Effect of increasing the level of alfalfa hay in finishing beef heifer diets on intake, sorting, and feeding behavior[1]

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ABSTRACT: Eight rumen cannulated Simmental heifers (BW = 281.4 ± 7.28 kg) were randomly assigned to one of four experimental treatments in a 4 × 4 replicated Latin square design to ascertain the effects of increasing levels of alfalfa hay on intake, sorting, and feeding behavior in comparison to barley straw as forage source. Treatments tested were four total mixed rations with: 1) 10% barley straw **(10BS)** with 7.0% NDF from forage, 2) 13% alfalfa hay (**13AH**) and less NDF from forage (5.7%) than 10BS, 3) 16% alfalfa hay (**16AH**) and the same NDF from forage (7.0%) as 10BS, and 4) 19% alfalfa hay (**19AH**) and more NDF from forage (8.3%) than 10BS. Each experimental period consisted of 3 wk for adaptation and 1 wk for sampling. Increasing the proportion of alfalfa hay in the diet linearly increased ($P < 0.05$) total DMI, CP intake, water consumption, intake of long, medium and fine particle size, extent of sorting of fine particle size, and time spent rumination, but linearly decreased ($P < 0.05$) extent of sorting of short particle size. Intake of DM was higher in heifers fed 16AH and 19AH than in heifers fed 10BS ($P < 0.001$). Intake of NDF and physically effective NDF (peNDF) was greater in 13AH, 16AH, and 19AH than in 10BS ($P < 0.01$). The DMI of medium and short particle size was greater in 13AH, 16AH, and 19AH than in 10BS ($P < 0.05$), whereas DMI of long particle size was greater in 16AH and 19AH compared to 10BS ($P < 0.001$). Heifers fed 13AH, 16AH, and 19AH diets sorted against fine particle size and sorted for or tended to sort for short, medium, and long particle sizes. Meal length was greater in heifers fed 16AH and 19AH than 10BS ($P < 0.05$). Time spent eating was not affected by diet but time spent ruminating was greater in heifers fed 19AH than in 10BS ($P < 0.05$). Results indicate that the inclusion of alfalfa hay at 19% of incorporation caused an increase in DM, NDF, and peNDF intake, in comparison to the 10BS diet. In the same way, intake of long, medium, and short particle size was greater in this diet. Moreover, heifers fed 19AH sorted for medium particle size and tended to sort for long and short particles size, and against fine particle size. Sorting behavior and meal length increased in the 19AH diet, which leads us to think that sorting feed ingredients requires time and therefore lengthens the meal. Time spent ruminating was greater in heifers fed 19AH, thus reducing the risk of ruminal acidosis when animals are fed high concentrate diets.

Key words: alfalfa hay, barley straw, beef heifers, sorting behavior

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INTRODUCTION

Adding a low percentage of roughage to high-concentrate diets helps to prevent digestive upsets and maximize energy intake (Galyean and Derfoor, 2003). With regard to the amount and type of forage to be used, availability and price determine the choice. Samuelson et al. (2016) reported that 8% to 10% was the typical range of forage inclusion used in feedlot finishing diets, with corn silage and alfalfa being the forages most commonly used (Vasconcelos and Galyean, 2007). When using cereal straw, animals consumed 10% of DMI as roughage when concentrate and straw were offered in different feed bunks (González et al., 2008a). In this intensive production system, increasing the amount of forage intake, as a way to reduce digestive troubles, could help enhance performance and animal well-being.

Total mixed rations (**TMR**) are designed to be a homogeneous mixture to minimize the selective consumption of individual components (Coppock et al., 1981). However, sorting behavior has been detected in growing calves (Miller-Cushon et al., 2013; Groen et al., 2015; Gordon and DeVries, 2016) and growing heifers (Greter et al., 2008; DeVries et al., 2014). Heifers fed with either alfalfa hay or barley straw at 8% of DM inclusion showed preferential consumption for the concentrate when straw was included, which did not occur in heifers fed alfalfa (Madruga et al., 2017a). We hypothesized that considering the low nutritive value of straw, sorting behavior could vary if it is replaced with increased levels of alfalfa, and this replacement is based on the proportion of NDF from forage rather than on a similar DM. This approach is considered more appropriate to replace forages in beef finishing diets (Galyean and Defoor, 2003; Salinas-Chavira et al., 2013, Swanson et al., 2017). Therefore, the objective was to study the effects of increasing the proportion of alfalfa hay in the TMR on intake, sorting, and feeding behavior when offered to beef heifers at finishing.

MATERIALS AND METHODS

Animal procedures were approved by the Institutional Animal Care and Use Committee (reference CEEAH 1585) of the Universitat Autònoma de Barcelona (Spain) in accordance with the European directive 2010/63/EU.

Animals, Experimental Design, and Housing

Eight ruminally cannulated Simmental heifers (212 ± 4.8-d old and with an average initial BW of 281.4 ± 7.28 kg) were randomly assigned to one of four experimental treatments in a replicated 4 × 4 Latin square design. The experiment was performed on the Experimental Farm of the Universitat Autònoma de Barcelona from February to June 2015, in four 28-d periods, and sampling was carried out in the last week of each period, after 3 wk of diet adaptation. Heifers were weighed before feeding on 2 consecutive days at the beginning and the end of the experiment, and on the last day of the sampling week. Treatments were 4 TMR: 1) with 10% barley straw as forage source **(10BS)**, which was used as a control diet, 2) TMR with 13% alfalfa hay as forage source (**13AH**), 3) TMR with 16% alfalfa hay as forage source **(16AH)**, and 4) TMR with 19% alfalfa hay as forage source **(19AH)**. Animals were allotted in a roofed open barn with separate individual pens. Each pen had a concrete floor and was 5 m long and 2.5 m wide (12.5 m^2/pen) and was equipped with a feed bunk and a water trough. The adjacent pens were separated by a metal fence with a bar design that allowed contact between animals.

In order to record feed intake, an automated system was used. Feedbunks (120-liter capacity) were mounted on waterproof digital platform scales in each stall (model DI-160, DIGI I's Ltd, Maesawa-cho, Isawa-gun, Iwate, Japan). Each scale was programmed to transmit the feed weight at intervals of 5 s. The information was downloaded onto a computer with appropriate data capture software (LabView, National Instruments Corporation, Austin, TX). To register water consumption, individual drinking cups with direct reading flow meters were used (B98.32.50, Invensys model 510 C, Tashia S.L., Artesa de Segre, Spain). Animal behavior was continuously recorded using a digital video-recording device (model VS-101P VioStor NVR, QNAP Systems Inc., Xizhi City, Taipei County, Taiwan). A digital color camera (model VIVOTEK IP7142, VIVOTEK INC., Chung-HO, Taipei County, Taiwan) was set up in front of the feeding area of each pen at a height of 3 m, permitting a full view of the pen. An infrared light with photoelectric cells was set up at each end of the paddock to allow video-recording at night (λ = 830 nm and 500 W; Dennard 2020, Hants, UK).

Feed and Data Collection

All diets were offered on an ad libitum basis as TMR and designed according to NRC (2000) to be isoenergetic and isonitrogenous for a targeted gain of 1.2 kg/day. Barley straw chemical composition was 69.9% NDF and 4.6% CP (DM basis), whereas for alfalfa hay the composition was 43.7% and 17.6% (DM basis) for NDF and CP,

Table 1. Ingredients and chemical composition of the diets

	Diet[1]			
Item	10BS	13AH	16AH	19AH
Ingredient composition, % of DM				
Barley straw	10.0	-	-	-
Alfalfa hay	-	13.0	16.0	19.0
Corn	35.0	30.0	35.0	41.5
Barley	43.0	48.0	40.5	31.5
Soybean meal, 44%CP	9.0	6.0	5.5	5.0
Salt	0.7	0.7	0.7	0.7
Sodium bicarbonate	1.0	1.0	1.0	1.0
Calcium carbonate	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.4	0.4	0.4	0.4
Vitamin-mineral premix[2]	0.4	0.4	0.4	0.4
Chemical composition, % DM				
CP	11.9	12.6	12.4	12.8
NDF	24.4	19.3	19.6	20.3
ADF	7.7	6.7	7.4	8.8
Ether extract	2.0	1.8	2.0	2.0
Ash	4.8	6.8	6.2	7.5
NFC[3]	56.9	59.5	59.8	57.4
ME[4], Mcal/kg of DM	2.83	2.85	2.82	2.81
Particle size, %				
Long	4.8	1.8	0.9	0.7
Medium	3.5	3.1	2.7	3.1
Short	46.9	44.9	45.8	44.8
Fine	44.8	50.2	50.6	51.4

[1]10BS = TMR with 10% of barley straw; 13AH = TMR with 13% of alfalfa hay; 16AH = TMR with 16% of alfalfa hay; 19AH = TMR with 19% of alfalfa hay.

[2]Nutral Terneros (NUTRAL, S.A., Colmenar Viejo, Madrid, Spain): vitamin and mineral premix contained per kg premix (as fed): 1,500 kIU vitamin A, 500 kIU vitamin D_3, 3.75 g vitamin E, 0.5 g vitamin B1, 0.5 g vitamin B2, 0.25 g vitamina B6, 1.25 mg vitamin B12, 15.0 g Zn, 2.5 g Fe, 83.3 g S, 55.0 mg Co, 2.5 g Cu, 7.5 g Mn, 100.0 mg I, 100.0 mg Se.

[3]NFC = nonfiber carbohydrates calculated as 100 – (CP + ash + NDF + Ether extract).

[4]According to NRC (2000).

respectively. Alfalfa diets were designed to provide less (5.7% for 13AH), similar (7.0% for 16AH), and more (8.3% for 19AH) NDF from forage than the 10BS diet (7.0%). Table 1 reflects the ingredients of the diets and their chemical composition after analysis. Ingredients, except forage source and vitamin-mineral premix, were ground through a 5-mm screen before mixing. Forage sources were mechanically chopped before their incorporation in the TMR with a forage chopper machine (Seko SpA, Curtarolo, Italy). After chopping, the mean (Mean ± SD) particle size of barley straw was 15.5 ± 2.88, and 6.3 ± 2.92 for alfalfa hay. Feeders were cleaned and orts collected at 0830 h each morning, and feed offered once daily at 0930 h, and increased by 15% in relation to the previous day's intake. Feed offered and refusal samples of each heifer were collected daily for 7 d in the sampling week for DM determination, chemical and particle size analyses. Particle size separation was performed using the 3-screen Penn State Particle Separator, obtaining four different fractions: 1) long particles (> 19 mm), 2) medium particles (<19 to > 8 mm), 3) short particles (< 8 to > 1.18 mm), and 4) fine particles (<1.18 mm). The proportion of each particle size in the diets is described in Table 1. Physically effective NDF (peNDF) was calculated as the sum of DM intakes of fractions greater than 1.18 mm multiplied by the NDF content of the diet. Sorting was calculated as the actual intake of each fraction size expressed as a percentage of the predicted intake, where predicted intake of each fraction equals the product of as-fed intake and as-fed fraction in the diet. Values less than 100% indicate selective refusals, >100% is preferential consumption, and =100% is no sorting (Leonardi and Armentano, 2003).

Chemical Analyses

Feed offered and refusal samples were dried in a forced air oven at 60 °C for 48 h for later chemical analysis. Samples were ground in a hammer mill through a 1-mm screen (P. PRAT SA, Sabadell, Spain) and retained for analysis. Dry matter content was determined by drying samples for 24 h at 103 °C in a forced-air oven, and ash according to AOAC (1990; ID 950.05). Nitrogen content was determined by the Kjeldahl procedure (AOAC, 1990; ID 976.05). Ether extract was performed according to AOAC (1990; ID 920.39). The NDF and ADF contents were determined sequentially by using an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY) in accordance with the methodology provided by the company. This is based on the methods described by Van Soest et al. (1991), using a thermostable alpha-amylase and sodium sulfite, and expressed on an ash-free basis. Dry matter intake and daily nutrient intake were calculated as the difference between amounts offered and refused based on chemical analysis of the composited sample within heifer and period.

Ruminal Fermentation

Before the experiment started, two heifers per treatment were used for the evaluation of the effects of diets on ruminal fermentation (Table 2). Samples were taken after 2 wk of diet adaptation on 3 non-consecutive days. During rumen sampling week,

Table 2. Characterization of the diets based on their effects on ruminal fermentation

	Diet[1]			
Item	10BS	13AH	16AH	19AH
Ruminal pH				
Average	6.43 ± 0.07[2]	6.66 ± 0.11	6.43 ± 0.05	6.74 ± 0.10
Lowest	5.51 ± 0.11	5.88 ± 0.15	5.62 ± 0.12	5.99 ± 0.18
Highest	7.51 ± 0.09	7.51 ± 0.11	7.42 ± 0.17	7.58 ± 0.11
Hours under pH < 5.8	4.70 ± 1.42	1.43 ± 0.95	3.44 ± 1.08	0.98 ± 0.90
Hours under pH < 5.6	2.06 ± 0.80	0.91 ± 0.74	1.99 ± 0.90	0.80 ± 0.74
Total VFA, mM	92.3 ± 6.79	83.7 ± 4.34	87.0 ± 5.93	83.1 ± 5.36
VFA, mol/100mol				
Acetate	50.04 ± 0.84	62.71 ± 1.21	62.50 ± 0.94	65.71 ± 1.01
Propionate	39.16 ± 1.09	23.75 ± 1.18	25.92 ± 0.88	18.61 ± 1.18
Butyrate	7.35 ± 0.33	9.72 ± 0.37	8.24 ± 0.39	12.08 ± 0.65

[1]10BS = TMR with 10% of barley straw; 13AH = TMR with 13% of alfalfa hay; 16AH = TMR with 16% of alfalfa hay; 19AH = TMR with 19% of alfalfa hay.

[2]Mean ± SE.

peNDF intake was calculated, being on average 0.74, 1.24, 1.13, and 1.12 kg/day for 10BS, 13AH, 16AH, and 19AH, respectively. Ruminal samples were taken with an electric vacuum pump connected to a 1-m iron tube that was introduced through the cannula to reach different locations within the rumen and obtain a 300-mL sample. Sampling times were as follows: immediately before feeding and at 4, 8, 12, 16 and 24 h after feeding. The ruminal fluid was squeezed through four layers of cheesecloth and pH was measured immediately with a glass electrode pH meter (model 507; Crisson Instruments SA, Barcelona, Spain). Samples were taken for VFA analyses. Four mL of the ruminal fluid was added to 1 mL of a solution made up of 1% (wt/wt) solution of mercuric chloride, to prevent microbial growth, 2% (vol/vol) orthophosphoric acid and 0.2% (wt/wt) 4-methylvaleric acid as an internal standard in distilled water and frozen at −20 °C (Jouany, 1982). Samples were thawed and centrifuged at 15,000 g for 20 min, and diluted 1:1 in distilled water for subsequent analysis using gas chromatography (model 6890; Hewlett Packard, Palo Alto, CA). A capillary column treated with polyethylene glycol TPA (BP21; SGE Europe Ltd, Buckinghamshire, UK) at 275 °C in the injector and a 29.9 mL/min total gas flow rate were used in the chromatograph. The number of hours during which ruminal pH remained below 5.8 and 5.6 were calculated assuming that the change in pH between two consecutive measures was linear.

Feeding and Animal Behavior

Feeding behavior data were recorded during 5 d in each sampling week and analyzed as described by González et al. (2008b). Briefly, the length of all inactive intervals in which feeding did not occur were log-transformed and used to calculate the meal criterion, which is the minimum time required to consider two periods of eating activity as separate events. The Mixed Distributions Package of the R software was used for this purpose, and model fitting was done according to Yeates et al. (2001). Meal frequency (meals/day) was the number of intervals where eating activity was registered and that exceeded the meal criterion. Meal length (min/meal) was calculated as the time from the first eating observation before an inactive interval that exceeded the meal criterion. Meals were further characterized by DM ingested (meal size; g DM/meal) and rate of DM ingested per meal (eating rate; g DM/min), calculated as the ratio of amount of feed ingested and the corresponding meal length.

Animal behavior was video-recorded for 24 h on 3 nonconsecutive days of each sampling week. In accordance with Madruga et al. (2017b), data processing was carried out by a time sampling method at intervals of 5 min. The behavioral activities recorded were eating and ruminating. Data for each activity are presented as the total time, expressed in minutes, in which the animal maintained this specific activity. An observation was recorded as eating when the animal had its muzzle in the feed bunk or was chewing or swallowing food with its head over it. Ruminating included the regurgitation, mastication and swallowing of the bolus. Eating and ruminating time, expressed as min/kg total DM and min/kg NDF, were calculated taking into account both time spent eating and ruminating and total DM and NDF intake recorded on the 3 days of behavior observation.

Statistical Analyses

Each heifer fed a given treatment diet at each period was considered the experimental unit in all the analyses. In each experimental period, the daily mean value was calculated as the average either of 7 d for DM and nutrient intake, DM intake by particle size, and sorting behavior, of 5 d for feeding behavior data, or of 3 d for video recording eating and ruminating activities. All these data were statistically analyzed using a mixed-effects linear regression model in the MIXED procedure of SAS (v. 9.3; SAS Institute Inc., Cary, NC, 2011). The model contained the fixed effects of Latin square, treatment and period, and the random effect of heifer nested within Latin square, the day being included as a repeated measure. The Dunnett's two-tailed *t* test was performed to test if any alfalfa diets were significantly different from 10BS. Orthogonal contrasts were used to determine linear and quadratic effects of increasing the proportion of alfalfa hay in the diet. To determine if heifers sorted against or for each particle size, sorting behavior was tested for a difference from 100 using a *t* test. Significance was declared at $P < 0.05$, and tendencies are discussed at $P < 0.10$.

RESULTS

Final BW of heifers, after the experimental period of 112 d, was on average 437.5 ± 11.42 kg. None of the variables studied in the present experiment were quadratically affected by the increased proportion of alfalfa hay in the diet, thus no comments have been included.

Intake

Dry matter intake and CP intake was greater in 16AH and 19AH than in 10BS, but there were no differences between 13AH and 10BS ($P = 0.001$; Table 3). Intake of NDF and peNDF was greater in 13AH, 16AH, and 19AH than in 10BS ($P = 0.001$ for NDF, and $P = 0.01$ for peNDF). Water consumption differed from 10BS only in 19AH ($P = 0.013$). Increasing the alfalfa hay proportion in the diet, total DMI, CP intake, and water consumption linearly increased ($P = 0.028$, $P = 0.040$, and $P = 0.017$, respectively).

When DM intake was separated by particle size, intake of long particles was greater in 16AH and 19AH than in 10BS, but the intake was not different between 13AH and 10BS ($P = 0.001$; Table 4). Intake of medium and short particles was greater in 13AH, 16AH, and 19AH than in 10BS ($P = 0.001$ for medium, and $P = 0.04$ for short particle size). Intake of fine particles tended to be affected by diet, but differences were only detected between 13AH and 10BS, the intake being lesser in 13AH ($P = 0.07$). A linear effect was found for intake of long ($P = 0.001$), medium ($P = 0.027$), and fine particle size ($P = 0.004$), which increased when increasing the proportion of alfalfa hay in the diet.

Sorting Behavior

Extent of sorting of long and medium particle sizes was greater in 13AH, 16AH, and 19AH than in 10BS ($P < 0.05$; Table 5). For short particle size, extent of sorting in heifers fed 13AH differed from 10BS ($P = 0.001$), while for fine particle size extent of sorting in 10BS differed from 13AH, 16AH, and 19AH ($P = 0.006$). Increasing the proportion of alfalfa hay resulted in a linear decrease in the extent of sorting of short particle size ($P = 0.001$), a linear increase in the case of fine particle size ($P = 0.015$), and a tendency to a linear decrease in the case of medium particle

Table 3. Intake and water consumption of heifers fed diets offered as TMR including 10% barley straw (10BS) or different levels of alfalfa hay (13% = 13AH; 16% = 16AH; 19% = 19AH)

	Diet					*P*-value		
	10BS	13AH	16AH	19AH	SEM	Overall effect	Alfalfa effect[1]	
Item							L	Q
Intake, kg/day								
Total DM	8.16[b,2]	7.89[b]	9.01[a]	8.80[a]	0.221	0.001	0.028	0.112
CP	0.94[b]	0.92[b]	1.02[a]	1.05[a]	0.026	0.001	0.040	0.555
NDF	1.66[b]	1.82[a]	2.02[a]	1.94[a]	0.053	0.001	0.266	0.245
peNDF[3]	0.92[b]	1.10[a]	1.15[a]	1.17[a]	0.070	0.010	0.409	0.935
Water consumption, liter/day	29.5[b]	29.3[b]	30.0[b]	32.7[a]	1.17	0.013	0.017	0.431

[1]Effect of alfalfa hay level: L = linear, Q = quadratic.

[2]Means in rows with different superscript than 10BS differed statistically ($P < 0.05$) from this treatment.

[3]Physically effective NDF = Sum of intakes of particle sizes bigger than 1.18 mm determined by Penn State Particle Separator × NDF content of the diet.

Table 4. Dry matter intake by particle size of heifers fed diets offered as TMR including 10% barley straw (10BS) or different levels of alfalfa hay (13% = 13AH; 16% = 16AH; 19% = 19AH)

	Diet					*P*-value		
	10BS	13AH	16AH	19AH	SEM	Overall effect	Alfalfa effect[1]	
Item							L	Q
Intake by particle size[2], kg/day								
Long	0.38[b,3]	0.39[b]	0.56[a]	0.64[a]	0.049	0.001	0.001	0.177
Medium	0.27[b]	0.44[a]	0.56[a]	0.59[a]	0.046	0.001	0.027	0.584
Short	3.68[b]	4.07[a]	4.22[a]	4.05[a]	0.188	0.040	0.562	0.351
Fine	3.83[a]	3.32[b]	3.68[a]	3.97[a]	0.211	0.070	0.004	0.567

[1]Effect of alfalfa hay level: L = linear, Q = quadratic.

[2]Particle size determined by Penn State Particle Separator.

[3]Means in rows with different superscript than 10BS differed statistically ($P < 0.05$) or tended to differ ($P < 0.10$) from this treatment.

Table 5. Effect of diet on sorting behavior of heifers fed diets offered as TMR including 10% barley straw (10BS) or different levels of alfalfa hay (13% = 13AH; 16% = 16AH; 19% = 19AH)

	Diet					*P*-value		
	10BS	13AH	16AH	19AH	SEM	Overall effect	Alfalfa effect[1]	
Item							L	Q
Particle size[2]								
Long	92.7[b,3]	105.5[a]	108.7[a,*,4]	105.8[a,+]	5.43	0.032	0.834	0.730
Medium	92.5[b]	108.1[a,**]	106.0[a,+]	106.3[a,**]	4.77	0.015	0.087	0.288
Short	100.7[b]	102.4[a,**]	101.1[b,+]	100.4[b,+]	0.36	0.001	0.001	0.197
Fine	100.3[a]	95.6[b,**]	96.6[b,*]	97.8[b,*]	1.17	0.006	0.015	0.616

[1]Effect of alfalfa hay level: L = linear, Q = quadratic.

[2]Particle size determined by Penn State Particle Separator.

[3]Means in rows with different superscript than 10BS differed statistically ($P < 0.05$) from this treatment.

[4]Values equal to 100% indicate no sorting, <100% selective refusals (sorting against), and >100% preferential consumption (sorting for). Statistical differences from 100% are expressed as: $^{**}P < 0.01$, $^{*}P < 0.05$, and $^{+}P < 0.10$.

Table 6. Feeding behavior of heifers fed diets offered as TMR including 10% barley straw (10BS) or different levels of alfalfa hay (13% = 13AH; 16% = 16AH; 19% = 19AH)

	Diet					*P*-value		
	10BS	13AH	16AH	19AH	SEM	Overall effect	Alfalfa effect[1]	
Item							L	Q
Meal frequency, meals/day	12.9	13.8	13.9	12.6	0.64	0.341	0.857	0.265
Meal length, min/meal	27.2[b,2]	28.6[b]	28.9[a]	30.3[a]	0.678	0.030	0.139	0.643
Meal size, g/meal	680	620	700	690	34.0	0.544	0.321	0.264
Eating rate, g/min	24	22	24	23	1.0	0.776	0.248	0.164

[1]Effect of alfalfa hay level: L = linear, Q = quadratic.

[2]Means in rows with different superscript than 10BS differed statistically ($P < 0.05$) from this treatment.

size ($P = 0.087$). Heifers fed 10BS did not show any sorting behavior. Heifers fed 13AH sorted for medium and short particle sizes ($P < 0.01$), and against fine particle size ($P < 0.01$). Heifers fed 16AH sorted for long particle size ($P < 0.05$), and against fine particle size ($P < 0.05$), and tended to sort for medium and short particle sizes ($P < 0.10$). Finally, heifers fed 19AH sorted for medium and against fine particle sizes ($P < 0.05$), and tended to sort for long and short particle sizes ($P < 0.10$).

Feeding Behavior

Meal criterion did not differ among diets, being on average 18.9 ± 1.36 min (Table 6). Number of meals per day, size per meal, and eating time were similar across diets. Length of meal was greater in 16AH and 19AH than in 10BS ($P = 0.03$). Time spent eating was not affected by diet when expressed as minutes/day, min/kg DM or min/kg NDF (Table 7). Heifers fed 19AH spent a longer time ruminating, when expressed as minutes/day, min/

Table 7. Behavioral activities of heifers fed diets offered as TMR including 10% barley straw (10BS) or different levels of alfalfa hay (13% = 13AH; 16% = 16AH; 19% = 19AH)

	Diet					*P*-value		
	10BS	13AH	16AH	19AH	SEM	Overall effect	Alfalfa effect[1]	
Item							L	Q
Eating								
min/day	130.4	121.6	136.9	119.2	8.47	0.111	0.735	0.067
min/kg DM	14.8	15.4	15.2	14.6	1.14	0.830	0.442	0.711
min/kg NDF	67.2	66.8	67.8	71.8	5.32	0.660	0.471	0.881
Ruminating								
min/day	330.0[b,2]	315.2[b]	368.5[b]	371.7[a]	20.99	0.018	0.045	0.245
min/kg DM	37.6[b]	39.9[b]	40.9[b]	45.5[a]	2.51	0.020	0.005	0.255
min/kg NDF	170.4[b]	173.0[b]	182.3[b]	223.4[a]	11.65	0.001	0.079	0.539

[1]Effect of alfalfa hay level: L = linear, Q = quadratic.

[2]Means in rows with different superscript than 10BS differed statistically ($P < 0.05$) from this treatment.

kg DM and min/kg NDF, than heifers fed 10BS, but heifers fed 13AH and 16AH did not differ from 10BS. Increasing the alfalfa hay proportion in the diet, time spent ruminating linearly increased when expressed as minutes/day and min/kg DM ($P = 0.045$ and $P = 0.005$, respectively), and tended to increase when expressed as min/kg NDF ($P = 0.079$).

DISCUSSION

The main risk factors involved in ruminal acidosis in feedlot cattle are high grain, low roughage diets because of their high rate and extent of degradation by rumen microbes, and low saliva production due to low chewing time (González et al., 2012). In regard to the forage component, diet formulation should take into account the proportion, fiber concentration and particle size of this forage. Some studies suggest that substituting dietary forage on the basis of NDF rather than on a similar DM basis might be more appropriate when formulating finishing diets (Galyean and Defoor, 2003; Salinas-Chavira et al., 2013, Swanson et al., 2017). In the present experiment, we formulated three alfalfa hay diets to provide less content of NDF from forage (13AH), the same content (16AH), and greater content (19AH) than the barley straw diet (10BS). In addition, the ratio of NDF from forage to total NDF from diet was 29% for 10BS and 13AH, and 36% and 41% for 16AH and 19AH, respectively. Thus, the results are discussed in separate sections on the basis of the inclusion level of NDF from forage.

13% Alfalfa Hay vs. 10% Barley Straw

Dry matter intake, CP intake, and water consumption were not different in heifers fed 13AH than those fed 10BS diet. This result disagrees with those obtained by Freeman et al. (1991), who evaluated the effects of alfalfa hay or wheat straw at either 10% or 6% in steam-flaked and high-moisture corn-based diets on feedlot performance, and reported that steers consuming wheat straw diets tended to eat more dry matter than those eating alfalfa-based diets. Although these authors did not describe the proportion of fiber supplied by the forage included in the diets, it is possible to make an estimation assuming the nutritive value of both forages in accordance with NRC (2000), and to conclude that their alfalfa diets also provided less fiber than the straw diets, as in the 13AH diet used in the present experiment in comparison with the 10BS diet. Freeman et al. (1991) argued that wheat straw probably did stimulate intake by increasing rumination time and the corresponding particle size reduction, causing an increase in rate of digesta passage and allowing for increased intake. However, rumination time recorded in the present experiment did not differ between heifers fed 13AH and 10BS.

Given that there were no differences in DMI between 10BS and 13AH diets but a greater NDF content in 10BS, greater NDF and peNDF intakes were expected in the former diet, but the opposite occurred. This contradictory result can be only explained by the sorting behavior detected in heifers fed 13AH diet, with a preferential consumption of the medium and short particles and against fine particles. This sorting behavior resulted in a greater DMI by means of medium and short particles for heifers fed 13AH, in no differences in long particles between 13AH and 10BS, and in a lesser DMI with fine particles for heifers fed 13AH. Surprisingly, heifers fed 10BS did not show any sorting behavior, although numerical values of less than 100% were recorded in heifers fed this diet for long and medium particle size. Including barley straw at 8%

of incorporation in a TMR fed to young growing heifers, Madruga et al. (2017a) observed that animals sorted against long particle size and for short particle size, which did not occur in the present experiment. Both the heifers' age and the duration of time consuming the TMR diet could explain these different results, as heifers in the present experiment were older, and therefore more experienced, and had more time to adapt to diet ingredients and feeding method.

16% Alfalfa Hay vs. 10% Barley Straw

Intake of DM, CP, NDF, and peNDF were greater in 16AH than 10BS, despite the fact that both diets contained similar NDF content from forage. Shain et al. (1999) found that DMI of steers was not different, when evaluating the effect of using alfalfa hay (10%) or wheat straw (5.2%) in high concentrate diets based on dry-rolled corn, and balanced to provide equal NDF concentrations, on animal performance. Swanson et al. (2017) obtained no differences in DMI comparing alfalfa hay (10%) and wheat straw (7.8%) when diets, based on dry-rolled corn and offered as a TMR, were offered to provide the same concentration of NDF from forage, in agreement with the present experiment.

Dry matter intake recorded by particle size showed that the intake of long, medium and short particle sizes were greater in 16AH than in 10BS. Once again, sorting behavior could help to explain this result because heifers fed 16AH sorted for long particle size, tended to sort for medium and short particle size, and sorted against fine particle size. In addition to this sorting behavior, meals were longer in the 16AH diet than in 10BS, which might suggest that sorting feed ingredients required time, thus increasing meal length. In addition, time spent eating and ruminating was not different between diets. These results suggest that in this comparison the main factor controlling animal response is essentially controlled by the fact that the NDF from forage in both diets was the same, reinforcing the idea that substituting dietary forage on the basis of NDF rather than on a similar DM basis might be more appropriate when formulating finishing diets (Galyean and Defoor, 2003; Salinas-Chavira et al., 2013, Swanson et al., 2017).

19% Alfalfa Hay vs. 10% Barley Straw

Increasing forage inclusion in high-concentrate finishing diets increases DMI (Bartle et al., 1994; Galyean and Defoor, 2003). However, other authors recommend not exceeding 10% (Hales et al., 2003) or 15% (Swanson et al., 2017) of forage in high concentrate finishing diets. In the present experiment, we substituted dietary forage on the basis of NDF using a diet with 19% of forage (19AH) with a greater proportion of NDF from forage than 10BS, and the response was that heifers fed 19AH had a greater DM, CP, NDF, and peNDF intake, as well as a greater water consumption, than heifers fed 10BS. Additionally, this proportion of forage resulted in a greater consumption of long, medium and short particle sizes, whereas there were no differences in the consumption of fine particles. Once again, with regard to the particle sizes, heifers fed 19AH sorted for medium or tended to sort for long and short particle size, and sorted against fine particle size, which could be related with the lengthening of the meals in heifers fed this diet.

Physically effective NDF contributes to maintaining the equilibrium in the rumen, avoiding the lowering of ruminal pH and the risk of ruminal acidosis because it is a measure that reflects fiber physical characteristics and its ability to stimulate chewing and saliva buffering in the rumen (Yang et al., 2006). In the present experiment, heifers fed the 19AH diet, the only one with a higher content of NDF from forage than 10BS, showed a longer ruminating activity. The relation between this chewing activity and its effect on the rumen was reflected when the diets were tested to know their effects on mean ruminal pH and fermentation profile. The 19AH diet showed the greatest average and lowest pH, and the minimum number of hours under pH under 5.8 and 5.6 thresholds. In addition, this diet had the greatest proportion of acetate and butyrate and the smallest proportion of propionate, which is in agreement with the fermentation profile of a ruminant fed a diet with higher forage content (France and Dijkstra, 2005) than that which is usually offered to cattle feedlot at finishing.

Effect of Alfalfa Hay Inclusion

Swanson et al. (2017), working with Angus and Simmental steers fed dry-rolled corn-based diets, compared different levels of forage inclusion (5%, 10%, 15%, or 20% of a mixture of bromegrass hay and corn silage) and found that DMI decreased linearly with increasing forage inclusion and recommended including less than 15% of forage in high-concentrate finishing diets. Hales et al. (2013) also showed a quadratic effect of increasing forage proportion in the diet on DMI during the finishing period, and reported an increase in DMI with alfalfa hay inclusion of up to 10%, and then a decrease with 14% alfalfa hay. These results, however, were not confirmed in the present experiment, where increasing the proportion of alfalfa hay included in the diet, from 13% to 19%, linearly increased DMI. In addition, intake of long, medium, and fine particle size also increased with increasing inclusion of alfalfa hay. The increased intake of the bigger particle size would explain the linear increase of time spent in rumination. Because an increase in chewing activity is believed to increase saliva flow and rumen pH (Beauchemin et al., 1997), these results would suggest that the range of alfalfa inclusion tested in the present experiment would help to reduce the incidence of ruminal acidosis. In this sense, the ruminal characteristics recorded when experimental diets were tested would indicate better health conditions with a greater minimum pH and fewer hours under critical rumen pH.

CONCLUSION

In conclusion, increasing the proportion of alfalfa hay, from 13% to 19% (DM basis), in high-concentrate diets linearly increased total DMI, CP intake, water consumption, intake of long, medium and fine particle size, extent of sorting of fine particle size, and time spent ruminating, but linearly decreased extent of sorting of short particle size. Intake of NDF and peNDF was greater in heifers fed alfalfa diets than 10BS. In comparison with 10BS, the increase in DMI with the long particle size was achieved for 16AH and 19AH, but not for 13AH, whereas the increase in DMI was obtained for the three alfalfa diets in medium and short particle sizes. Sorting behavior was detected in 13AH, 16AH, and 19AH, but not in 10BS, because heifers fed alfalfa diets sorted or tended to sort for long, medium and short particle sizes, and against fine particle size, which could be related with the lengthening of the meals, which was only detected in 16AH and 19AH. Therefore, level of inclusion of alfalfa hay did not affect sorting behavior as was hypothesized. Both proportion of fine particle size and its quality could have a differential effect on sorting behavior but with the present results we are unable to confirm or reject this hypothesis. The longer rumination time was achieved with heifers fed 19AH diet, the only alfalfa diet providing greater NDF from forage than 10BS, a result that could contribute to reduce the risk of ruminal acidosis.

Conflict of interest statement. None declared.

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