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Effects of the density of extruded pellets on starch digestion kinetics, rumen fermentation, fiber digestibility and enteric methane production in dairy cows

Ghulam Q. Khan[1] | Egil Prestløkken[1] | Peter Lund[2] | Anne L. F. Hellwing[2] | Mogens Larsen[2]

[1]Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences (NMBU), Ås, Norway

[2]Department of Animal Science, Aarhus University, Tjele, Denmark

Correspondence

Ghulam Q. Khan, Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences (NMBU), P. O. Box 5003, N-1432 Ås, Norway.
Email: ghulam.khan@nmbu.no

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Abstract

Dynamics of starch digestion in dairy cows fed extruded pellets differing in physical functional properties were investigated by measuring starch digestibility, post-prandial rumen fermentation patterns, and post-prandial duodenal starch appearance. Additionally, starch digestion effects on neutral detergent fiber (NDF) digestibility and methane (CH_4) emission were studied. Pure barley was extruded to produce three treatments having pellets of either low-density (LD), medium-density (MD) or high-density (HD). The experiment was conducted in a 3 × 3 Latin square design using three lactating Danish Holstein cows fitted with ruminal, duodenal and ileal cannulas. After the allocation of experimental concentrate directly into the rumen through the rumen cannula, cows were fed a basal diet low in starch. Eight samples were collected on equal time intervals (9 h) from duodenal digesta, ileal digesta and feces (grab sample) to determine digestibility. For post-prandial rumen fermentation patterns, four sample sets of rumen dorsal, medial and ventral fluid were taken from each cow, whereas for post-prandial duodenal starch appearance, 14 samples of duodenal chyme were obtained from each cow relative to morning feeding of experimental concentrate at 07:00 h. Ruminal, small intestinal, hindgut and total tract digestibility of starch did not differ among treatments. Similarly, NDF digestibility and CH_4 emission also remained unaffected by treatments. However, compared with the LD and MD treatments, the HD treatment showed higher acetate: propionate ratio at all positions in the rumen and a higher post-prandial duodenal starch appearance. This indicates lower ruminal starch degradation (RSD) and higher starch flow into the small intestine for HD treatment. In conclusion, the current study indicates that pellets' physical properties can manipulate RSD, where pellets with high density and fluid stability can partly shift starch digestion from the rumen to the small intestine. Indeed, further investigations are needed.

KEYWORDS

extrusion, in vivo, methane, ruminal degradation, starch

1 | INTRODUCTION

High-producing dairy cows have high demands for the supply of energy, especially in early lactation. In that period, feed intake, despite increasing, cannot match milk production demands (Allen et al., 2005). Usually, the need for energy is met by increasing the amount of concentrate fed. However, high levels of rapidly fermentable starch from concentrates may negatively affect the rumen environment and increase the risk of rumen acidosis (Owens et al., 1998). It has been demonstrated that the energy efficiency is about 42% higher for small intestinal starch digestion compared with starch degraded in rumen (Brake & Swanson, 2018). Thus, partly shifting the starch digestion site from the rumen to the small intestine could optimize feed intake and feed utilization in high-producing ruminants. Most commonly, the site of nutrient digestion is shifted by altering the rate of rumen digestion either by the selection of feed ingredients or the application of various feed processing methods. However, since rumen actual degradation results from the concurrent rate of ruminal degradation and rate of passage, increasing the rate of passage, especially if combined with a lower rate of degradation, will result in the shift of the digestion site from the rumen to the small intestine. Therefore, manipulating passage kinetics also could be an alternative approach to alter the site of nutrient digestion.

Although intrinsic physical properties of feed particles influencing their outflow from reticulorumen were identified a long time ago (Campling & Freer, 1962; King & Moore, 1957), altering the digestion site by manipulating the passage rate of feed particles is scarcely studied. Based on studies using either inert plastic particles or labeled indigestible plant fiber particles, it is revealed that the rate of passage of rumen particulate matter is mainly dependent upon particle density (Lechner-Doll et al., 1991). High-density (HD; sinking) particles have a higher passage rate from reticulorumen than particles with low density (LD; floating). However, controlling the density of feed particles during processing is not easy. Since concentrate feedstuffs are being increasingly pelletized by conventional pelleting to ease on-farm allocation, feed pellets with some specific density may be used to manipulate the passage rate and thereby alter the site of nutrient digestion in ruminants. Conventional pellets exhibit high density and have low water stability (Larsen & Raun, 2018) and therefore may disintegrate rapidly in the rumen, losing their physical properties. Extrusion feed processing is being extensively used in the fish feed industry to obtain compound feed pellets with functional physical properties like density with varying sinking velocities in seawater and high water stability (Sørensen, 2012; Welker et al., 2018). Recently, the effect of extruded pellets' physical properties on rumen environment variables and post-prandial starch appearance in the duodenum was studied in vivo (Larsen et al., 2019). They compared conventional pellets with extruded pellets of wheat, maize and mixtures of them and soybean meal having either LD or HD based on their bulk densities. They could not observe any apparent effect of treatments on the variables studied but suggested that feed pellets with HD and high liquid stability could influence rumen digestion kinetics and thus increase post-prandial duodenal flow. However, a major challenge is analyzing the behavior of extruded pellets in rumen fluid. During an in vitro study, Khan (2021) demonstrated that bulk density did not correlate with specific density for HD feed pellets. Thus, the determination of specific density is important when feed pellets are intended for increased passage from reticulorumen.

The present study's objective was to investigate the effects of pellets' physical properties on starch digestion kinetics along the gastrointestinal tract, rumen fermentation patterns, post-prandial duodenal starch flow, fiber digestion, and methane emission by using extruded barley. We hypothesized that the HD (or high sinking velocity) pellets would increase the passage rate, resulting in less rumen digestion and more rumen escape of starch.

2 | MATERIALS AND METHODS

The present experiment complied with Danish Ministry of Justice Law no. 382 (10 June 1987), Act no. 726 (9 September 1993), concerning experiments with animals and experimental animals' care.

2.1 | Processing of experimental treatments

The experimental concentrate was composed of pure barley grain and was processed at Fôrtek (Center for Feed Technology), NMBU, Ås, Norway, to obtain three treatments. The barley was ground using a hammer mill equipped with a 6 mm screen (HM 21.115; Münch-Wuppertal), and the meal was subsequently divided into three portions. All portions of the meal were pre-conditioned in a double shaft conditioner (BCTC 10; Bülher) and then extruded (Twin Screw BCTG 62 Extruder; Bülher) using a 6 mm die. Three different extruder processing settings were used to get pellets of either LD, medium density (MD) or HD. LD treatment was produced using high screw speed (300 rpm), giving an exit temperature of 120°C, which resulted in extrudate expansion. MD treatment was obtained by reducing the extent of expansion with low screw speed (210 rpm), giving an exit temperature of 113°C. HD treatment was produced by limiting the extrudate expansion using low screw speed (210 rpm) with cooling of the last section of the extruder barrel, intended to keep the exit temperature around 90°C as maximum. The maximum temperatures obtained during processing were 122°C, 116°C, 109°C for LD, MD and HD treatments respectively. The extruded pellets were dried in a fluid bed continuous dryer (Fôrtek; NMBU) at ~100°C with a retention time of 7–10 min and afterward cooled at room temperature. When steady-state processing conditions were achieved, a sample was taken for each treatment at the start, the middle and the end of production. These three samples were pooled into one sample for each treatment and used to analyze physical properties.

2.2 | Analysis of physical properties

Bulk densities were determined in triplicate as described by Sørensen (2012), in which the weight of pellets was measured

using a 1-L steel cylinder. Radial expansion of pellets was calculated as a ratio of a pellet's diameter, measured with an electronic sliding caliper, to the diameter of the die and expressed in percentage. The reported value is the average of 30 measurements. Hardness was determined on a texture analyzer HK5T (Tinius Olsen) fitted with a 100 N load cell using a flat knob and 10 mm/min compression speed. The force (N) used to make the first crack in the pellet was used as a hardness value. Each reported value is the average of 30 measurements. Specific density, sinking velocity and fluid stability index (FSI) were performed as described in Khan (2021). In short, specific density was determined by measuring the weight of five selected pellets and then the pellets' volume by volumetric displacement method using 0.5 mm glass beads in tapped density analyzer (AUTOTAP; Quantachrome Instruments; 1900 Corporate Drive). Each reported value is the average of five measurements. The sinking velocity test was performed by measuring the time taken by a pellet to pass a distance of 220 mm in a transparent glass cylinder (310 mm high and 35 mm inner diameter), filled with rumen fluid of approximately 39°C. Each value is the average of 30 pellets measurements. The FSI of pellets was determined in triplicate by measuring the dry matter (DM) remaining in 2 mm mesh net ball-shaped baskets after incubation in rumen fluid at 39 °C for 30, 60 and 120 min.

The physical properties of treatments and their chemical composition are reported in Table 1. The anticipated differences in physical functional properties of pellets were obtained as treatments varied in their bulk and specific densities from 384 to 602 g/L and 0.66 to 1.04 g/ml, respectively giving pellets with floating (LD), slow sinking (MD) and fast sinking (HD) properties. Moreover, all treatments showed an FSI higher than 850 g/kg DM after 120 min incubation (Table 1; Figure 1). The experimental treatments did not vary in their chemical composition, except DM.

TABLE 1 Physical properties and chemical composition of experimental concentrate pellets

Item	Experimental concentrate pellets			SEM	*p* Values
	LD	MD	HD		
Physical properties*					
Pellet size (length × diameter), mm	13 × 9	8 × 7	7 × 6	–	–
Bulk density, g/L	384 ± 10.08	497 ± 12.58	607 ± 12.47	–	–
Radial expansion, %	33.3 ± 2.33	20.8 ± 2.18	12.3 ± 2.75	–	–
Hardness, *N*	107 ± 24.6	67.2 ± 14.1	141 ± 18.3	–	–
Specific density, g/ml					
Dry pellets	0.66 ± 0.05	0.80 ± 0.01	1.04 ± 0.01	–	–
Wet pellets†	0.88 ± 0.04	0.99 ± 0.04	1.22 ± 0.03	–	–
Sinking velocity‡, mm/s	–	27.6 ± 13.8 (60)	110 ± 3.45 (100)	–	–
Fluid stability index (FSI), g/kg DM§					
30 min incubation	989 ± 12.4	994 ± 5.98	995 ± 8.24	–	–
60 min incubation	973 ± 6.44	984 ± 14.1	990 ± 4.45	–	–
120 min incubation	882 ± 6.19	863 ± 3.37	921 ± 3.21	–	–
Chemical composition					
Dry matter (DM), g/kg	919[b]	928[a]	919[b]	2.25	0.04
Starch, g/kg DM	633	622	625	2.58	0.28
Crude protein, g/kg DM	115	115	114	0.90	0.85
aNDFom, g/kg DM	180	179	170	5.66	0.45
Ash, g/kg DM	20.6	20.7	20.8	0.17	0.71
Crude fat, g/kg DM	29.2	29.5	30.0	0.36	0.32

Note: SEM ($n = 3$).

Abbreviations: aNDFom, neutral detergent fiber corrected for residual ash; HD, high density; LD, low density; MD, medium density; SEM, standard error of the mean.

[a,b]Indicate significant differences among the treatments at $p \leq 0.05$.

*Average values with standard deviations.

†Determined after soaking pellets in rumen fluid for 30 min at 39°C.

‡Numbers in the parenthesis represent percentages of sinking pellets measured up to 20 min after dropping.

§g DM retained pellets per kg DM intact pellets after incubation in rumen fluid at 39°C for 30, 60 or 90 min.

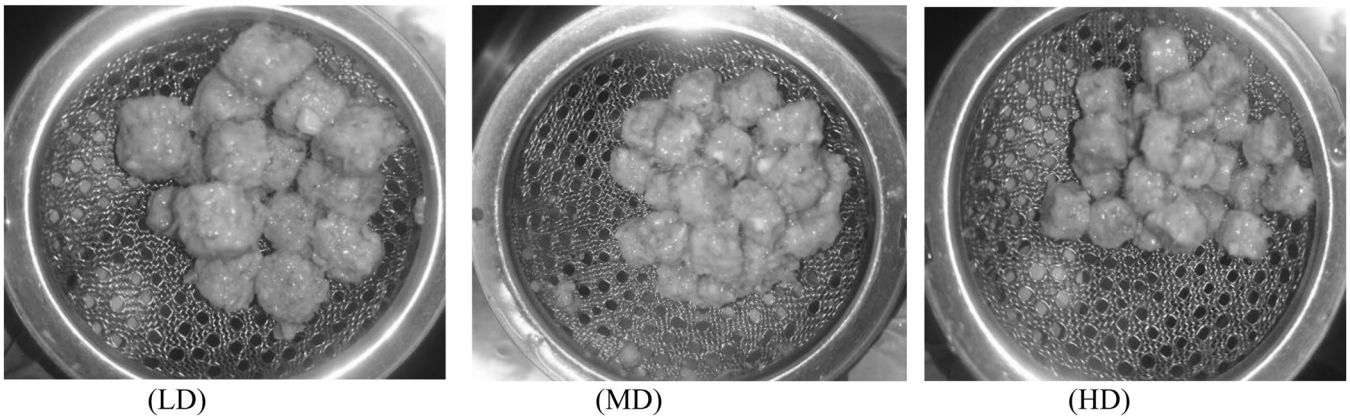

FIGURE 1 Example of fluid stability of extruded pellets with low-density (LD), medium-density (MD), and high-density (HD) after 120 min of incubation in rumen fluid

2.3 | Animal experiment

The three treatments were tested in a 3 × 3 Latin square experiment with 21-day periods having 11 days of adaptation and 10 days of sampling. Three lactating Danish Holstein cows (weighing 700 ± 52 kg, 253 ± 146 days in milk, and yielding 33 ± 5 kg milk/day) fitted with ruminal (#1C; Bar Diamond), duodenal (open T-piece placed 60 cm caudal to the pylorus) and ileal (open T-piece placed 20 cm cranial to the caecum) cannulas were used. Cows were housed in tie stalls with mattresses and had free access to water. A total of 4.8 kg/day of each treatment was fed into two equally divided portions at 7:00 and 16:30. Due to low palatability observed during pretrial testing, all treatments were fed directly via the rumen cannula. To simulate the entrance into the reticulo-rumen by eating, pellets were emptied from small plastic bags for 10 min as close as possible to the esophageal opening. The external digesta flow marker (13 g of TiO_2) was placed into the rumen dorsal sac at the end of concentrate feeding. Thirty min after concentrate feedings, a partial mixed ration (PMR) (Table 2) was allocated ad libitum with 60% of the daily allowance in the morning. Residual PMR was removed and weighed just before morning milking. Cows were milked at 06:00 and 16:00, and milk volume was recorded each time.

Samples for the determination of nutrient digestibility were collected from Day 12 to 15 in each period. The samples of concentrate, PMR, and residual PMR were subsampled for DM determination and subsequently stored frozen at −20°C until preparation for chemical analysis. PMR samples were pooled within the period, whereas one sample of each concentrate was taken in each period. Eight samples were collected on equal time intervals (on Day 12 at 18:00; Day 13 at 0:300, 12:00 and 21:00; Day 14 at 06:00, 15:00 and 24:00; Day 15 at 09:00) from duodenal digesta (500 ml), ileal digesta (300 ml) and feces (~250 ml grab sample) and pooled within cow and period. The duodenal and ileal samples were collected in tube-shaped plastic bags mounted to the cannula with plastic knees. The pooled samples of digesta and feces were stored frozen at −20°C until preparation for chemical analysis.

To determine the diurnal rumen fermentation pattern, eight samples of rumen fluid were taken from the ventral rumen at time-points corresponding to digesta samplings. For the determination of the post-prandial fermentation pattern, rumen fluid samples were taken on Day 15. Both diurnal and post-prandial samples were drawn with the same procedure using a suction strainer (#RT; Bar Diamond) equipped with a 50 ml syringe. For post-prandial samples, rumen fluid was taken from dorsal, medial and ventral rumen at 2, 4, 6 and 8 h relative to the feeding of the experimental pellets at 07:00. At first, about 40 ml of rumen fluid

TABLE 2 Composition and chemical analysis of partial mixed ration (g/kg DM unless otherwise stated)

Item	
Ingredients	
Soybean meal	119
Rapeseed cake, rolled	36.0
Sugar beet pulp, dried, rolled	119
Grass/clover- silage (first cut)*	716
Mineral mix, Type 1, granulated†	9.00
Nutrients	
DM, g/kg	380
Crude protein (CP)	216
Starch	6.01
Crude fat	28.3
aNDFom	309
Ash	86.9

Abbreviations: aNDFom, neutral detergent fiber corrected for residual ash; DM, dry matter.

*Chemical analysis (Eurofins A/S): DM, 357 g/kg; ash 88.4 g/kg DM; aNDFom, 333 g/kg DM; CP, 174 g/kg DM and in vitro digestible organic matter (OM), 799 g/kg OM.

†Pre-mix lactation (VM 2; Vitfoss) containing (per kg): 160 g of Ca, 50 g of P, 65 g of Mg, 90 g of Na, 0.5 g of S, 600 kIU of vitamin A, 190 kIU of vitamin D, 4000 IU of vitamin E, 4000 mg of Mn, 1500 mg of Cu, 25 mg of Co, 4500 mg of Zn, 225 mg of I and 50 mg of Se.

was sucked into a 50 ml syringe from the rumen's ventral sac and transferred to a 50 ml Falcon tube. Then the strainer was pulled upward about 25–30 cm to get a sample from the medial rumen. The sample from the dorsal rumen was taken through or just below the upper fiber mat. The pH in rumen fluid samples was measured immediately using a combination electrode (PHC2002-8; Hach Lange ApS) and a pH meter (PHM240 pH/ION Meter, MeterLab; Radiometer Analytical) calibrated at pH 4.000 and 7.000. Each rumen fluid sample was then subsampled into three Sarstedt tubes (10 ml in each) and stored at −20 °C until the analysis of volatile fatty acids (VFAs).

For post-prandial duodenal starch flow, chromium ethylenediaminetetraacetic acid (Cr-EDTA) was used as digesta flow marker as follows: 22 h before first sampling, a priming dose of 400 ml Cr-EDTA infusate (3.1 ± 0.06 g Cr/L) was administered to the ventral rumen followed by continuous infusion at a rate of 60 ± 3 ml/h using a peristaltic pump. On Day 19, samples of duodenal chyme were obtained from each cow at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 and 16 h relative to morning feeding of concentrate at 07:00. Samples (200 ml) were immediately transferred to plastic containers and weighed. About 13 ml of fluid from each sample was transferred to a Sarstedt tube and centrifuged to extract liquid. An 8 ml of supernatant was transferred to another tube and stored at −20°C for Cr determination. The remaining supernatant and precipitate were transferred back into the respective sample. The duodenal digesta samples were then dried at 100°C in a forced air oven for 48 h, DM was determined, and the dried samples were stored for later grinding and starch analysis.

On Days 20 and 21, the exchange of CH_4, CO_2 and H_2 was measured in respiration chambers. The system and equipment are described in detail by Hellwing et al. (2012). In the two first periods, there were technical problems with the CH_4 sensor (Horiba VIA-510 infrared CH4 sensor (Horiba). Therefore, the CH_4 emission in all three periods was measured by an infrared sensor from Guardian Range (Edinburgh sensors). Methane in Period 3 was measured with both the Guardian Range sensor and Horiba CH_4 sensor. The recovery for the Horiba sensor is usually between 99% and 100%. The Guardian Range sensor produced lower numbers compared with the Horiba sensor, and the Guardian numbers were corrected to the same level as the Horiba sensor for all three periods. In the experiment, the cow and chamber were confounded. Recovery tests for the CO_2 and CH_4 (measured with Horiba VIA-510) were performed after the experiment and averaged 99.4 ± 1.1% and 99.0 ± 0.6% for CH_4 and CO_2 respectively. Reported numbers are corrected using these recovery factors. Hydrogen was corrected with 99.2 ± 0.83%, which is an average of the recovery for CH_4 and CO_2. Feeding and milking were done similarly as before. Samples of PMR and PMR residues were taken for DM determination. Gas data are reported at standard temperature and pressure (0°C [273.15 K] and 101.325 kPa).

2.4 | Chemical analysis

The DM content of concentrates, PMR and PMR residues was determined by drying for 48 h at 60°C in a forced-air oven. Dried samples were subsequently ground using a Retch cutter mill with a 1 mm screen to analyze nutrients except for starch analysis, where a 0.5 mm screen was used. Starch content in concentrates, PMR, duodenal digesta, ileal digesta and feces was determined enzymatically (Kristensen et al., 2007) using the immobilized glucose oxidase electrode technique for glucose measurements (Mason, 1983). Starch was partially hydrolyzed at 100°C with thermostable α-amylase, followed by complete hydrolysis with amyloglucosidase at 60°C, and released glucose was determined by YSI 2900D analyzer (YSI). Starch content was corrected for the content of free glucose in the original sample by incubation without enzymes. Nitrogen content in concentrates, PMR, duodenal digesta, ileal digesta and feces was determined according to the Dumas method (Hansen, 1989) by using a Vario Max CN (Elementar Analysesysteme GmbH). Neutral detergent fiber (NDF) in concentrates, PMR, duodenal digesta, ileal digesta and feces was determined using Fibertec 2010 (Foss) equipment after treatment with a heat-stable amylase and corrected for residual ash (Mertens, 2002) and expressed as neutral detergent fiber corrected for residual ash (aNDFom). Ash content in concentrates and PMR was determined by combustion for 6 h at 525°C (method 923.03; AOAC, 1990). The TiO_2 content in PMR, duodenal digesta, ileal digesta and feces was analyzed as described by Myers et al. (2004). The Cr in the supernatant (from duodenal flow samples) was analyzed by atomic absorption spectroscopy at 357.9 nm, as described by (Williams et al., 1962). Concentrations of VFA and L-lactate were analyzed by gas chromatography (Kristensen et al., 1996) and immobilized L-lactate oxidase electrode technique (YSI 2900D; YSI), respectively after stabilizing rumen fluid with 25% m-phosphoric acid (MPA)/2-EB solution.

2.5 | Calculations and statistical analysis

The DM flow of duodenal and ileal digesta and fecal output was calculated from daily TiO_2 doses and concentrations at their respective sites. The flow of nutrients was calculated from DM flow and chemical analysis of DM at each site. The apparent digestibility of nutrients in each section of the gastrointestinal tract was calculated based on the inflow and outflow of nutrients at each respective section. The post-prandial duodenal DM flow was calculated using Cr as an indigestible marker, assuming constant hourly rumen Cr outflow as rumen infusion was continuous. Subsequently, the post-prandial duodenal starch flow was calculated from DM flow and the percentage of starch present in duodenal DM at each time point.

Feed intake, nutrient digestibility and methane emission data were statistically analyzed using the general linear model procedure in SAS (2013) and period, treatment (experimental concentrate) and cow as fixed effects in the model. The post-prandial and diurnal rumen fermentation variables and post-prandial duodenal DM and starch flow were statistically analyzed using the MIXED procedure of SAS, 2013 for repeated measurements with the period, treatment (Trt), Time and Trt × Time as fixed effects, and cow as a random factor. Time within cow × period was considered a repeated measurement using

the autoregressive covariance structure. The Kenward–Roger method was used to calculate denominator degrees. The results are reported as least square means (LS means) with standard error of the mean for each treatment. Significance was claimed when $p \leq 0.05$ and tendencies were considered at $0.05 < p \leq 0.10$.

3 | RESULTS

3.1 | Intake, flow and digestibility

Total dry matter intake (DMI) was not affected by treatments ($p = 0.68$; Table 3). However, due to higher DM (Table 1), a higher DMI from the MD treatment than the LD and HD treatments was observed in the concentrate part ($p = 0.01$; Table 3) of the diet. Intake of nutrients also did not differ among treatments ($p \geq 0.33$). Milk yield was not affected ($p = 0.17$) by treatments and averaged 23.1 ± 0.8 kg/day.

The flow of glucose and starch in the duodenum, ileum and feces did not differ among treatments ($p \geq 0.15$; Table 4). The ruminal starch degradation (RSD) was not affected by treatments ($p = 0.43$). Similarly, small intestinal, hindgut and total tract digestibility of starch (TTSD) did not differ ($p \geq 0.23$) among treatments. Only a small fraction of starch escaping the small intestinal digestion was degraded in the hindgut, and TTSD was 99 ± 0.1% for all treatments.

The ruminal, small intestinal, hindgut and total tract digestibility of DM and aNDFom did not differ among treatments ($p \geq 0.28$; Table 5). However, the small intestinal digestibility of aNDFom was negative for all treatments. Ruminal and total tract digestibility of crude protein (CP) did not differ ($p \geq 0.24$) among treatments. However, the small intestinal digestibility of CP was higher for the HD treatment than for LD and MD treatments ($p = 0.02$).

TABLE 3 Nutrient intake (kg/day)

Item	Experimental concentrate pellets			SEM	*p* Values
	LD	MD	HD		
Dry matter intake					
PMR	14.9	14.5	14.8	0.34	0.68
Experimental concentrate	4.40[b]	4.45[a]	4.40[b]	0.01	0.01
Total	19.3	19.0	19.2	0.33	0.74
Starch	2.87	2.85	2.84	0.01	0.33
Crude protein	3.71	3.66	3.71	0.06	0.79
aNDFom	5.41	5.25	5.34	0.14	0.76
Ash	1.38	1.35	1.38	0.03	0.74
Organic matter	17.9	17.6	17.8	0.30	0.74

Note: SEM ($n = 3$).

Abbreviations: aNDFom, neutral detergent fiber corrected for residual ash; HD, high density; LD, low density; MD, medium density; PMR, partial mixed ration; SEM, standard error of the mean.

3.2 | Rumen variables

Post-prandial ruminal pH and concentration of total VFA in the dorsal, medial or ventral part of the rumen did not differ among treatments ($p_{Trt} \geq 0.32$; Table 6). However, compared with the ventral rumen, pH was lower and the concentration of total VFA was higher in the dorsal and medial rumen for all treatments ($p < 0.001$). The pH was the lowest at 4 h post-feeding in the dorsal and medial rumen ($p_{Time} \leq 0.02$; Table 6). The acetate to propionate (Ac:Pr) ratio was higher ($p_{Trt} = 0.03$) for the HD treatment in the medial rumen and tended ($p_{Trt} \leq 0.08$) to be higher in the dorsal and ventral rumen compared to the LD and MD treatments. The Ac:Pr ratio increased from 2 to 8 h after feeding ($p_{Time} \leq 0.03$) for all treatments in all sections of the rumen.

Diurnal pH and total VFA concentration in the ventral rumen were not affected by treatments ($p_{Trt} \geq 0.23$; Table 7) but were affected by the time of sampling ($p_{Time} < 0.01$). Diurnal propionate proportion was lower ($p_{Trt} = 0.04$), and Ac:Pr ratio tended to be higher ($p_{Trt} = 0.09$) for HD treatment compared with MD treatment but did not differ from LD treatment.

TABLE 4 Flow and apparent digestibility of starch along the gastrointestinal tract

Item	Experimental concentrate pellets			SEM	*p* Values
	LD	MD	HD		
Flow, g/day					
Duodenal starch	442	488	659	104	0.45
Duodenal glucose	15.1	4.13	16.3	5.16	0.37
Ileal starch	63.8	90.2	121	12.2	0.15
Ileal glucose	8.07	7.06	7.82	1.56	0.90
Fecal starch	26.4	36.4	40.3	3.89	0.23
Fecal glucose	25.1	18.1	21.2	3.05	0.43
Digestibility					
Rumen digestibility, % of intake	84.6	82.9	76.9	3.54	0.43
Small intestine digestibility					
% of entering	86.2	79.7	80.0	5.35	0.68
% of intake	13.2	13.9	18.9	3.65	0.58
Hindgut digestibility					
% of entering	58.8	51.8	63.9	14.4	0.85
% of intake	1.29	1.90	2.83	0.53	0.32
Total tract digestibility, % of intake	99.1	98.7	98.6	0.14	0.23

Note: SEM ($n = 3$).

Abbreviations: HD, high density; LD, low density; MD, medium density; SEM, standard error of the mean.

[a,b]Indicate significant differences among the treatments at $p \leq 0.05$.

TABLE 5 Apparent digestibility of dry matter (DM), neutral detergent fiber corrected for residual ash (aNDFom) and crude protein (CP) along the gastrointestinal tract

Item	Experimental concentrate pellets			SEM	*p* Values
	LD	MD	HD		
DM					
Duodenal flow, kg/day	14.2	13.9	14.9	0.60	0.58
Rumen digestibility, % of intake	26.0	26.9	22.2	3.42	0.65
Small intestine digestibility					
% of entering	56.3	55.6	59.6	1.29	0.28
% of intake	41.7	40.7	46.4	2.54	0.41
Hindgut digestibility					
% of entering	16.9	24.3	24.8	6.99	0.71
% of intake	5.52	7.88	7.81	2.47	0.77
Total tract digestibility, % of intake	73.3	75.5	76.4	1.61	0.50
aNDFom					
Duodenal flow, kg/day	1.52	1.55	1.61	0.06	0.57
Rumen digestibility, % of intake	71.8	70.8	69.5	1.43	0.59
Small intestine digestibility					
% of entering	−31.4	−23.3	−27.9	12.2	0.90
% of intake	−8.80	−6.79	−8.79	3.58	0.91
Hindgut digestibility					
% of entering	17.3	27.7	32.4	15.3	0.79
% of intake	7.67	9.84	13.7	6.38	0.81
Total tract digestibility, % of intake	70.7	73.8	74.4	2.95	0.69
CP					
Duodenal flow, kg/day	4.95	4.82	5.28	0.14	0.26
Rumen digestibility, % of intake	−33.6	−31.8	−43.4	3.52	0.24
Small intestine digestibility					
% of entering	73.2[b]	71.2[b]	76.2[a]	0.36	0.02
% of intake	97.8[a,b]	93.8[b]	109[a]	2.39	0.08
Hindgut digestibility					
% of entering	−3.02	13.6	12.8	7.84	0.41
% of intake	−1.01	5.14	4.36	2.81	0.41
Total tract digestibility, % of intake	63.2	67.2	70.3	2.54	0.33

Note: SEM ($n = 3$).

Abbreviations: HD, high density; LD, low density; MD, medium density; SEM, standard error of the mean.

[a,b]Indicate significant differences among the treatments at $p \leq 0.05$.

3.3 | Post-prandial duodenal flow

During the post-prandial sampling day, DMI did not differ among the treatments ($p_{Trt} = 0.13$; Table 8). Overall, post-prandial duodenal DM flow was greater with LD and HD treatments than with MD treatment ($p_{Trt} = 0.01$; Figure 2a). Concerning the first post-prandial sequence, duodenal starch flow and concentration did not differ among treatments ($p_{Trt} \geq 0.14$) up to 9 h after morning feeding. However, when both post-prandial sequences were taken into consideration, duodenal starch flow and concentration increased towards the evening ($p_{Time} \leq 0.02$; Figure 2b,c), where both post-prandial duodenal starch flow and concentration were highest for HD treatment ($p_{Trt} \leq 0.05$) as compared with LD and MD treatments.

TABLE 6 Post-prandial rumen pH and VFA patterns (until 8 h after morning feeding)

Item	Experimental concentrate pellets			SEM	*p* Values		
	LD	MD	HD		Trt	Time	Trt × Time
Dorsal							
pH	5.80	5.84	5.78	0.10	0.90	<0.01	0.44
Total VFA, mM	147	151	155	7.33	0.58	0.07	0.57
Acetate, % of total	58.0	57.7	58.8	1.26	0.16	<0.01	0.30
Propionate, % of total	22.0	22.0	20.8	0.81	0.23	0.01	0.68
Butyrate, % of total	15.0	15.1	15.2	1.43	0.92	0.05	0.41
Isobutyrate, % of total	0.70	0.75	0.73	0.04	0.41	0.26	0.65
Valerate, % of total	2.19	2.37	2.25	0.16	0.50	0.01	0.07
Isovalerate, % of total	1.48	1.54	1.57	0.20	0.74	0.43	0.64
Caproate, % of total	0.50	0.53	0.56	0.10	0.24	0.03	0.22
Acetate:propionate ratio	2.66[b]	2.63[b]	2.84[a]	0.13	0.06	<0.01	0.52
L-lactate, mM	3.15	1.69	1.70	0.62	0.19	0.23	0.58
Medial							
pH	5.63	5.67	5.68	0.11	0.86	0.02	0.85
Total VFA, mM	165	155	162	7.75	0.50	0.56	0.98
Acetate, % of total	57.2[a]	58.0[a,b]	59.1[b]	1.09	0.03	0.02	0.88
Propionate, % of total	22.4	21.8	20.9	0.90	0.16	0.04	0.79
Butyrate, % of total	15.3	15.0	14.9	1.35	0.85	0.36	0.95
Isobutyrate, % of total	0.71	0.76	0.72	0.03	0.35	0.69	0.85
Valerate, % of total	2.30	2.37	2.27	0.14	0.89	0.07	0.97
Isovalerate, % of total	1.54	1.53	1.55	0.22	0.99	0.53	0.82
Caproate, % of total	0.52	0.53	0.56	0.11	0.27	0.16	0.95
Acetate:propionate ratio	2.58[b]	2.67[b]	2.84[a]	0.13	0.03	0.01	0.81
L-lactate, mM	0.31[b]	0.84[a]	0.27[b]	0.20	0.06	0.07	0.81
Ventral							
pH	6.57	6.46	6.51	0.08	0.32	0.30	0.62
Total VFA, mM	116	121	121	4.55	0.54	0.45	0.71
Acetate, % of total	59.3[b]	59.2[b]	60.6[a]	1.47	0.08	0.44	0.95
Propionate, % of total	21.6	21.5	20.4	0.75	0.16	0.04	0.90
Butyrate, % of total	14.1	14.2	14.0	1.62	0.92	0.59	0.96
Isobutyrate, % of total	0.88	0.84	0.86	0.02	0.63	0.28	0.28
Valerate, % of total	2.05	2.17	2.00	0.14	0.65	0.06	0.78
Isovalerate, % of total	1.58	1.54	1.60	0.19	0.91	0.38	0.60
Caproate, % of total	0.44	0.48	0.49	0.10	0.13	0.16	0.64
Acetate:propionate ratio	2.77[b]	2.77[b]	2.98[a]	0.13	0.08	0.03	0.95
L-lactate, mM	0.08	0.94	0.24	0.25	0.12	0.07	0.10

Note: SEM ($n = 3$).

Abbreviations: HD, high density; LD, low density; MD, medium density; SEM, standard error of the mean; Trt, treatment; VFA, volatile fatty acid.

[a,b]Indicate significant differences among the treatments at p a,bIndicate significant differences among the treatments at $p \leq 0.05$.

TABLE 7 Diurnal pH and VFA pattern in ventral rumen

Item	Experimental concentrate pellets			SEM	*p* Values		
	LD	MD	HD		Trt	Time	Trt × Time
pH	6.49	6.39	6.44	0.07	0.23	<0.01	0.77
Total VFA, mM	127	127	128	5.55	0.95	<0.01	0.80
Acetate, % of total	61.3	60.5	61.6	0.91	0.42	<0.01	0.94
Propionate, % of total	20.9[a,b]	21.2[a]	20.3[b]	0.66	0.04	<0.01	0.64
Butyrate, % of total	13.2	13.5	13.5	1.11	0.85	<0.01	0.99
Isobutyrate, % of total	0.80	0.77	0.76	0.02	0.22	<0.01	0.43
Valerate, % of total	1.93	2.07	1.93	0.07	0.34	<0.01	0.78
Isovalerate, % of total	1.49	1.48	1.46	0.16	0.52	<0.01	0.52
Caproate, % of total	0.41	0.45	0.44	0.08	0.16	<0.01	0.93
Acetate:propionate ratio	2.97[a,b]	2.87[b]	3.05[a]	0.10	0.09	<0.01	0.96
L-lactate, mM	0.87	0.87	1.34	0.35	0.48	<0.01	0.82

Note: SEM (n = 3).

Abbreviations: HD, high density; LD, low density; MD, medium density; SEM, standard error of the mean; Trt, treatment; VFA, volatile fatty acid.

[a,b]Indicate significant differences among the treatments at $p \leq 0.05$.

TABLE 8 Post-prandial duodenal dry matter (DM) and starch flow

Item	Experimental concentrate pellets			SEM	*p* Values		
	LD	MD	HD		Trt	Time	Trt × Time
Intake*							
DM, kg/day	17.2	15.6	18.2	0.50	0.13	–	–
Starch, kg/day	2.87	2.85	2.84	0.01	0.33	–	–
Digesta flow up to 9 h after feeding							
DM, g/h	549[a]	477[b]	498[b]	111	0.01	0.28	0.74
Starch, g/h	13.6	11.8	14.9	3.94	0.53	0.02	0.36
Starch, g/kg DM	25.5	24.2	29.6	4.30	0.14	<0.01	0.16
Digesta flow up to 16 h after feeding							
DM, g/h	546[a]	488[b]	522[a]	105	0.01	0.11	0.71
Starch, g/h	14.4[b]	14.0[b]	18.3[a]	4.21	0.05	<0.01	0.56
Starch, g/kg DM	25.8[b]	28.1[b]	34.1[a]	4.32	0.01	<0.01	0.39

Note: SEM (n = 3).

Abbreviations: HD, high density; LD, low density; MD, medium density; SEM, standard error of the mean; Trt, treatment.

[a,b]Indicate significant differences among the treatments at $p \leq 0.05$.

*Intake on the day for post-prandial sampling.

3.4 | Methane measurements

The total daily methane emission and methane emission/kg DMI did not differ among treatments (Table 9).

4 | DISCUSSION

Barley starch is an easily digestible starch source, with an average RSD and TTSD of about 87% and 96% respectively (Moharrery et al., 2014; Nocek & Tamminga, 1991). However, these values vary greatly depending upon differences between barley varieties, amount of starch intake, degree of processing and feeding level. For current treatments, it was assumed that highly stable extruded pellets differing in densities would ferment at different positions in the rumen and give different patterns of duodenal starch appearance. Thus, it was hypothesized that HD pellets with fast sinking behavior combined with high fluid stability would have lower fermentation in the ventral rumen compartment and would have the greatest likelihood of passing out of the rumen, leading to reduced RSD. Indeed, the RSD did not differ among treatments, but both the post-prandial duodenal starch appearance and ruminal Ac:Pr ratio were

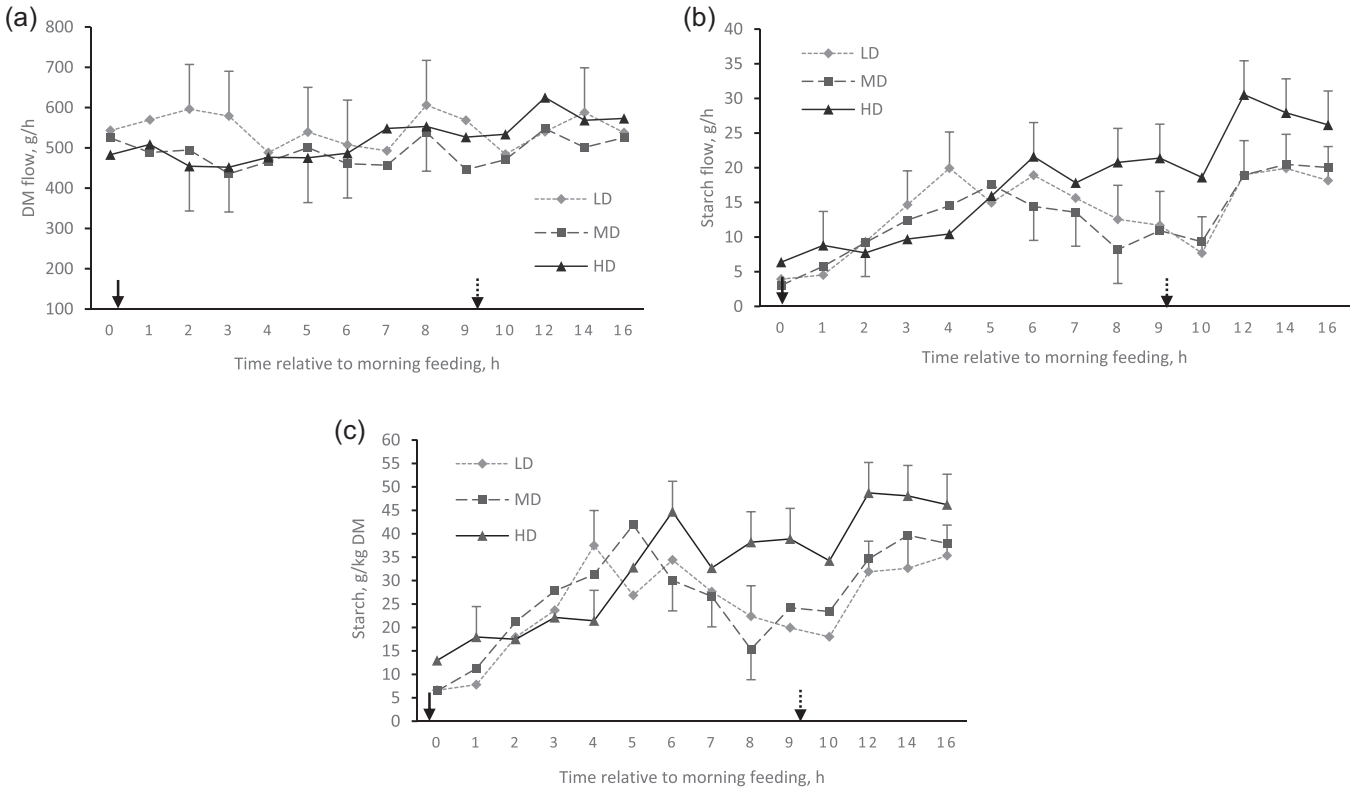

FIGURE 2 Post-prandial duodenal flow of dry mater (DM) (a) and starch (b) and starch concentration (c) for extruded pellets with low-density (LD), medium-density (MD), and high-density (HD). The solid arrow indicates morning feeding, and the dashed arrow indicates afternoon feeding of experimental concentrate pellets.

TABLE 9 Daily gas exchange

Item	Experimental concentrate pellets			SEM	*p* Values
	LD	MD	HD		
CH_4, L/day	595	535	536	13.9	0.14
CO_2, L/day	7070	6844	6612	114	0.20
H_2, L/day	5.12	4.62	4.56	0.53	0.74
CH_4, L/kg DMI	31.8	30.5	32.4	1.70	0.74

Note: SEM (n = 3).

Abbreviations: DMI, dry matter intake; HD, high density; LD, low density; MD, medium density; SEM, standard error of the mean.

higher for HD treatment compared with LD and MD treatments indicating reduced RSD with HD treatment.

Experiments with plastic particles have revealed that particles with a specific density between 1.1 and 1.42 g/ml have a lower mean retention time in the rumen (desBordes & Welch, 1984; Dufreneix et al., 2019; Seyama et al., 2017) and thus would have higher passage rate from the reticulorumen compared with particles having a specific density below 1.1 g/ml. However, contrary to inert plastic particles, feed particles change their specific densities once they are in the rumen, where particle-specific density may first increase due to hydration by rumen fluid and then decrease due to entrapment of fermentation gases (Wattiaux et al., 1992). Dufreneix et al. (2019) suggested that a particle density close to 1.3 g/ml will take more time to decrease its density below 1.1 g/ml and, therefore, will have a higher chance to leave the rumen. After soaking in rumen fluid for 30 min, the specific density of HD treatment was increased from 1.04 to 1.22 g/ml, whereas the specific density of LD and MD treatments, despite increasing remained below 1 g/ml (Table 1). This demonstrates that the functional specific density of HD treatment after entering the rumen was in the optimal range and thus resulting in higher starch flow to the duodenum compared with LD and MD treatments. Larsen et al. (2019) fed cows with extruded pellets of pure wheat and pure maize having either LD with an average bulk density of 443 ± 15 g/L or HD with an average bulk density of 658 ± 25 g/L. Contrary to our findings, they could not observe a clear difference in rumen fermentation variables and duodenal starch appearance, despite marked differences in the bulk densities of their extruded treatments. However, they did not determine the specific densities of the pellets. Moreover, the difference in functional specific density of pellets within the rumen may be smaller than the difference in bulk density due to physical forces by motility, digesta and fermentation gasses. This might explain similar effects of pellet densities on rumen fermentation variables and duodenal starch appearance as observed by Larsen et al. (2019) and in the present study for LD and MD treatments.

The overall post-prandial duodenal flow of starch increased at a lower rate after feeding and has a lower mean starch appearance than observed by Larsen et al. (2019) for LD and HD treatments based on 100% wheat or maize. In their study, duodenal starch flow peaked at

2.5 h post-feeding and then followed an exponential decline. Indeed, the digesta flow markers applied differed between the two studies, but the duodenal samples' current starch concentrations also indicate lower starch flow rates. Previously, Tothi et al. (2003) observed that post-prandial duodenal starch flow increased at lower rates reaching peak flow at 4–6 h post-feeding by feeding barley (ground or expander-treated conventionally pelleted) as a pulse dose. However, the functional physical properties of pellets used in Tothi et al. (2003) and the current study might differ in density and fluid stability as conventional pellets typically have HD and disintegrate quickly in liquid. Using 24 commercial pelletized concentrates, Larsen and Raun (2018) observed that the water stability index (WSI) varied from 21 to 198 g/kg DM, whereas FSI in the present study was more than 850 g/kg DM after 120 min incubation. Extruded pellets used in the present study can be considered highly stable in the rumen, as rumen fluid at 39°C instead of water at 25°C and more vigorous agitation was used to determine FSI compared with WSI. In addition to differences in physical properties, pellets were fed into both morning and evening feeding in the current study; thus, giving two peaks of post-prandial duodenal starch flow. Nevertheless, post-prandial duodenal starch flow for HD treatment increased gradually as the day progressed towards evening compared with LD and MD treatments (Figure 2b,c). It is evident that starch outflow from reticulorumen did not follow an exponential decline. Therefore, it did not follow first-order kinetics, which is generally assumed for starch, and indicated a lag time of newly ingested starch before passage. The starch outflow for LD and MD treatments was probably delayed due to their lower densities and slow disintegration. In contrast, HD treatment took a long time to attain the necessary density and perhaps size for passage out of the reticulorumen. A large peak after evening feeding, particularly for HD pellets, suggests increased starch flow due to new starch intake combined with a pulse of undigested starch either from the rumen or from the abomasum (Tothi et al., 2003). It can be speculated that duodenal starch flow was highest towards the evening, where few samples were taken, giving a lower mean starch flow than observed by Larsen et al. (2019).

Apart from density, feed particle size can also influence passage from the reticulorumen (Offer & Dixon, 2000). Since current extruded pellets are very stable, these can be considered particles. A 6 mm die size was used in the present study compared with Larsen et al. (2019), where extruded pellets were produced using a 2.4 mm die size. Therefore, the pellets' size also differs between the two studies which could influence passage from the reticulorumen. Based on wet sieving analysis of digesta particles leaving the reticulorumen, the probability of passage is negatively related to particle size (Poncet, 1991). However, experiments using inert plastic particles have provided inconclusive results on the relationship between particle size and passage kinetics. Kaske et al. (1992) using plastic particles with different lengths (1, 5, 10 and 20 mm), observed a decrease in particle passage as the particle size increased; however, they demonstrated that particles with 10 mm size could substantially pass from reticulorumen. Seyama et al. (2017) observed a higher passage rate for spherical particles with diameters of 6.35 and 7.95 mm than particles with a diameter of 3.97 mm. In contrast, Dufreneix et al. (2019) recently suggested that particles with a diameter between 3–4 mm would have a higher flow out of the reticulorumen than particles having other sizes. In the present study, the effect of particle (pellet) size on passage appeared minimal as MD treatment gave similar duodenal starch flow as LD treatment although pellet size was closer to HD treatment. Nevertheless, higher duodenal starch flow rates observed by Larsen et al. (2019) compared with the current study can be attributed to the smaller pellet size used in their study. Despite that the way pellets were fed into the animals differed between the two studies, experiments with plastic particles, administered orally or directly into the cows' rumen, revealed similar effects regarding passage kinetics and rates independent of the administration method (desBordes & Welch, 1984). Thus, using a smaller die size (e.g., 3 mm) will give pellets that could probably give higher duodenal starch flow as long as the optimal density is maintained.

The increased HD treatment density was obtained by decreasing expansion, giving increased compaction of particles and high hardness. The increased particle compaction limited the microbial penetration and consequently degradation of pellets as also supported by relatively a high FSI after 120 min incubation for HD treatment. In contrast, LD and MD treatments have either high expansion or low hardness; thus, making starch more susceptible to microbial breakdown (Giuberti et al., 2014; Huntington, 1997). As the proportion of propionate increases relative to acetate's proportion with the increase in starch degradation in the rumen (Sjaastad et al., 2016), the Ac:Pr ratio decreases. This trend is evident by comparing the post-prandial rumen Ac:Pr ratio with the corresponding first post-prandial sequence of duodenal starch flow. During this period, duodenal starch flow did not differ among treatments, but Ac:Pr ratio was higher for cows fed HD treatment than cows fed LD and MD treatments, indicating lower starch degradation for HD treatment. Thus, high density and high fluid stability, like in the current HD treatment, are essential pellet properties resulting in higher rumen escape of starch.

It is vital that starch escaping the reticulorumen is digested in the small intestine and absorbed as glucose to achieve the actual energetic potential of shifting the starch digestion site (Huntington et al., 2006; Mills et al., 2017). Small intestinal starch digestibility (SISD) remained unaffected among the treatments. However, it seemed that SISD followed the same pattern as RSD, especially for LD and HD treatments, that is, LD treatment with a numerically higher RSD has numerically higher SISD and vice versa. This agrees with Larsen et al. (2009) suggested that both RSD and SISD are affected by similar processes. However, on average, 97 ± 0.8% of starch intake was digested up to the distal ileum, and free glucose in ileal contents did not differ among treatments. Moreover, SISD was above 80% and thereby above 75%, which is the minimum threshold value demonstrated by Huntington et al. (2006) to increase energy yield by shifting the site of starch digestion from the rumen to the small intestine. Thus, no negative impact on SISD was observed. Regarding protein, duodenal flow relative to intake was high compared with what could be expected according to Titgemeyer (1997). However, the flow was consistent among animals and periods, and therefore results should reflect treatment effects. A higher small intestinal digestibility of CP for HD treatment than LD and MD treatments is compelling as, based on current starch observations, a greater rumen outflow of dietary CP could be expected for HD treatment and needs further investigation.

An increase in RSD has been observed to be accompanied by a decrease in ruminal and total tract digestibility of fiber (Chibisa et al., 2015; McCarthy et al., 1989). Ruminal and total tract digestibility of NDF did not differ among treatments, despite an increased rumen escape of starch for HD treatment. Besides, both rumen and total tract NDF digestibility remained above 70% for all treatments. Cellulolytic bacteria's ability to digest fiber is sensitive to pH changes, where pH below 6.0 is recognized to impair the growth of these bacteria (Dijkstra et al., 2012; Van Kessel & Russell, 1996). Despite that pH remained below 6.0 in the dorsal and medial part of the rumen, ruminal pH was not affected by the treatments, and the average ruminal pH (calculated as the average across the dorsal, medial and ventral parts of the rumen) and particularly pH in the ventral rumen was above 6.0. It can be speculated that during the current conditions, ruminal pH did not drop drastically due to an overall slow degradation of extruded pellets, thus favoring optimal ruminal conditions.

Since increased ruminal starch fermentation resulting in lower Ac:Pr ratio reduces methane emission (Mills et al., 2001), the effects of expected alterations in patterns of RSD on methane emission were also studied. A higher Ac:Pr ratio for HD treatment could be an indicator of a higher methane emission for this treatment; however, both methane emissions as L/day and L/kg DMI did not differ among treatments. The methane emission is not only affected by the fermentation pattern but also by the amount of fermented nutrients in the rumen and the use of hydrogen for other processes in the rumen. Overall, the treatments' effects had been too small to be detected in the methane data despite differences in rumen fermentation patterns.

The voluntary intake of experimental feeds was a challenge and varied among cows in pretrial. Larsen et al. (2019) reported similar intake problems for extruded pellets, especially LD pellets of pure wheat and pure maize, but not for grain–soybean meal mixes. They observed difficulty in swallowing due to the stickiness of pellets during chewing. In the present study, the stickiness of pellets was not observed. However, the pellets used were hard and big (6–13 mm), with sharp fiber particles protruding on the pellets' edges compared with Larsen et al. (2019). It was observed that the cows were troubled in chewing while eating the pellets. Primdal et al. (2014) found that large size (8 mm) and high hardness of pellets could decrease intake. Hence, the intake problems could be related to the size and physical shape of the extruded pellets, and the use of smaller pellets might have solved the problem for the extruded barley feeds.

5 | CONCLUSION

The study indicated that the density of pellets could manipulate starch passage kinetics from reticulorumen. Although RSD did not differ among the treatments, the high Ac:Pr ratio and rumen escape of starch for HD treatment all point towards the support of the hypothesis that the high density combined with the high fluid stability of extruded pellets could reduce RSD by decreasing the rate of degradation and increasing the rate of passage. However, further investigations are needed with optimal pellet size and relevant composition of concentrates.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Ghulam Q. Khan http://orcid.org/0000-0003-4162-1615

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