Running head: Forage level in feedlot diets

Using nineteen percent of alfalfa hay in beef feedlot finishing diets did not modify meat quality but increased feed intake and average daily gain[1]

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ABSTRACT: To evaluate the effects of including extra alfalfa hay in high concentrate diets fed to beef heifers on intake, average daily gain (**ADG**), gain to feed ratio (**G:F**), and carcass and meat quality, we used 24 Simmental heifers (initial BW 235.6 ± 4.19 kg). Heifers were blocked in 4 BW blocks and allotted in groups of 3 in a randomized block design with 2 treatments and 12 heifers per treatment. Treatment diets offered as total mixed ration (**TMR**) were: a) TMR with 10% barley straw (**BS**), considered the control diet, and b) TMR with 19% alfalfa hay (**AH**). The experiment was performed over 4 28-d experimental periods, and we took measurements in the last week of each period. After this period of performance control, heifers were fed the corresponding diet until each BW block reached the target weight of 400 kg on average. Feed intake and ADG were greater for AH than BS (9.5 vs 8.4 kg/d, and 1.45 vs 1.29 kg/d, respectively; $P < 0.05$), but G:F was unaffected by diet ($P > 0.10$). Diet did not affect hot carcass weight, dressing percentage, backfat color, pH and meat color, or carcass grade. The sixth rib was dissected to determine the proportion of fat, lean and bone, which were unaffected by diet. Diet did not affect the Longissimus muscle composition in water, protein, collagen, intramuscular fat, and cholesterol. The intramuscular fat proportion of C18:1 *n*-7 was greater in BS than in AH ($P = 0.016$), whereas the proportion of C18:3 *n*-3 tended to be greater in AH than in BS ($P = 0.09$). When fatty acid concentration was expressed as g per 100 g of Longissimus muscle, these differences disappeared, and only the content of C15:0 tended to be greater ($P = 0.08$) in BS than in AH. Meat characteristics evaluated by trained panelists did not differ in toughness, chewiness, juiciness, odor, taste and overall acceptability, and there were no differences between diets in Warner-Bratzler shear force values after 3 or 10 d of ageing ($P > 0.10$). In summary, heifers fed TMR with alfalfa hay at 19% of inclusion showed a greater feed intake and ADG than those fed barley straw at 10% of inclusion, but without affecting

G:F ratio. However, this extra alfalfa hay was not sufficient to cause any relevant change in the carcass and meat quality of the heifers fed this diet.

Key Words: beef cattle, forage source, meat quality, performance

INTRODUCTION

Animal production in the future must consider the compromise between animal performance, in terms of feed efficiency and economic profitability, and animal welfare, something increasingly demanded by consumers, to obtain quality meat with special attention to health aspects of this food. To prevent digestive upsets and maximize energy intake in high-concentrate finishing diets fed to beef cattle, Galyean and Derfoor (2003) recommend adding a percentage of roughage. However, more information is needed about the optimal concentration and type of forage required to reduce digestive disorders without compromising animal performance. Samuelson et al. (2016) reported that 8 to 10 % was the typical range of forage inclusion used in feedlot finishing diets, and elsewhere, when growing heifers were offered free-choice of concentrate and straw provided in separate feedbunks, González et al. (2018) recorded barley straw intake ranging from 10 to 12 %. A decrease in DMI has been reported with a level of forage inclusion greater than 10 % (Hales et al., 2013) or 15 % (Swanson et al., 2017). However, in a previous experiment Madruga et al. (2018) reported increased DMI and time spent ruminating with an inclusion of 19% of alfalfa hay in comparison with 10% barley straw, because more forage fiber was provided, thus helping to prevent ruminal acidosis.

In recent years, there has been an abundance of literature comparing the effect of pasture-based or forage-based diets with concentrate-based or grain-based diets, on

carcass and meat quality. Number of days at pasture (Noci et al., 2005), amount of grass intake (O'Sullivan et al., 2003), pre-finishing grazing period (Moran et al., 2017), type of forage (Duckett et al., 2013), and concentrate supplementation (French et al., 2000) has been studied. French et al. (2000) stated that decreasing the proportion of concentrate in the diet caused a linear increase in the polyunsaturated to saturated fatty acid ratio. Taking into account the previous results recorded by Madruga et al. (2018), we wondered if it would be possible to confirm the increase in DMI when a 10% barley straw is substituted by alfalfa hay in a greater proportion of forage than that usually used in finishing feedlot diets, and in addition to improve meat quality. Thus, our aim here was to evaluate the effects of including 19 % alfalfa hay compared to 10 % barley straw in the diet offered to beef heifers on performance, carcass and meat quality.

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

Twenty four Simmental heifers (188.9 ± 2.06 d old and with an average initial BW of 235.6 ± 4.19 kg) were blocked in 4 BW groups (260, 241, 230, and 209 kg) with 6 heifers per block, and randomly assigned to 1 of 2 experimental treatments. Thus, there were 12 heifers per treatment allotted in 4 pens with 3 heifers per pen. Treatment diets offered as total mixed ration (**TMR**) were (Table 1): a) TMR with 10% barley straw (**BS**), considered the control diet, and b) TMR with 19% alfalfa hay (**AH**). We

designed the experiment with 4, 28-d experimental periods, and took measurements in the last week of each period. Heifers were allotted in a roofed open barn. 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. Adjacent pens were separated by a metal fence with a bar design that allowed contact between animals.

To record feed intake, we used an automated system. Feed bunks (120 L capacity) were mounted on waterproof digital platform scales in each stall (model DI-160, DIGI I's Ltd, Maesawa-cho, Isawa-gun, Iwake, Japan). We were able to measure individual feed intake each time that a heifer ate because each heifer was tagged with an electronic ear tag (Allflex HDX ULTRA HP ISO 982, Azasa, Madrid, Spain), which was detected by an antenna (Allflex panel reader, Azasa, Madrid, Spain) placed next to each feed bunk. Each scale was programmed to transmit the feed weight at intervals of 5 s. The information was downloaded onto a computer with data capture software (LabView, National Instruments Corporation, Austin, TX, USA).

Animal and Feed Data Collection

Heifers were weighed before feeding on two consecutive days at the beginning and the end of the experiment, and every week during the experiment. The weights recorded were used to calculate ADG, and subsequently the gain to feed ratio (**G:F**).

We offered the diets on an ad libitum basis as TMR, and formulated them to be isoenergetic and isonitrogenous for a targeted gain of 1.2 kg/d (NRC, 2000). Table 1 reflects the ingredients and chemical composition of the diets after analysis. The fatty acid profile of the diets is shown in Table 2. We formulated two different concentrates, one for the BS and another for the AH diet. The ingredients of the concentrates, except minerals and premix, were ground through a 5-mm screen. Forages were mechanically

chopped (Seko SpA, Curtarolo, Italy) before their incorporation in the TMR. After chopping, the mean (mean ± SD) particle size of barley straw was 15.5 ± 2.90 mm, and 5.92 ± 2.98 mm for alfalfa hay. Total mixed rations were manually prepared every day before their distribution by mixing each concentrate with the corresponding forage source. The leftover feed was collected at 0830 each morning, then feed offered once daily at 0930h. After calculating each day's feed intake from the difference between feed offered and refused, we increased the feed offered by 15% in relation to the previous day's intake to allow ad libitum consumption. Feed intake, expressed on as-fed basis, was individually monitored every 5 s for 24 h during 7 d in each sampling wk.

Feed Chemical Analysis

Feed samples were dried in a forced air oven at 60ºC 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 103ºC in a forced-air oven, and ash content 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 the procedure of Van Soest et al. (1991) using a thermostable alpha-amylase and sodium sulfite, and expressed on an ash-free basis.

Measurement of Carcass Quality

Heifers were allocated to treatments and fed the corresponding diet until each BW block reached the target weight of 400 kg on average. Heifers from each BW block were then transported to a commercial slaughterhouse (Sabadell, Spain) located 5.8 km

from the UAB experimental farm. Heifers were slaughtered using standard procedures in an EU-licensed abattoir. Each animal's BW was registered immediately before transfer to the abattoir. After slaughter, HCW was recorded, and carcass back fat and conformation were classified according to the EU classification system into 1, 2, 3, 4 and 5 and S, E, U, R, O, P categories, respectively (EU Regulation No 1234/2007 and No 1249/2008). Dressing percentage was calculated as HCW divided by BW measured on the farm. Instrumental color of back fat was recorded at three places on the loin region for L* (measures darkness to lightness), a* (measures redness), and b* (measures yellowness) with a colorimeter HunterLab MiniScan EZ 45/0 LAV (Hunter Associates Laboratory, Inc, Reston, Virginia, USA), using illuminant D65 and observer 10^{o}, and an aperture size of 25 mm. These data were used to calculate Chroma ($C^* = \sqrt{(a^{*2} + b^{*2})}$) and Hue angle value ($H^{o} = \arctan(a^*/b^*)$).

Meat Quality Sampling

After 24 h of carcass chilling under commercial conditions, a 5 cm bone-in rib section at the anterior end of the sixth rib was removed from each left and right carcass and transported to the laboratory for subsequent analysis. On arrival at the laboratory, Longissimus muscle (**LM**) was excised from the sixth right rib and used for immediate measurements of pH and color. We measured pH using a Crisson portable pH-meter (model 507; Crisson Instruments SA, Alella, Spain) with a xerolyt electrode. Instrumental color measurements were recorded after 30 minutes blooming for L*, a*, and b* with a colorimeter HunterLab MiniScan EZ 45/0 LAV (Hunter Associates Laboratory, Inc, Reston, Virginia, USA), using illuminant D65 with a 10^{o} standard observer, and an aperture size of 25 mm. We used these data to calculate Chroma and Hue angle values. After that, this sample and the sixth left rib were vacuum-packed and

frozen 72h post mortem at -20 ± 2 ºC until further analysis. The LM sample taken from the sixth right rib, once thawed at room temperature (22-23ºC), was used to determine intramuscular fat, protein, collagen, and water content by near infrared transmission technique using a FoodScan™ analyzer (Type 78800, FOSS, Hilleroed, Denmark).

Intramuscular Fatty Acid Profile

A subsample of 2 g from the right LM was used to determine the fatty acid profile of intramuscular fat. Fat was extracted as described by Folch et al. (1957). The subsample was homogenized in 100 ml of 2:1 (vol:vol) chloroform:methanol. After being agitated for 2h, the mixture was filtered and re-extracted twice in a separator funnel. The filtrate was mixed at a ratio of 2:5:1 with 10% NaCl (vol/vol) and 4mL and 2mL of internal standard (C13:0 and C19:0, respectively) to quantify individual fatty acids (FA). After being left overnight, the layer containing lipid in chloroform was decanted and dried in a rotary evaporator at 40 ºC. Chloroform remaining was evaporated with a N_2 stream. Fatty acids were separated and quantified as FA methyl esters (FAME) prepared using the AOAC (1990) method. The extracted fat was mixed with 2 mL of 2N KOH and 1 mL of 14% (wt/vol) boron trifluoride in methanol. The sample was methylated by incubation at 80ºC for 60 min and, after cooling to room temperature, was extracted with 5 mL of hexane and 2mL of 10%NaCl. The FAME in the hexane layer were analyzed by GC (5890 Series II GC, Hewlett Packard, S.A., Barcelona, Spain). All samples were methylated in duplicate, and 0.1 µL was introduced by split injection into a fused silica capillary column (30 m x ID 0.25 mm, BPX 70; 0.25-microm film thickness; VWR International Eurolab S.L., Llinars del Vallès, Barcelona, Spain). Hydrogen was the carrier gas at 41 cm/sec. Column temperature was initially 80ºC for 1 min, then increased by 3ºC per min to 210ºC, and

finally held at 215ºC for 10 min. Individual FAME were identified by retention time with reference to FAME MIX C4-C24 standards (N.18919-1AMP, Sigma Aldrich Co LLC, St Louis, MO). The *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA isomers were identified with reference to methyl esters of CLA (O-5507, Sigma-Aldrich, St. Louis, MO). The FA profile was expressed as g per 100 g of total FA, and FA content as g per 100 g of LM.

Cholesterol Analysis

In addition, another LM subsample of 0.750 g, also subjected to total lipid extraction by the procedure of Folch et al. (1957), was used to determine the cholesterol content using 1 mL of acetone:acetonitrile (40:60, v/v), and 250 µl of 5α-cholestane added to each sample as internal standard. Samples were saponified with 5.5 mL of KOH 11.5% in methanol (55:45, v/v) for 1 hour at 80ºC. After cooling to room temperature, 2 mL of hexane, 1.5 mL of NaCl 10% and 3 mL of ethanol were added. The tubs were vortexed for 2 min and left overnight. The upper phase was recovered (1 mL) and evaporated to dryness under a stream of nitrogen. After that, 1 mL of acetone:acetonitrile (40:60, v/v) was added. Cholesterol content was analyzed by HPLC with detection by refractive index (HPLC-IR, Waters 515, Waters Corporation, Milford, USA). The column used was the Agilent Poroshell 120 EC-C18 Threaded Column (Agilent, Santa Clara, USA).

Instrumental Texture

The sixth left ribs were also thawed for 24 h at 2 ± 2 ºC and lean, bone (including tendons and cartilage) and fat were dissected, and their respective weights were expressed as percentage of total rib weight. To determine the texture at 3 and 10 d of

227 ageing, Latissimus dorsi muscles were excised from the sixth right and left ribs.
228 Samples 2.5 cm thick were wrapped in aluminum foil and cooked in a convection oven
229 (Spider 5, Novosir, Spain), pre-heated at 200 °C, until reaching a core temperature of 71
230 °C, monitored with a data logger and a thermocouple probe (Comark, Oregon, USA)
231 inserted horizontally at the steak midpoint. We allowed steaks to cool, at room
232 temperature (22-23˚C), before five or six 1.27-cm-diameter cores were removed from
233 each steak parallel to the longitudinal orientation of the muscle fibers. All cores were
234 sheared perpendicular to the long axis of the core using a Texture Analyser TA.HD plus
235 (Stable Micro Systems Ltd., Surrey, UK) equipped with a Warner-Bratzler blade with
236 crosshead speed set at 2 mm/s. The maximum peak force (kg) was recorded and results
237 were expressed as the average of all sub-samples.
238
239 ***Sensory analyses***
240 To carry out the sensory analysis, samples of right rib LM aged 10 d were
241 thawed at 2 ± 2˚C for 36 h and cooked first in a double hot-plate grill and after in the
242 oven preheated to 200˚C until the final internal temperature reached 45 ^{o}C and 60 ^{o}C,
243 respectively, which was determined using individual thermocouples inserted into the
244 geometric center of each steak. Cooked steaks were trimmed of external fat and
245 connective tissue, then cut in 6 subsamples, wrapped individually in coded aluminum
246 foil using 3 random digits and were tested immediately. Two replicated sessions with 6
247 trained panelists were carried out in a sensory room (ISO 8589, 1988) equipped with
248 individual cabins and red lighting. Sample order was designed to avoid any first sample
249 and carry over effects (MacFie et al., 1989). Panelists evaluated beef in blind conditions
250 of 24 LM samples corresponding to the 2 diets and 10 d of ageing. They ate unsalted
251 toasted bread and drank mineral water to rinse their palate between samples. Panelists

evaluated each steak for tenderness, juiciness, chewiness, odor, flavor, and overall acceptability using a unipolar, semi-structured scale of 10 cm. Each line scale was suitably anchored on the left (0 cm = tender for toughness; easy to chew; dry for juiciness; none detectable for odor or taste intensity; and unacceptable for overall acceptability) as well as the right (10 cm = tough for toughness; difficult to chew; juicy for juiciness; pronounced for odor or taste intensity; and very desirable for overall acceptability). The data from each panelist were entered into a computer software program. Scores of individual panelists were averaged per treatment to obtain a single value for the statistical analysis.

Statistical Analyses

All data were screened for normality using the UNIVARIATE procedure of SAS (v. 9.3; SAS Institute Inc, Cary, NC, USA). For the statistical analyses, we considered pen to be the experimental unit. Daily means for intake were calculated as the average of 7 d in each experimental period and statistically analyzed using the MIXED procedure of SAS (v. 9.3; SAS Institute Inc., Cary, NC, USA). The model for intake and performance data contained the fixed effects of treatment and block, and random effect of pen. We included period as a repeated measure. In addition, the treatment x period and block x period interactions were also included in the model. The model for carcass data, meat quality and fatty acid profile contained the final BW as covariate, fixed effect of treatment, and random effect of pen except for sensory analysis, where panelists and replication were specified as a random effect. For categorical variables not normally distributed (fatness and conformation), we used rank transformation prior to the analysis. Analysis of rank-transformed data were analyzed by the Tukey adjust Multiple Comparisons test of the PROC GLM procedure of SAS (v. 9.3.; SAS Institute

Inc., Cary, NC, USA). Untransformed data are presented as Mean ± SE. Significance was declared at $P < 0.05$ and tendencies discussed at $P < 0.10$.

RESULTS

Performance

Initial BW was not different between diets but final BW was greater in heifers fed AH than BS ($P = 0.035$; Table 3). Average daily gain and average feed intake were affected by diet, being greater for AH than BS ($P = 0.036$ and $P = 0.049$, respectively). However, the average G:F ratio was unaffected by diet ($P > 0.10$; Table 3). Hot carcass weight and dressing percentage were not affected by diet ($P > 0.10$; Table 3). Conformation grade and fatness grade of carcasses were not different between treatments. Back fat color did not differ between diets ($P > 0.10$; Table 3).

Meat Quality

Meat color and pH of the meat at 24 h after slaughter were not different between diets ($P > 0.10$; Table 4). After dissection of the sixth right rib, the proportion of fat, lean and bone was not different between diets ($P > 0.10$; Table 4), being on average 22.1%, 55.8%, and 22.3 %, respectively. Meat composition in water, protein, collagen, intramuscular fat, and cholesterol was unaffected by diet ($P > 0.10$; Table 4).

Fatty Acid Profile and Fatty Acid Content of Intramuscular Fat

Fatty acid profile did not differ between diets except for C18:1 *n*-7 and C18:3 *n*-3 (Table 5). The proportion of C18:1 *n*-7 was greater in BS than in AH ($P = 0.016$), whereas the proportion of C18:3 *n*-3 tended to be greater in AH than in BS ($P = 0.09$). When fatty acid content was expressed as g per 100 g of LM (Table 6), these

differences detected between diets disappeared and diets only tended to differ in C15:0. The content of C15:0 tended to be greater in BS than in AH ($P = 0.08$).

Sensory Panel

Meat characteristics evaluated by trained panelists were not different between diets (Table 7). Meat samples did not differ in toughness, chewiness, juiciness, odor, taste and overall acceptability ($P > 0.10$). In addition, there were no differences between diets in Warner-Bratzler shear force values (WBSF) after 3 or 10 d of ageing ($P > 0.10$; Table 7).

DISCUSSION

Increasing forage proportion in high-concentrate finishing diets increases DMI (Bartle et al., 1994; Galyean and Defoor, 2003). Zinn (1986) evaluated three proportions of alfalfa hay (10, 15 and 20 %) fed to crossbred steers and found only a numerical increase in feed intake and weight gain. Net energy values were not different among diets in the study by Zinn (1986), suggesting a possible associative effect of forage level on nutrient utilization. Salinas-Chavira et al. (2013), working with Holstein steers, tested a steam-flaked corn-based diet containing 9.6 or 19.2 % (DM basis) of alfalfa hay, and did not detect any effect on DMI or weight gain, but feed efficiency tended to decrease with a greater proportion of alfalfa hay. However, other authors recommended not exceeding 10% (Hales et al., 2003) or 15% (Swanson et al., 2017) of forage in high concentrate finishing diets to avoid a decrease in DMI. The results obtained in the present experiment showed that the inclusion of alfalfa hay at 19% (DM basis) increased feed intake in comparison with the diet in which barley straw was supplied at 10% (DM basis). These results agree with those obtained by Madruga et al. (2018) with

beef heifers fed diets with 13 to 19 % of alfalfa hay. Increased DMI led to an increased ADG, although feed efficiency was unaffected. At slaughter, there were no differences between diets in HCW or dressing percentage, and carcasses did not show a different conformation grade or fatness grade.

Carotenoids provided by the diet are absorbed and deposited into adipose tissue (Yang et al., 1992). Since grains contain low level of carotenoids compared with forage, it is not surprising that the yellow pigmentation of fat declines as the amount of grain increases. However, Muir et al. (1998) stated that there was no significant effect of forage- or grain-based feeding systems on fat color in five of the nine experiments, as was the case between BS and AH in the present study.

Differences in meat pH values at 24 h post-mortem are mainly related to differences in muscle glycogen content at slaughter or to differences in stress susceptibility in pre-slaughter handling. Meat from steers fed grass-based diets have been found to present higher pH values than steers fed concentrate-based diets (French et al., 2000; del Campo et al., 2008). In the present experiment, however, in which transport and slaughter handling was the same for all animals involved, we detected no differences in meat pH suggesting that there were no differences in muscle glycogen at slaughter. This result is in agreement with those obtained by Leheska et al. (2008), comparing the effect of conventional and grass-feeding systems on meat pH, and by Arnett et al. (2012), working with Jersey steers fed steam-flaked, corn-based diets supplemented with 12 and 24 % forage (DM basis). In addition, meat pH was in the interval considered to be normal (between 5.4 and 5.8) for beef (Mach et al., 2006).

The study of the effect of diet on meat color has produced contradictory results. The LM muscle color of Angus-cross steers allotted to a pasture finishing system was darker (lower L*) than those fed a concentrate diet supplemented with 18% of corn

352 silage (Duckett et al., 2007). Other authors have also described darker-colored LM from
353 steers finished on forages vs. concentrates (Realini et al., 2004; Dunne et al., 2006;
354 Duckett et al. 2013). In addition, a redder meat has been related to forage-based diets
355 (Dunne et al., 2006), although the opposite has been reported by Duckett et al. (2007) or
356 with no relationship according to other authors (Realini et al., 2004; Kerth et al., 2007;
357 Duckett et al., 2013). With regard to the yellowness of the meat, LM b* values did not
358 differ between forage-based and concentrate-based diets (Realini et al., 2004; Duckett et
359 al., 2013), values were higher (Kerth et al., 2007, French et al., 2000) or lower (Dunne
360 et al., 2006; Duckett et al., 2007) in forage-based diets. On the contrary, and in
361 agreement with the results of the present experiment, other authors reported no effect on
362 meat lightness, redness and yellowness (Cerdeño et al., 2006; Blanco et al., 2010;
363 Arnett et al., 2012). Because both meat color and water-holding capacity are affected by
364 the acidification that takes place post-mortem (Warris, 2010), the absence of effects on
365 color found in the present experiment could be related to the fact that there were no
366 differences in final pH.
367 The proportions of muscle and bone tissues obtained after rib dissection are
368 usually greater in animals fed forage-based diets, whereas fat tissue is greater in
369 concentrate-based diets (Duckett et al., 2007 and 2013; Blanco et al., 2010). Cerdeño et
370 al. (2006) assessing the effect of finishing strategy on rib composition, did not find
371 differences in muscle and bone tissues when comparing Brown Swiss x Limousine bulls
372 fed concentrate and barley straw offered on ad libitum basis versus bulls fed 4 kg of
373 concentrate and alfalfa hay offered ad libitum. However, subcutaneous and
374 intermuscular fat were greater in animals fed the diet based on concentrate and barley
375 straw (Cerdeño et al., 2006). We did not find differences in any of the tissues dissected
376 from the 6^{th} rib. With regard to the chemical composition of LM, no differences were

377 recorded in moisture, protein and intramuscular fat (**IMF**). Similar results were reported
378 by French et al. (2000) and Arnett et al. (2012) when comparing animals fed forage-
379 based or concentrate-based diets. The lack of differences between diets in the
380 cholesterol and collagen content of the present study agrees with Leheska et al. (2008)
381 for cholesterol. However, Duckett et al. (2007) reported greater collagen for Angus-
382 cross steers allotted to pasture than those fed a high-concentrate diet.
383 Due to the amount and composition of their fatty acids, forages can help
384 improve the nutritional quality of meat (Glasser et al., 2013), because plants are the
385 primary source of *n*-3 PUFA (Dewhurst et al., 2006). Feeding grass increases the
386 content of linolenic, eicosapentanoic and docasahexanoic acids in beef muscle and
387 adipose tissue, resulting in a lower *n*-6:*n*-3 ratio (Scollan et al., 2006). Although we
388 found a tendency for a greater proportion of C18:3 *n*-3 in the AH diet, this effect
389 disappeared when the amount of this FA in 100 g of muscle was calculated. It is known
390 that haymaking induced a slight decrease in total fat and C18:3 *n*-3 (Glasser et al.,
391 2013). This finding, together with the particular proportion of alfalfa hay included in
392 our AH diet, could explain the limited differences between diets in the FA profile and
393 FA content of the IMF. In addition, increasing the forage to concentrate ratio resulted in
394 a linear decrease in the concentration of SFA, and a linear increase in PUFA:SFA ratio
395 (Woods and Fearon, 2009). Although in the present experiment this ratio changed from
396 10 to 90 in the BS diet to 19 to 81 in AH, this change was insufficient to cause these
397 effects.
398 Kerth et al. (2007) reported that the meat from steers grazing on ryegrass was
399 less tender, juicy, flavorful, and with a lesser acceptability score than meat from steers
400 fed a diet containing 85% corn, 7.5% cottonseed and 7.5% of a commercial premix.
401 However, there is abundant literature where meat quality from animals fed forage-based

diets did not differ from animals fed concentrate-based diets (French 2000; Cerdeño; Arnett 2012), as occurred in the present experiment. In addition to the analysis made by the trained sensory panel, the instrumental tenderness evaluation also confirmed that there was no difference between diets in the WBSF values recorded. These WBSF values, obtained 3 d and 10 d post-mortem, were below the threshold of 4.6 kg proposed by Schackelford et al. (1991) to consider beef meat tender.

In conclusion, alfalfa hay as forage source for finishing heifer diets offered as TMR at 19% of inclusion allowed greater feed intake and ADG than diets using barley straw at 90:10 of concentrate:forage ratio without affecting G:F ratio. However, this level of forage inclusion was not sufficient to cause any relevant change in the carcass and meat quality of the heifers fed this more forage-based diet in which in addition, barley straw was replaced by alfalfa hay.

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544 **Table 1.** Ingredients and chemical composition of the diets

Item	Diets[1]	
	BS	AH
Ingredient composition, % of DM		
Barley straw	10.0	-
Alfalfa hay	-	19.0
Corn, ground	35.0	41.5
Barley, ground	43.0	31.5
Soybean meal, 44%CP	9.0	5.0
Salt	0.7	0.7
Sodium bicarbonate	1.0	1.0
Calcium carbonate	0.5	0.5
Dicalcium phosphate	0.4	0.4
Vitamin-mineral premix[2]	0.4	0.4
Chemical composition, % DM		
CP	12.0	13.0
NDF	23.8	21.2
ADF	7.7	8.8
Ether extract	2.0	2.0
Ash	4.8	7.5
NFC[3]	57.4	56.3
ME[4], Mcal/kg of DM	2.83	2.81

545 [1] BS = total mixed ration with 10% of barley straw; AH = total mixed ration with 19%

546 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 + EE)

[4]According to NRC (2000)

554 **Table 2.** Fatty acid profile of the diets

Fatty acid	Diets[1]	
	BS	AH
	----------g/100 g of fatty acid methylesters[2]----------	
16:0	17.42	16.68
18:0	2.29	2.17
18:1, *cis*-9	21.58	22.57
18:2, *cis*-9, *cis*-12	51.60	50.66
18:3, *cis*-9, *cis*-12, *cis*-15	4.21	4.65
SFA[3]	21.85	20.65
MUFA[4]	22.00	23.10
PUFA[5]	54.95	55.30

555 [1] BS = total mixed ration with 10% of barley straw; AH = total mixed ration with 19%

556 of alfalfa hay

557 [2]Only fatty acids with a proportion greater than 1 g/100 g have been included

558 [3]SFA = ∑ C12:0, C13:0, C14:0, C16:0, C17:00, C18:0, C20:0, C22:0, C24:0

559 [4]MUFA = ∑ C16:1, C17:1, C18:1 *n*-9, C18:1 *n*-7, C20:1 *n*-9, C22:1

560 [5]PUFA = ∑ C18:2 *n*-6, C18:3 *n*-3, C20:2 *n*-6

561 **Table 3.** Live weight, average daily gain (ADG), feed intake, gain to feed ratio, and
562 carcass characteristics of beef heifers fed 10% barley straw or 19% alfalfa hay

Item	Diets[1]		SEM	*P*-value
	BS	AH		
Performance variables				
Initial BW, kg	234.0	237.2	2.31	0.345
Final BW, kg	364.3	383.9	6.13	0.035
ADG, kg/d	1.29	1.45	0.051	0.036
Feed intake, kg/d	8.40	9.51	0.392	0.049
Gain to feed ratio, kg/kg	0.15	0.17	0.013	0.632
Carcass characteristics				
HCW, kg	212.0	217.1	4.42	0.292
Dressing percent	53.5	52.9	0.56	0.535
Conformation grade[2]	3.0 ± 0.0[3]	2.9 ± 0.08		0.285
Fatness grade[4]	2.9 ± 0.08	2.8 ± 0.11		0.505
Backfat color				
Lightness (L*)	71.0	68.4	1.62	0.702
Redness (a*)	4.8	4.3	0.30	0.343
Yellowness (b*)	12.1	10.8	0.51	0.377
Chroma	13.1	11.7	0.55	0.344
Hue angle	1.2	1.2	0.02	0.830

563 [1] BS = total mixed ration with 10% of barley straw; AH = total mixed ration with 19%
564 of alfalfa hay
565 [2]Conformation grade: 6 = Superior; 5 = Excellent; 4 = Very good; 3 = Good; 2 = Fair; 1
566 = Poor

[3]Mean ± standard error

[4]Fatness grade: 1 = Low; 2 = Slight; 3 = Average; 4 = High; 5 = Very high

570 **Table 4** Meat quality of beef heifers fed 10% barley straw (BS) or 19% alfalfa hay

571 (AH)

Item	Diet		SEM	*P*-value
	BS	AH		
Longissimus muscle				
pH	5.47	5.46	0.033	0.868
Color				
Lightness (L*)	36.5	35.4	1.25	0.561
Redness (a*)	14.4	15.0	0.45	0.375
Yellowness (b*)	12.2	12.3	0.31	0.738
Chroma	18.8	19.4	0.35	0.311
Hue angle	0.70	0.69	0.020	0.605
6^{th} rib dissection, %				
Fat	23.5	21.1	1.42	0.326
Lean	53.8	56.9	3.25	0.555
Bone	22.7	22.0	1.96	0.817
Meat composition				
Water, %	71.9	71.3	0.29	0.180
Protein, %	22.6	22.4	0.19	0.550
Collagen, %	1.34	1.42	0.040	0.189
Intramuscular fat, %	4.34	5.01	0.386	0.235
Cholesterol, mg/100g	61.6	61.2	2.63	0.920

572

573 **Table 5.** Fatty acid profile of the LM of beef heifers fed 10% barley straw (BS) or 19%

574 alfalfa hay (AH)

Item	Diet		SEM	*P*- value
	BS	AH		
	----g/100 g total fatty acids----			
C14:0	2.29	2.57	0.151	0.199
C14:1	0.43	0.54	0.061	0.284
C15:0	0.44	0.39	0.030	0.355
C16:0	23.83	25.50	0.551	0.124
C16:1	2.92	3.16	0.145	0.283
C17:0	1.92	1.45	0.353	0.100
C17:1	1.04	0.89	0.066	0.210
C18:0	16.78	16.58	0.543	0.797
C18:1 *trans*-9	0.94	0.94	0.015	0.951
C18:1 *trans*-11	2.50	2.06	0.307	0.333
C18:1 *n*-9	38.04	36.89	0.689	0.257
C18:1 *n*-7	2.28	2.07	0.055	0.016
C18:2 *n*-6	4.54	4.75	0.269	0.638
C18:3 n-6	0.12	0.12	0.029	0.814
C18:3 *n*-3	0.23	0.28	0.017	0.090
C20:0	0.24	0.25	0.015	0.678
CLA *cis*-9 *trans*-11	0.22	0.23	0.022	0.920
C20:3 *n*-6	0.41	0.43	0.036	0.641
C20:4 *n*-6	1.09	1.03	0.111	0.724
C22:2	0.26	0.11	0.092	0.280

SFA[1]	44.54	46.12	0.777	0.170
MUFA[2]	44.71	45.55	0.728	0.275
PUFA[3]	6.53	6.59	0.386	0.908
PUFA:SFA	0.15	0.14	0.009	0.755
n-6:*n*-3	27.93	24.63	2.260	0.314

575 [1]SFA = ∑ C14:0, C15:0, C16:0, C17:00, C18:0, C20:0

576 [2]MUFA = ∑ C14:1, C16:1, C17:1, C18:1 *trans-9*, C18:1 *trans-11*, C18:1 *n*-9, C18:1 *n*-7

577 [3]PUFA = ∑ CLA *cis-9 trans-11*, C22:2; *n*-6 = C18:2 *n*-6, C18:3 *n*-6, C20:3 *n*-6, C20:4

578 *n*-6; *n*-3 = C18:3 *n*-3

579 **Table 6.** Fatty acid content of the Longissimus muscle (LM) of beef heifers fed 10%

580 barley straw (BS) or 19% alfalfa hay (AH)

Item	Diet		SEM	*P*- value
	BS	AH		
	----g/100 g of LM----			
C14:0	0.38	0.42	0.019	0.168
C14:1	0.07	0.09	0.007	0.100
C15:0	0.07	0.06	0.004	0.080
C16:0	3.94	4.20	0.225	0.450
C16:1	0.48	0.53	0.034	0.372
C17:0	0.32	0.24	0.075	0.166
C17:1	0.17	0.15	0.014	0.302
C18:0	2.77	2.72	0.195	0.832
C18:1 *trans*-9	0.16	0.16	0.010	0.994
C18:1 *trans*-11	0.43	0.34	0.055	0.305
C18:1 *n*-9	6.28	6.20	0.479	0.914
C18:1 *n*-7	0.38	0.35	0.028	0.456
C18:2 n-6	0.76	0.79	0.077	0.765
C18:3 *n*-6	0.02	0.02	0.004	0.708
C18:3 *n*-3	0.04	0.05	0.003	0.158
C20:0	0.04	0.04	0.003	0.812
CLA *cis*-9 *trans*-11	0.04	0.04	0.005	0.976
C20:3 *n*-6	0.07	0.07	0.007	0.732
C20:4 *n*-6	0.19	0.18	0.031	0.882
C22:2	0.04	0.02	0.013	0.284

SFA[1]	7.45	7.63	0.435	0.775
MUFA[2]	7.54	7.47	0.557	0.933
PUFA[3]	1.57	1.50	0.143	0.713
PUFA:SFA	0.21	0.20	0.013	0.482
n-6:n-3	27.80	24.78	2.228	0.354

581 [1]SFA = ∑ C14:0, C15:0, C16:0, C17:00, C18:0, C20:0

582 [2]MUFA = ∑ C14:1, C16:1, C17:1, C18:1 *trans-9*, C18:1 *trans-11*, C18:1 *n*-9, C18:1 *n*-7

583 [3]PUFA = ∑ CLA *cis-9 trans-11*, C22:2; *n*-6 = C18:2 *n*-6, C18:3 *n*-6, C20:3 *n*-6, C20:4

584 *n*-6; *n*-3 = C18:3 *n*-3

585

586 **Table 7.** Least squares means for trained sensory panel on LM and Warner-Bratzler
587 shear force (kg) of *Latissimus dorsi* muscle of beef heifers fed 10% barley straw (BS) or
588 19% alfalfa hay (AH)

	Diets			
Item	10BS	19AH	SEM	*P*-value
Toughness	3.99	3.81	0.266	0.643
Chewiness	4.74	4.44	0.465	0.685
Juiciness	5.05	5.24	0.499	0.646
Beef odor	4.65	3.58	0.873	0.447
Blood odor	1.48	1.68	0.190	0.457
Fat odor	2.66	2.73	0.238	0.850
Beef flavor	5.01	4.72	0.246	0.469
Fat flavor	2.42	2.49	0.211	0.823
Liver flavor	2.35	2.30	0.271	0.922
Acid flavor	3.05	2.84	0.242	0.547
Overall acceptability	4.49	4.91	0.208	0.251
WBSF[1], kg				
3 d post-mortem	4.40	4.28	0.198	0.684
10 d post-mortem	4.10	4.01	0.204	0.786

589 [1]Warner-Bratzler shear force