particles of cereal grain in dairy cattle results in higher rumen capacity and muscle compared with those treated with grain flour or pellets. This suggests that the degree of grain processing and/or particle size, influence rumen capacity and muscular tissue stimulation (23). Lesmeister and Heinrichs (4) reported that dried steam-rolled maize grain, roasted-flaked, and steam-rolled maize had a significant effect on the rumen's papillae length in dairy calves. In this light, the longest and shortest papillae lengths were related to the treatments with steam-rolled maize and dried steam-rolled maize, respectively. Heinrichs (8) demonstrated that grains with larger particles, compared with milled or plated grains, resulted in a greater increase in the rumen capacity and its muscular tissue, implying the effectiveness of processing extent or particle size on its ability to stimulate rumen capacity and muscular tissue.

Highly intensive sheep production needs special nutria management strategies. In this study, we used different barley-based processed treatments in Lory breed sheep. Overall, our research showed that replacing ground barley with soaked barley increased the thickness of the rumen epithelial tissue and also the number of ruminal cellulolytic bacteria.

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Authors' contributions

All the authors commented on the early and final versions of the manuscript.

Conflict of interest

The authors declare no conflicts of interest

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