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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,¹ A. Palmonari, L. Mammi, G. Canestrari, M. Fustini, A. Formigoni.**

20
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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 ± 30 d, BW 346 ± 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).

107 RuminAct[®], SCR Heatime, Israel).
108

109 Reticulorumen pH and temperature values were monitored continuously via an indwelling
110 pH and temperature sensor (SmaXtech[®], 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[®]; 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 ± 1.7 kg/d. DIM: 251 ± 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 O₂-free CO₂. 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 CO₂
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 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_{240h} 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_{240h} 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 uNDF₂₄₀ 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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332

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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 ¹ | 58.8 | 60.2 | 0.66 |
| ADF | 40.7 | 41.4 | 0.80 |
| ADL | 8.1 | 8.4 | 0.42 |
| IVNDFD 24h ² | 45.3 | 46.2 | 1.50 |
| IVNDFD 240h ² | 78.4 | 77.3 | 0.73 |
| uNDF ₂₄₀ ³ | 12.4 | 14.1 | 0.61 |
| Starch | 15.7 | 15.6 | 1.07 |

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

501 ²IVNDFD = in vitro NDF digestibility.

502 ³uNDF₂₄₀ = 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, % ¹ | | | | |
| 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 ² | 20.1 | 66.1 | 5.90 | <0.01 |
| Physically effective NDF ³ , % of DM | 11.8 | 39.8 | 3.58 | <0.01 |

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

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

511 ³Physically 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 | | |
| DMI | | | | |
| 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 |
| aNDFom¹ intake | | | | |
| kg/d | 6.34 | 5.03 | 0.267 | <0.01 |
| % of BW | 1.69 | 1.34 | 0.059 | <0.01 |
| uNDF₂₄₀² intake | | | | |
| kg /d | 1.33 | 1.18 | 0.059 | <0.01 |
| % of BW | 0.36 | 0.32 | 0.013 | <0.01 |
| peNDF³ intake | | | | |
| kg /d | 1.21 | 3.45 | 0.118 | <0.01 |
| % of BW | 0.32 | 0.92 | 0.024 | <0.01 |
| Water intake | | | | |
| L/d | 55.00 | 45.00 | 3.229 | <0.01 |
| L/kg of DMI | 5.01 | 5.13 | 0.245 | 0.31 |

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

518 ² uNDF₂₄₀ = unavailable NDF estimated via 240h in vitro fermentation.

519 ³peNDF= 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 | | |
| Rumination | | | | |
| 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 |
| Rumen condition¹ | | | | |
| 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 ¹ 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 ¹ , % 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 ₂₄₀ | 47.38 | 52.12 | 0.748 | <0.01 |
| pdNDF | 22.18 | 17.14 | 0.817 | <0.01 |
| NDF digestibility, % of aNDFom | | | | |
| IVNDFD 24h ² | 11.41 | 10.70 | 0.724 | 0.51 |
| IVNDFD 240h ² | 31.82 | 24.72 | 1.128 | <0.01 |
| TTdpdNDF ³ , % of pdNDF | 86.82 | 90.25 | 0.652 | <0.01 |

530 ¹aNDFom = amylase- and sodium sulfite-treated NDF, corrected for ash residue, uNDF₂₄₀=
 531 unavailable NDF estimated via 240h in vitro fermentation, pdNDF= potentially digestible NDF.

532 ²IVNDFD = in vitro NDF digestibility.

533 ³TTdpdNDF = total tract digestibility of the potentially digestible NDF.