Feeding method, feed sorting, digestibility

Complete feed versus concentrate and straw fed separately: effect of feeding method on eating and sorting behavior, rumen acidosis, and digestibility in crossbred Angus bulls fed high-concentrate diets

Sandra Genís[123], Marçal Verdú[1], Jordi Cucurull[1], Maria Devant[2*]

[1]bonÀrea Agrupa, 25210 Guissona, Spain

[2]Ruminant production, IRTA, Torre Marimon, 08140, Caldes de Montbui, Spain

[3] Global Biologics Research, VMRD, Zoetis Manufacturing & Research Spain S.L., 17813, La Vall de Bianya, Spain

[*]Maria.devant@irta.cat

ABSTRACT

The aim of this study was to evaluate the effect of feeding method on eating and sorting behavior, rumen acidosis, and apparent total tract digestibility of crossbred Angus bulls fed a high-concentrate diet. Twenty-one Angus beef bulls (497 ± 7.7 kg of initial BW, and 324 ± 3.0 d of age) were housed individually and fed *ad libitum*. Three experimental treatments were tested: complete feed of pellet and chopped straw in a single feeder (TMR), pellet and chopped straw fed separately in two feeders (SS); pellet and long unprocessed straw fed separately in two feeders (LS). Feed consumption, fecal and bloat scoring were recorded daily. Every 2 wk TMR sorting, eating behavior, general activity, social and oral behaviors, and BW were recorded. At days 14 and 35 apparent total tract digestibility during one week was measured. At days 28 and 42 rumen samples were collected via rumenocentesis to measure rumen pH and determine ruminal volatile fatty acids (VFA) concentrations. At the study end (day 57) animals at slaughterhouse liver and rumen wall color and lesions were recorded by a macroscopic inspection. No differences among treatments in total DMI, and fecal and bloat scoring were observed. The straw to concentrate ratio was smaller in the SS and LS treatments (8 to 92) than in

the TMR (15 to 85), and sorting analyses indicated that TMR bulls refused large particles (> 4 mm) and small particles (< 1.5 mm). TMR bulls spent less time eating ($P < 0.01$) and tended to perform more self-grooming ($P = 0.06$), oral non-nutritive behaviors ($P < 0.01$) and stereotypes ($P < 0.01$) than bulls fed straw separately. Animals fed TMR had a greater ($P < 0.01$) pH than SS and LS, however rumen pH was above 5.6 in all treatments and rumen wall lesions did not differ among treatments. Feeding TMR increased ($P < 0.05$) the rumen acetate to propionate ratio. Bulls fed LS had greater total apparent DM ($P < 0.05$) and CP digestibility ($P < 0.01$), but no differences among treatments were observed in starch digestibility. In conclusion, even if straw to concentrate ratio and NDF intake was smaller in the SS and LS treatments than in the TMR, feeding pellet and straw separately, independently of straw length, did not predispose animals to suffer rumen acidosis as indicated by rumen pH, feed consumption, animal behavior, fecal and bloat scoring and rumen wall macroscopic evaluation.

Keywords*:* feeding method, ruminal acidosis, sorting behavior, total tract digestibility.

List of abbreviations

SS: pellet and chopped straw fed separately in two feeders; LS: pellet and long unprocessed straw fed separately in two feeders; TMR: total mixed ration; VFA: volatile fatty acids

1. Introduction

Feeding method in beef production varies depending on the production system. Complete feed (total mixed ration: TMR) is common in most of European countries or in North America. However, in intensively fattening cattle, from Mediterranean beef production systems, the concentrate is mainly in pellet presentation form, and long unprocessed straw are usually fed *ad libitum* in separate self-feeders (Devant et al., 2000; Mach et al., 2009).

TMR allows for the mixing of all feed ingredients together based on a prescribed amount of each ingredient to meet the specific requirements, blended thoroughly to theoretically prevent separation and selection, fed as a sole source of nutrients formulated in a desired proportion (NRC, 2001). Ruminants require sufficient fiber and an adequate particle length to maintain proper rumen function (Allen, 1997; Zebeli et al., 2012; Devant et al., 2016; Beauchemin, 2018). Therefore, hypothetically one practical feeding management to avoid rumen acidosis is feeding TMR compared with feeding concentrate and forage separately; TMR vs. a free choice feeding method theoretically encourages straw consumption (sufficient fiber) as it is mixed, complicating the selection of dietary ingredients and reducing the risk of rumen acidosis. To facilitate a good ingredient mixture the forage of the TMR is usually chopped. However, TMR fed animals in contrary to expected may select and, consequently, consume feeds or ingredients with particle sizes that are different from the offered ones (Coon et al., 2019); this may affect their rumen health or increase their risk of suffering rumen acidosis .

In growing beef animals, when comparing TMR vs. concentrate and straw fed separately, Iraira et al., (2012) observed in animals fed TMR as expected that forage intake and rumination time per kg of DMI increased ~~and~~, while total DMI and eating rate decreased. These effects may lead to beneficial effects on rumen health, reducing the risk of ruminal acidosis; however, in the cited study rumen pH was not recorded. Moreover, in this previous mentioned study, straw was chopped and only particles above 8 mm were used to feed animals; this small particle size of the straw in the free choice treatment is not a common commercial practice, where straw is usually fed unprocessed. It is known that forage particle size affects eating (mastication) and rumination time, consequently affecting salivary secretion (Allen, 1997), that is one of the principal factors that regulates ruminal pH (Bailey and Balch, 1961).

77 One of the novelties of the present study is that the TMR is a complete feed resulting
78 from the mixture of chopped straw and concentrate in pellet presentation form. Pellet is
79 not a common concentrate presentation form used in a TMR. One of the main concerns
80 when feeding pellet is the potential risk of rumen acidosis as starch exposure is increased
81 (Gruyer, 1996; Castillo et al., 2006; Devant et al., 2016). Therefore, when concentrate is
82 in pellet presentation form the advantages of ensuring fiber intake in a TMR feeding
83 method should be more pronounced. Another novelty is the comparison in a free choice
84 feeding strategy between two fiber source (straw) sizes. As mentioned previously, particle
85 size of fiber source may be an easy way to control the chewing, eating and ruminating
86 time (Khan et al., 2014), and therefore rumen function.
87 Finally, there is published literature that describes the effect of feed presentation form,
88 mainly grain processing, and its impact on total tract digestibility (Theurer, 1986; Secrist
89 et al., 1995; Huntington, 1997; Devant et al., 2019). However, in beef cattle there is little
90 published literature of the effect of the feeding method (TMR or free choice) and of the
91 forage particle size when fed in a free choice system on nutrient digestibility. The possible
92 effect of this feeding method on digestibility cannot be overseen as it may have a great
93 impact not only on animal efficiency, it also may have an environmental impact by
94 altering N excretion and methane production.
95 Therefore, the objective of the present study was to evaluate the effect of feeding method:
96 mixed or in separated feeders (TMR vs. separated), and the size of straw (short or long)
97 when fed separately from concentrate on eating pattern, sorting, behavior, rumen pH and
98 health, and apparent total tract digestibility of crossbred Angus bulls fed a high-
99 concentrate diet.

100 **2. Materials and methods**

101 *2.1 Animals, housing, experimental design, and diets*

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

Twenty-one crossbred Angus beef bulls (497 ± 7.7 kg of initial BW, and 324 ± 3.0 d of age) were kept in individual slatted pens (1.9 x 3.4 m) at the experimental station of the Corporación Alimentaria Guissona, S.A. - bonÀrea Agrupa (bonÀrea Agrupa, Guissona, Lleida, Spain), and randomly assigned to 1 of 3 treatments in a complete randomized experimental design. The 3 treatments consisted of: complete feed of pelleted concentrate and chopped straw in a single feeder (TMR), pellet and chopped straw fed separately in two feeders (SS, chopped with a particle length of 5 cm); pellet and long straw fed separately in two feeders (LS, long unprocessed straw with a particle length from 15 to 20 cm). All treatments were fed *ad libitum* with the same concentrate (nutritional formulation) and straw source throughout 57 days of study. The concentrate to total intake ratio in the TMR treatment was 0.85 based on previous studies (Mach et al., 2009; Marti et al. 2011, Devant et al., 2015), and expecting that with age the straw intake would increase decreasing the concentrate to total intake ratio. The concentrate presentation was a concentrate pellet form and was formulated according to (FEDNA, 2008) recommendations (Table 1). Before pelleting, all ingredients of the concentrate were ground, through a hammer mill with screen openings of 2.75 mm. The pellet was manufactured as described by Verdú et al. (2017) and had a uniform diameter (3.5 mm) and length (70 mm). Before starting the experimental period, all animals were fed free-choice diet with concentrate and long straw *ad libitum* in separate feeders. In SS and LS treatments straw and concentrate were fed in two separate troughs (0.6 × 1.2 × 0.3 m),

and the TMR in a single trough (0.6 × 1.2 × 0.3 m) The pens were also equipped with a water bowl drinker.

2.2. Feed consumption

Feed offers (concentrate and straw, or TMR) were recorded daily at 0900 h, and feed orts were also registered daily at 0800 h. Animal BW was recorded fortnightly.

2.3. Pellet quality, TMR and straw particle size

Pellet quality was performed as described by Verdú *et al*., 2016. To summarize, 500g sample was collected and hardness, density and percentage of fines were determined. The percentage of fines content was analyzed using a sieve with 2.5 mm pore sizes. A total of 300 g of concentrate were placed on a 2.5 mm sieve and shaken for 30 s. The acceptable reference value of fines at the silo by feed manufacturer was below 10% of particles < 2.5 mm. The pellet hardness (kg) was determined using a Kahl device, which measures the compression force required to fragment a pellet into smaller particles and fines. To determine pellet hardness, the uniform feed pellets were chosen, prior to hardness analysis, by visually measuring their length and diameters. Hardness was expressed as an average of 10 measurements. Density (kg/m^3) of the concentrate was estimated by weighing the feed necessary to fill a test tube of 100 cm^3 striking off level with the top surface.

To analyze TMR and straw particle size a 1,500 g sample for each dietary treatment (free-choice, either chopped and whole straw, and mixed-ration) were collected every 2 wk to assess the forage particle size using the 3-screen (19, 8, and 1.18 mm) Penn State Particle Separator (Kononoff and Heinrichs, 2005), which separates particles into 4 fractions: long (>19 mm), medium (<19 and >8 mm), short (<8 and >1.18 mm), and fine (<1.18 mm) particles.

2.4 Sorting of TMR

At day 2, 6, 17, 20, 34, 35, 44, and 49 of study samples of TMR, from feed offer and refusal, were collected to analyze feed sorting through particle size distribution of feed offer and orts (Leonardi and Armentano, 2003). To determine particle size distributions of TMR, samples were placed on a series of stacked sieves (sizes 0.5, 1, 1.7, 2.5, 3.35, and 4 mm; CISA, Barcelona, Spain) contained in a CISA RP 200N sieve shaker (CISA, Barcelona, Spain) and were sieved for 3 min at 1.9-mm amplitude. Particles retained on each sieve were then weighed to determine their proportion of total sample.

2.5 Fecal and bloat scoring.

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

2.6 Rumen pH and volatile fatty acids.

Rumenocentesis was conducted in two identical sampling periods at d 28 and d 42 of the study between 1 and 2 hours after morning feed offer. Rumenocentesis was conducted with a 14 cm 14-gauge needle inserted into the ventral sac of the rumen approximately 15 to 20 cm caudal and ventral to the costocondral junction of the last rib. After sampling,

177 rumen fluid pH was measured immediately with a portable pHmeter (model 507, Crisson
178 Instruments SA, Barcelona, Spain). Besides, 4 mL of ruminal fluid were mixed with 1
179 mL of a solution containing 0.2% (wt/wt) mercuric chloride, 2% (wt/wt) orthophosphoric
180 acid, and 4-methylvaleric acid (internal standard) in distilled water based on (Jouany,
181 1982) and stored at -20°C until subsequent VFA analysis.

182 *2.7 Eating and animal behavior*

183 Each pen was filmed for 24 h every 14 d throughout the study using digital cameras
184 (model CSM-UTM824, Casmar S. A., Barcelona, Spain) installed approximately 3 m
185 above the ground. Each camera filmed simultaneously 2 pens. Videotapes were processed
186 by continuous recording of the activities performed by animals at feeding area and
187 drinker. Time (min) devoted to eating concentrate or forage (when an animal had its head
188 into the feeder and was engaged in chewing), and drinking (when animal had its mouth
189 in the water bowl) was recorded. Unfortunately, only data from 0700 to 2100 were
190 analyzed as image quality outside this frame time was not good, and other activities like
191 rumination time could not be recorded with the quality that would guarantee that all
192 animals were observed under the same conditions.

193 A scan sampling procedure was used to analyze the general activity (standing, lying,
194 eating concentrate or forage, drinking, and ruminating), and oral and social behaviors
195 (self-grooming, social, oral non-nutritive, flehmen, stereotypies-tongue-rolling and
196 scratching) for each pen. Records correspond to total count of each activity in a pen
197 (Mounier et al., 2005). Animal behavior (selfgrooming, social, oral non-nutritive, eating,
198 drinking, ruminating, lying, standing, scratching) was recorded every 14 d throughout the
199 study from 0830 to 1100 h by scan sampling as described previously by (Mach et al.,
200 2008) and (Marti et al., 2010). The scan sampling method describes a behavior exhibited
201 by an animal at a fixed time interval (Colgan, 1978). Between four and eight pens were

observed at the same time. Pens were scored during 2 continuous sampling periods of 15 min; general activities were scored using 2 scan samplings of 10 s at 5 min intervals (Mach et al., 2008). This recording procedure (15 min) was repeated twice during the morning.

2.8 Apparent total tract digestibility

A diet digestibility trial was conducted daily during the third (from d 14 to 21) and sixth wk (from d 35 to 42) of the study. These two identical sampling periods were performed in order to reduce the animal variability. Therefore, the sampling periods were considered as blocks in statistical analysis. All feeds (concentrate and mixed ration) was thoroughly mixed with chromium oxide (1 g / kg DM), and were offered for *ad libitum* consumption to each animal. During these days an offer feed sample (concentrate, and mixed ration), and refusals from each animal were collected. Fecal grab samples were collected the last 3 d throughout the wk and dried at 103°C for 48h and composited by animal (Titgemeyer, 1997).

2.9 Rumen macroscopic and liver macroscopic evaluation

On day 57 of the study, bulls were transported to a commercial slaughterhouse (La Closa, bonÀrea Agrupa, Guissona, Spain) by truck. Transport distance was less than 1 km. The entire ruminal epithelium was examined and presence of clumped papillae (Nocek et al., 1984), ulcers, hair presence, and parakeratosis (presence and location) was recorded. Also, rumens were classified from 1 to 5 depending on the color, with "5" indicative of a black colored color rumen and "1" a white colored rumen (González et al., 2001).

Liver abscesses were graded following (Brown et al., 1975) who described a scoring system to assess the incidence of liver abscesses (0 = no abscess, A- = one or two small abscesses or inactive scars, A = one or two large abscesses or several small abscesses, and A+ = multiple large abscesses often involving collateral tissue).

227 *2.10 Chemical Analyses*

228 Feed samples of each dietary treatment were collected every 2 wk for determination of
229 nutrient composition. Samples were analyzed for DM (24h at 103°C; method number
230 925.04; AOAC, 1995), ash (4h at 550°C; method number 642.05; AOAC, 1995), CP by
231 the Kjeldahl method (method number 988.05; AOAC, 1995), NDF according to (Van
232 Soest et al., 1991) using sodium sulfite and alpha-amylase, and fat using a Soxhlet
233 apparatus after an acid hydrolysis preparation (method number 942.05 AOAC, 1995).
234 Total starch content was analyzed using the polarimetric method according to the EU
235 Regulation for feed analyses (n° 152/2009). Chromium concentration of feed and fecal
236 samples were determined based to the procedure of Le Du and Penning (1982). Digestion
237 were carried out on duplicates weighing 0.5 g of sample. Two digestion steps were made.
238 First digestion step was performed with 4 mL HNO_3 concentrated at 220° during 15 min,
239 in a microwave oven (Ultrawave model, Milestone, Sorisole, Italy); uncolored solutions
240 were obtained with a green solid at the bottom of the digestion tube. That solid is
241 attributed to Cr_2O_3(s). In the second step, 3 mL of H_2SO_4, 0.5 mL of $HClO_4$ and 2 mL of
242 hydrofluoric acid at the same digestion tube were added and new digestion procedure was
243 made at 260°C during 15 min. Finally, the Cr content was determined by inductively
244 coupled plasma optical emission spectrometry (model Optima 4300D, Perking-Elmer,
245 Shelton, CT, USA). Rumen volatile fatty acids (VFA) concentration was analyzed with a
246 semicapillary column (15 m by 0.53 mm i.d. and 0.5 µm film thickness; TRB-FFAP;
247 Teknokorma, Barcelona, Spain) composed by 100% polyethylene glycol esterified with
248 nitroterephtalic acid, using a CP-3800 Gas Chromotograph (Varian Inc., Walnut Creek,
249 CA) based on Jounay (1982).

250 *2.11 Calculations and statistical analyses*

TMR sorting data by particle size distribution was calculated contrasting the particle size distribution between feed offer and refusal. Actual intake was expressed as a percentage of the predicted intake of particle size distribution. The actual intake of each particle size was calculated as the difference between the amount of each particle size on the offered feed and in the refusal. The predicted intake of each particle size fraction was calculated as the product of total DMI multiplied by the DM percentage of that fraction in the feed ration. Values equal to 100% indicate no sorting, <100% indicate selective refusals (sorting against), and >100% indicate preferential consumption (sorting for) according to Miller-Cushon et al., 2013. Scan samples of each general behaviors (in a total of 30 min of observation) were converted to a percentage of the total time observed (Mitlöhner et al., 2001), and lastly these percentages were transformed to the root of percentage plus 1 to achieve a normal distribution. The frequency of each social behavior indicator was obtained summing by day, pen and scan, and then were transformed into the root of the sum of each activity plus 1 to achieve a normal distribution. Data from apparent total tract digestibility were calculated estimating total fecal output, which was estimated as the ratio of chromium intake to chromium concentration in the feces.

Intake and behavior data were analyzed using a mixed-effects model with repeated measures (SAS Inst. Inc., Cary, NC). The model included initial BW as a covariate; treatment, period (7-d period or sampling day), and the interaction between treatment and period as fixed effects; and animal as random effect. Period was considered a repeated factor, and for each analyzed variable, animal nested within treatment (the error term) was subjected to 4 variance-covariance structures: compound symmetry, variance components, autoregressive order one, and heterogeneous autoregressive order one. The covariance structure that minimized Schwarz's Bayesian information criterion was considered the most desirable analysis. Rumen pH and VFA concentration as well as

276 apparent total tract digestibility data were analyzed using a mixed-effect model (SAS Inst.
277 Inc,. Cary, NC). The model included initial BW as a covariate, treatment and sampling
278 period (block) as fixed effects, and animal as random effect. Two sampling periods were
279 done to reduce animal variability; therefore, these sampling periods were considered as
280 blocks, and in the statistical model no interaction between treatment and period was
281 analyzed. Particle size sorting data of the TMR treatment were analyzed as described
282 using mixed-effects model with repeated measures (SAS Inst. Inc., Cary, NC). The model
283 for each particle size fraction included, period (sampling day), as fixed effect; and animal
284 as random effect. Period was considered a repeated factor, and for each analyzed variable,
285 animal nested within treatment (the error term) was subjected to the 4 variance-covariance
286 structures described before. Last, a Chi-square test was conducted to evaluate the effects
287 of treatment on liver and rumen wall macroscopic evaluation (categorical variables). For
288 all analyses, significance was declared at $P \leq 0.05$ and tendencies were discussed at 0.05
289 $< P \leq 0.10$.

290 **RESULTS**

291 One animal from the SS treatment was removed from the analysis due to pneumonia. All
292 data corresponding to this animal was removed prior to statistical analysis.

293 The percentage of fines (< 2.5 mm) of the pellet was less than 10% (12 ± 0.38 g/kg), the
294 average hardness was 10.9 ± 0.93 kg and the average density was 61.95 ± 1.25 kg/m^3
295 (data not shown). Seventy-five point seven per cent of TMR had a particle size between
296 3.35 and 4 mm, whereas only 7.29% had a superior size than 4 mm (Table 1).

297 *3.1 Total intake, eating and animal behavior, and TMR sorting behavior*

298 Treatments did not affect total intake (Table 2). Straw length did not affect concentrate
299 or straw intake, and the resulting straw to concentrate ratio was 0.80 to 0.92 which was
300 lesser than the predicted ratio in the TMR (0.15 to 0.85). The period effect was significant

301 for all variables, except on straw consumption where only a tendency was observed.
302 Finally, an interaction between treatment and period was observed in CV on total intake
303 ($P = 0.03$). Animals fed TMR and SS had an increased variation in consumption during
304 the last period of the trial, whereas animals fed LS showed a greater intake variation
305 during the second period. Time eating concentrate and Straw did not differ between SS
306 and LS treatments (Table 3)). Animals fed LS and SS spent significantly more time eating
307 concentrate and straw (total eating) than animals fed TMR (Table 3, $P = 0.01$). No
308 differences were observed in time drinking water among treatments.
309 Treatment did not affect the proportion of time devoted to standing, lying, eating, or
310 drinking (Table 4). Treatment did also not affect the proportion of time for rumination or
311 total chewing time (eating plus ruminating time). LS and TMR animals groomed
312 themselves more often compared with SS being the differences among treatments greater
313 as the study advanced, for example selfgrooming activites doubled in TMR and LS (1.23
314 ± 0,165 times/15 min) compared with SS (0.52 ± 0,165 times/15 min) calves (treatment
315 by day interaction; $P < 0.01$). Bulls fed LS tended ($P = 0.06$) to exhibit more social
316 behaviors than SS fed bulls. Regarding the non-nutritive behaviors, bulls fed TMR
317 showed ($P = 0.03$) more oral behaviors than SS, being LS intermediate. A treatment by
318 day interaction was observed in stereotypy ($P < 0.01$). In TMR bulls more tongue-rolling
319 behaviors were recorded at day 17 than SS or LS bulls (0.33, 0.05, and 0.05 ± 0.06
320 times/15 min, respectively; $P < 0.01$). After day 17 tongue-rolling records decreased in
321 TMR and no differences among treatments were observed.
322 There is no effect of day on particle size TMR sorting ($P > 0.10$) and the mean for sorting
323 were 99.1% for particles bigger than 4 mm, 107% for particles between 3.35 and 4 mm,
324 109.7 for particles between 2.5 and 3.35mm, 107.2% particles between 1.7 and 2.5mm,
325 99.5% for particles between 1 and 1.7mm, 87.3% for particles between 1 and 0.5mm, and

73.3% for particles smaller than 0.5mm. So, animals fed TMR sorted particles with a size between 1.7 and 4 mm (mean above 100%), while sorted against particles bigger than 4 mm, or shorter than 1.7 mm (mean below 100%).

3.2 Rumen fermentation parameters, bloat and fecal scores, and macroscopic evaluation of liver and rumen wall

Rumen fermentation parameters are presented at Table 5. Treatment affected rumen pH when collected via rumenocentesis. Animals fed TMR (pH = 6.50 ± 0.15), had a greater (P = 0.01) rumen pH than animals fed SS (pH = 5.82 ± 0.15) or LS (pH = 6.02 ± 0.15). Total rumen VFA concentration followed the inverse pattern of rumen pH. Rumen molar percentage of VFA was affected by treatment. Animals fed TMR had greater molar percentage of acetate and lesser of propionate than SS or LS fed animals, and in consequence the acetate to propionate ratio (1.80 ± 0.11) was greater (P < 0.05) in TMR bulls compared with SS or LS bulls (1.41 ± 0.11 and 1.48 ± 0.11, respectively). In addition, in TMR bulls the molar percentage of branched chain VFA (isobutyrate and isovalerate) was greater (P < 0.05) than in bulls fed SS or LS. .The rumen concentration of n-butyrate (10.1 ± 0.71 mol/100 mol), and n-valerate (2.6 ± 0.22 mol/100 mol) were greater (P < 0.05 and P = 0.08) in animals fed SS than animals fed TMR or LS. No differences among treatments were observed in fecal score which records were "1" (normal) and in the bloat score which records were "0" (no bloat). No treatment effect was observed in liver macroscopic evaluation. Treatment did not affect rumen wall macroscopic evaluation parameters presented in Table 6.

3.3 Apparent total tract digestibility

Apparent total tract digestibility of DM and CP were greater (P = 0.03 and P < 0.01, respectively) in LS bulls than in TMR and SS bulls (Table 7). Moreover, bulls fed LS tended (P = 0.10) to have greater OM digestibility compared with TMR and SS bulls. No

351 differences were observed on EE, NDF, or starch digestibility. NDF intake was greater in
352 TMR bulls compared the other treatment bulls (P = 0.01, Table 7), probably as the
353 consequence of the greater straw to concentrate ratio (0.15:0.85) compared with the SS
354 and LS treatments (0.08:0.92; Table 2). NDF in feces also tended (P = 0.09) to be greater
355 in TMR bulls compared with SS and SL bulls.

356 **4. Discussion**

357 The ratio of concentrate to total DMI in the TMR treatment was set to be 85 and, even if
358 animals performed feed selection, this ratio was close to the expected one based on NDF
359 intake (Table 7). The ratio of concentrate to total DMI when animals were in a free choice
360 situation (SS and LS) was greater than the ratio in TMR being around 90 (Table 2), and
361 moreover it was greater than expected. In previous studies with Holstein bulls younger
362 than 1 year of age at the finishing phase this ratio was 90 (Devant et al., 2016, 2015; Mach
363 et al., 2009; Marti et al., 2011). The present study started with crossbred Angus with 1
364 year of age and as animals get older the concentrate to total DMI intake tends to decrease
365 (Devant et al., 2015; Mach et al., 2009; Marti et al., 2011) therefore a reduction in this
366 ratio was expected. The greater rumen acetate to propionate ratio observed in the TMR
367 fed animals compared with the free choice fed animals is probably the consequence of a
368 greater straw and NDF intake. Furthermore, Bharanidharan et al. (2018) feeding TMR at
369 a restricted level observed a greater daily methane production compared with feeding
370 concentrate and forage separately; this could be related to the greater rumen acetate to
371 propionate ratio observed at 4.5 h post-feeding, as in the present study. The production
372 of acetate from pyruvate is accompanied by the production of H_2, whereas the production
373 of propionate utilizes H_2, which is the major substrate for methane.

374 In the present study ruminal pH was greater (Table 5) in bulls fed TMR than animals fed
375 diets with the free choice feeding system (SS and LS). This greater pH of TMR animals

376 was probably due to a greater straw (Table 2) and NDF (Table 7) intake than the other
377 two treatments. NDF intake or forage NDF intake together with medium (from 8 to 19
378 mm) particle sizes are the main factors affecting rumination time (Forbes and Provenza,
379 2000; Beauchemin, 2018) and regulating ruminal pH. However, surprisingly in the
380 present study, no differences among treatments in rumination activity were observed.
381 Perhaps, rumination time data recorded in the present study, were only a partial picture
382 of daily total rumination time, as they were recorded by scan sampling during the
383 morning; so, they need to be interpreted with caution and this may be the reason why no
384 differences among treatments were observed even there was a different forage NDF
385 intake or despite the particle size selection of TMR bulls. Furthermore, TMR animals
386 avoided small particles and this may help to modulate rumen pH. Small particles have a
387 great surface area increasing the microbes attach points and fermentability of the diet, so
388 avoiding them may have reduced the risk to suffer rumen acidosis. Moreover, Yang et al.
389 (2001) suggested that rumen pH and the extent of subclinical ruminal acidosis cannot be
390 predicted only using the physical characteristics of the diet, rumen fermentability of
391 starch may have larger effects on pH than physical characteristics of feeds. In this sense,
392 in the present study, apparent total tract starch digestibility did not differ among
393 treatments, but the lower consumption of NDF of LS and SS bulls (Table 7) may have
394 increased the rumen molar proportion of propionic acid (Table 5). Propionic acid has a
395 lower pKa compared with acetic acid, and this, together with the increased VFA
396 concentration, may have decreased rumen pH in LS and SS compared with TMR animals.
397 Nevertheless, although the pH in SS and LS was lesser, it was still greater than 5.6, a
398 value commonly considered as a threshold for ruminal acidosis diagnosis (Nagaraja and
399 Titgemeyer, 2007). Moreover, in the present study no clinical signs were observed and
400 neither severe lesions on the ruminal epithelium, and liver, both indicators of ruminal

401 acidosis (Snyder and Credille, 2017) were detected. Often, we focus on rumination time
402 when analyzing factors that affect rumen pH and forget that chewing time is composed
403 by rumination and eating time, and the total chewing time is important for salivation, diet
404 particle size reduction and digestion. When analyzing the total eating time, when animals
405 were fed straw separately from the concentrate total eating time was 24% greater than the
406 eating time of the TMR animals (Table 3), this may explain why rumen pH was not below
407 5.6 despite the low NDF intake.
408 LS bulls tended to present more concentrate intake daily variation and less time eating
409 concentrate (Table 2 and 3). This fluctuance in concentrate consumption has been already
410 observed in animals offered a choice feeding, while animals fed TMR like in the present
411 study have a more constant eating pattern among days (Atwood et al., 2001).
412 Unexpectedly, animals fed TMR conducted more oral behaviors than SS and SL animals,
413 these behaviors are indicative of poor welfare (oral non-nutritive and stereotypes). As
414 discussed in Devant et al. (2016), Bergeron et al. (2006) reviewed stereotypic or abnormal
415 oral behavior in captive ungulates and described in cattle 2 abnormal behaviors: tongue
416 rolling (designated as stereotypy in the present study) and object-licking (designated as
417 non-nutritive oral behaviors herein). These authors indicated that ruminants restrictively-
418 fed low-fiber diets display these abnormal behaviors. There are 3 hypotheses (Bergeron
419 et al., 2006) that could explain the origin of oral stereotypies in ungulates: a) a deficiency
420 of some nutrient (fiber) for which cattle are inherently motivated to obtain; b) insufficient
421 time devoted to chewing and ruminating leaving animals with unfulfilled motivations;
422 and c) a consequence of gut dysfunction (such as rumen acidosis). In the present study,
423 the behaviors that are indicative of poor welfare were observed in TMR fed bulls, which
424 had increased straw and NDF intake compared with free choice feeding systems, similar
425 results were observed by Iraira et al. (2012). However, in the present study in SS and LS

bulls despite that rumen pH and NDF intake were lesser, these bulls devoted 24% more the time to eat, and this may have had a positive effect reducing abnormal behaviors.

One of the most unexpected results were the effects of feeding method on apparent total tract protein digestibility, which was greater in LS than in SS and TMR treatments. Bharanidharan et al. (2018) when feeding steers chopped forage and concentrate mixed or separately, in disagreement to the present study, did not observe an effect of the feeding method on total tract digestibility of crude protein. However, this cited study did not evaluate unprocessed long straw. One hypothesis of why in the present study there was an increase of CP digestibility in LS could be due to an improvement in starch digestibility. The increased availability of energy in the rumen facilitates the degradation of proteins into peptides and aminoacids, this finally being converted into microbial protein (Bach et al., 2005). However, starch digestion in pelleted concentrate was high, over 95%, (Table 7), and no improvements among treatments were observed. Another hypothesis could be that the increase of total protein digestibility could be explained by differences in rumen passage rate; greater straw particles may have enhanced the retention time of feed in the rumen and in consequence the time that the microbes have to access to protein sources and digest them may be increased (Pino et al., 2018). However, this hypothesis cannot be contrasted in the present study as passage rate has not been measured. One consequence of this improvement in protein digestibility could be the lesser N excretion in feces and, therefore, in the environment. Thus, the advantage of feeding unprocessed straw separately from concentrate in the N excretion should be further studied as reduction of N excretion is priority to be implemented to improve animal production or livestock sustainability.

5. Conclusions

To conclude, mixing chopped straw with a pellet concentrate increases rumen pH compared with feeding straw and concentrate in separate feeders; however, animals fed in this straw free choice feeding method do not show signs of rumen acidosis. Straw size (unprocessed vs. chopped) rather than feeding method (TMR vs. forage and concentrate separately) has shown an impact on the apparent total tract protein digestion.

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611 **Table 1:** Ingredient, nutrient composition, and particle size distribution (Penn State and

612 granulometry) of the dietary treatments.

			Straw	
Items	TMR	Concentrate[1]	Chopped straw	Long unprocessed straw
Ingredients, g/ kg				
Corn	300.5	353.7		
Barley	159.2	187.1		
Chopped barley straw	149.9	-		
Corn gluten feed	127.3	149.9		
Wheat middlings	71.3	83.9		
Corn flour	68.4	80.4		
Beet pulp	50.1	58.9		
Palm oil	27.3	32		
Soybean meal 47 % CP	23.3	27.5		
Calcium soaps	10.7	12.5		
Sodium bicarbonate	5.1	6.0		
Urea	3.6	4.2		
Salt	1.7	2.0		
Vitamin-mineral premix[2]	1.7	2.0		
Nutrients, g/ kg DM				
DM	875	871	904	903
Starch	399	469	-	-
CP	129	145	44	45
Ether extract	54	60	10	15
NDF	286	196	695	655
Penn State Particle Separator sieving retained on the sieve, g/ kg				
> 19 mm	1.3		201.1	713.0
< 19 mm to > 8 mm	39.4		212.3	83.3
< 8 mm to >1.18 mm	946.1		536.3	74.1
< 1.18 mm	0		0	0
Dry sieving, retained on sieve, g/ kg				
4 mm	72.9			
3.35, mm	756.7			
2.5 mm	12.2			
1.7 mm	34.2			
1 mm	43.2			
0.5 mm	42.1			
< 0.5 mm	31.7			

613 [1] Concentrate had a pellet size of 3.5 mm diameter and 70 mm length

614 [2] Nucleous for finisher concentrate (CAG, Guissona, Spain): vitamin and mineral

615 contained per kg of DM: 3,575.8 kIU of vitamin A, 858.6 kIU of vitamin D_3, 101 g of

616 vitamin E, 2.3 g of vitamin B_1, 0.2 g of Co, 2.5 g of Cu, 0.26 g of I, 15.7 g of Mn, 0.15

617 g of Se, 20.6 g of Zn, 7.2 g of Fe, 75.8 g of etoxiquine, and 1 kg of barley as excipient.

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Table 2. Intake and initial and final BW of crossbred Angus bulls fed high-concentrate diets as a complete feed system (TMR) or fed concentrate and straw (chopped o long unprocessed) separately

	Treatment[1]				*P*-value[2]		
	TMR	SS	LS	SEM	Treatment	Period	Treatment x Period
Number of animals	7	6	7				
Initial age, d	323	324	324	3.0	0.94	-	-
Initial BW, kg	498	496	499	7.7	0.96	-	-
Final BW, kg	567	561	562	6.5	0.76	-	-
DMI, kg/d							
Concentrate[3]	-	10.1	10.2	0.51	0.16	< 0.001	0.95
Straw[3],	-	0.90	0.81	0.121	0.57	0.07	0.15
Total DMI	11.3	11.0	11.0	0.58	0.92	< 0.001	0.98
Concentrate to total DMI ratio, (kg/kg) [3]	-	0.92	0.93	0.010	0.52	0.41	0.12
Coefficient of variation, %							
Concentrate	11.4	11.8	16.4	1.61	0.06	0.056	0.06
Straw[3]	.	54.7	68.5	12.81	0.43	< 0.001	0.40
Total	11.4	13.8	15.7	1.62	0.14	< 0.001	0.03

[1] Treatments: complete feed of pelleted concentrate and chopped straw (TMR); pellet and chopped straw fed separately in two feeders (SS); pellet and long unprocessed straw fed separately in two feeders (LS); all treatments fed ad libitum.

[2] Treatment effect; Period effect; Interaction between treatment and period

[3] Only SS and LS treatments were compared.

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637 **Table 3.** Eating time (min) from 0700 to 2100 of crossbred Angus bulls fed high-
638 concentrate diets as a complete feed system (TMR) or fed concentrate and straw (chopped
639 o long unprocessed) separately.

	Treatment[1]				*P*-value[2]		
	TMR	SS	LS	SEM	Treatment	Day	Treatment x
Number of animals	7	6	7				
Concentrate	-	38.9a	33.5b	4.47	0.53	0.08	0.15
Straw	-	33.2	36.6	5.30	0.61	0.73	0.70
Total	54.4b	72.1a	70.1a	4.29	0.01	0.05	0.07
Drinking	18.2	16.1	16.9	4.79	0.96	0.35	0.68

640 [ab] Rows with different superscripts differ ($P < 0.05$).

641 [1] Treatments: complete feed of pelleted concentrate and chopped straw (TMR); pellet
642 and chopped straw fed separately in two feeders (SS); pellet and long unprocessed straw
643 fed separately in two feeders (LS); all treatments fed ad libitum.

644 [2] Treatment effect; Day effect; Interaction between treatment and day
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Table 4. General behavior (percentage of the activity during the scan sampling of 30 min) and social behavior (number of occurrences/15 min) performed from 830 to 1100 h of crossbred Angus bulls fed high-concentrate diets as a complete feed system (TMR) or fed concentrate and straw (chopped o long unprocessed) separately.

	Treatment[1]				*P* – value[2]		
	TMR	SS	LS	SEM	Treatment	Day	Treatment x Day
Number of animals	7	6	7				
General behavior, %							
Standing	33.9	30.9	34.5	9.69	0.92	0.05	0.46
Lying	54.7	51.2	49.4	6.37	0.72	0.03	0.51
Eating	5.95	8.93	7.14	2.62	0.72	0.72	0.13
Drinking	0.60	1.79	1.79	1.26	0.58	0.66	0.88
Ruminating	5.95	7.14	6.55	3.30	0.97	0.24	0.14
Chewing	11.9	16.1	13.7	3.69	0.72	0.61	0.15
Social behavior, number of occurrences/15 min							
Self-grooming	0.61	0.38	0.40	0.131	0.30	< 0.001	< 0.001
Social	0.18	0.12	0.33	0.072	0.06	0.58	0.44
Oral	0.40a	0.19b	0.30ab	0.070	0.03	0.00	0.58
Butting	0.02	0.01	0.01	0.010	0.67	0.03	0.78
Stereotypy	0.13	0.04	0.08	0.041	0.19	0.11	0.01
Scratching	0.07	0.11	0.08	0.050	0.62	0.01	0.69

[ab] Rows with different superscripts differ ($P < 0.05$).

[1] Treatments: complete feed of pelleted concentrate and chopped straw (TMR); pellet and chopped straw fed separately in two feeders (SS); pellet and long unprocessed straw fed separately in two feeders (LS); all treatments fed ad libitum.

[2] Treatment effect; Day effect; Interaction between treatment and day

669 **Table 5.** Rumen fermentation parameters of crossbred Angus bulls fed high-concentrate
670 diets as a complete feed system (TMR) or fed concentrate and straw (chopped o long
671 unprocessed) separately.

	Treatment[1]				*P* – value[2]	
	TMR	SS	LS	SEM	Treatment	Day
Number of animals	7	6	7			
pH	6.50[a]	5.82[b]	6.01[b]	0.150	0.01	0.61
Total VFA[3], m*M*	106[b]	130[a]	130 [a]	8.4	0.03	0.16
Individual VFA[3], mol/100 mol						
Acetate	55.3[a]	48.8[b]	51.5[b]	1.49	0.03	0.49
Propionate	32.4[b]	37.0[a]	36.6[a]	1.45	0.03	0.33
Isobutyrate	0.81[a]	0.53[b]	0.61[b]	0.071	0.02	0.15
n-Butyrate	7.82[b]	10.08[a]	7.85[b]	0.710	0.01	0.43
Isovalerate	1.54[a]	0.85[b]	1.04[b]	0.141	0.01	0.29
n-Valerate	1.96[b]	2.61[a]	2.33[ab]	0.225	0.08	0.13
Acetate:propionate, mol/mol	1.80[a]	1.41[b]	1.48[b]	0.110	0.05	0.29

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

673 [1] Treatments: complete feed of pelleted concentrate and chopped straw (TMR); pellet
674 and chopped straw fed separately in two feeders (SS); pellet and long unprocessed straw
675 fed separately in two feeders (LS); all treatments fed ad libitum.

676 [2] Treatment effect; Day effect; Interaction between treatment and day
677 [3] VFA: volatile fatty acids
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679 **Table 6.** Rumen macroscopic evaluation of the rumen of crossbred Angus bulls fed high-
680 concentrate diets as a complete feed system (TMR) or fed concentrate and straw (chopped
681 o long unprocessed) separately.

	Treatment[1]			*P* – value[2]
	TMR	SS	LS	Treatment
Number of animals	7	6	7	
Color, %				
1	0.0	0.	0.0	0.41
2	14.3	0.0	0.0	
3	14.3	16. 7	14.3	
4	57.1	83.3	57.1	
5	14.3	0.00	28.6	
Color homogeneity, %				0.81
Homogenous	28.6	33.3	42.9	
Heterogenous	71.4	66. 7	57.1	
Papillae fusion, %				0.81
no	42.9	33.3	42.9	
yes	57.1	66.7	57.1	
Presence of hairs, %				
no	57.1	83.3	71.4	0.81
yes	42.9	16.7	28.6	
Baldness, %				
no	85.7	50.0	42.9	0.48
yes	14.3	50.0	57.1	
Papillae length, %				
normal	85.7	83.3	57.1	0.29
long	14.3	0.0	28.6	
short	0.0	16. 7	14.3	

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683 [1] Treatments: complete feed of pelleted concentrate and chopped straw (TMR); pellet
684 and chopped straw fed separately in two feeders (SS); pellet and long unprocessed straw
685 fed separately in two feeders (LS); all treatments fed ad libitum.

686 [2] Treatment effect
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691 **Table 7.** Nutrient intake, feces nutrient excretion, and apparent total tract digestibility of

692 crossbred Angus bulls fed high-concentrate diets as a complete feed system (TMR) or fed

693 concentrate and straw (chopped o long unprocessed) separately.

	Treatment[1]				*P* value[2]	
	TMR	SS	LS	SEM	Treatment	Day
Number of animals	7	6	7			
Intake, kg/d						
DM	10.36	10.08	9.82	0.611	0.82	0.89
CP	1.13	1.28	1.24	0.080	0.32	0.34
Ether extract	0.53	0.58	0.57	0.041	0.63	0.22
OM	9.79	8.83	8.56	0.542	0.25	0.73
NDF	2.76[a]	2.13[b]	2.08[b]	0.141	0.01	0.25
Starch	4.53	4.62	4.48	0.283	0.94	0.37
Feces, kg/d						
DM	2.51	2.32	2. 10	0.181	0.15	0.20
CP	0.37	0.38	0.31	0.029	0.14	0.15
Ether extract	0.17	0.21	0.19	0.028	0.63	0.21
OM	2.21	1.91	2.38	0.174	0.17	0.15
NDF	1.54	1.28	1.15	0.121	0.09	0.06
Starch	0.09	0.11	0.07	0.016	0.28	0.20
Apparent total tract digestibility, g/ kg						
DM	742[b]	754[b]	790[a]	12.1	0.03	0.14
CP	670[b]	705[b]	758[a]	16.0	<0.001	0.11
Ether extract	688	638	675	43.9	0.69	0.38
OM	757	749	787	12.3	0.10	0.09
NDF	442	401	476	36.3	0.35	0.11
Starch	981	977	987	03.5	0.22	0.23

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

695 [1] Treatments: complete feed of pelleted concentrate and chopped straw (TMR); pellet

696 and chopped straw fed separately in two feeders (SS); pellet and long unprocessed straw

697 fed separately in two feeders (LS); all treatments fed ad libitum.

698 [2] Treatment effect; Day effect

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