Effect of particle size of a mash concentrate on behavior, digestibility, and macroscopic and microscopic integrity of the digestive tract in Holstein bulls fed intensively[1]

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ABSTRACT: Twenty-four individually housed Holstein bulls (456 ± 6.9 kg of body weight and 292 ± 1.4 d of age) were enrolled in a complete randomized experiment involving four dietary treatments to evaluate the potential effect of mash particle size of diets in finishing beef diets on behavior, digestibility, and macro- and microscopic changes of the digestive tract. The four treatments were all ingredients sieved at 2 mm (HM2), all ingredients sieved at 3 mm (HM3), all ingredients, but corn, sieved at 2 mm and corn at 10 mm (HM210), and all ingredients, but corn, sieved a 3 mm and corn at 10 mm (HM310). For the HM210 and HM310 mashes, corn ground at 10 mm was mixed with the remaining concentrate ingredients ground at 2 or 3 mm, respectively. Concentrate (36% corn, 19% barley, 15% corn gluten feed, 8.4% wheat; 14% crude protein, 3.28 Mcal of ME/kg) consumption was recorded daily and straw consumption weekly. To register behavior, animals were filmed for 24 h on a weekly basis. At day 49 of study nutrient digestibility was estimated. Bulls were slaughtered after 56 d of exposure to treatments. Digestive tract and hepatic lesions were recorded, and tissue samples from the digestive tract collected. Geometric mean particle size was 0.61 ± 0.041, 0.76 ± 0.041, 0.62 ± 0.041, 0.73 ± 0.041 mm, and percentage of particles between 0.5 and 1 mm were 68 ± 2.9, 46 ± 1.7, 46 ± 5.0, and 39 ± 3.3 g/100 g for HM2, HM210, HM3, and HM310, respectively. Performance, total tract digestibility, or digestive tract integrity did not differ when ingredients were ground at 2 or 3 mm. Grinding corn with a hammer mill sieve size of 10 mm reduced feed efficiency and decreased total tract apparent dry matter, and organic matter digestibility compared with treatments from which all ingredients were ground at 2 or 3 mm. Straw intake was greatest and starch digestibility was least in the HM210 treatment. Last, only minor differences among treatments in rumen wall color, rumen papillae fusion, and histological conformation were observed. In summary, to improve feed efficiency, grinding corn at 10 mm is not recommended. In the present study, grinding procedure did not have a great effect on behavior and/or digestive tract health; however, under commercial conditions (group housing), grinding procedures that cause small mean particle sizes or particle size heterogeneity may increase the risk to suffer digestive tract lesions.

Key words: beef, behavior, digestive tract morphology, particle size of mash

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INTRODUCTION

Intensive beef production systems rely on cereal grains as the primary energy source (Svihus et al., 2005). The improvement of grain starch availability enhances animal growth, efficiency, and profitability (Wondra et al., 1995; Amerah et al., 2007; Moya et al., 2015; Vukmirović et al., 2017). Reduction of particle size (rolling or grinding) is one of the most common mechanical processing methods used to increase starch availability because increases the surface area facilitating the activity of microbial or digestive tract enzymes (Offner et al., 2003; Svihus et al., 2005). However, negative side effects of reducing particle size may increase the risk of rumen acidosis and gastric ulcers (Galyean et al., 1979; Rebhun et al., 1982; Wang et al., 2003) and altering animal behavior (rumination and stereotypies) and compromise animal welfare (Devant et al., 2016). Therefore, animal behavior and digestive health should be also evaluated when the particle size is reduced to improve digestibility.

The most common mechanical processing methods used to reduce particle size of grains are the hammer (grinding) and the roller (rolling) mills. The first is common in Europe, whereas the second in North America. When grinding is the procedure used there are no guidelines for the optimum particle size, therefore in the present study, the potential effects of two common sieve sizes (2 and 3 mm) in concentrate in meal form (mash) manufacture on animal performance and integrity of the digestive tract were evaluated. Moreover, to our knowledge, whether grinding all ingredients of the same sieve size has not been explored. Corn is commonly used in the rations of beef cattle, and grinding has much greater effect on starch digestibility in corn than in other cereals such as wheat or barley (Offner et al., 2003). The hypothesis of the present study was that grinding all ingredients in a mash at a small particle size (sieve size of 2 or 3 mm) and mixing them with corn ground at a greater sieve size (10 mm) would reduce the risk of digestive disorders resulting in improvements in growth performance and digestive health, even at the expense of a potential decrease in starch availability. Thus, the objectives of the present study were to evaluate the effects of particle size of mash on behavior, apparent total tract digestibility, and on macro- and microscopic changes in the digestive tract of finishing bulls.

MATERIALS AND METHODS

Animals, Diets, and Housing

All experimental protocols were approved by the Institutional Animal Care Committee of the Institut de Recerca i Tecnologia Agroalimentàries (Barcelona, Spain), and the study was conducted in accordance with the Spanish guidelines for experimental animal protection (Royal Decree 53/2013 of 1 February on the protection of animals used for experimentation or other scientific purposes; Boletín Oficial del Estado, 2013).

Twenty-four Holstein bulls (456 ± 6.9 kg of body weight [BW] and 292 ± 1.4 d of age) were kept in individual partially slatted pens (1.9 × 3.4 m) at the experimental station of the Cooperativa Agraria de Guissona (Guissona, Lleida, Spain) and randomly assigned to one of four treatments in a complete randomized experimental design. The four treatments consisted of all ingredients sieved at 2 mm (HM2), all ingredients sieved at 3 mm (HM3), all ingredients were sieved at 2 mm except for corn that was ground at 10 mm (HM210), and all ingredients were sieved at 3 mm except for corn that was ground at 10 mm (HM310). For the HM210 and HM310 concentrates in meal form (mash), corn ground at 10 mm was mixed with the remaining concentrate ingredients ground at 2 or 3 mm, respectively. All treatment concentrates had the same ingredient composition (35.7% corn, 18.7% barley, 8.0% wheat, 8.4% wheat middlings, 15.0% corn gluten feed, 5.9% beet pulp, 2.3% soybean meal [47% crude protein (CP)], 3.2% palm oil, 1.2% calcium carbonate, 0.6% sodium bicarbonate, 0.2% vitamin/mineral premix, 0.5% urea, 0.2% white salt), and thus, nutrient composition was also the same in all concentrates (5.3% ash, 13.9% CP, 19.3% neutral detergent fiber [NDF], 6.3% ether extract [EE], 55.2% nonfiber carbohydrates, 3.3 Mcal of ME/kg; dry matter [DM] basis) and was formulated to meet FEDNA (2008) recommendations. Barley straw (long particles unprocessed around 20 to 30 cm, 3.5% CP, 1.6% EE, 76.9% NDF, and 6.1% ash, on a DM basis) and concentrate were fed in separate troughs (0.6 × 1.2 × 0.3 m) both ad libitum until 56 d of the experiment when animals reached a target final BW of approximately 530 kg. Concentrate and straw offers and orts of concentrate were recorded daily, and orts from straw were recorded weekly at 0730 except for the last week of the study when they were recorded daily. Animal BW was recorded every 14 d.

Measurements and Sample Collection

Every 14 d, feed samples of each treatment were collected for particle size analyses. Feed particle size (granulometry) was determined by dry sieving of a 100-g sample (Baker and Herrman, 2002). The feed sample was passed through a sieve stack (0.5, 1, 1.7, 2.5, 3.35, 4 mm inside diameter) on a shaker (RP-200-N, Cisa, Barcelona, Spain) for 10 min. The quantity of particles retained on each screen size was then weighed. Feed particle size distribution was assessed as the weight of the different fractions and expressed as a percentage of total sample weight (g of fraction/g of total sample). Particle size geometrical mean was determined as described by ASAE (1983).

Fecal and bloat scoring were recorded weekly during the study. Fecal scoring was based on Heinrichs et al. (2003), where "1" was normal, "2" was soft to loose, "3" was loose to watery, "4" was watery, mucous, slightly bloody, and "5" was watery, mucous, bloody. Bloat scoring was determined according to the following description scale as defined by Johnson et al. (1958), where "0" corresponded to absence of bloat and thus no distension in left paralumbar fossa, "1" corresponded to a slight distension in left paralumbar fossa, "2" corresponded to a mild, marked distension in left paralumbar fossa; well rounded out, "3" corresponded to a well rounded out on left side, drum like; full on right side; restless, "4" corresponded to severe, both sides badly distended; left hip nearly hidden; skin tight; defecation; urination; incoordination; protruding anus; mild respiratory distress, and "5" corresponded to terminal, extreme abdominal distension; severe respiratory distress; cyanosis; prostration; death unless treated.

The behavior of four bulls from each treatment randomly selected was filmed continuously for 24 h on days 0 to 1, 7 to 8, 14 to 15, 21 to 22, 28 to 29, 35 to 36, 42 to 43, and 49 to 50 of the study using a digital video-recording device (model CSM-UTM824, Casmar S.A., Barcelona, Spain) and tubular D&N cameras (model CSM-BFN420, Casmar S.A.) fitted with 1/4 Sony CDD image sensor, 420 TVL, LEDs IR 15 m, and 4- to 9-mm varifocal lenses that were installed approximately 3 m above the ground. Each camera filmed simultaneously two pens. Videotapes were processed by scan-sampling at 10-min interval to represent behavior over an entire hour. Behaviors recorded were as follows: consumption (when an animal had its head into the feeder and was engaged in chewing) of concentrate, and straw, drinking (when an animal had its mouth in the water bowl), ruminating (including regurgitation, mastication, and swallowing of the bolus), self-grooming (nonstereotyped licking of its own body), social behavior (when a bull was licking or nosing a cohort with the muzzle or social horning defined as a head play when animals were rubbing their heads together), and oral non-nutritive behavior (the act of licking or biting the fixtures), flehmen response (upper lip reversed), and tongue rolling (swinging of the tongue outside the mouth, from one side to the other, or, repetitively, rolling the tongue).

Between 0 and 56 d of study, pendant data loggers (HOBO Pendant G Acceleration Data Loggers, Onset Computer Corp., Pocasset, MA) were placed on the right hind leg of each bull by using cohesive bandages (Eurimexflex, Divasa Farmavic, Barcelona, Spain) as described by Ledgerwood et al. (2010). Data loggers were programed to record measurements at 1-min intervals.

From day 49 to day 55, the concentrate was thoroughly mixed with chromium oxide (1 g/kg DM). During these days, a daily sample of feed (concentrate and straw) offered, and refusals were collected from each animal. From day 53 to day 55, a fecal grab sample was collected from the rectum at 1 h before and 3 and 5 h after feeding and dried at 55 °C during 48 h, and these samples were later composited by animal on an equal DM basis.

On day 56 of the study, bulls were transported to a commercial slaughterhouse (Guissona, Lleida, Spain) by truck. Transport distance was less than 1 km. Immediately following slaughter, a liquid sample from rumen and jejunum was obtained from homogeneous contents strained with a cheesecloth and a sample of cecal content was also collected and pH was measured using a portable pH meter (model 507, Crisson Instruments SA, Barcelona, Spain). Following the procedures of Jouany (1982), 4 mL of ruminal, jejunal, and cecal fluid were mixed with 1 mL of a solution containing 0.2% (wt/wt) mercuric chloride, 2% (wt/wt) orthophosphoric acid, and 2 mg/mL of 4-methylvaleric acid (internal standard) in distilled water and stored at −20 °C until subsequent volatile fatty acid (VFA) analysis.

The entire ruminal epithelium was examined for the presence of clumped papillae (Nocek et al., 1984), ulcers, hair presence, and parakeratosis (presence and location). Also, rumen walls were classified from 1 to 5 depending on the color, with "5" indicative of a black-colored rumen and "1" a white-colored rumen (González et al., 2001). The presence of cecal wall petechia and the color of cecal wall (0 = white pink, 1 = light pink, 2 = pink, 3 = dark pink) were also recorded. Liver abscesses were graded as described by Brown et al. (1975). In addition, 1-cm^2 sections of the ruminal (left side of the cranial ventral sac), jejunal, and cecal epithelia from the end of the cecum were sampled, washed with a 0.9% (wt/vol) NaCl solution, and preserved in a 10% formalin solution until subsequent histological analyses.

Biological and Chemical Analyses

Samples of feed were analyzed for DM (24 h at 103 °C; method number 925.04; AOAC, 1995), ash (4 h at 550 °C; method number 642.05; AOAC, 1995), CP by the Kjeldahl method (method number 988.05; AOAC, 1995), NDF assayed according to Van Soest et al. (1991) using sodium sulfite and heat stable amylase and expressed inclusive of residual ash, and EE by Soxhlet with a previous acid hydrolysis (method 920.39; AOAC, 1995). Total starch content was analyzed using the polarimetric method according to the EU Regulation for feed analyses (no. 152/2009). Chromium concentration of feed and fecal samples was determined based on the procedure of Le Du and Penning (1982). Digestion was carried out in duplicates. Two digestion steps were performed. The first digestion step was performed with 4 mL of concentrated HNO_3 at 220 °C for 15 min in a microwave oven (Ultrawave model, Milestone, Sorisole, Italy). After this step, two fractions were obtained in the digestion tube; an uncolored solution at the top and a green solid at the bottom of the tub. That solid was attributed to Cr_2O_3(s). In the second step, 3 mL of H_2SO_4, 0.5 mL of $HClO_4$, and 2 mL of hydrofluoric acid were added in the same digestion tube and digested at 260 °C during 15 min. Finally, the Cr content was determined by inductively coupled plasma optical emission spectrometry (model Optima 4300D, Perkin-Elmer, Shelton, CT). Total tract apparent digestibilities were calculated estimating total fecal output based on the ratio of chromium intake to chromium concentration in the feces.

Ruminal, jejunal, and cecal VFA concentrations were determined with a semicapillary column (15 m × 0.53 mm ID, 0.5 μm film thickness, TRB-FFAP, Teknokroma, Barcelona, Spain) composed of 100% polyethylene glycol esterified with nitroterephtalic acid, bonded, and crosslinked phase (method number 5560; APHA–AWWA–WPCF, 2005), using a CP-3800-GC (Varian, Inc., Walnut Creek, CA).

For the histological analysis of ruminal papillae, jejunal, and cecal epithelia, tissue samples were dehydrated and embedded in paraffin wax, sectioned at 4 μm, and stained with hematoxylin and eosin. Morphometric measurements were performed with an optical microscope (BHS, Olympus, Barcelona, Spain) using a linear ocular micrometer (Olympus, Microplanet, Barcelona, Spain) with 2× and 20× magnification. For ruminal samples, papillae length and width, number of papillae, and keratin layer thickness were measured on a 1-cm section. All morphometric measurements were performed by the same person (who was blinded to treatments). Mean papillae density, papillae width, and papillae length were used to calculate papillae surface area following the methods of Hill et al. (2005). Based on Nocek et al. (1984), a scale of 1 to 5 was used to characterize the tissue with "1" being an unvacuolated cytoplasm, particularly in the stratum granulosum and "5" was an epithelium with highly vacuolated stratum granulosum. Gradations "2" to "4" were gradations of the two extremes. In the cecum samples, crypt depth, number of goblet cells, number of intraepithelial lymphocytes (IEL), and number of mitosis were measured in a 1-cm section. In a 1-cm section in the jejunum samples, villus and crypt depth were measured, and in the cecum samples, crypt depth, number of goblet cells, number of IEL, and number of mitosis were measured.

Calculations and Statistical Analysis

To represent each behavior over an entire hour, videotapes were processed by scan-sampling every 10-min interval (Mitlöhner et al., 2001) and the total behavior duration in a day was analyzed. An intraclass correlation coefficient with a 95% confidence interval was used to determine interrater and intrarater reliability for four different observers who were blind to the treatments. Data from the data loggers were recovered using HOBOwarePro software (Onset Computer Corporation, Bourne, MA) and exported into a text file, which was processed according to the method of Ledgerwood et al. (2010). Briefly, a Python script was used to determine when the animals were standing up by using data from the y-axis, and if the animals were lying, the side of recumbence was determined using data from the z-axis. The same script was used to calculate daily lying time, number of lying bouts per day, and laterality (side of recumbence). The average bout duration was calculated by dividing daily lying time by the number of bouts per day. Total daily lying time, daily number of lying bouts, lying bout duration, and laterality were averaged for each 14-d period within each bull.

Normality of the data prior conducting statistical analyses was evaluated by the frequency histogram distribution and a Shapiro–Wilk test. Then, performance and behavior data were analyzed using a mixed-effects model with repeated measures (SAS Inst. Inc., Cary, NC). The model included initial BW as a covariate; effects of treatment, time (14-d period), and the interaction between treatment and time as fixed effects, and animal as a random effect. Time was considered a repeated measure, and for each analyzed variable, animal nested within treatment (the error term) was subjected to three variance–covariance structures: compound symmetry, autoregressive order one, and unstructured. The covariance structure that minimized Schwarz's Bayesian information criterion was considered the most desirable analysis.

Rumen, jejunum, and cecum data (VFA, pH, and histological data) were analyzed using ANOVA, with a model that included initial BW as a covariate and treatment as the main effect. Particle size analysis data were analyzed using a mixed-effects model. The model included the effects of treatment, time as fixed effects, and sampling day as a random effect. Last, a χ^2 test was conducted to evaluate the effects of treatment on rumen macroscopic classification, and fecal and bloat scoring data (categorical variables). For all analyses, significance was declared at $P < 0.05$ and tendencies established at $0.05 \le P < 0.10$.

RESULTS

Particle Size Distribution

Particle size distribution of the different treatments is presented in Table 1. Geometric mean of particle size did not differ between HM2 and HM3 treatments (0.61 ± 0.031 mm). However, when all ingredients were ground with a hammer mill sieve size of 2 mm, most particles (71.0 ± 2.59 g/100 g) were retained ($P < 0.001$) between the sieve size of 1 and 0.5 mm, and when all ingredients were ground with a hammer mill sieve size of 3 mm, main particles were retained ($P < 0.001$) in the sieves between 1 and 0.5 mm (50.5 ± 2.59 g/100 g) and 1.7 and 1 mm (29.7 ± 0.93 g/100 g). Moreover, in HM2 and HM3 treatments, the percentage of particles retained in the sieve above a 2.5 mm did not differ and were low (<0.5 g/100 g). Independent of the sieve size used to grind the main ingredients (2 or 3 mm), geometric mean particle size increased ($P < 0.001$) when corn was ground with a sieve size of 10 mm (0.74 ± 0.031 mm) compared with HM2 and HM3 treatments. When corn was ground with a hammer mill sieve size of 10 mm and the remaining ingredients at 2 mm, the percentage of particles retained in a sieve above 2.5 mm were greater ($P < 0.001$) compared with the mash where corn was ground with a hammer mill sieve size of 10 mm and the remaining ingredients at 3 mm.

Table 1. Particle size distribution of the concentrate in meal form with all ingredients or all ingredients except corn ground with a hammer mill using different sieve sizes

	Treatment[1]					
Item	HM2	HM210	HM3	HM310	SEM	*P* value[2]
Mean, mm	0.61[b]	0.76[a]	0.62[b]	0.73[a]	0.041	<0.001
Particle size distribution, g/100 g						
>4 mm	0.1[c]	1.9[a]	0.1[c]	1.4[b]	0.14	<0.001
4 to 3.35 mm	0.1[c]	3.5[a]	0.1[c]	2.5[b]	0.24	<0.001
3.35 to 2.5 mm	0.3[c]	6.5[a]	0.1[c]	4.6[b]	0.41	<0.001
2.5 to 1.7 mm	1.2[c]	9.3[a]	5.6[b]	9.3[a]	0.62	<0.001
1.7 to 1 mm	17.2[c]	18.5[c]	29.7[a]	24.3[b]	0.93	<0.001
1 to 0.5 mm	71.0[a]	48.0[b]	50.5[b]	44.9[b]	2.59	<0.001
<0.5 mm	10.1	12.3	13.9	13.0	1.193	0.16

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]HM2 = all ingredients ground with a hammer mill with a sieve size of 2 mm; HM210 = all ingredients ground using a hammer mill with a sieve size of 2 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm, HM3 = all ingredients ground with a hammer mill with a sieve size of 3 mm, HM310 all ingredients ground using a hammer mill with a sieve size of 3 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm.

[2]Treatment effect.

Animal Health, Bloat, and Fecal Scoring

One bull of HM310 treatment was removed at day 42 of the study because intake was low, although animal examination did not reveal any clear diagnosis. All data from this bull were removed from the database. The only bloat score recorded throughout the study was "0" (no bloat). Fecal score did not differ among treatments ($P = 0.40$); the most common fecal score registered was "1" (normal), with the exception of one HM2 bull that at day 14 ($P = 0.40$) and day 35 ($P < 0.05$) of the study scored "2" (was soft to loose) and "3" (was loose to watery), respectively, and at day 35, one HM3 bull and at day 42, one HM210 scored "2" (data not shown).

Intake and Animal Performance

Effects of treatments on feed consumption are summarized in Table 2. No interaction between treatment and time (14-d periods) was observed, and treatment did not concentrate or total DM intake (DMI). An interaction between treatment and time ($P < 0.05$) was found for straw intake as well as the ratio of concentrate to total DMI. After the first period (14 d), straw intake was greater in bulls fed HM210 followed by HM3 and HM310 compared with bulls fed HM2; the ratio of concentrate to total DMI followed consequently the opposite trend.

Total Tract Apparent Digestibility

Total tract apparent digestibility of DM and organic matter (OM) decreased ($P < 0.05$) when hammer mill sieve size of corn was 10 mm (HM210 or HM310) compared with grinding corn like the remaining ingredients (HM2 or HM3; Table 3). When hammer mill sieve size of corn was 10 mm and main sieve size was 2 mm, total tract digestibility of starch decreased ($P < 0.001$); however, this decrease was not observed when main sieve size was 3 mm.

Animal Behavior

Flehmen and tongue-rolling incidences were infrequent; thus, these behaviors were not statistically analyzed and were not reported. Treatment did not affect most of the behavior measurements recorded (Table 4) except for HM3 bulls, which tended ($P = 0.08$) to lie less and had a higher within-period coefficient of variation of lying bouts ($P = 0.09$) compared with HM2 bulls in some periods (inconsistent pattern).

Rumen Wall, Cecum, and Liver Lesions

Treatment tended ($P = 0.08$) to affect rumen wall color and affected ($P < 0.05$) papillae fusion (Table 5). No differences in rumen color were observed when main sieve size was 2 mm independent of corn sieve size, and the rumen walls of the bulls fed these treatments had a lighter color compared with HM3 and HM310 treatments. Moreover, in HM310 bulls, 40% of the rumen walls were classified as "5" (score corresponding to the darkest color), whereas in HM3 bulls, no rumens walls were classified as "5." Half of the rumen walls of HM3 bulls had papillae fusion in

Table 2. Performance during the finishing period (from 10 to 12 mo of age) in Holstein bulls fed high-concentrate diets with all ingredients or all ingredients except corn ground with a hammer mill using different sieve sizes

	Treatment[1]					P value[2]		
Item	HM2	HM210	HM3	HM310	SEM	Treatment	Time	Treatment × time
Initial age, d	291	292	292	294	5.97	0.91		
Initial BW, kg	457	456	456	455	13.3	0.99		
Final BW, kg	535	521	530	528	5.97	0.51		
Concentrate, kg DM/d	7.5	7.7	7.9	7.6	0.28	0.56	<0.001	0.41
Straw, kg DM/d	0.5	0.7	0.6	0.6	0.059	0.07	<0.001	<0.05
Total, kg DM/d	8.0	8.4	8.5	8.2	0.27	0.57	<0.001	0.36
Ratio of concentrate to total DMI, kg/kg	93.5	91.5	92.1	93.0	0.71	0.22	<0.001	0.05

[a–b]Means within a row with different superscripts differ ($P < 0.05$).

[1]HM2 = all ingredients ground with a hammer mill with a sieve size of 2 mm; HM210 = all ingredients ground using a hammer mill with a sieve size of 2 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm; HM3 = all ingredients ground with a hammer mill with a sieve size of 3 mm; HM310 = all ingredients ground using a hammer mill with a sieve size of 3 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm.

[2]Treatment effect, time effect (14-d period), and treatment by time interaction effect.

Table 3. Total tract nutrient apparent digestibility during the finishing period (from 10 to 12 mo of age) of Holstein bulls fed high-concentrate diets with all ingredients or all ingredients except corn ground with a hammer mill using different sieve sizes

Item	Treatment[1]					
Nutrient, %	HM2	HM210	HM3	HM310	SEM	*P* value[2]
DM[3]	69.1[a]	61.1[b]	70.4[a]	62.2[b]	2.12	<0.01
OM	70.6[a]	62.4[b]	72.2[a]	64.1[b]	2.32	<0.01
Starch	95.7[a]	84.0[b]	96.3[a]	92.6[a]	1.84	<0.001
CP	61.5	60.5	65.3	61.1	2.12	0.41
EE	81.5	76.7	82.2	74.8	2.42	0.16
NDF	39.7	46.5	48.5	45.5	3.78	0.47

[a–b]Means within a row with different superscripts differ ($P < 0.05$).

[1]HM2 = all ingredients ground with a hammer mill with a sieve size of 2 mm; HM210 = all ingredients ground using a hammer mill with a sieve size of 2 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm; HM3 = all ingredients ground with a hammer mill with a sieve size of 3 mm; HM310 = all ingredients ground using a hammer mill with a sieve size of 3 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm.

[2]Treatment effect.

[3]CP = crude protein; DM = dry matter; EE = ether extract; NDF = neutral detergent fiber; OM = organic matter.

Table 4. Daily performance of different behaviors (min/d) during the finishing period (from 10 to 12 mo of age) of Holstein bulls fed high-concentrate diets with all ingredients or all ingredients except corn ground with a hammer mill using different sieve sizes

	Treatment[1]					*P* value[2]		
Item	HM2	HM210	HM3	HM310	SEM	Treatment	Time	Treatment × time
Digital video-recording device records, min/d[3]								
Concentrate consumption	84.5	64.8	76.0	66.9	6.98	0.25	0.36	0.49
Straw consumption	60.4	69.7	59.6	68.8	7.22	0.71	<0.01	0.77
Drinking	41.5	42.7	47.5	46.9	7.24	0.30	0.37	0.63
Ruminating	408.0	422.8	454.8	430.2	30.8	0.74	0.49	0.39
Self-grooming	36.8	31.4	29.9	27.3	7.46	0.71	0.20	0.62
Social behavior	128.0	128.6	148.2	162.8	15.0	0.42	0.06	0.28
Non-nutritive oral behavior	29.1	38.3	45.0	36.5	9.10	0.60	0.05	0.87
Pendant data loggers records[4]								
Total lying, min/d	953	926.1	873.1	899.2	28.10	0.22	0.75	0.63
CV total lying, %	15.3	17.5	21.6	17.8	1.83	0.08	0.32	0.28
Lying bouts, n/d	10.7	10.9	9.5	10.1	1.31	0.88	0.03	0.84
CV lying bouts, %	18.6	20.8	23.1	22.2	1.76	0.27	0.95	0.36
Lying duration, min/lying	99.0	90.6	105.4	106.7	12.39	0.79	0.04	0.09
CV lying duration, %	33.9	34.5	36.2	38.1	3.28	0.69	0.15	0.30
Laterality, % lying right	51	57	48	44	5	0.39	0.15	0.62
CV laterality, %	23.6	19.7	43.4	35.8	11.96	0.45	0.27	0.32

[1]HM2 = all ingredients ground with a hammer mill with a sieve size of 2 mm; HM210 = all ingredients ground using a hammer mill with a sieve size of 2 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm; HM3 = all ingredients ground with a with a hammer mill with a sieve size of 3 mm; HM310 = all ingredients ground using a hammer mill with a sieve size of 3 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm.

[2]Treatment effect, time effect (14-d period), and treatment by time interaction effect.

[3]Behavior was analyzed at scan intervals of 10 min. To represent behavior over an entire hour, scan samples were multiplied by 10, and duration of each behavior was summed and analyzed as the time in minute that an animal devoted to each behavior within day.

[4]The average bout duration was calculated by dividing daily lying time by the number of bouts per day. Total daily lying time, daily number of lying bouts, lying bout duration, and laterality were averaged for the entire 10 d within each bull. CV means the interday coefficient of variation.

contrast to HM2 bulls ($P < 0.05$). Moreover, no rumen papillae fusion was observed in HM210 bulls, whereas some rumen walls of HM2 bulls had hairs attached to the wall (0 and 16.7% hair presence for bulls fed HM210 or HM2, respectively, $P < 0.05$). Last, no cecum petechia was observed in HM3 bulls; however, 20%, 66.7%, and 66.7% of cecum samples had petechiae in HM3, HM2, and HM210 bulls ($P < 0.05$). No liver abscesses were observed in this study.

Table 5. Rumen and cecum macroscopically evaluation at slaughterhouse of Holstein bulls fed high-concentrate diets with all ingredients or all ingredients except corn ground with a hammer mill using different sieve sizes

	Treatment[1]				
Item	HM2	HM210	HM3	HM310	*P* value[2]
Rumen color[3], %					0.08
1					
2					
3	33.4	16.7	—	.	
4	64.6	83.7	100	60.0	
5	—	—	—	40.0	
Rumen papillae fusion[4], %					<0.05
Yes	16.7	—	50	60.0	
No	83.3	100	50	40.0	
Cecum petechia presence, %					
Yes	33.3	33.3	—	80.0	<0.05
No	66.7	66.7	100	20.0	
Cecum color[5], %					0.74
0	66.7	83.3	83.3	60.0	
1	33.3	16.7	16.7	40.0	

[1]HM2 = all ingredients ground with a hammer mill with a sieve size of 2 mm; HM210 = all ingredients ground using a hammer mill with a sieve size of 2 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm; HM3 = all ingredients ground with a with a hammer mill with a sieve size of 3 mm; HM310 = all ingredients ground using a hammer mill with a sieve size of 3 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm.

[2]Treatment effect.

[3]Adapted from González et al. (2001): Rumen color: 1 = white; 5 = black.

[4]Adapted from Nocek et al. (1984).

[5]0 = white pink, 1 = light pink, 2 = pink, 3 = dark pink.

Rumen, Jejunum, and Cecum Morphometric Measures

Mean papillae number in the rumen was higher ($P < 0.05$) in HM210 than in HM3, with HM2 and HM310 treatments being intermediate (Table 6). In the jejunum, the crypt depth was double in bulls of HM310 treatment compared with HM3 bulls, whereas the crypt depth of the jejunum of HM2 and HM210 bulls was intermediate between HM310 and HM3 ($P < 0.05$). In the cecum, the goblet cell number was greater ($P < 0.01$) in bulls fed a concentrate ground with a hammer mill with a sieve size of 2 mm compared with the bulls fed HM210, HM3, and HM310 concentrates (Table 6).

Rumen, Jejunum, and Cecum pH and VFA

Ruminal, jejunum, and cecal fermentation variables are presented in Table 7. Treatment did not affect rumen pH, which was above 5.6 in all treatments, or total or molar proportions of VFA. However, in the jejunum, HM210 bulls tended ($P = 0.06$) to have a lower molar proportion of acetate and higher ($P < 0.05$) molar proportion of isovalerate compared with HM2, HM3, and HM310 bulls.

DISCUSSION

To our knowledge, for beef animals, there are no clear grinding recommendations available for mash feed. When corn was ground separately with a hammer mill with sieve size of 10-mm mean particle size increased (0.71 ± 0.031 mm) independent of the hammer mill sieve size used for the remaining ingredients (2 or 3 mm) and no differences were observed between grinding all ingredients with a sieve size of 2 or 3 mm (0.61 ± 0.031 mm). Even if mean particle size did not differ greatly between hammer mill sieve sizes, particle size distribution differed greatly among treatments. For example, in HM310, a great particle size dispersion was observed, as no particle size fraction had more than 45% of the particles compared with HM210, HM3, or HM2 treatments.

Theoretically, when mean particle size of mash is greater, starch digestibility is lesser as enzyme access to the nutrient can be limited reducing nutrient digestion; however, in the literature, as in the present study, contradictory data have been reported. Galyean et al. (1979) evaluated the effect of grinding corn particles at different sieve sizes in steers fed diets containing 72% ground corn. The hammer

Table 6. Rumen (left side of the cranial ventral sac), jejunum, and cecum morphometric measures of Holstein bulls fed high-concentrate diets with all ingredients or all ingredients except corn ground with a hammer mill using different sieve sizes

	Treatment[1]					
Item	HM2	HM210	HM3	HM310	SEM	*P* value[2]
Rumen papillae						
Length, µm	5,409	6,254	6,568	5,098	694	0.45
Width, µm	286	282	290	299	15.3	0.87
Vacuole[3], grading	1.0	1.13	0.78	0.67	0.231	0.53
Keratin, µm	29.7	28.6	28.8	32.3	2.71	0.73
Papillae number, per cm	8.9[b]	10.8[a]	7.7[c]	8.3[b]	0.75	<0.05
Jejunum						
Villus height, µm	2,525	2,453	1,537	2,820	359	0.11
Crypt depth, µm	860[b]	809[b]	530[c]	1,094[a]	120	<0.05
Cecum						
Crypt depth, µm	465	379	407	388	28.9	0.19
Goblet cells, number	62.6[a]	45.4[b]	43.2[b]	46.4[b]	3.54	<0.01
Intraepithelial lymphocyte, number	7.2	7.3	6.4	8.8	1.48	0.75
Mitosis, number	0.96	0.88	0.84	1.08	0.160	0.76

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]HM2 = all ingredients ground with a hammer mill with a sieve size of 2 mm; HM210 = all ingredients ground using a hammer mill with a sieve size of 2 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm; HM3 = all ingredients ground with a with a hammer mill with a sieve size of 3 mm; HM310 = all ingredients ground using a hammer mill with a sieve size of 3 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm.

[2]Treatment effect.

[3]Based on Nocek et al. (1984). A quantitative morphological analysis was used to determine rumen epithelial integrity. A scale of one to five was used to characterize the tissue with "one" being an epithelium that had light staining unvacuolated cytoplasm, particularly in the stratum granulosum. "Five" was an epithelium with highly vacuolated granulosum, and often the very thick corneum was in various stages of sloughing. The four layers of the epithelium stained densely and were differentiated easily. Grades "two" to "four" were gradations of the two extremes.

mill sieve sizes that these authors evaluated were 3.18, 4.75, and 7.94 mm, and the consequent mean geometric diameters of corn were 0.510, 0.588, and 0.833 mm, respectively. These authors observed neither an effect of hammer mill sieve size in total DM digestibility nor in total starch digestibility, rumen pH, or VFA profile. In contrast, when feeding steers a diet containing 86% corn rolled at different roller mill clearances, Secrist et al. (1995) reported an increase in starch digestibility when particle size was reduced from 1.54 to 0.75 mm. In the present study, as described previously, hammer mill sieve size (2 or 3 mm) did have a low impact on mean particle size (0.61 ± 0.031 mm) and affected mainly particle size distribution between 2.5 and 0.5 mm (Table 1). In the present study, hammer mill sieve size did not have an impact on total tract apparent digestibility, behavior (except of tendencies observed in lying time), or rumen, jejunum, and cecum morphology or rumen fermentation characteristics. May be to observe differences in digestibility, behavior, and gut health among treatments when comparing different hammer mill sieve sizes, mean particle size and/or particle size distribution should differ greatly among treatment like in the study by Secrist et al. (1995).

Grinding an ingredient like corn with a hammer mill sieve size of 10 mm increased mean particle size from 0.61 to 0.74 mm, and mainly percentage of the particles retained at sieves greater than 1.7 mm. The increase of mean particle size to 0.74 mm (HM210 and HM310) decreased DM and OM digestibilities compared with HM2 and HM3 that had a mean particle size of 0.61 mm. Surprisingly, starch digestibility of HM310 was not reduced compared with HM210 may be because the particle size dispersion was lesser in HM210 even if mean particle size was similar. As suggested by Amerah et al. (2007), particle size distribution (percentages), rather than mean particle size, is probably the more critical criterion when assessing the effect of hammer mill sieve size on total tract apparent digestibility, and integrity of the digestive tract. Starch content changes among the different particle fractions of corn steam flakes resulting from different bulk densities (Hales et al., 2010). In the present study, the nutrient content of the different fractions have not been analyzed, in the future they should be analyzed as they could provide some explanations for the differences observed in starch digestibilities among treatments. Moreover, the

Table 7. Rumen, jejunum, and cecum fermentation parameters of Holstein bulls fed high-concentrate diets with all ingredients or all ingredients except corn ground with a hammer mill using different sieve sizes

	Treatment[1]					
Item	HM2	HM210	HM3	HM310	SEM	*P* value[2]
Rumen						
pH	6.56	6.81	6.86	6.60	0.201	0.66
Total VFA[3], mM	92.4	76.9	77.8	81.5	11.61	0.77
Individual VFA, mol/100 mol						
Acetate	57.6	59.4	60.7	57.1	3.31	0.86
Propionate	27.3	25.3	25.4	27.4	3.24	0.94
Isobutyrate	1.3	1.5	1.4	1.2	0.15	0.70
n-Butyrate	9.3	9.2	8.1	9.1	0.99	0.83
Isovalerate	2.7	2.7	2.6	2.7	0.43	0.99
Valerate	1.7	1.8	1.7	2.3	0.32	0.56
Acetate:propionate	2.26	2.50	2.50	2.70	0.426	0.92
Jejunum[4]						
pH	7.31[a]	6.72[b]	7.11[a]	7.24[a]	0.166	0.12
Total VFA, mM	4.7	1.6	5.3	3.2	1.51	0.34
Individual VFA, mol/100 mol						
Acetate	97.5[a]	71.7[b]	91.0[a]	94.1[a]	6.10	0.06
Propionate	1.8	ND	0.9	ND	0.50	0.14
Isobutyrate	ND	ND	ND	ND	—	—
n-Butyrate	0.6	ND	2.6	0.9	0.98	0.34
Isovalerate	ND	28.3	5.4	5.0	6.2	<0.05
Valerate	ND	ND	ND	ND	—	—
Acetate:propionate	54.1	—	99.9	—	6.17	0.93
Cecum						
pH	6.44	6.34	6.53	6.69	0.158	0.57
Total VFA, mM						
Individual VFA, mol/100 mol	114	117	105	83	11.09	0.26
Acetate	70.9	69.8	69.4	74.2	1.52	0.23
Propionate	16.3	15.4	17.3	15.9	0.55	0.12
Isobutyrate	0.4	0.3	0.5	0.4	0.06	0.39
n-Butyrate	11.3	13.3	11.4	7.9	1.37	0.14
Isovalerate	0.3	0.4	0.4	0.3	0.05	0.58
Valerate	0.8	0.7	0.9	1.1	0.17	0.55
Acetate:propionate	4.39	4.57	4.01	4.67	0.216	0.21

[a–b]Means within a row with different superscripts differ ($P < 0.05$).

[1]HM2 = all ingredients ground with a with a hammer mill with a sieve size of 2 mm; HM210 = all ingredients ground using a hammer mill with a sieve size of 2 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm; HM3 = all ingredients ground with a with a hammer mill with a sieve size of 3 mm; HM310 = all ingredients ground using a hammer mill with a sieve size of 3 mm with the exception for corn, which was ground using hammer mill with a sieve size of 10 mm.

[2]Treatment effect.

[3]VFA = volatile fatty acid.

[4]ND = not detected.

bulls of HM210 treatment also consumed more straw compared with the other treatments. Reasons behind the increased straw intake in HM210 bulls are unknown. The increase of roughage intake when bulls are fed high-concentrate diets may be indicative of the need of fiber to ruminate and to regulate rumen pH. Forbes and Provenza (2000) asserted that ruminants in a free-choice situation are capable of adjusting their intake to minimize metabolic discomfort. Maybe, the great particle size dispersion of HM210 mash herein may have caused erratic rumen pH episodes, but the lack of continuous rumen pH recording in the present study precludes supporting this hypothesis. So, differences in the remaining ingredients like straw in the present study, or cotton seed hulls, alfalfa, and soybean meal in the study by Galyean et al. (1979) and cottonseed hulls in the study by Secrist et al. (1995) may influence the effect of particle size reducing methods on starch digestibility. These remaining dietary ingredients may affect rumination, as discussed previously, rumen particle distribution, or rumen microbiota composition, or passage rate. In summary, differences among particle size reducing methods in total tract starch apparent digestibility can be related to particle size distribution and/or to the remaining ingredients of the diet rather than to particle size geometrical mean. Moreover, HM210 bulls had greater jejunum molar proportions of acetate and isovalerate compared with the other treatments. In ruminants, the effect of the VFA in the jejunum has not been deeply studied in contrast to monogastrics. In swine, the beneficial effect of short-chain fatty acids, mainly butyrate, is not restricted to the colon, and short-chain fatty acids also stimulate cell proliferation and growth of small intestine (Liu, 2015).

There are few published studies that describe the effect of mash particle size on the integrity of the digestive tract. Hironaka et al. (1979) analyzed the effect of different particle sizes (from 0.47 to 1.52 mm) in barley-based diets in fattening bulls on digestive tract morphology; papillae crumpling, abnormal growth of papillae, and rumenitis were greater when mean particle size was decreased (0.67 vs. 0.46 mm). Moreover, in theory, fine particles adhere to the ruminal wall between papillae, and larger particles slide across the surface and cause abrasion (Greenwood et al., 1997). We expected large differences in digestive tract integrity among treatments; however, only some differences were observed in indices of tissue health (Table 5), which are difficult to interpret when coupled with other observations. For example, the HM310 bulls had the darkest rumens and largest percentages of papillae fusion and cecum petechial. Also, HM3 bulls had the lowest jejunum villus height and crypt depth compared with the other treatments, and the cecum of the HM2 bulls had a greater number of goblet cells compared with the other treatments was observed. These data may be indicative that a mash with a small particle size (HM2 and HM3) or a greater particle size dispersion (HM310) may potentially impair digestive tract health compared with HM210. These data could be also related to the reduced total tract apparent starch digestibility observed in the HM210 bulls compared with the other treatments. Moreover, no clinical signs of bloat (bloat scoring) or rumen acidosis (low intakes, very loose feces, rumen pH < 5.6) were observed. However, in the present study, animals were penned individually, whereas in group-fed animals with greater competition to access the feed and thus an increased stress, the severity of lesions observed might have increased.

When corn was ground at 10 mm, no propionic acid in the jejunum was detected. To our knowledge, there is scarce literature regarding the effects of short-chain fatty acids, and specifically of propionic acid, in the jejunum in ruminant animals. As mentioned previously, short-chain fatty acids may stimulate enterocyte proliferation in the small intestine (Liu, 2015).

In summary, when grinding all ingredients with a hammer sieve size from 2 or 3 mm, no differences in mean particle size were observed although distribution of between 2.7 and 0.5 mm differed and did not affect total tract apparent digestibility. However, grinding corn with a hammer mill sieve size of 10 mm increased mean particle size from 0.61 to 0.74 mm and decreased OM digestibility. Moreover, starch digestibility only decreased when hammer mill corn sieve size was 10 mm and the remaining ingredients were ground at 2 mm, and therefore should not be recommended. Finally, although in the present study grinding procedure did not affect at all behavior and/or digestive tract health, when animals are group-housed in commercial farms and competition is present, grinding procedures that cause small mean particle sizes or greater particle size heterogeneity may increase the risk suffering digestive tract lesions.

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