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Effect of different corn processing methods on enzyme producing bacteria, protozoa, fermentation and histomorphometry of rumen in fattening lambs

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Abstract

The purpose of this study was to investigate the effect of different corn processing methods on rumen microbial flora, histomorphometry and fermentation in fattening male lambs. Twenty male lambs (average age and weight of 90 days and 25.00 ± 1.10 kg, respectively) were used in a completely randomized design including four treatments and five replicates each over 80 days long period: 1) Lambs fed ground corn seeds; 2) Lambs fed steam-rolled corn; 3) Lambs fed soaked corn seeds (24 hr) and 4) Lambs fed soaked corn seeds (48 hr). At the end of the experiment, three lambs of each treatment were slaughtered and samples were collected for pH, volatile fatty acids, amylolytic, proteolytic, cellulytic and heterophilic bacteria and protozoa assessment. The number of proteolytic bacteria in soaked corn seeds was significantly increased in comparison with other treatments. The thickness of wall, papillae and muscular layers of rumen in the soaked corn seeds treatment was significantly increased. Overall, from a practical point of view, soaked corn processing could be generally used in lambs fattening system.

اثر روش‌های مختلف فرآوری ذرت بر روی باکتری‌های تولید کننده‌ی آنزیم، تک یاخته‌ها ، تخمیر و هیستومورفومتری شکمبه در بره‌های پرواری

چکیده

هدف این پژوهش بررسی اثر روش‌های مختلف فرآوری ذرت بر فلور میکروبی، هیستومورفومتری و تخمیر شکمبه در بره‌های پرواری نر بود. بیست بره نر به ترتیب با میانگین سنی و وزنی ۹۰ روز و ۱/۱۰ ± ۲۵/۰۰ کیلوگرم در طرحی کاملاً تصادفی با چهار تیمار و پنج تکرار هر یک به مدت ۸۰ روزمورد استفاده قرار گرفتند: ۱) بره‌های تغذیه شده با دانه‌های ذرت آسیاب شده؛ ۲) بره‌های تغذیه شده با ذرت پرک شده با بخار؛ ۳) بره‌های تغذیه شده با دانه‌های ذرت ۲۴ ساعت خیس خورده و ۴) بره‌های تغذیه شده با دانه‌های ذرت ۴۸ ساعت خیس خورده. در پایان آزمایش، از هر تیمار سه بره کشتار گردید و نمونه ها جهت بررسی pH، اسیدهای چرب فرار، باکتری‌های آمیلولیتیک، پروتئولیتیک، سلولیتیک و هتروفیلیک و تک‌یاخته‌ها جمع آوری شد. شمار باکتری های پروتئولیتیک در دانه های ذرت خیس خورده در مقایسه با دیگر تیمارها به طور معنی‌داری افزایش یافت. ضخامت دیواره ، پرزها و لایه های ماهیچه ای شکمبه در تیمار دانه‌های ذرت خیس خورده به طور معنی‌داری افزایش یافت. در مجموع، از منظر کاربردی، فرآوری ذرت به روش خیساندن می‌تواند در سیستم پرواربندی بره‌ها مورد استفاده قرار گیرد.

واژه های کلیدی: باکتری، بافت شناسی، بره های پرواری، شکمبه ، فرآوری ذرت

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Introduction

The microorganisms (bacteria, protozoa and fungi) digest large amounts of food and make energy available for the animals.[1] Epithelial tissues have several important physiological functions including nutrients absorption and transfer, short chain fatty acids metabolism and urea recycling.[2] The rumen epithelial tissues normal growth and development are performed by nutrients utilization e.g. volatile fatty acids.[3] Starch ruminal fermentation and its digestion by animal could produce volatile fatty acids stimulating rumen tissue growth and development. Although excessive production of volatile fatty acids would increase absorption by rumen papillae, sudden accumulation of volatile fatty acids in the rumen could lead to rumen pH reduction and tissue changes.[4,5] On the other hand, larger amounts of volatile fatty acids produced by fermentation could reduce ruminal pH, movements and growth and animal performance.[6] One way to increase the utilization and efficiency of feeds is increasing their digestibility. In this regards, with the various methods of physical and chemical feed processing, the digestibility of feed nutrients could be increased. Although ruminants have greater ability to consume whole grains such as barley and corn, feed processing e.g. grinding grain, could improve feed nutritional values and digestibility.[7] The first duty of corn seeds mechanical processing is to soften the outer coating of starch promoting microbial feed availability to starch and therefore, increasing starch degradability in the rumen and/or entire gastrointestinal tract[7]. The processing of corn grain, particularly, will help rapid development of rumen papillae. The mechanical methods are including pellet grain processing, roasting, steaming-soaking and laminating.[7] The rough concentrate diets or well-milled ones would improve rumen capacity and muscular tissue far better than flour or pelleted concentrates.[8] The dietary processed feed containing coarser particles may be desirable for the overall development of the rumen due to ability to stimulate the development of rumen epithelial and muscular tissues and capacity.[9] Soaking corn seeds could disrupt and gelatinized starch granules, reduce their viscosity and conflict with protein matrix surrounding the starch grains. Soaking corn seeds can also increase the amylolytic bacteria accessibility increasing the fermentation process.[10] These changes would increase the percentages of fatty acids acetate, propionate and butyrate and the possibility of bacterial growth and development in rumen. Corn seeds processing through soaking causes normal amylose and amylopectin starch granules semi-crystallization and gelatinization. Seed soaking disrupts the crystalline arrangement of amylose and amylopectin, increases starch degradation in the rumen microbial mass and leads to amylolytic bacteria increases.[11] Hence, corn processing to increase the starch resistant to digestion in the rumen can be used as a solution to prevent gastrointestinal disorders during lambs feeding with processed corn grain.

The aim of this study was to compare effects of various processing methods such as corn soaking and steamrolling on rumen normal flora, fatty acids and morphological characteristics in fattening lambs.

Materials and Methods

Animals. In this study, twenty male lambs (average age and weight of 90 days and 25.00 ± 1.10 kg, respectively) were used in a completely randomized design including four treatments and five replicates each over 80 days long period. Before the experiment, feeding and management conditions were same for a month as an adaptation period and vaccinations against some pathogens were prescribed. Treatments were ground corn (*Zea mays var. indentata*) seed, steam-rolled corn, soaked corn seeds (24 hr) and soaked corn seeds (48 hr). The diets were formulated using Small Ruminant Nutrient Requirements Table (Table 1). Lambs were fed three times a day (total mixed ration) and at the end of experiment and following 5 hr starvation, three lambs of each treatment were slaughtered.[4] The protocol for animal research was approved by the Ethics Committee of the University of Yasouj (10240; 29.08.2016).

Bacterial tests. Lambs were slaughtered; all of the rumen contents were mixed with sterile gloves and 1 kg of samples were filtered under anaerobic conditions (with carbon dioxide) and stored at 4 ˚C for following the practice. For this purpose, tenfold serial dilution was prepared using sterile phosphate buffer saline (in sterile conditions). For the isolation and enumeration of cellulytic, amylolytic, proteolytic and heterotrophic bacteria, 100 µL of each dilution were inoculated and cultured on carboxy methyl cellulose agar medium, starch agar, peptides gelatin agar and tryptone soy agar, respectively. The above mentioned media were purchased from Merck (Darmstadt, Germany). Carboxy methyl cellulose medium was incubated for 24 hr at 37 ˚C. At the end of incubation, 2 mL of Congo red dye was purred on media and washed with phosphate buffer saline after 15 min. Starch Agar medium was incubated in anaerobic conditions (Gas pack A) for 48 hr at 37 ˚C, then one mL of lugol was poured on medium and washed with phosphate buffer saline. Peptides Gelatin Agar and Tryptone Soy Agar were incubated in anaerobic conditions for 48 hr at 37 ˚C. Finally, colonies were counted by colony counter (Safarn, Iran) and the numbers of bacteria per gram of rumen suspensions were calculated and analyzed base on Log_{10} CFU g^{-1} formula.[12]

For counting protozoa, 1 mL of rumen fluid was mixed with 1 mL of methyl-formaldehyde solution (MFS) including 0.60 g of methyl green (Merck) 8.00 g NaCl (Merck) and 100 mL formaldehyde (Gameron Petro

Table 1. Composition and analysis of experimental diets (formulated based on kg).

Composition	Ground corn	Steam-rolled corn	Soaked corn (24 hr)	Soaked corn (48 hr)
Components of the diet (dry matter)				
Chopped alfalfa	29.90	29.90	29.70	29.70
Wheat straw	11.00	11.00	11.00	11.00
Corn seed	19.45	19.45	19.45	19.45
Soybean meal	23.25	23.25	23.25	23.25
Wheat bran	9.70	9.70	9.70	9.70
Protected fat powder	4.70	4.70	4.70	4.70
Vitamin supplements	1.00	1.00	1.00	1.00
Mineral supplements	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00
The ratio of forage in the diet (%)	39.85	39.85	39.85	39.85
The ratio of concentrate in the diet (%)	60.15	60.15	60.15	60.15
The nutrient composition (based on the dry matter)				
Metabolizable energy (MCal kg^{-1} of dry matter)	2.50	2.50	2.50	2.50
Crude protein (%)	14.00	14.00	14.00	14.00
Acid detergent fiber (%)	56.27	60.29	61.69	58.34
Neutral detergent fiber (%)	24.72	23.21	23.39	21.21

Industrial Complex, Bandar-E Abbas, Iran) were mixed with 25 mL of phosphate buffer saline) and after coloring (about 20 min), protozoa were counted with hemocytometer slide using an optical microscope (Olympus, Tokyo, Japan) with 100× magnification.[12]

Determination of fatty acids, lactate and pH. At the first, pH of 10 mL of rumen solution was measured and then mixed with 1 mL metaphosphoric acid (Merck) and the concentration of rumen fluid volatile fatty acids (acetate, propionate, butyrate, valeric and isovaleric) was measured via gas chromatography (model GC-PU4410; Philips, Amsterdam, The Netherlands) and lactate was measured with turbidimetry (UV-VIS; Shimadzu, Tokyo, Japan) assay.[13] Lactic acid concentration was calculated with the following formula: lactic acid concentration**=** amount of absorption multiplied by 50 and results divided by 0.774.

Histomorphological assay. Samples were taken from rumen dorsal sac and stored in 10% buffered formalin. Once the tissue was fixed and after preparing paraffin sections (5 µm thickness) and staining with hematoxylin and eosin (four samples in each group, four sections in each sample), at least four microscopic fields were surveyed in different magnifications using a digital microscope (A.423; Dino-Lite, Hsinchu, Taiwan), digital lens and DinoCapture software (version 1.0.0; Dino-Lite). Micrometrical analyses were included height and thickness of papillae and thickness of epithelial tissue, muscular layer and whole wall.[14]

Statistical analysis. Analysis of microbial data, tissue changes, pH and volatile fatty acids was performed in the form of statistical model-based completely randomized design with generalized linear model (GLM) procedures (version9.10; SAS Institute, Cary, USA). Mean comparison with Duncan test was carried out at 95 confidences. Data analysis model equations were as follows:

$$Yij = \mu + Ti + Eij$$

where, *Yij* is the observation in the treatment; μ is the population mean; *Ti* is the treatment effect and *Eij* is the residual error.

Results

Rumen microbial flora. There were no significant differences between cellulytic bacteria and protozoa counts in all treatments ($p > 0.05$), but a significant difference was seen in the number of amylolytic ($p < 0.05$) and heterophilic ($p < 0.05$) bacteria and to some extent proteolytic ($p < 0.05$) bacteria. A number of amylolytic bacteria in the ground corn and soaked corn seeds (24 hr) treatments was significantly higher (Fig. 1). Heterophilic bacteria in ground corn treatment were lower than other treatments. The count of proteolytic bacteria in soaked corn seeds (24 hr) was increased ($p < 0.05$) in comparison with other treatments (Table 2).

Bacteria. The results showed that numbers of amylolytic bacteria in ground and soaked corn (24 hr) treatments were increased in comparison with rolled-steam and soaked corn (48 hr), in this way, processing

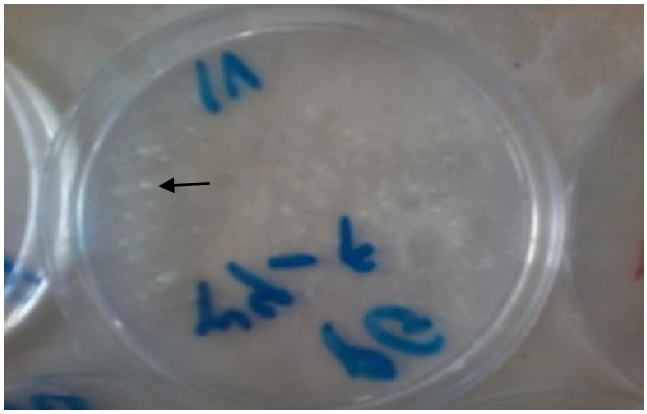

Fig. 1. Formation of bright halo (black arrow) around colonies due to growth of proteolytic bacteria.

Table 2. The effect of treatments on the number of rumen different microbial strains (Log_{10} CFU g^{-1}).

Microorganisms	Ground corn	Steam-rolled corn	Soaked corn seeds (24 hr)	Soaked corn seeds (48 hr)	*p*-value	SEM
Cellulytic	6.91	7.33	6.59	6.54	0.223	0.22
Amylolytic	8.74[a]	7.49[b]	8.29[a]	7.58[b]	0.001	0.18
Proteolytic	8.02[b]	7.37[b]	8.96[a]	8.05[b]	0.008	0.23
Heterophilic	8.68[b]	11.13[a]	10.76[a]	11.85[a]	0.001	0.36
Protozoa	5.97[a]	5.97[a]	5.95[a]	5.85[a]	0.861	0.11

[ab] Columns with different letters are significantly different ($p < 0.05$).

methods had no significant effect on the cellulytic bacteria, however, in the ground corn treatment, the amount of amylolytic bacteria was increased in comparison with rolled-steam corn treatment.

Assaying of fatty acids, lactate and pH. The results showed that there were no significant differences between acetic, butyric, isovaleric and lactic acid ($p > 0.05$), But there were significant differences between the means of propionic and valeric acids ($p < 0.05$). Steam-rolled corn showed a lower propionic acid compared to ground corn treatment; however, there was not a significant difference between ground corn and soaked corn seeds (24 and 48 hr) and within soaked corn seeds (24 and 48 hr). The valeric acid percentage in the soaked corn (48 hr) was higher than other treatments ($p < 0.05$). Furthermore, rumen pH in fattening lambs fed ground corn compared to steam-rolled and soaked corn seeds (24 and 48 hr) was reduced (Table 3) and rumen pH in lambs fed soaked corn seeds (24 hr) was increased ($p < 0.05$).

Histological findings. No significant difference was observed in papillae height due to applied treatments, however, a substantial statistical difference in papillae thickness ($p < 0.001$) was observed in lambs fed ground corn. In this study, the thickness of epithelial tissue in the soaked corn seeds treatment (48 hr) was higher than others. Table 4 shows that the use of grain diets with easy-accessed starch would increase papillae thickness and rumen epithelial tissue. The results showed that ground corn compared to steam-rolled and soaked corn seeds (24 hr) had no significant effects on epithelial tissue thickness, however, in soaked corn seeds (48 hr) a greater epithelial tissue thickness was observed compared to ground and soaked corn seeds (24 hr). Also, steam-rolled corn treatment had no significant effect on epithelial tissue thickness. Lambs fed steam-rolled corn showed higher wall and muscular layer thicknesses than other treatments (Figs. 2 and 3).

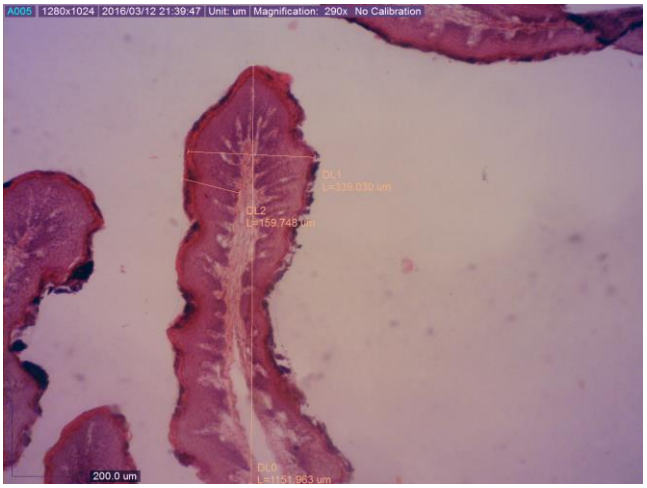

Fig. 2. Photomicrograph of a rumen section at 4.50 cm CRL (crown–rump length) in lambs fed steam-rolled corn. High and width of papillae were noted (H & E, 250×).

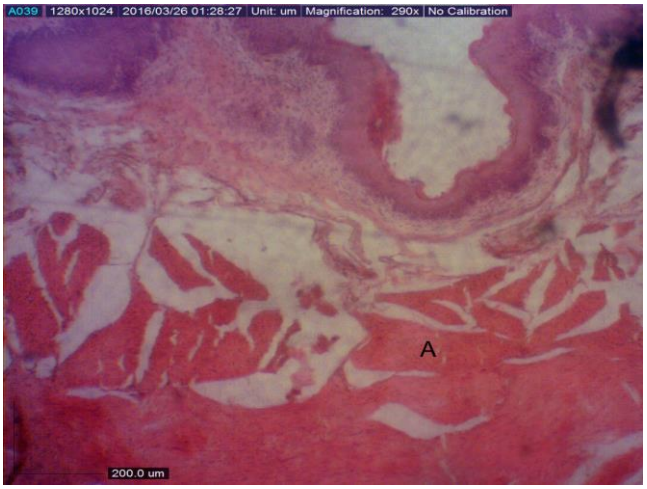

Fig. 3. Photomicrograph of a rumen section at 4.50 cm CRL. Lambs fed steam-rolled corn showed higher wall and muscular layer thicknesses than other treatments (A), (H & E, 250×).

Table 3. Amount of volatile fatty acids (mM) and rumen fluid pH in the lambs fed experimental diets.

Parameters	Ground corn	Steam-rolled corn	Soaked corn seeds (24 hr)	Soaked corn seeds (48 hr)	*p*-value	SEM
Acetic acid	44.25[a]	42.30[a]	48.83[a]	52.63[a]	0.350	4.13
Propionic acid	17.76[ab]	18.70[ab]	16.90[b]	21.30[a]	0.140	1.22
Butyric acid	19.65[a]	27.30[a]	20.93[a]	28.20[a]	0.273	3.46
Valeric acid	1.95[b]	2.10[b]	2.33[b]	2.83[a]	0.004	0.12
Isovaleric acid	1.55[a]	1.65[a]	1.53[a]	22.23[a]	0.184	0.23
Lactic acid	1.07[a]	0.40[a]	1.08[a]	1.21[a]	0.220	0.27
pH	5.60[b]	5.98[a]	5.87[b]	5.09[c]	0.0001	0.05

[ab] Columns with different letters are significantly different ($p < 0.05$).

Table 4. The effect of treatments on histomorphometrical characteristics (μm) of rumen.

Parameters	Ground corn	Steam-rolled corn	Soaked corn seeds (24 hr)	Soaked corn seeds (48 hr)	*p*-value	SEM
Papillae height	2430.30	2404.90	3369.50	2654.80	0.572	534. 50
Papillae thickness	222.12[c]	281.22[b]	469.02[a]	299.15[b]	0. 0001	17.33
Thickness of epithelium	79.20[b]	104.00[b]	206.00[a]	139.30[ab]	0.027	24.17
Thickness of muscle	1604.90[a]	1279.40[ab]	1349.60[ab]	1219.90[b]	0.129	106. 22
Total thickness of wall	2796.10[a]	2012.50[b]	2145.20[b]	1856.90[b]	0.010	149. 84

[ab] Columns with different letters are significantly different ($p < 0.05$).

Discussion

Our results showed that numbers of amylolytic bacteria in ground and soaked corns (24 hr) treatments were increased in comparison with rolled-steam and soaked corns (48 hr), in this way, processing methods had no significant effect on the cellulytic bacteria, however, in the ground corn treatment, the amount of amylolytic bacteria was increased in comparison with rolled-steam corn treatment. The amount of propionic acid in rolled-steam corn treatment was lower than ground corn one. This might be due to reduction of starch degradability in rolled-steam and soaked treatments.[7] Even though, the amylolytic bacteria counts in soaked (24 hr) corn treatment were similar to ground corn and also number of these bacteria in rolled-steam one was similar to soaked corn (48 hr), but no significant difference was seen regarding to propionic acid assay. It has been reported previously that degradability of rolled-steam barley treatment is lower than ground-dried barley in calves rumen.[15] It has been shown that accumulation of volatile fatty acids can increase the height, width and number of papillae and folds leading to nutrients absorption increases and acute and subacute acidosis prevention in animals.[3] In an experiment using Holestein dairy cattle fed two types of processed diet i.e. ground and high moisture corns, it has been shown that the number of amylolytic and proteolytic bacteria and starch degradability increase with the latter diet.[11] It was also found that feeding a breeding cow with dried rolled-steam compared to whole corn leads to a reduction of acetic acid and promotion of propionic and butyric acids in rumen. [16]

The valeric acid percentage in the ground corn treatment was higher than other treatments ($p < 0.05$). In this way, the results showed no sign of discrepancy between steam-rolled with soaked corn seeds (24 and 48 hr). It has been revealed that if the production rate of volatile fatty acids supersedes their absorption rate which might happen due to accumulation of primary substances fermentable in the rumen, a significant reduction in rumen pH will be occurred.[17] Also, through using dairy cow fed two types of processing grain including fine rolled-steam corn (thin roll) and rolled-steam corn with larger particles (roller thresholds) it has been concluded previously that with intensifying processing rate and grain particle size refinement downwardly, a sharp reduction in rumen pH is observed.[18] It has been suggested that discrepancy among fermentation parameters in different diets treatment could be addressed by their chemical compositions and ruminal degradability.[19] Reportedly, using dairy cow fed types of processed treatments including rolled-steam and dry rolled-steam corns, it has been demonstrated that diet processing type does not have any significant effect on ruminal various volatile fatty acids.[20] It has been also reported that feeding breeding cows with rolled-steam corn compared to whole grain corn results in the reduction of acetic acid, but an increasing trend of ruminal propionic and butyric acids is observed. [16] In the current study, a reduction of corn grains starch resistance to degradability was observed in animals fed ground corn compared to rolled-steam and soaked corn diets, and thereby, a reduction of pH level was observed in the rumen of animals fed ground corn diet. Similar findings were observed previously suggesting bacterial fiber-degrading functional performance reduction.[21]

The results of present study showed that diets containing processed corn (soaked for 48 hr), increased papilla and epithelial tissue thicknesses compared to ground corn. Such increases could be attributed to the slower releasing of carbohydrates in soaked corn. Another possible reason for higher thickness of papilla and epithelial tissue in lambs fed soaked corn may be the higher abrasion ability of soaked corn.[14] In lambs fed aforementioned diets, a non-significant papillae height was observed in comparison with lambs fed high-rubbed diets.[22] It has been reported that feeding dry rolled-steam, roasted-steam and rolled-steam corns has a significant effect on the ruminal papillae height in the calves; however, in the rolled-steam corn treatment a maximum height of papillae is observed, the shortest height of papillae is seen in animals treated with dried rolled-steam corn, though, papillae thickness is remained unaffected.[9,23] It has been indicated that use of large-drilled particles of cereal grain in dairy cattle results in higher rumen capacity and muscle than those treated with grain flour or pellets. This would show that the degree of grain processing and/or particle size affect rumen capacity and muscular tissue stimulation.[9,24]

In conclusion, processing corn grain, ground corn, improved the propionate concentration and ruminal pH normal range; this could be addressed as a better starch accessibility to ruminal environment. Also, the current study showed that swap of ground grain with soaked corn seeds (48 hr), improves some morphological, biological

and fermentative features of rumen. Therefore, from practical point of view, the soaked corn processing could be generally used in lambs producing system.

Acknowledgments

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Conflict of Interest

The authors declare that there is no conflict of interests.

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