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RESEARCH ARTICLE

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Performance, carcass and ruminal fermentation characteristics of heifers fed concentrates differing in energy level and cereal type (corn *vs.* wheat)

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Abstract

A total of 144 beef heifers (218 ± 26.4 kg body weight) were housed in 24 pens (6 animals each) and used in a 168-day feedlot study to evaluate the influence of cereal type and energy level on performance, carcass quality and ruminal fermentation. Four concentrates were formulated according to a 2×2 factorial arrangement of treatments, with two energy levels (1,452 *vs.* 1,700 kcal net energy/kg) and two main cereals (wheat *vs.* corn). Concentrate and straw were offered *ad libitum*. Concentrate intake and body weight were recorded on days 42, 84, 126 and 168. Ruminal fluid was obtained by ruminocentesis from 3 heifers per pen on days 1, 84 and 168; and carcass weight, classification and yield, were determined in the same animals. Heifers fed high-energy diets had lower intake (6.97 *vs.* 7.29 kg fresh matter/d; p=0.011), and lower concentrate to gain ratio (5.15 *vs.* 5.66 kg/kg; p=0.002) than those fed low energy concentrates, and tended (p=0.069) to be heavier along the time. Neither carcass yield and classification, nor ruminal pH, volatile fatty acids nor NH_3-N concentrations were affected (p>0.050) by energy level. Total volatile fatty acids concentration tended (p=0.070) to be greater in heifers fed corn-based than wheat-based concentrates. No energy level x cereal type interactions were observed. These results indicate that high energy concentrates decreased feed intake and feed conversion but had minor effects on carcass performance. Cereal type had no effects on performance and ruminal fermentation and no interactions between cereal type and energy were detected.

Additional keywords: beef cattle; ruminal pH; volatile fatty acids; *in vitro* fermentation.

Abbreviations used: ADG (average daily gain); BW (body weight); cADI (concentrate average daily intake); DM (dry matter); F:G (concentrate to gain ratio); FM (fresh matter); NDF (neutral detergent fibre); NE (net energy); SARA (subacute ruminal acidosis); VFA (volatile fatty acids).

Authors' contributions: Conceived and designed the experiments: CC, PM, AF and MDC. Performed the experiments and contributed reagents/materials/analysis tools: CC, AF and MDC. Analyzed the data: CC and PM. Wrote the paper: CC, PM and MDC.

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Introduction

A large part of Spanish beef production is based on feeding *ad libitum* cereal concentrates and straw, with the concentrate reaching over 85% of total dry matter intake (Gimeno *et al.*, 2014; Verdú *et al.*, 2015). High-concentrate diets favour beef growth, but an excessive amount of rapidly fermented starch in the rumen could result on subacute ruminal acidosis (SARA), which is commonly stated as a reduction in ruminal pH below 5.8, mainly due to volatile fatty acids (VFA) and lactate accumulation in the rumen (Calsamiglia *et al.*, 2012).

The structure and composition of cereal starches and the physical interactions between starch and protein can influence the digestibility and feeding value of cereal grains for ruminants (Rooney & Pflugfelder, 1986). Ruminal degradation of starch from corn grain is limited by its vitreousness, which reflects the association between starch and protein in the endosperm. In the vitreous endosperm, starch granules are surrounded by proteins and embedded in a dense matrix that limits the accessibility of microbial enzymes to starch (Corona *et al.*, 2006). In contrast, the protein matrix of wheat is more diffuse and does not hamper the access of ruminal

microbes to starch granules (McAllister *et al.*, 2006). Although the endosperm in different wheat types differs in hardness, all wheat types are digested more rapidly than corn in the rumen (Yang *et al.*, 2014), and greater *in situ* disappearance rates and theoretical degradability values for wheat compared to corn have been reported (0.302 *vs.* 0.058%/h and 90.1 *vs.* 58.4%, respectively; Bacha, 1991).

Therefore, a high proportion of wheat in the concentrate could favour SARA incidence compared with corn, especially in diets with low effective neutral detergent fibre (NDF) content (Owens *et al.*, 1997). However, most of scientific research to evaluate the dietary effects of corn and wheat has been carried out in dairy cows (reviewed by Ferraretto *et al.*, 2013). Some comparative studies have also been conducted with beef cattle but, in most of them, the concentrates also included monensin, which is currently banned in Europe and can mask the effects of different cereals on ruminal fermentation (Yang *et al.*, 2014). In addition, the response of beef cattle to corn and wheat-based concentrates, as measured by feedlot performance, may be influenced by the rest of the ingredients in the diet, which, in turn, determine its energy level. Our hypothesis is that the use of wheat instead of corn in high energy diets, with low NDF content and without monensin, may increase the risk of SARA and have an impact on growth performance and ruminal fermentation, whereas the effect of cereal type could be insignificant in low energy diets.

Therefore, the objective of the current study was to assess the interaction of cereal type (corn *vs.* wheat) and energy level of the diet on growth performance, carcass quality and ruminal fermentation characteristics of beef cattle.

Material and methods

The experimental protocols were approved by the institutional Animal Care Committee of the Technical University of Madrid (Madrid, Spain), and the study was conducted in accordance with the Spanish guidelines for experimental animal protection (BOE, 2013).

Animals, diets and experimental design

A total of 144 Charolaise cross heifers were used. Upon arrival to the experimental farm (Comercial Pecuaria Segoviana SL, Coca, Spain), each animal was treated for endo- and ecto-parasites with ivermectin (1 mL per 50 kg body weight (BW), Ivomec, Merial Laboratorios SA, Barcelona) and vaccinated against infectious bovine rhinotracheitis, parainflueza-3 and bovine viral diarrhoea (2 mL per animal, Cattlemaster-4, Zoetis Spain SLU, Madrid), enterotoxemia and carbuncle (2 mL per animal, Miloxan, Merial Laboratorios SA, Barcelona, Spain). A booster dose was given 3 weeks later.

Before starting the trial, all heifers were fed an adaptation diet consisting of concentrate and barley straw for 7 days. Concentrate was provided twice a day, and the amount provided was recorded. Fresh water and straw was freely accessible at all times. The main ingredients in the concentrate were sunflower seed, olive pulp, soybean hulls, corn grain, corn distiller's dried grains with solubles (DDGS), barley straw pelleted, sugar beet pulp, rice bran, soybean meal, palm kernel meal, peas meal, calcium carbonate, sepiolite, wheat middling, sodium chloride and animal fat (189, 100, 100, 100, 100, 100, 70, 60, 50, 30, 28, 25, 24, 15, 7 and 2 g/kg fresh matter (FM), respectively). Calculated chemical composition based on FEDNA (de Blas *et al.*, 2010) was 88.0, 16.9, 38.6 and 4.9% of organic matter, crude protein, neutral detergent fibre and starch, respectively (dry matter (DM) basis).

At the beginning of the experimental period, heifers were weighted and assigned to 24 pens (6 heifers each) according to their BW (average 218 ± 26.4 kg). The pens were 5.0 × 7.0 m, had continuous concrete floor, and barley straw was used as bedding material. Pens were randomly allotted to one of the four experimental treatments (6 pens per treatment) following a totally randomized experimental design. Four concentrates were formulated following NRC (2000) requirements for growing/finishing heifers of 350 kg BW and 1.5 kg average daily gain (ADG) according to a 2 × 2 factorial arrangement of treatments. The concentrates had energy levels of 1,452 kcal of net energy (NE)/kg (low energy; 713 and 739 kcal of NE for maintenance and growth per kg, respectively) or 1,700 kcal NE/kg (high energy; 835 and 865 kcal of NE for maintenance and growth per kg, respectively), and the main cereal type was either corn or wheat. Ingredient and chemical composition of the concentrates are shown in Table 1. Feed ingredients were ground (3 mm diameter pore sieve) and particle size was determined by dry sieving of a 400 g representative sample of each concentrate before pelleting. Samples were passed through sieves of 4.76, 2.36, 1.40, 0.80, 0.50 and 0.30 mm, and the amount of particles retained on each screen size was calculated.

Concentrate and full-length barley straw were provided separately *ad libitum* twice a day (0700 and 1800 h), and their supply was recorded daily throughout the trial. Concentrates were pelleted (3.5 mm diameter) to reduce feed selection and refusals, and supplied into

Table 1. Ingredients, chemical composition, durability and particle size distribution of the experimental concentrates[1]

	High corn		High wheat	
	Low energy	**High energy**	**Low energy**	**High energy**
Ingredients, g/kg as fed basis				
Wheat grain	-	-	405	405
Barley grain	100	100	105	105
Corn grain	400	400	-	-
Wheat middlings	156	65.0	160	65.0
Sunflower seed	96.0	76.1	96.0	80.0
Soybean meal (47% CP)	74.9	59.2	50.7	32.6
Palm kernel meal	63.5	80.0	65.0	80.0
Barley straw[2]	62.7	35.6	64.8	35.6
Peas meal	-	120	-	125
Sepiolite	20.0	-	20.0	-
Calcium carbonate	19.3	11.0	19.4	11.3
Oleine[3]	-	14.8	8.8	15.0
Calcium soap[4]	-	30.0	-	30.0
Fat, rumen inert[5]	-	-	-	9.4
Monocalcium phosphate	2.6	2.2	-	-
Sodium chloride	2.9	3.6	2.8	3.6
Mineral/vitamin premix[6]	2.5	2.5	2.5	2.5
Chemical composition[7]				
Dry matter (g/kg)	878 (5.3)	895 (3.2)	888 (9.2)	900 (1.5)
Organic matter (g/kg DM)	930 (5.2)	934 (5.2)	931 (6.6)	943 (7.8)
Crude protein (g/kg DM)	170 (9.1)	167 (7.9)	171 (4.5)	177 (4.9)
Ether extract (g/kg DM)	35.8 (4.6)	74.1 (4.9)	36.3 (13.9)	75.1 (15.4)
Neutral detergent fibre (g/kg DM)	241 (12.9)	216 (9.5)	250 (8.7)	222 (7.9)
Starch (g/kg DM)	395 (18.8)	403 (29.3)	383 (5.9)	390 (17.5)
Durability	97.97 (0.47)	98.28 (0.21)	98.25 (0.41)	98.41 (0.18)
Particle size (%)[8]				
> 4.76 mm	0.87	0.37	1.62	0.87
2.37 – 4.76 mm	1.50	1.24	1.24	1.25
1.41 – 2.36 mm	8.50	11.41	12.22	13.00
1.01 – 1.40 mm	17.75	15.90	18.42	18.50
0.81 – 1.00 mm	8.25	11.06	8.97	11.00
0.51 – 0.80 mm	18.88	20.25	16.70	19.13
0.31 – 0.50 mm	22.87	22.38	18.68	21.12
0.00 – 0.30 mm	21.38	17.39	22.15	15.13
Total > 1 mm	28.62	28.92	33.50	33.62
Total < 1 mm	71.38	71.08	66.50	66.38

[1]Low energy: 1,453 kcal NE/kg; High energy: 1,700 kcal NE/kg [fresh matter basis, according to FEDNA (de Blas *et al.*, 2010)]. [2]A product based on barley straw treated with NaOH and pelleted. [3]A product based on olive soapstock (Refinación Industrial Oleícola SA, Ibros, Spain): Contained 980 g of ether extract/kg DM. Fatty acid profile (g/kg ether extract): 640 of C18:1, 160 of C18:2, 110 of C16:0, and 38 of C18:0. [4]Mixed fat calcium soap (Comercial Pecuaria Segoviana SL, Coca, Spain): Contained 850 g of ether extract/kg DM. Fatty acid profile (g/kg ether extract): 475 of C16:0, 370 of C18:1, 85 of C18:2 and 45 of C18:0. Ca content: 90 g/kg of DM. [5]Hydrogenated fat (Mateos SL, Cabezón de Pisuerga, Spain) containing 985 g of ether extract/kg of DM. Fatty acid profile (g/kg of ether extract): 470 of C16:0, 430 of C18:0 and 80 of C18:1. [6]Mineral and vitamin premix (Comercial Pecuaria Segoviana SL, Segovia, Spain) contained: 8,000 IU of vitamin A (retinyl acetate), 1,600 IU of vitamin D_3 (cholecalciferol), 6,000 IU of vitamin E (α-tocopheryl acetate), 7.9 mg of Cu (copper sulfate), 0.7 mg of Co (cobalt (II) sulfate), 0.2 mg of Se (sodium selenite), 50 mg of Zn (zinc oxide), 35 mg of Mn (manganese oxide), 438 mg of Mg (magnesium oxide), 100 mg of S (potassium sulfate), 20 mg of Fe (iron sulfate) and 0.8 mg of I (potassium iodide). [7]Mean values (± standard deviation) of five samples. [8]Particle size of ground feed ingredients before pelleting.

a 1.5 × 0.2 m line feed bunk. Concentrate supply was controlled by registering the number of packs provided to each pen per day. In addition, feed refusals were observed daily at feeding time to adjust the amount supplied to allow for 5% refusals, and orts were removed and weighed when any spoilage was detected. Straw was offered in a separate feed bunk. Straw was provided equally to each pen, adjusted daily at feeding time to allow for 5% average refusals in the feeder. However, some straw refusals dropped on the floor and formed part of bedding material. Therefore, the actual straw intake per pen could not be quantified.

Data collection and carcass measurements

On days 42, 84, 126 and 168, all animals were individually weighed and concentrate refusals in each pen were determined to calculate the following productive variables: ADG (kg/d), concentrate average daily intake (cADI, kg FM/d) and concentrate to gain ratio (F:G).

On day 168 of trial, animals were slaughtered at a commercial slaughterhouse (MACRISA Matadero de Castilla Rioseco SA; Medina de Rioseco, Valladolid, Spain). Three heifers per pen, which had been ruminally sampled as described below, were selected to determine individual hot carcass weight and carcass characteristics. The dressing percentage was calculated as the relationship between hot carcass weight and BW at 168 days of trial. Carcass compactness index was calculated as the relationship between carcass length and chest depth. Carcass length was determined on the hanged hot left mid-carcass as the length from the cranial border of the first rib to the point of the pubic symphysis, and chest depth was measured from the ventral surface of the spinal canal (at fifth rib level) to the lowest point of the sternum. Leg compactness index was calculated as the relationship between leg length and perimeter. Leg length was measured from the inner side of the tarsus-metatarsus joint to the point of the pubis symphysis, and leg perimeter was measured at the level of the crest of the ileum. Carcass conformation was determined following the European carcass grading system (OJEU, 2006) according to the following conformation classes: S (superior), E (excellent), U (very good), R (good), O (fair) and P (poor), and to five degrees of fat cover: 1 (poor), 2 (slight), 3 (average), 4 (high) and 5 (very high).

The procedure used to evaluate rumen papillae characteristics has been described by Carrasco *et al.* (2012). Briefly, a 30 × 30 cm rumen wall sample was collected from the ventral area after evisceration and rumen content evacuation. Ventral area of the rumen was identified using the esophagus and spleen as physical references. Samples were stored at 5ºC until visual evaluation within 4 h after slaughtering. Rumen samples were washed with saline solution (85 g NaCl/L) and displayed on a white surface under an intense and homogeneous light to evaluate their colour (1, pale; 2, pale-pink; 3, pink-green; 4, green; 5, dark green) and papilla length (1, very short; 2, short; 3, medium; 4, long). The evaluation was performed by four trained judges, and the average score was used for the statistical analysis.

Ruminal fermentation characteristics

On days 1, 84 and 168, individual ruminal fluid samples of three representative heifers per pen (heifers having maximum and minimum initial BW were excluded) were obtained between 2 and 3 hours after morning feeding by rumenocentesis. A needle of 12.5 cm long and 1.6 mm diameter was inserted into the ventral sac of the rumen in the centre of the triangle between last rib, wing of ileum and transverse process of spine, and an aliquot (about 20 mL) of rumen fluid was obtained. The time of feed supply was adjusted to the expected time of ruminal fluid sampling, and the pens of each treatment were consistently distributed across the collection period. Ruminal samples were homogenized, the pH was immediately measured (Crison Basic 20, Crisson Instruments, Barcelona), and fermentation was stopped by swirling the samples in iced water. Five millilitres of fluid were then added to 5 mL of deproteinizing solution (20 g of metaphosphoric acid and 0.6 g of crotonic acid per L) for VFA analysis and 2 mL were added to 2 mL of 0.5 M HCl for NH_3-N and lactate determination. Samples were immediately frozen (-20ºC) until analyses. Unfortunately, lactate analyses could not be performed due to a problem during the handling of samples.

In vitro fermentation of concentrates

Five samples of each experimental concentrates were collected throughout the experiment. They were mixed within each experimental treatment, ground through a 1 mm pore sieve and weighed (400 mg DM) into 120 mL serum bottles. Ruminal fluids from other eight heifers fed high-grain concentrates (60% cereals, being a mixture of barley, wheat and corn) and straw were taken in pre-warmed thermal flasks and immediately transported to the laboratory. The contents of randomly selected pairs of heifers were mixed, and strained through four layers of cheesecloth into an Erlenmeyer flask with an O_2-free headspace to obtain four ruminal fluids. Particle-free fluid was mixed with the buffer solution (no trypticase added) of Goering &

Van Soest (1970) in a proportion 1:4 (v/v) at 39ºC under continuous flushing with CO_2. Four bottles with each concentrate and eight bottles without substrate (blanks; two per inoculum) were incubated. Forty millilitres of buffered rumen fluid were added into each bottle under CO_2 flushing. Bottles were sealed with rubber stoppers and aluminium caps and incubated at 39ºC. After 17 h of inoculation (corresponding to a rumen passage rate of 0.06/h), total gas production was measured using a pressure transducer (HD2304.0 pressure gauge, DELTA OHM, Italy) and a plastic syringe. Bottles were then uncapped and the pH was measured immediately with a pH meter (Crison Basic 20, Crisson Instruments, Barcelona). Fermentation was stopped by swirling the bottles in ice, and the contents of the bottles were sampled for VFA, NH_3–N and lactate analysis as described above.

Analytical procedures

Dry matter (ID 934.01), ash (ID 942.05), nitrogen (ID 984.13), starch (ID 920.40) and ether extract (ID 920.39) content of concentrates were determined (n=5) according to the AOAC (1999). Neutral detergent fibre analyses were carried out according to Van Soest *et al.* (1991) using an ANKOM^{220} Fibre Analyzer unit (ANKOM Technology Corporation, Fairport, NY, USA). Sodium sulphite and heat-stable amylase were used in the analysis of NDF and results were expressed inclusive of residual ash.

Durability was measured using a durabilimeter (Mabrik, Barcelona). A sample of 500 g of pellets was inserted. After tumbled for 10 min, the pellets were passed through a 1.4 mm pore sieve, and the amount of particles retained measured. Durability was expressed as the ratio of retained particles to the sample weight before tumbling, multiplied by 100.

Samples of ruminal fluid were defrosted at 4ºC, centrifuged (13,000 × g, 20 min, 4ºC) and the supernatant used for analyses. Analysis of VFA were performed as described by Carro *et al.* (1999) using a Shimadzu GC 2010 gas chromatography (Shimadzu Europa GmbH, Duisburg, Germany) heated at 150ºC and equipped with a flame ionization detector and a TR-FFAP column (30 m × 0.53 mm × 1 µm; Supelco, Madrid). Helium and crotonic acid were used as carrier gas and internal standard, respectively, and peaks were identified by comparison with standards of known individual VFA concentrations. Total lactate and NH_3-N concentrations were determined by the colorimetric methods described by Taylor (1996) and Weatherburn (1967), respectively.

Statistical analysis

The experimental unit for BW, ADG, cADI and F:G measurements was the pen (averaged data for 6 heifers). Data were analysed as Repeated Measures procedure within General Lineal Model of IBM SPSS Statistics v. 19. A compound symmetry was used to model the covariance structure for the repeated measures. The statistical model included cereal type (corn *vs.* wheat), energy level (low *vs*. high), time (sampling day) and their interactions as main effects, and initial BW was used as a covariate. Data on ruminal characteristics were analysed with the same model, using the pen as the experimental unit (averaged data for 3 heifers) and excluding the BW as a covariate.

The experimental unit for carcass weight, dressing percentage and carcass characteristics (carcass compactness index and leg compactness index) was also the pen (averaged data of 3 heifers). Data were analysed through an ANOVA. The statistical model included cereal type, energy level, and the cereal type x energy level interaction as main effects, and final body weight as a covariate. Regardless the experimental unit was the pen, carcass quality data (quality and fat cover grade) and rumen colour and papillae length scores the experimental unit was the individual heifer as they were discrete variables, and were analysed using the Chi-square independence test for categorical variables of SPSS.

In vitro data were analysed as a mixed model (n=4), with cereal type, energy level, and the cereal type × energy level interaction as fixed effects, and inoculum as a random effect. For all statistical analyses, significance was declared at $p<0.050$, and $0.050<p<0.10$ values were considered to be a trend.

Results

Intake and growth performance

Chemical analysed composition, durability and particle size of experimental concentrates are shown in Table 1. The effect of treatments on performance is shown in Table 2. Cereal type did not affect ($p>0.100$) BW, ADG or cADI, but heifers fed corn tended to have greater F:G ratio than those fed wheat (5.52 *vs*. 5.28 kg/kg, respectively; $p=0.089$). The energy level did not affect BW or ADG, but heifers fed the high-energy concentrates had lower cADI ($p=0.011$) and F:G ratio ($p=0.002$) than those fed the low-energy ones (6.97 *vs*. 7.29 kg/d, and 5.14 *vs*. 5.66 kg/kg, respectively). No cereal type × energy level interactions ($p>0.100$) were detected for any of these variables. Although the actual intake of straw could not be quantified, the amount of straw supplied per pen was registered and there were no differences among experimental treatments.

As expected, time significantly affected BW ($p=0.010$), but ADG, cADI and F:G ratio were not affected by time

Table 2. Effect of cereal type (CT) and energy level (EL) in the concentrate and time on body weight (BW), average daily gain (ADG), concentrate average daily intake (cADI) and feed:gain ratio (F:G) of heifers during the experimental period[1]

Item and period (days of feed)	Corn		Wheat		SEM[2]	*p*-value					
	Low energy	High energy	Low energy	High energy		CT	EL	CT × EL	Time	CT × time	EL × time
BW (kg)											
0	214	215	215	214							
42	273	270	269	271	7.1	0.978	0.364	0.971	0.010	0.691	0.069
84	336	338	335	335							
126	387	395	390	394							
168	440	449	442	452							
ADG (kg/d)											
0–42	1.39	1.33	1.31	1.36	0.065	0.731	0.199	0.979	0.286	0.722	0.629
43–84	1.49	1.62	1.56	1.52							
85–126	1.23	1.36	1.32	1.39							
127–168	1.15	1.18	1.13	1.28							
cADI (kg FM/d)											
0–42	5.88	5.60	5.57	5.48	0.173	0.130	0.011	0.728	0.107	0.299	0.022
43–84	7.47	7.32	7.24	6.94							
85–126	7.82	7.72	7.96	7.46							
127–168	8.26	7.64	8.12	7.56							
F:G (kg/kg)											
0–42	4.26	4.27	4.27	4.06	0.207	0.089	0.002	0.830	0.281	0.859	0.097
43–84	5.02	4.55	4.66	4.56							
85–126	6.50	5.71	6.05	5.39							
127–168	7.26	6.58	7.23	6.04							

[1] Low energy: 1,452 kcal NE/kg; High energy: 1,700 kcal NE/kg (fresh matter basis). No CT × EL × time interactions were detected (*p* values between 0.176 and 0.662). [2]SEM: standard error of the mean of CT × EL interaction (n=6).

(*p*>0.100). There were no cereal type × time or cereal type × energy level × time interactions (*p*>0.100). However, there was an energy level × time interaction for cADI (*p*=0.022): low energy cADI increased along the time while cADI of high energy concentrate was depressed at the end of the trial (8.19 *vs.* 7.60 kg in period 127-168 d). In addition, heifers fed high energy diets tended to show a greater BW from d 96 of trial than low energy ones (*p*=0.069); and heifers fed high energy diets tended to have a lower F:G ratio (*p*=0.097) than heifers fed low energy diets from d 85 of trial.

Carcass characteristics

Hot carcass weight, dressing percentage and carcass characteristics (carcass compactness index, leg compactness index, carcass conformation and fat cover grade) were not affected either by cereal type or energy level, and no cereal type × energy level interactions were detected for any of these variables (*p*>0.100; Table 3).

Ruminal fermentation characteristics

Rumen fermentation characteristics are shown in Table 4. The cereal type did not affect ruminal pH, NH_3-N concentrations or molar proportions of individual VFA, with the exception of valerate proportion, which tended to be lower for corn compared with wheat (2.33 *vs.* 2.73 mol/100 mol, respectively; *p*=0.052). Heifers fed corn concentrates tended to show greater total VFA concentrations than those fed wheat (140 *vs*. 132 m*M*, respectively; *p*=0.070). There were not differences (*p*>0.050) due to energy level in either ruminal pH or concentrations of total VFA and NH_3-N, but heifers fed the high-energy concentrates had greater isovalerate proportions and lower caproate proportions than those fed low-energy concentrates (1.39 *vs*. 1.02 mol/100 mol, *p*=0.019; and 0.55 *vs*. 0.76 mol/100 mol, *p*=0.040; respectively). Cereal type × energy level interactions were detected for molar proportions of acetate (*p*=0.048), propionate (*p*=0.020) and acetate/propionate ratio (*p*=0.020). Whereas the low energy wheat-based concentrate promoted greater

Table 3. Effect of cereal type (CT) and energy level (EL) in the concentrate on carcass characteristics of heifers[1].

Item	High corn		High wheat		SEM[6]	*p*-value		
	Low energy	High energy	Low energy	High energy		CT	EL	CT × EL
Hot carcass weight (kg)	247	249	248	250	4.3	0.639	0.373	0.998
Dressing proportion (%)	53.8	54.4	54.1	54.7	0.93	0.622	0.327	0.970
Carcass characteristics								
Carcass compactness index[2]	1.96	1.95	1.94	1.96	0.040	0.745	0.744	0.601
Leg compactness index[3]	1.45	1.45	1.47	1.44	0.031	0.697	0.489	0.562
Carcass conformation (%)[4]								
U - Very good	50.00	38.89	33.33	33.33	N/A	0.281	0.655	0.658
R – Good	50.00	61.11	66.67	66.67				
Fat cover grade (%)[5]								
2 – Slight	62.50	55.56	56.25	62.50	N/A	0.964	0.964	0.960
3 – Average	37.50	44.44	43.75	37.50				

[1] Low energy: 1,452 kcal NE/kg; high energy: 1,700 kcal NE/kg (fresh matter basis). [2] Relationship between carcass length (from the cranial border of the first rib to the point of the pubic symphysis) and chest depth (from the ventral surface of the spinal canal, at fifth rib level, to the lowest point of the sternum). [3] Relationship between leg length (from the inner side of the tarsus –metatarsus joint to the point of the pubis symphysis) and perimeter (measured at the level of the crest of the ileum). [4] Determined according to European carcass grading system for conformation: 1 (S, superior), 2 (E, excellent), 3 (U, very good), 4 (R, good), 5 (O, fair), and 6 (P, poor). [5] Determined according to European carcass grading system for fat cover: 1 (poor), 2 (slight), 3 (average), 4 (high) and 5 (very high). [6]SEM: standard error of the mean of CT × EL interaction (n=6).

acetate and lower propionate proportions compared with the high energy one, the opposite was observed for the corn-based concentrates. As a consequence, high energy wheat-based diets and low energy corn-based diets had the highest acetate/propionate values.

All ruminal variables measured were significantly affected by time (sampling day along the trial). Ruminal pH increased (p=0.003) with time, whereas NH_3-N and total VFA concentrations decreased (p=0.001 and p<0.001, respectively). No energy level × time interactions were detected, with the exception of a trend observed for isovalerate proportion (p=0.057), which continuously increased along time in high energy diet, whereas isovalerate concentration of low energy diets increased only at d 168. In contrast, cereal type × time interactions were detected for NH_3-N concentrations (p=0.013), that decreased on d 84 more in corn diets than in wheat ones. Also, molar proportions of acetate and propionate tended to increase or decrease, respectively, more in corn than wheat diets on d 84 of trial (p=0.087 and 0.097, respectively). No cereal type × energy level × time interactions were observed for any ruminal parameter (p>0.100).

Rumen papillae characteristics are shown in Table 5. Heifers fed corn had shorter papilla (p=0.013) and tended to show lighter rumen colour (p=0.064) than those fed wheat, whereas heifers fed the high-energy concentrates had shorter (p=0.048) and tended to have darker papilla (p=0.057) than those fed the low-energy ones.

In vitro fermentation characteristics

Data on *in vitro* fermentation are shown in Table 6. There were no differences (p>0.067) due to cereal type in any of the variables determined in the *in vitro* incubations, but pH tended to be greater for wheat compared with corn (6.56 *vs.* 6.53 respectively; p=0.067) and acetate proportion tended to be greater for corn compared with wheat (55.85 *vs*. 55.35 mol/100 mol, respectively; p=0.068). The fermentation of high-energy concentrates resulted in lower (p<0.001) gas production, total VFA production and acetate molar proportions, but in greater (p<0.050) pH, NH_3-N concentration, and molar proportions of butyrate, isovalerate, valerate and caproate, than the low-energy concentrates. Cereal type × energy level interactions were observed for pH (p=0.030), gas production (p=0.003) and acetate proportions (p=0.025), as those values were similar in corn and wheat low-energy diets, but high-energy wheat-based diets obtained the highest pH and lowest gas and acetate production rates, whereas high energy corn-based diets showed intermediated values.

Discussion

This study was designed to compare diets based on the most typical cereals in beef diets in the range

Table 4. Effect of cereal type (CT) and energy level (EL) on ruminal fermentation characteristics of heifers over the experimental period[1]

Item and period (days of feed)	High corn		High wheat		SEM[2]	*p*-value					
	Low energy	High energy	Low energy	High energy		CT	EL	CT × EL	Time	CT × time	EL × time
pH											
d 1	5.97	5.86	5.95	6.01	0.134	0.154	0.112	0.323	0.003	0.914	0.244
d 84	5.84	5.96	5.79	6.32							
d 168	6.16	6.31	6.34	6.37							
NH_3-N (mg/L)											
d 1	94.9	80.2	64.5	64.1	13.30	0.836	0.362	0.908	0.001	0.013	0.985
d 84	40.8	36.4	61.7	54.4							
d 168	64.1	59.0	66.1	54.9							
Total volatile fattyacids (VFA; mM)											
d 1	165	174	150	163	7.6	0.070	0.614	0.816	<0.001	0.743	0.241
d 84	140	135	137	117							
d 168	117	109	113	109							
Individual VFA (mol/100 mol)											
Acetate											
d 1	54.2	51.3	52.5	54.2	1.35	0.307	0.944	0.048	0.004	0.087	0.279
d 84	51.0	48.0	51.3	52.6							
d 168	53.7	54.7	52.5	54.6							
Propionate											
d 1	25.9	29.8	30.1	27.7	1.82	0.728	0.756	0.020	<0.001	0.097	0.718
d 84	37.0	39.5	36.8	32.7							
d 168	32.0	32.5	34.8	32.3							
Butyrate											
d 1	13.7	12.9	10.9	12.9	0.125	0.839	0.706	0.196	<0.001	0.689	0.491
d 84	8.00	8.78	7.22	9.48							
d 168	10.2	8.51	8.00	8.71							
Isobutyrate											
d 1	0.57	0.57	0.61	0.67	1.072	0.197	0.395	0.087	<0.001	0.508	0.155
d 84	0.57	0.20	0.24	0.33							
d 168	0.67	0.83	0.71	0.94							
Valerate											
d 1	3.53	3.66	3.66	3.67	0.331	0.052	0.327	0.652	<0.001	0.244	0.396
d 84	2.00	1.81	2.74	2.62							
d 168	1.63	1.37	2.21	1.47							
Isovalerate											
d 1	1.17	1.02	0.78	0.90	0.246	0.597	0.019	0.204	0.002	0.585	0.057
d 84	0.88	1.30	0.61	1.67							
d 168	1.42	1.68	1.28	1.76							
Caproate											
d 1	0.95	0.71	0.93	0.85	0.170	0.289	0.040	0.611	<0.001	0.568	0.721
d 84	0.66	0.51	1.05	0.58							
d 168	0.44	0.33	0.54	0.30							

Table 4. Continued

Item and period (days of feed)	High corn		High wheat		SEM[2]	*p*-value					
	Low energy	High energy	Low energy	High energy		CT	EL	CT × EL	Time	CT × time	EL × time
Acetate/propionate (mol/mol)											
d 1	2.23	1.79	1.86	2.08	0.150	0.782	0.963	0.020	<0.001	0.258	0.628
d 84	1.43	1.23	1.41	1.67							
d 168	1.74	1.71	1.54	1.72							

[1] Low energy: 1,452 kcal NE/kg; high energy: 1,700 kcal NE/kg (fresh matter basis). No CT × EL × time interactions (*p* values between 0.412 and 0.921) were detected for any measured variable. [2]SEM: standard error of the mean of CT × EL interaction (n=6).

of energy used, in order to determine the effect of both cereals and energy, and the possible interactions between them on performance, carcass and ruminal fermentation characteristics.

In vivo trial

In agreement with the results of Bock *et al.* (1991) and Philippeau *et al.* (1999) in beef cattle and Gozho & Mutsvangwa (2008) in dairy cows, the type of cereal (corn *vs.* wheat) did not affect cADI in our experiment. In contrast, greater intakes for corn-based concentrates than for wheat-concentrates have been reported in other studies with beef cattle (Fulton *et al.*, 1979; Kreikemeier *et al.*, 1987). Studies comparing the ruminal fermentation of cattle fed different cereals have also produced contrasting results. In agreement with our results, Fulton *et al.* (1979) observed that steers fed corn tended to have greater VFA concentrations than those fed wheat, without differences in the molar proportions of the main VFA. In contrast, Oltjen *et al.* (1966), Philippeau *et al.* (1999) and Liu *et al.* (2016) in beef cattle and Kreikemeier *et al.* (1987) in lambs observed significantly greater VFA concentrations in the rumen of animals fed wheat than in those fed corn, and Gozho & Mutsvangwa (2008) reported no differences in dairy cows fed either corn or wheat.

It was expected that the greater fermentability of wheat compared with corn should decreased significantly the rumen pH, as it has been previously

Table 5. Effect of cereal type (CT) and energy level (EL) in the concentrate on rumen wall characteristics[1].

Item	High corn		High wheat		SEM[4]	*p*-value		
	Low energy	High energy	Low energy	High energy		CT	EL	CT × EL
Papilla length[2] (%)								
Very short	6.25	11.11	6.25	25.00	N/A	0.013	0.048	0.010
Short	12.50	44.44	6.25	12.50				
Medium	43.75	16.67	62.50	62.50				
Long	37.50	27.78	25.00	0.00				
Colour[3] (%)								
Pale	18.75	0.00	6.25	0.00	N/A	0.064	0.057	0.001
Pale-pink	6.25	0.00	18.75	0.00				
Pink	31.25	5.56	0.00	18.75				
Pink-Green	12.50	5.56	25.00	50.00				
Green	25.00	50.00	31.25	25.00				
Dark green	6.25	38.89	18.75	6.25				

[1] Low energy: 1,452 kcal NE/kg; High energy: 1,700 kcal NE/kg (fresh matter basis). [2] Papilla length was determined according to a subjective scale: 1 (very short), 2 (short), 3 (medium) and 4 (long). [3]Colour was scored as: 1 (pale), 2 (pale-pink), 3 (pink-green), 4 (green) and 5 (dark green). [4]SEM: standard error of the mean of CT × EL interaction (n=6); N/A: not applicable.

Table 6. Effect of cereal type (CT) and energy level (EL) on *in vitro* fermentation characteristics of the experimental concentrates after 17 h of incubation[1]

Item	High corn		High wheat		SEM[2]	*p*-value		
	Low energy	High energy	Low energy	High energy		CT	EL	CT × EL
pH	6.51	6.55	6.51	6.60	0.015	0.067	<0.001	0.030
Gas (µmol)	3060	2823	3185	2545	50.2	0.163	<0.001	0.003
NH_3-N (mg/L)	163	180	163	175	3.2	0.472	0.002	0.467
Lactate (mg/L)	10.4	10.0	10.3	11.8	1.86	0.670	0.753	0.635
Total volatile fatty acids (VFA; µmol)	2656	2428	2708	2331	42.8	0.611	<0.001	0.115
Individual VFA (mol/100 mol)								
Acetate	56.7	55.0	56.9	53.8	0.26	0.068	<0.001	0.025
Propionate	23.1	21.7	22.9	21.4	0.99	0.814	0.164	0.937
Butyrate	15.0	16.8	14.9	17.8	0.77	0.565	0.015	0.445
Isobutyrate	1.53	1.60	1.37	1.73	0.113	0.905	0.099	0.241
Isovalerate	1.62	1.83	1.54	1.88	0.055	0.814	<0.001	0.270
Valerate	1.75	2.58	2.06	2.83	0.170	0.127	0.001	0.840
Caproate	0.25	0.54	0.32	0.59	0.047	0.255	<0.001	0.829
Acetate/propionate (mol/mol)	2.47	2.56	2.49	2.56	0.117	0.904	0.525	0.963

[1] Low energy: 1,452 kcal NE/kg; high energy: 1,700 kcal NE/kg (fresh matter basis). Samples of 400 mg DM of concentrates were incubated with ruminal fluid from heifers fed a high-grain diet. [2]SEM: standard error of the mean of CT × EL interaction (n=6).

reported by others (Fulton *et al.*, 1979; Philippeau *et al.*, 1999). However, the type of cereal did not affect significantly rumen pH in our study, supporting the lack of differences reported by Liu *et al.* (2016) in beef steers and by Gozho & Mutsvangwa (2008) in dairy cows. The lack of differences could partly be explained by the high individual variability of pH values, as some authors have reported a high variability inter- and intra-individuals along the day when using indwelling pH meter probes that continuously measure pH (Beauchemin & Penner, 2009; Danscher *et al.*, 2015). As pH meter probes were not available, rumenocentesis was selected as the best method for ruminal sampling in the conditions of our trials. In fact, Duffield *et al.* (2004) concluded that ruminocentesis was the most accurate field technique for ruminal sampling after comparing this technique and the use of an oral stomach tube with direct sampling through a rumen cannula and continuous electronic pH measurement. All sampling days, time of feed supply in each pen was adjusted to the expected time of ruminal sampling and experimental treatments were consistently distributed across the day (*i.e.*, one pen per treatment was successively sampled). Although these precautions were taken, it was impossible to know the actual time of feed intake in each heifer, and individual variations may have affected the results.

Rumen wall characteristics related to papillae length are in agreement to those reported by Khan *et al.* (2008), who reported that corn diets increased papillae length and width compared to wheat, although animals had similar papillae concentration and rumen mucosa colour, which was described as dark brown. Those authors performed their study on post-weaning calves at the early stages of rumen development. The early exposure to high-starch concentrates could promote the dark brown colour of papillae, as it is associated to keratinized tissue, resulted from rapid growth and acid pH (Nockels *et al.*, 1966; Gelberg, 2016). On the contrary, in this study, the high-starch concentrates were provided in older animals which had a post-weaning transition into pasture prior to the experimental period. This management could palliate the effect of the high-concentrate diets and prevent parakeratosis incidence, resulting on lighter rumen papilla colour, especially in low energy diets.

Responses of cattle to different cereal grains depends, among other factors, on the level of dietary inclusion, the basal ration, physical processing of the cereal grains, the composition of a given batch of cereal grain, and the level of dietary intake, and therefore direct comparisons among studies are often not possible (Philippeau *et al.*, 1999). Bock *et al.* (1991) conducted two experiments to compare wheat and corn as the grain source for beef steers and reported that feeding wheat decreased ADG compared with corn in the first trial, but improved ADG in the second trial. This was attributed to differences in the cereal processing; as wheat was dry-rolled in the first trial and steam-rolled

in the second one, and steam-rolled wheat usually results in improved performance compared with dry-rolled wheat (Bock *et al.*, 1991). These results indicate that processing can markedly affect the comparison of cereals, and the effects of cereal processing can be even more evident than those derived from cereal type as reported by Gimeno *et al.* (2014). Pellet durability can influence feed efficiency and growth (Devant *et al.*, 2015) because low durability pellets promotes feed selection and higher spoilage, as cattle rejects low particle size, whereas high durability increases chewing and rumen buffering activity. In the current study, concentrates were pelleted and durability was similar among treatments (98%) so no differences were expected due to this factor. Grinding cereal grains reduces particle size and increases degradation rate, but the magnitude of this effect on starch utilization is greater with corn than with wheat, due to disrupting the protein matrix which encapsulates the starch granules (McAllister *et al.*, 1993). Liu *et al.* (2016) reported that corn starch in particles smaller than 1 mm was extensively fermented in the rumen, whereas in wheat was highly fermented in particles smaller than 2 mm. In our study both cereals were ground and more than 70% of the particles were lower than 1.00 mm in the corn-concentrate (71.38% and 71.08% for low- and high-energy corn concentrates, respectively) and more than 66% in the wheat-concentrate (66.50 and 66.38% for low- and high-energy wheat concentrates, respectively). This may have resulted in an extensive fermentation of corn grains, which could help to explain the trend to greater total VFA concentration observed for corn-fed heifers.

The lack of impact of cereal type in the current study could also be explained by the feeding management, as heifers were fed twice daily and had enough feeding space (25 cm per animal), and these conditions could have resulted in low social competition and a more uniform eating pattern (specially in wheat diets). Previous studies in beef cattle fed high-grain diets have shown that feeding space per animal has an impact in eating behaviour. Gonzalez *et al.* (2008a,b) conducted several trials to investigate the effects of increased social pressure caused by a reduced number of concentrate feeding places on performance and behaviour of beef cattle, and reported that reducing the feeding space (25 *vs.* 12.5 cm per animal) reduced animal performance, increased concentrate eating rate and reduced straw eating time during peak feeding times, which may result in lower ruminal pH. Different studies have reported straw intakes in beef cattle fed high-grain concentrates and straw on a feed-choice system ranging from 3.9 to 10.2% (Devant *et al.*, 2000; Gimeno *et al.*, 2014; Iraira *et al.*, 2015). Unfortunately straw intake could not be measured in our study, but ingested straw should have increased the effective NDF intake, which may have increased rumination and chewing, contributing to mask the possible differences in corn and wheat fermentation.

As expected, the increase in energy supply of the concentrate reduced beef cADI and F:G ratio, as ruminants fed on high-grain diets usually eat trying to maintain constant energy intake (Krehbiel *et al.*, 2006). Despite the cADI decrease, in our study the average daily NE intake of heifers fed the high-energy concentrates was 11.5% greater than that of heifers fed the low-energy ones. That increase in NE intake had no impact on ADG or carcass traits in the present study. This lack of effect could be associated to the high fat content of the high-energy concentrates (74.1 and 75.1 g/kg DM for corn and wheat diets, respectively), as Krehbiel *et al.* (2006) reviewed the relationships between dietary energy density and animal performance in finishing diets for beef cattle, and reported that supplementing fat above 60 to 70 g/kg of DM resulted in decreased intake to a level at which F:G is maintained or decreased. Those authors also reported that maximum ADG was achieved at the equivalent of 39.9 g/kg of supplemental fat, that is close to fat content of low energy diets (38.8 and 36.3 g/kg DM for corn and wheat diets, respectively).

Carcass characteristics results agree with the results in BW and ADG, with no differences regarding to cereal type or energy level. Even though when cereal type had an effect on productivity of mixed crossbred yearling cattle, as reported by Kreikenmier *et al.* (1987), there was not an impact in carcass conformation. Those authors only reported an increase in back fat depth in diets with high corn content compared to wheat-diets, ranging from 117 mm in 100% corn-diets to 93 mm in 100% wheat-diets, but those differences were smaller in 67:33 and 33:67 corn:wheat diets (114 mm *vs* 108 mm, respectively). As in our study the experimental diets contained other cereals, as barley, we assumed that the expected effect should be similar to mixed corn:wheat diets. Therefore, the difference in 6 mm of back fat reported by Kreikenmier *et al.* (1987) could have not been high enough to affect fat cover carcass grading in our study. Regarding to the effect of energy level on carcass characteristics, Li *et al.* (2014) reported no effect of energy level of corn-based concentrates on carcass weight and dressing, carcass length, chest depth and leg depth. However, those authors reported a high medium and top grade cuts yield and increased intramuscular fat of high energy diets compared to low energy ones. Unfortunately those variables were not measured in our study, so we could not evaluate the effect of energy level on them.

In vitro incubations

The *in vitro* trial was conducted to analyze the fermentation potential of the experimental concentrates under identical conditions (pH, concentrate to ruminal fluid ratio, retention time, etc.). Straw was not included as part of the incubated substrate because actual straw intake by heifers could not be determined. *In vitro* values were in the range of those previously reported for *in vitro* fermentation of high-concentrate diets (Tejido *et al.*, 2005; Mateos *et al.*, 2013). The lack of differences in fermentation due to the cereal type agrees quite well with the results observed *in vivo*. In contrast, more differences due to concentrate energy levels were detected *in vitro* than *in vivo*. Gas and total VFA production were 14.0 and 11.3% lower, respectively, for high-energy concentrates compared with the low-energy ones, which is consistent with the lower pH values observed for low-energy concentrates. These results indicate that high-energy concentrates were less fermented. Although low and high-energy concentrates had similar starch content, the high ether extract content of high-energy concentrate (74.5 *vs*. 35.5 g/kg DM for high- and low-energy concentrates, respectively) could affect its fermentation. High levels of fat (>50-60 g/kg DM) can negatively affect ruminal fermentation, as the free fatty acids resulting from the triglyceride hydrolysis are toxic for the cellulolytic bacteria (Maia *et al.*, 2007). This is in agreement with the lower acetate proportions observed for the high-energy concentrates (54.4 *vs*. 56.8 mol/100 mol for high and low-energy concentrates, respectively), as acetate is the main VFA resulting from fibre degradation. The greater isobutyrate, isovalerate and valerate proportions observed for the high-energy concentrates may also indicate a lower abundance and/or activity of the cellulolytic bacteria, as these minor VFA are specific nutrients for these bacteria and are required for cellulose digestion (Muller, 1987). Differences between *in vivo* and *in vitro* fermentation characteristics have been attributed to the high buffering in the batch cultures, lack of absorption and differences in solids retention time, among other factors (Mateos *et al.*, 2015). Moreover, absolute amounts of feed input (no straw was included in the *in vitro* fermentations) and solid/liquid ratios were different *in vivo* and in the *in vitro* cultures, and that may have also contributed to the differences in the variables measured.

Altogether, the results indicate that, under the conditions of this trial, substitution of wheat by corn had a minimal impact on animal performance and ruminal fermentation regardless of the energy level of the concentrate. Increasing the energy level of concentrates from 1,452 to 1,700 kcal NE/kg reduced significantly the concentrate to gain ratio, but had no significant impact on daily gain or carcass traits.

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