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Effects of a completely pelleted diet on growing performance of Holstein heifers.

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1 **INTERPRETIVE SUMMARY**

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3 **Effects of a completely pelleted diet on growing performance of Holstein heifers**

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5 **Bonfante**

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7 The pelletizing process, along with several potential benefits causes an important reduction of fiber  
8 particle size compared to a common total mixed ration. The aim of the current study was to evaluate  
9 the effects on rumen conditions (temperature and pH), fiber digestibility and animal performance of  
10 a pelleted diet fed to growing heifers. The results show that a pelleted diet, well designed to  
11 guarantee an adequate amount of NDF, could be fed to growing ruminants without causing dietary  
12 dysfunction.

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15 **COMPLETE DIET IN PELLET FOR GROWING ANIMALS**

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17 **Effects of a completely pelleted diet on growing performance of Holstein heifers**

18  
19 **E. Bonfante,<sup>1</sup> A. Palmonari, L. Mammi, G. Canestrari, M. Fustini, A. Formigoni.**

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## ABSTRACT

26  
27 The attributes of forage NDF content and particles size are recognized as important factors that  
28 impact rumen function. The aim of the current study was to evaluate the effects of pelleting a  
29 forage-based diet on rumen health, NDF digestibility, and animal performance. Eight Holstein  
30 heifers (age  $336\pm 30$ d, BW  $346\pm 35$ kg) were randomly assigned to a repeated cross-over design.  
31 Animals were housed in tie-stalls and fed for ad libitum intake. The study last four 3-wk periods,  
32 having the initial 2-wk for adaptation to the diet and 1 for data collection. Diets had the same  
33 ingredients but differed in physical forms: total mixed ration (treatment TMR) and PELLET  
34 ( $\emptyset=8$ mm; treatment P). The physically effective NDF (peNDF) differed among the two treatments  
35 (39.8 and 11.8% of NDF in TMR and P, respectively). During the trial DMI, water intake,  
36 rumination time, rumen temperature and pH were evaluated daily. Fecal samples were collected on  
37 the third wk of each period for total tract digestibility of the potential digestible NDF (TTdpdNDF).  
38 Average daily gain (ADG) and feed conversion ratio (FCR) were calculated at the end of each  
39 period. The DMI, DMI/BW, and water consumption were higher during the feeding of pelleted diet.  
40 There was no significant difference in ADG and FCR. Rumination time was lower for P than TMR  
41 treatment (241 vs. 507 min/d, respectively). Diet had no effect on rumen temperature and rumen  
42 pH. TTdpdNDF was greater in the TMR compared to P treatment (90.25 vs. 86.82 %pdNDF,  
43 respectively). The results of the current study suggest that a complete-feed pelleted diet was well  
44 accepted by the animals, as demonstrated by higher DMI. Rumination time was reduced with the P  
45 diet treatment, but rumen pH was not different than with the TMR diet treatment. The pdNDF  
46 digestibility was high for both diet treatments, with the TMR treatment significantly higher. Given  
47 the similar animal performance between the two treatments, which differed in DMI and apparent  
48 extent of fiber digestion, we might hypothesize different retention times of the two diets, related to  
49 their respective physical form. In conclusion, a complete-feed pelleted diet formulated to provide a  
50 sufficient level of NDF from forages, could be fed to growing ruminants without any apparent  
51 negative impact on rumen health and animal productivity, at least for a short period of time. More

52 researches considering a longer growing period are needed before recommending this feeding  
53 strategy for growing heifers.

54 **Key Words:** pellet, fiber particle size, pdNDF digestibility.

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## INTRODUCTION

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Fiber particle size and its NDF content have been considered important factors influencing rumen health (Allen, 1997; Krause et al., 2002b; Kononoff et al., 2003). This lead to a new concept introduced by Mertens (2000) who estimated the physically effective NDF (**peNDF**) to be the product of NDF concentration and physical effectiveness factor (**pef**). The latter represents the percentage of particles retained on a 1.18-mm sieve, considered highly resistant to passage out of the rumen (Poppi et al., 1985).

Fiber particle size influences chewing time and saliva secretion thereby affecting ruminal pH. It might also impact the retention time of particles in the rumen, and the extent of rumen fermentation and fiber degradation (Kaske et al., 1992; Teimouri Yansari et al., 2004; Kammes and Allen, 2012).

The reduction of fiber particle size in feeds has been used as an effective strategy to increase dry matter intake (**DMI**). Several studies demonstrate the difference between two or more feed chop lengths on animal performance (Yang and Beauchemin, 2006, 2009; Kammes and Allen, 2012); however, few trials have focused on pelletizing as a strategy to achieve this effect (McCroskey et al., 1960; Cullison, 1961; Burt, 1966). Through this method, controlled amounts of pressure and heat are applied to the combined aggregate of feed to increase its density (Mani et al., 2006). Pelleting offers many technical advantages including improved stability (owing to very low moisture content), easier handling management, storage and transportation.

Pelleting reduces fiber particle size and thus it might promote an increased rate of passage out of the rumen, and a subsequent decrease in fiber digestibility (Van Soest, 1994). Conversely, reduced particle size might serve to increase the surface area available for bacterial attachment (Miron et al., 2001), thereby increasing fiber digestibility. This reduction of fiber particle size could impact rumen fermentation and promote the development of subacute rumen acidosis (**SARA**; Khafipour et al., 2009).

81 The objective of this study was to evaluate the effects of a complete pelleted diet,  
82 formulated for growing heifers, on eating behavior, rumen fermentation, fiber digestibility, and  
83 animal performance. The hypothesis was that peNDF is not the only factor able to maintain an  
84 healthy rumen, but a diet high in NDF content can overcome the risk related with a low pH due to a  
85 lack in coarse forages.

## 86 MATERIALS AND METHODS

### 87 *Animals and Treatments*

88 The experimental procedures were approved by the Scientific Ethical Committee for animal  
89 experimentation at Bologna University. Eight Holstein heifers were used in a repeated cross-over  
90 design. The duration of the study was 12 wk, with four 3-wk periods. Heifers were adapted to the  
91 diet during the first two wk, and samples and data were collected during the last wk (experimental  
92 wk) of each period. The heifers had similar characteristics of age ( $336 \pm 30$  d) and body weight  
93 ( $346 \pm 35$  kg) at the beginning of the experiment, and were divided in two homogeneous groups.

94 Diet composition was the same for both treatments, but different in physical form (Table 1  
95 and 2). Diet for treatment one (**TMR**) was prepared as total mixed ration with a horizontal auger,  
96 trailer-type TMR feed-mixer (Zago 13-m<sup>3</sup>, ZAGO srl, PD, Italy). Treatment two (**P**) was produced  
97 as a complete-feed pelleted diet with the forages (grass hay and barley straw) chopped at 12-mm  
98 theoretical length of cut and then incorporated with the other ingredients (corn meal, sunflower  
99 meal, NaCl), mixed and pelleted (8-mm diameter).

### 100 *Data and Samples Collection*

101 Throughout the duration of the experiment, heifers were housed in tie-stalls bedded with  
102 sawdust, and fed their respective diets once daily in the morning (0830 h). The amount of feed  
103 offered and the refusals (orts) were weighed daily for each heifer. Feed was given for ad libitum  
104 intake based on orts quantity (10% of the DMI of the day before). Feed samples and orts were  
105 collected twice a wk for chemical and physical composition analysis. Daily water consumption was  
106 also recorded.

107 RuminAct<sup>®</sup>, SCR Heatime, Israel).  
108

109 Reticulorumen pH and temperature values were monitored continuously via an indwelling  
110 pH and temperature sensor (SmaXtech<sup>®</sup>, Animal Care, Austria) instilled in the reticulorumen  
111 region of the stomach. Data were transmitted to an external receiver via a Wi-Fi signal every 10  
112 min.

113 Heifers were weighed at the beginning of the study and at the end of each 3-wk period.

114 Fecal samples were collected every 6 h at d 5 (starting at 0000 am) and d 6 (starting at 0300  
115 am) of the experimental wk so that eight samples were taken for each heifer, each period,  
116 representing every 3 h of a 24-h period to account for diurnal variation. In each period fecal  
117 samples belonging to the same heifer were composited and then analyzed for nutrient chemical  
118 composition.

### 119 *Samples Analysis and Calculations*

120 Feed and fecal samples were dried in a forced-air drying oven (M700-VF, MPM instrument,  
121 Bernareggio, IT) at 65°C for 48 h to determine DM content. Particle size distribution of the dried  
122 diet was determined using a sieve-type shaker (Ro-Tap<sup>®</sup>; WS Tyler, Mentor, OH) consisting of six  
123 sieves having 6.70, 4.75, 3.35, 2.36, 1.18, 0.15 mm apertures plus a bottom pan. The fraction of DM  
124 retained on the 1.18-mm screen, or larger, was used to calculate the physical effectiveness factor  
125 (**pef**) of the diets.

126 For purposes of the analysis, dried diets, individual feed ingredients, and fecal samples were  
127 each ground separately in a Cyclone mill (1-mm screen; model SM100; Resch GmbH, Haan,  
128 Germany). Feed samples were analyzed for ash, determined after 4 h combustion at 550°C in a  
129 muffle furnace (Vulcan 3-550, Dentsply Neytech, Burlington, NJ); aNDFom, in according with  
130 Mertens (2002), with addition of sodium sulfite; ADF, ADL (AOAC, 1990; method 973.18); and  
131 CP (AOAC, 1990; method 976.06 and 984.13).



132 In vitro NDF digestibility at 24h and 240h (**IVNDFD24h** and **IVNDFD240h**) was  
133 performed using the Tilley and Terry modified technique (Robertson and Van Soest, 1981; Tilley  
134 and Terry, 1963). Rumen fluid was collected from two lactating cows fed a hay-based diet (milk  
135 production =  $33.2 \pm 1.7$  kg/d. DIM:  $251 \pm 2$ ) through the rumen cannula, mixed and placed in a  
136 thermally controlled bottle (PYREX, SciLabware, Staffordshire, UK). Rumen contents were filtered  
137 through four layers of cheese cloth under constant O<sub>2</sub>-free CO<sub>2</sub>. 10 ml rumen fluid was added to  
138 each 150-ml Erlenmeyer flasks that had been placed in a heated (39.3°C) water bath under CO<sub>2</sub>  
139 positive pressure to ensure anaerobiosis. 0.5 g of ground sample was weighed into each flask before  
140 the addition of 40 ml of the buffer, as described by Goering and Van Soest (1970). Each sample  
141 was analyzed in triplicate, in two separate in vitro incubations. Sample preparation, donor cows and  
142 their diets were the same for both assays. At the end of the fermentation, the contents of each flask  
143 was analyzed to determine the aNDFom content of the residue, and filtered through crucibles (40-  
144 µm porosity) with the addition of microfiber glass filters. Residues were then treated following the  
145 procedure described by Goering and Van Soest (1970), after a 3 h drying in a forced – air oven  
146 (105°C), and hot weigh recorded of crucibles. Ash correction was made after incineration of the  
147 residue at 495°C for 3 h, followed by a second crucible hot – weigh.

148 Digestibility was then calculated as described in equation 1:

$$149 \quad \text{IVNDFD, \% aNDFom} = [1 - (aNDFom_r - aNDFom_b) / aNDFom_i] * 100 \quad [1]$$

150 where  $aNDFom_r$  is the residual aNDFom,  $aNDFom_b$  is the blank correction, and  $aNDFom_i$   
151 represents the initial NDF. All the described terms are expressed in grams. The unavailable NDF  
152 fraction was determined after 240h in vitro fermentations, and calculated as expressed in equation  
153 2:

$$154 \quad \text{uNDF}_{240}, \% \text{DM} = (100 - \text{IVNDFD}_{240h}) * aNDFom / 100 \quad [2]$$

155 where  $aNDFom$  is the aNDFom content of the sample, on DM basis. Potentially digestible NDF  
156 was calculated as the difference between aNDFom and  $\text{uNDF}_{240}$ , on DM basis. Total tract  
157 digestibility of the pdNDF was then computed as described in the equation 3:

158 
$$TTdpdNDF, \%pdNDF = [100 - (uNDF_{240} diet / feces) * (pdNDF feces / diet)] * 100 \quad [3]$$

159 where  $uNDF_{240} diet / feces$  is the ratio among dietary and fecal  $uNDF_{240}$ , and  $pdNDF feces / diet$   
160 represents the ratio between fecal and dietary  $pdNDF$ .

161 In addition to that described above, length of fermentations was based on previous studies  
162 indicating 240h as the maximum extent of fiber digestion in an anaerobic environment in vitro (Fox  
163 et al., 2004; Raffrenato and Van Amburgh, 2011; Palmonari et al., 2014, 2016). For these  
164 fermentations, both rumen fluid and buffer were re-inoculated after 120h to preserve the microbial  
165 activity during the whole process, as described by Palmonari et al., (2014). A final volume of 100  
166 ml was treated for aNDFom determination as described above.

167 Rumination time data (rumination/DMI, rumination time/aNDFom intake, rumination  
168 time/forage aNDFom intake, rumination/peNDF intake) were used to calculate the average daily  
169 rumination time in the period.

170 Rumen pH data were used to evaluate mean pH, area under the curve (the area between the  
171 observed pH and a line draw at pH 5.8 and 5.5), and time (min) under pH 5.8 and 5.5. Rumen pH of  
172 5.8 was chosen as threshold for a subacute rumen acidosis (SARA) status and 5.5 for sever SARA.  
173 The duration (min/d) and total area (pH x min, area under the curve: AUC) that pH was below each  
174 SARA threshold were calculated to evaluate the severity of rumen acidosis. AUC was calculated by  
175 adding the absolute value of negative deviations in pH from 5.5 or 5.8 for each 10-min interval.  
176 (Dohme et al., 2008)

177 Body weight (BW) was used to calculate average daily gain (ADG) using the formula  
178 reported below:

179 
$$(\text{final weight (kg)} - \text{initial weight (kg)}) / \text{period length (d)}$$

180 the calculation was made at the end of all four periods.

181 Feed efficiency was computed as feed consumption adjusted for differences in gain (feed  
182 conversion ratio, FCR).

183 ***Statistical Analysis***

184 Data recorded in the third wk of each period were analyzed using the statistical program JMP-12  
185 software (SAS Institute Inc., Cary NC). DMI, water intake, rumination time, rumen pH and  
186 temperature, and NDF digestibility were carried out according with a mixed effects model for  
187 repeated measures. Treatment (T), period (P), day (D), treatment x period (TP), treatment x day  
188 (TD) were used as fixed effect and heifers (H) as random. The following model was used:

$$189 Y_{ijkl} = \mu + T_i + P_j + D_k + H_l + TP_{ij} + TD_{ik} + e_{ijkl}$$

190 ADG and FCR were analyzed using a post hoc Tukey's adjustment.

191 Data were considered significant if  $P < 0.01$ .

## 192 **RESULTS AND DISCUSSION**

### 193 *Diet Characteristics, Fiber Particle Size, and Intakes*

194 The two diets used in this trial were similar in chemical composition (Table 1). The CP (%  
195 of DM) resulted lower compared to NRC (2001) suggestions for 300 kg heifers. However, diets in  
196 the current study were formulated according to the Cornell Net Carbohydrate and Protein System  
197 (CNCPS; Higgs et al., 2015 and Van Amburgh et al., 2015) software, in which the MP and ME  
198 requirements were covered (656.4 g/d and 16.1 Mcal/d, respectively with a DMI of 8.4 kg/d). The  
199 two diets had different distribution of fiber particles (Table 2). The amount of particles retained by a  
200 1.18-mm screen was greater in the TMR ration compared with the pelleted one (66.12% and  
201 20.12% respectively). We used the threshold of 1.18 mm to distinguish the particles that are highly  
202 resistant to passage and consequently are able to stimulate rumination (Cardoza, 1985; Poppi et al.,  
203 1985; Mertens, 2000). PeNDF was 39.78 and 11.82 % of DM in the TMR diet and in the P diet  
204 respectively. Measurement of peNDF is important to determine the size of particles that are retained  
205 in the rumen. The minimum peNDF recommendation is 21% of the ration DM (Mertens, 2000) with  
206 consideration to the previous study in which approximately 19.7% peNDF was needed to maintain  
207 milk fat percentage of Holstein cows at 3.4%, and 22.3% peNDF was needed to maintain an  
208 average rumen pH of 6.0 (Mertens, 1997). The values of peNDF recorded in the TMR ration of this  
209 study were more than adequate to guarantee a good chewing activity, saliva production and rumen

210 health. Conversely, P diet was created intentionally, to have a low peNDF (11.82%) compared to  
211 recommendations. Since the study involved only the use of pre-primiparous growing animals,  
212 observations were limited primarily to rumination time and rumen pH mainly.

213 A treatment effect was noted on DMI (Table 3). Differences were observed in DMI at 3 h  
214 post feeding (2.70 vs. 3.25 kg in P and TMR treatment, respectively.  $P < 0.01$ ). Greater daily DMI  
215 was noted during the consumption of the P treatment (10.80 vs. 8.40 kg;  $P < 0.01$ ). This difference  
216 was still significant (2.88 vs. 2.23 % of BW;  $P < 0.01$ ) even when the DMI was normalized for  
217 animal BW. The DMI resulted higher compared with values suggested by NRC (2001) for 300 kg  
218 growing heifers. This is not in line with literature, in which a low protein content could negatively  
219 affect DMI (Tedeschi et al., 2000). This could partly compensate the lower protein content in the  
220 diet, allowing similar or higher consumption of grams of protein per day compared to the NRC  
221 (2001) guidelines (756 vs. 972 g of CP/d in TMR and P diet respectively).

222 Water intake (L/d) was higher for the P diet (55.0 vs. 45.0 L;  $P < 0.01$ ), but the difference  
223 disappeared when corrected for DMI. This result suggests that water intake wasn't a treatment  
224 effect, but rather, was related to the amount of DMI.

225 The aNDFom intake (kg/d and % of BW) were greater in P treatment compared with TMR  
226 (6.34 vs. 5.03 kg/d, 1.70 vs. 1.35 % of BW;  $P < 0.01$ ); as were the corresponding uNDF intake  
227 (kg/d and % of BW; 1.33 vs. 1.18 kg/d, 0.36 vs. 0.32 % of BW;  $P < 0.01$ ). The peNDF intake,  
228 consistent with previous findings, was higher in the TMR treatment (3.45 vs. 1.21 kg/d and 0.92 vs.  
229 0.32 % of BW  $P < 0.01$ ). Results from this trial confirm that an increase in fiber particle size in the  
230 diet has a negative impact on DMI, as reported in other studies (Allen, 2000; Kononoff et al., 2003;  
231 Kammes and Allen, 2012). The reduction of dietary fiber particle size could be considered as a  
232 strategy to decrease the DMI limiting fill effect in the reticulum-rumen when diet fiber composition  
233 could, otherwise, prevent animals from attaining an adequate DMI to reach their energy  
234 requirements (Montgomery and Baumgardt, 1965).

235 The ratio between uNDF<sub>240h</sub> intake and BW (0.36 vs. 0.32 % of BW in P and TMR  
236 treatment, respectively) was similar to values reported by other authors (Cotanch et al., 2014). In  
237 this study done with dairy cows, the ratio was 0.36 for grass hay based diet and 0.48 with alfalfa  
238 hay. Based on these data it is possible to hypothesize a minimum requirement of uNDF<sub>240h</sub> to assure  
239 rumen health and function.

#### 240 ***Animal Performance***

241 Average daily gain (ADG) was similar in the two treatments (1.1 vs. 1.0 kg in the P and  
242 TMR diet, respectively;  $P = 0.94$ ); which was considered within the range of normality for breed,  
243 age, sex, and size of the cattle used in this study, even if it was referred to a short period of time.  
244 The optimal ADG for growing heifers is 0.8 kg/d (NRC, 2001). Higher values are associated with  
245 the delay of age at first conception and calving. Conversely, other authors (Gardner et al., 1977)  
246 reported that high ADG (1.1 kg/d) is not associated to reproductive problems, but with a reduction  
247 in milk production, primarily, in the first lactation. Still other studies reported no negative effect on  
248 milk production with an ADG higher than 0.9-1 kg/d (Gardner et al., 1988; Van Amburgh et al.,  
249 1998).

250 Feed conversion ratio (FCR) was similar in the two treatments (11.0 vs. 10.6 kg in P and  
251 TMR diet, respectively;  $P = 0.33$ ).

#### 252 ***Rumination Time and Rumen pH***

253 Rumination time and rumen attribute data are reported in Table 4. Cows fed with P and  
254 TMR treatment ruminated 241 and 507 min/d ( $P < 0.01$ ), respectively. Rumination time decreased  
255 during P treatment administration (-52%), as expected with the reduction of fiber particle size. This  
256 effect on rumination was observed when related to DMI, aNDFom, or forage-aNDFom (23.3 vs.  
257 58.5 min/kg; 41.0 vs. 94.0 min/kg; 23.0 vs. 58.5 min/kg in P and TMR respectively;  $P < 0.01$ ).  
258 Rumination time is a parameter closely related to physical and chemical characteristics of the diet  
259 (Grant et al., 1990). The difference in rumination time observed in the current study could be  
260 related to different **pef** of the diets (20.12 and 66.12% for P and TMR treatment, respectively). This

261 relationship was also reported in other studies (Woodford and Murphy, 1988; Mertens, 2000;  
262 Krause et al., 2002). Other authors (Teimouri Yansari et al., 2004) evaluated the impact of reduced  
263 fiber particles on chewing, rumination time, and rumen pH; however that study was conducted on  
264 dairy cows fed alfalfa hay – based diets. In the current study the reduction in rumination time had  
265 no effects on rumen pH. Recorded values were similar among the two treatments (6.10 vs. 6.11 in P  
266 and TMR, respectively;  $P = 0.79$ ). According to other authors this effect may be related to diet  
267 composition being low in starch, high in fiber, and with an adequate uNDF intake (Yang and  
268 Beauchemin, 2007; Cotanch et al., 2014).

269 Short particle size, as well as reduced rumination time and saliva production, is usually  
270 associated to metabolic disorders such as sub-acute rumen acidosis (SARA). The definition of  
271 SARA is based upon rumen fluid pH (Plaizier et al., 2008). For purposes of this study we  
272 considered two different thresholds of suboptimal ideal pH: a pH below 5.8 as an indicator of  
273 fibrolytic bacteria depression, and a pH below 5.5 as the cutoff value for SARA determination, in  
274 accordance with Kleen et al., (2003). In our study, the average daily pH values (recorded every ten  
275 minutes over the experimental wk) were  $> 6.0$  throughout the entire experimental wk. Furthermore,  
276 the pH value, expressed either as min under the critical pH thresholds (5.5 and 5.8) or the  
277 corresponding areas under the curve, did not demonstrate any significant differences between the  
278 two treatments or indicate any risk of SARA, defined to be likely to occur when rumen pH remain  
279 below 5.5 for at least 180 min/d (Plaizier et al., 2008; Kleen et al., 2003).

### 280 ***Neutral Detergent Fiber Digestibility***

281 Data reported in Table 5 specify chemical composition of feces and the corresponding  
282 calculation of fiber digestibility in the gastrointestinal tract.

283 Fecal chemical composition of the two diets, show a similar aNDFom, ADF and ADL  
284 content; however uNDF<sub>240</sub> content was higher in TMR compared to P treatment (52.12 vs. 47.38 %  
285 of DM;  $P < 0.01$ ). PdNDF results were lower in the TMR compared with the P diet (17.14 vs. 22.18  
286 % of DM;  $P < 0.01$ ).

287 In vitro NDF digestibility (**IVNDFD**) was conducted at two different time points (24 and  
288 240 h). The IVNDFD24h was not different among the treatment (11.41 vs. 10.70 % of aNDFom in  
289 P and TMR, respectively;  $P = 0.51$ ), while IVNDFD240h was higher in diet P compared to TMR  
290 diet (31.82 vs. 24.72 % of aNDFom;  $P < 0.01$ ). Considering the fecal IVNDFD24h as the aNDFom  
291 fraction with potential rapid digestibility, the difference between treatments observed in the  
292 IVNDFD240h rates could hypothetically, be assigned to a slowly degradable fraction. This result  
293 suggests that fiber particle size influenced digestibility of the slowly digestible aNDFom, while  
294 having no effects on the rapidly digestible aNDFom.

295 Fecal slowly digestible fraction represents that fibrous material not digested in the gastro –  
296 intestinal tract. Given that this fraction was lower in the TMR treatment, a higher total tract  
297 digestibility would be expected. Total tract digestibility of the potentially digestible aNDFom  
298 (TTdpdNDF) was indeed higher in the TMR compared to the P treatment (90.25 vs. 86.82 % of  
299 pdNDF;  $P < 0.01$ ). Data observed in the current study are consistent with those recorded by  
300 Kammes and Allen (2012). In that study, the animals were fed forage based diet chopped at two  
301 different lengths (19 vs. 10 mm). The calculated TTdpdNDF in the cited study was 90.6 and 88.7%  
302 for long and short particles based diets, respectively. However, no treatment effect was observed in  
303 that experiment. As reported in the current study, the pelletizing process could have had an effect  
304 on particle structure and density, increasing their respective passage rate.

305 Fiber particle size influences many aspects of rumen function and digestion kinetics. The  
306 passage rate of particles is related to their reduction in size and increase in density. The dynamic  
307 relationship between these factors define the egress from the forage mat and flow out from the  
308 rumen (Sutherland, 1988). By experimental design, the fiber particle size was higher in the TMR  
309 diet. This size difference could have resulted in an increase in rumen retention time, thereby  
310 improving de facto fiber digestion (Sejrsen et al., 2006). While the shorter particles of the P  
311 treatment could theoretically have increased surface area available for microbial attachment, and  
312 consequently more extensive rumen degradation, the same attribute of size may have also increased

313 the escape rate from the rumen, limiting potential degradation. (Lammers et al., 1996; Kaske et al.,  
314 1992).

## 315 **CONCLUSIONS**

316 This study demonstrates that reduction of fiber particle size is a potential strategy to increase  
317 DMI in young ruminants. The shorter particle size led to a reduction in rumination time, without  
318 causing an adverse effect on rumen pH; furthermore the use of a pelleted diet did not affect ADG.

319 The different particle size of the treatments would be expected to impact the rate of passage  
320 from the rumen, being faster for the P treatment. Due to this, total tract digestibility of pdNDF was  
321 remarkably high in both treatments, although the effect of larger particle size in the TMR diet  
322 resulted in a significant increase.

323 We can conclude that a complete pelleted diet, well designed to provide an adequate amount  
324 of NDF, could be fed to growing ruminants without any apparent negative impact on rumen health  
325 and animal productivity, at least for a short period of time. More researches considering a longer  
326 growing period are needed before recommending this feeding strategy for growing heifers.  
327 Moreover, future studies are required to evaluate the effectiveness of this strategy on dairy cows, in  
328 particular during the transition period, when the low DMI is not sufficient to meet the increasing  
329 animal requirement.

330

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## REFERENCES

- 335
- 336 Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the  
337 requirement for physically effective fiber. *J. Dairy Sci.* 80:1447–1462.
- 338
- 339 Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J.*  
340 *Dairy Sci.* 83:1598–1624.
- 341
- 342 Association of Official Analytical Chemists. 1990. *Official Methods of Analysis*. 15th ed. Off.  
343 *Anal. Chem.*, Arlington, VA.
- 344
- 345 Burt, A. W. A. 1966. The nutritive value of ground and pelleted barley straw. I. Ground and  
346 pelleted v. long straw for growing Ayrshire heifers. *J. Agric. Sci.* 66:131–137.
- 347
- 348 Cardoza, R. S. 1985. Threshold size and factors affecting fecal particle weight distribution. M.S.  
349 Thesis, Univ. Georgia, Athens.
- 350
- 351 Cotanch, K. W., R. J. Grant, M. E. Van Amburgh, A. Zontini, M. Fustini, A. Palmonari, and A.  
352 Formigoni. 2014. Applications of uNDF in ration modeling and formulation. Pages 114-131  
353 in *Proc. Cornell Nutr. Confe.*, Dept. Anim. Sci., Cornell Univ., Ithaca, NY.
- 354
- 355 Cullison, A. E. 1961. Effect of physical form of the ration on steer performance and certain rumen  
356 phenomena. *J. Anim. Sci.* 20:478–483.
- 357
- 358 Dohme, F., T. J. DeVries, and K. A. Beauchemin. 2008. Repeated ruminal acidosis challenges in  
359 lactating dairy cows at high and low risk for developing acidosis: ruminal pH. *J. Dairy Sci.*  
360 91:3554–3567.

361

362 Fox, D. G., L. O. Tedeschi, T. P. Tylutki, J. B. Russell, M. E. Van Amburgh, L. E. Chase, A. N.  
363 Pell, and T. R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for  
364 evaluating herd nutrition and nutrient excretion. *Anim. Feed Sci. Technol.* 112:29–78.

365

366 Gardner, R. W., J. D. Schuh, and L. G. Vargus. 1977. Accelerated growth and early breeding of  
367 holstein heifers. *J. Dairy Sci.* 60:1941–1948.

368

369 Gardner, R. W., L. W. Smith, and R. L. Park. 1988. Feeding and management of dairy heifers for  
370 optimal lifetime productivity. *J. Dairy Sci.* 71:996–999.

371

372 Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (Apparatus, Reagents, Procedures,  
373 and Some Applications). Agric. Handbook No. 379. USDA-ARS, Washington, DC.

374

375 Grant, R. J., V. F. Colenbrander, and J. L. Albright. 1990. Effect of particle size of forage and  
376 rumen cannulation upon chewing activity and laterality in dairy cows. *J. Dairy Sci.*  
377 73:3158–3164.

378

379 Higgs, R. J., L. E. Chase, D. A. Ross, and M. E. Van Amburgh. 2015. Updating the CNCPS feed  
380 library and analyzing model sensitivity to feed inputs. *J. Dairy Sci.* 98:6340-6360

381

382 Kammes, K. L., and M. S. Allen. 2012. Nutrient demand interacts with grass particle length to  
383 affect digestion responses and chewing activity in dairy cows. *J. Dairy Sci.* 95:807–823.

384

385 Kaske, M., S. Hatiboglu, and W. V. Engelhardt. 1992. The influence of density and size of particles  
386 on rumination and passage from the reticulo-rumen of sheep. *Br. J. Nutr.* 67:235–244.

387

388 Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009. Alfalfa pellet-induced subacute ruminal  
389 acidosis in dairy cows increases bacterial endotoxin in the rumen without causing  
390 inflammation. *J. Dairy Sci.* 92:1712–1724.

391

392 Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. T. M. Noordhuizen. 2003. Subacute ruminal  
393 acidosis (SARA): a Review. *J. Vet. Med. Ser. A.* 50:406–414.

394

395 Kononoff, P. J., A. J. Heinrichs, and H. A. Lehman. 2003. The effect of corn silage particle size on  
396 eating behavior, chewing activities, and rumen fermentation in lactating dairy cows. *J. Dairy*  
397 *Sci.* 86:3343–3353.

398

399 Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2002. Effects of forage particle size and grain  
400 fermentability in midlactation cows. II. Ruminal pH and chewing activity. *J. Dairy Sci.*  
401 85:1947–1957.

402

403 Lammers, B. P., D. R. Buckmaster, and A. J. Heinrichs. 1996. A simple method for the analysis of  
404 particle sizes of forage and total mixed rations. *J. Dairy Sci.* 79:922–928.

405

406 Mani, S., L. G. Tabil, and S. Sokhansanj. 2006. Effects of compressive force, particle size and  
407 moisture content on mechanical properties of biomass pellets from grasses. *Biomass*  
408 *Bioenergy.* 30:648–654.

409

410 McCroskey, J. E., L. S. Pope, D. F. Stephens, and G. Waller. 1960. Effect of pelleting per-se on the  
411 utilization of milo and high roughage rations by steer calves. *J. Anim. Sci.* 4:1275–1276.

412

413 Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. *J. Dairy*  
414 *Sci.* 80:1463–1481.

415

416 Mertens, D. R. 2001. Physical effective NDF and its use in formulating dairy rations. Pages 143-  
417 160 in *Int. Symp. on Dairy Cattle*. Vol. 2. Florida Dairy Ext., Univ. Florida, FL.

418

419 Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds  
420 with refluxing in beakers or crucibles: Collaborative study. *J. AOAC* 85:1217-1240.

421

422 Miron, J., E. Yosef, and D. Ben-Ghedalia. 2001. Composition and in vitro digestibility of  
423 monosaccharide constituents of selected byproduct feeds. *J. Agric. Food Chem.* 49:2322–  
424 2326.

425

426 Montgomery, M. J., and B. R. Baumgardt. 1965. Regulation of food intake in ruminants. 1. Pelleted  
427 rations varying in energy concentration. *J. Dairy Sci.* 48:569–574.

428

429 NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. National Academies Press,  
430 Washington, DC.

431

432 Palmonari, A., M. Fustini, G. Canestrari, E. Grilli, and A. Formigoni. 2014. Influence of maturity  
433 on alfalfa hay nutritional fractions and indigestible fiber content. *J. Dairy Sci.* 97:7729–  
434 7734.

435

436 Palmonari, A., A. Gallo, M. Fustini, G. Canestrari, F. Masoero, C. J. Sniffen, and A. Formigoni.  
437 2016. Estimation of the indigestible fiber in different forage types. *J. Anim. Sci.* 94:248–  
438 254.

439

440 Plaizier, J. C., D. O. Krause, G. N. Gozho, and B. W. McBride. 2008. Subacute ruminal acidosis in  
441 dairy cows: The physiological causes, incidence and consequences. *Vet. J.* 176:21–31.

442

443 Poppi, D. P., R. E. Hendricksen, and D. J. Minson. 1985. The relative resistance to escape of leaf  
444 and stem particles from the rumen of cattle and sheep. *J. Agric. Sci.* 105:9–14.

445

446 Raffrenato, E., and M. E. Van Amburgh. 2011. Technical note: Improved methodology for analyses  
447 of acid detergent fiber and acid detergent lignin. *J. Dairy Sci.* 94:3613–3617.

448

449 Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis and its application to  
450 human foods. Page 123 in *Analysis of Dietary Fiber in Food*. Vol. 3 W. P. T. James, O.  
451 Theander, ed. Marcel Dekker, Inc, New York, NY.

452

453 Sejrsen, K., T. Hvelplund, and M. O. Nielsen. 2006. Page 601 in *Ruminant Physiology: Digestion,*  
454 *metabolism and impact of nutrition on gene expression, immunology and stress.*  
455 *Wageningen Acad. Pub., Wageningen, Netherlands.*

456

457 Sutherland, T. M. 1988. Particle separation in the forestomachs of sheep. Pages 43–73 in *Aspects of*  
458 *Digestive Physiology in Ruminants.* Dobson A, ed. Cornell Univ. Press, Ithaca, NY.

459

460 Tedeschi, L. O., D. G. Fox, and J. B. Russell. 2000. Accounting for the effects of a ruminal nitrogen  
461 deficiency within the structure of the Cornell Net Carbohydrate and Protein System. *J.*  
462 *Anim. Sci.* 78:1648–1658.

463

- 464 Teimouri Yansari, A., R. Valizadeh, A. Naserian, D. A. Christensen, P. Yu, and F. Eftekhari  
465 Shahroodi. 2004. Effects of alfalfa particle size and specific gravity on chewing activity,  
466 digestibility, and performance of Holstein dairy cows. *J. Dairy Sci.* 87:3912–3924.  
467
- 468 Tilley, J. M. A., and R. A. Terry. 1963. A Two-stage technique for the in vitro digestion of forage  
469 crops. *Grass Forage Sci.* 18:104–111.  
470
- 471 Van Amburgh, M. E., E. A. Collao-Saenz, R. J. Higgs, D. A. Ross, E. B. Recktenwald, E.  
472 Raffrenato, L. E. Chase, T. R. Overton, J. K. Mills, and A. Foskolos. 2015. The Cornell Net  
473 Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. *J.*  
474 *Dairy Sci.* 98:6361-6380  
475
- 476 Van Amburgh, M. E., D. M. Galton, D. E. Bauman, R. W. Everett, D. G. Fox, L. E. Chase, and H.  
477 N. Erb. 1998. Effects of three prepubertal body growth rates on performance of Holstein  
478 heifers during first lactation. *J. Dairy Sci.* 81:527–538.  
479
- 480 Van Soest, P. J. 1994. Page 277 in *Nutritional Ecology of the Ruminant*. 2nd ed. Cornell Univ.  
481 Press, Ithaca, NY.  
482
- 483 Woodford, S. T., and M. R. Murphy. 1988. Effect of forage physical form on chewing activity, dry  
484 matter intake, and rumen function of dairy cows in early lactation. *J. Dairy Sci.* 71:674–686.  
485
- 486 Yang, W. Z., and K. A. Beauchemin. 2006. Increasing the physically effective fiber content of dairy  
487 cow diets may lower efficiency of feed use. *J. Dairy Sci.* 89:2694–2704.  
488
- 489 Yang, W. Z., and K. A. Beauchemin. 2007. Altering physically effective fiber intake through forage

490 proportion and particle length: chewing and ruminal pH. *J. Dairy Sci.* 90:2826–2838.  
491  
492 Yang, W. Z., and K. A. Beauchemin. 2009. Increasing physically effective fiber content of dairy  
493 cow diets through forage proportion versus forage chop length: Chewing and ruminal pH. *J.*  
494 *Dairy Sci.* 92:1603–1615.  
495

496 **Table 1.** Ingredients and chemical composition of a pelleted (P) and total mixed ration (TMR)  
 497 treatment diet fed to heifers for ad libitum intake; the diets were formulated to be similar in  
 498 chemical composition but different in physical form (evaluated as physical effectiveness factor and  
 499 physically effective NDF)

Item	Treatment		
	P	TMR	SEM
Ingredients, % AF			
Grass hay	41.8	41.8	-
Barley straw	27.4	27.4	-
Corn grain	16.4	16.4	-
Sunflower meal	13.7	13.7	-
Salt (NaCl)	0.7	0.7	-
Chemical composition, % DM			
DM, % as fed	92.0	88.0	1.02
Crude protein	8.7	9.0	0.36
Ash	9.6	7.9	0.38
aNDFom <sup>1</sup>	58.8	60.2	0.66
ADF	40.7	41.4	0.80
ADL	8.1	8.4	0.42
IVNDFD 24h <sup>2</sup>	45.3	46.2	1.50
IVNDFD 240h <sup>2</sup>	78.4	77.3	0.73
uNDF <sub>240</sub> <sup>3</sup>	12.4	14.1	0.61
Starch	15.7	15.6	1.07

500 <sup>1</sup>aNDFom = amylase- and sodium sulfite-treated NDF, corrected for ash residue.

501 <sup>2</sup>IVNDFD = in vitro NDF digestibility.

502 <sup>3</sup>uNDF<sub>240</sub> = unavailable NDF estimated via 240-h in vitro fermentation.

503



504 **Table 2.** Physical characteristics and particle size distribution of a pelleted (P) and total mixed  
 505 ration (TMR) treatment diet fed to heifers for ad libitum intake; the diets were formulated to be  
 506 similar in chemical composition but different in physical form (evaluated as physical effectiveness  
 507 factor and physically effective NDF)

Item	Treatment		SEM	P-value
	P	TMR		
Particle size distribution, % <sup>1</sup>				
6.70, mm	0	4.86	0.28	<0.01
4.75, mm	0	8.95	0.58	<0.01
3.35, mm	1.19	11.78	0.39	<0.01
2.36, mm	4.29	12.15	0.28	<0.01
1.18, mm	14.62	28.36	0.34	<0.01
0.15, mm	60.66	31.11	0.94	<0.01
Pan	19.23	2.77	0.27	<0.01
Physical effectiveness factor <sup>2</sup>	20.1	66.1	5.90	<0.01
Physically effective NDF <sup>3</sup> , % of DM	11.8	39.8	3.58	<0.01

508 <sup>1</sup>Particle size was measured using the Tyler Ro-Tap (W. S. Tyler, Mentor, OH).

509 <sup>2</sup>Physical effectiveness factor: determined as the proportion of fiber retained by the sieve with 1.18-  
 510 mm pore size.

511 <sup>3</sup>Physically effective NDF: measured as the NDF content of the forages (DM basis) multiply by the  
 512 physical effective factor.

513 **Table 3.** Intake characteristics of heifers (daily average) fed for ad libitum intake with diet as pellet  
514 (P) or total mixed ration (TMR); the diets were formulated to be similar in chemical composition  
515 but different in physical form (evaluated as physical effectiveness factor and physically effective  
516 NDF)

Item	Treatment		SEM	P-value
	P	TMR		
<b>DMI</b>				
3h post feeding, kg/d	2.70	3.25	0.439	<0.01
24h post feeding, kg/d	10.80	8.40	0.451	<0.01
% of BW	2.88	2.23	0.100	<0.01
<b>aNDFom<sup>1</sup> intake</b>				
kg/d	6.34	5.03	0.267	<0.01
% of BW	1.69	1.34	0.059	<0.01
<b>uNDF<sub>240</sub><sup>2</sup> intake</b>				
kg /d	1.33	1.18	0.059	<0.01
% of BW	0.36	0.32	0.013	<0.01
<b>peNDF<sup>3</sup> intake</b>				
kg /d	1.21	3.45	0.118	<0.01
% of BW	0.32	0.92	0.024	<0.01
<b>Water intake</b>				
L/d	55.00	45.00	3.229	<0.01
L/kg of DMI	5.01	5.13	0.245	0.31

517 <sup>1</sup>aNDFom = amylase- and sodium sulfite-treated NDF, corrected for ash residue.

518 <sup>2</sup> uNDF<sub>240</sub> = unavailable NDF estimated via 240h in vitro fermentation.

519 <sup>3</sup>peNDF= physically effective NDF, computed as the NDF content of the forages (DM basis)  
520 multiplied by the physical effectiveness factor.

521 **Table 4.** Rumination time and rumen condition of heifers (daily average) fed for ad libitum intake  
 522 with diet as pellet (P) or total mixed ration (TMR); the diets were formulated to be similar in  
 523 chemical composition but different in physical form (evaluated as physical effectiveness factor and  
 524 physically effective NDF)

	Treatment		SEM	<i>P</i> -value
	P	TMR		
<b>Rumination</b>				
Time, min/d	241.00	507.00	17.20	<0.01
Time/DMI per d, min/kg	23.30	58.50	1.86	<0.01
Time/NDF intake per d, min/kg	41.00	94.00	1.56	<0.01
Time/forage NDF intake per d, min/kg	23.00	58.50	0.96	<0.01
<b>Rumen condition<sup>1</sup></b>				
Mean rumen pH	6.10	6.11	0.07	0.79
Mean rumen Temperature, °C	38.87	38.84	0.07	0.34
Time below pH 5.8, min/d	188.00	176.00	124.90	0.33
Time below pH 5.5, min/d	3.40	4.60	5.58	0.67
Area below pH 5.8, minxpH units/d	24.40	22.80	15.60	0.51
Area below pH 5.5, minxpH units/d	0.21	0.31	0.34	0.59

525 <sup>1</sup> pH values evaluated as described by Kleen et al., (2003)

526 **Table 5.** Fecal composition and fiber digestibility of heifers fed for ad libitum intake with diet as  
 527 pellet (P) or total mixed ration (TMR); the diets were formulated to be similar in chemical  
 528 composition but different in physical form (evaluated as physical effectiveness factor and  
 529 physically effective NDF)

Item	Treatment			P-value
	P	TMR	SEM	
Chemical composition <sup>1</sup> , % of DM				
aNDFom	69.59	69.21	0.397	0.26
ADF	57.13	54.94	0.454	0.24
ADL	26.82	27.88	0.707	0.26
uNDF <sub>240</sub>	47.38	52.12	0.748	<0.01
pdNDF	22.18	17.14	0.817	<0.01
NDF digestibility, % of aNDFom				
IVNDFD 24h <sup>2</sup>	11.41	10.70	0.724	0.51
IVNDFD 240h <sup>2</sup>	31.82	24.72	1.128	<0.01
TTdpdNDF <sup>3</sup> , % of pdNDF	86.82	90.25	0.652	<0.01

530 <sup>1</sup>aNDFom = amylase- and sodium sulfite-treated NDF, corrected for ash residue, uNDF<sub>240</sub>=  
 531 unavailable NDF estimated via 240h in vitro fermentation, pdNDF= potentially digestible NDF.

532 <sup>2</sup>IVNDFD = in vitro NDF digestibility.

533 <sup>3</sup>TTdpdNDF = total tract digestibility of the potentially digestible NDF.