

## Influence of different feeding strategies on carcass and meat quality of grass-fed cull cows

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**Abstract.** Animal performance, carcass and meat quality characteristics of beef cull cows under different feeding strategies were compared. Cows were assigned to one of four grazing treatments combining different levels of forage allowance (FA) and supplementation rate (% of body weight, BW) using rice bran (RB): T1= FA 2 % + RB0, T2 = FA 4 % + RB0, T3= FA 2 % + RB 0.8 % and T4 = FA 2 % + RB 1.6 %. Cows from T1 presented lower ( $P < 0.05$ ) slaughter weight (SW) than the other three treatments. Additionally, T1 presented lower body weight ( $P < 0.05$ ) for rump and loin, striploin, sirloin, inside round and tri-tip, compared to T2, T3 and T4. Intramuscular fat (IMF), pH (48 h), Warner-Bratzler shear force (WBSF; aged for 7 or 21 days), lean colour, saturated fatty acid (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) concentrations did not differ among treatments ( $P > 0.05$ ). Nonetheless, differences between treatments were found in n-6 and n-3 fatty acid contents ( $P < 0.05$ ). The strategic use of low supplementation rates using rice bran on an oats forage crop improved certain meat and carcass quality traits of cull cows.

**Key words:** beef cattle; supplementation; grass-based feeding systems; valuable cuts and fatty acids.

## Influencia de diferentes estrategias de nutrición sobre la calidad de la canal y de la carne de vacas de descarte alimentadas con pasto

**Resumen.** Se comparó la producción, las características de la canal y la calidad de la carne de vacas de descarte bajo diferentes estrategias de alimentación fueran evaluadas. Las vacas fueron asignadas a uno de cuatro tratamientos que combinaban diferentes niveles de asignación de forraje (AF) y suplementación con afrechillo de arroz (AA), según el peso vivo (% PV): T1 = AF 2 %, T2 = FA 4 %, T3 = AF 2 % + AA 0.8 % y T4 = FA 2 % + AA 1.6 %. Las vacas de T1 presentaron menor ( $P < 0.05$ ) peso de faena que los restantes tratamientos. A su vez, T1 presentó menor peso ( $P < 0.05$ ) para el lomo, bife, nalga de adentro y colita de cuadril, en comparación con T2, T3 y T4. La grasa intramuscular, pH (48 h), fuerza de corte Warner-Bratzler (con periodos de maduración de 7 o 21 días), color de la carne y concentración de ácidos grasos (AG) saturados (AGS), monoinsaturados (AGM) y poliinsaturados (AGP) no difirieron entre tratamientos ( $P > 0.05$ ). Sin embargo, se observaron diferencias en el contenido de los ácidos grasos n-6 y n-3 ( $P < 0.05$ ). El uso estratégico de un bajo nivel de suplementación de AA en un verdeo de avena mejoró ciertas características de calidad de la canal y la carne de las vacas de descarte.

**Palabras clave:** ganado vacuno; suplementación; sistemas de alimentación forrajeros; cortes valiosos y ácidos grasos.

## Influência de diferentes estratégias de alimentação na qualidade da carcaça e qualidade da carne de vacas de descarte alimentadas com pastagem

**Resumo.** O desempenho animal, as características de carcaça e a qualidade da carne de vacas de corte (refugo) sob diferentes estratégias de alimentação foram avaliados. As vacas foram distribuídas em um dos quatro tratamentos de pastoreio direto combinando com diferentes níveis de oferta de forragem (FA) e taxa de suplementação (% do peso vivo, PC) usando farelo de arroz (RB): T1 = FA2 % + RB0, T2 = FA 4 % + RB0, T3 = FA 2 % + RB 0.8 % e T4 = FA 2 % + RB 1.6 %. As vacas do T1 apresentaram menor ( $P < 0.05$ ) peso de abate (PS) do que os outros três

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tratamentos. Além disso, T1 apresentou menor peso ( $P < 0.05$ ) para alcatra e lombo, contrafilé, pernil e tripa, em comparação com T2, T3 e T4. A gordura intramuscular (IMF), o pH (48 h), a força de cisalhamento Warner-Bratzler (WBSF; envelhecido por 7 ou 21 dias), a cor da carne, a quantidade de ácido graxo saturado (SFA), monoinsaturados (MUFA) e poliinsaturados (PUFA) não diferiram entre os tratamentos ( $P > 0.05$ ). No entanto, foram encontradas diferenças entre os tratamentos nos teores de ácidos graxos n-6 e n-3 ( $P < 0.05$ ). O uso estratégico de baixas taxas de suplementação com farelo de arroz em uma cultura forrageira de aveia melhorou algumas características de qualidade da carne e carcaça de vacas de descarte.

Palavras-Chave: bovinos de corte; suplementação; sistemas de alimentação baseados em capim; cortes valiosos e ácidos graxos.

## Introduction

The Uruguayan beef supply chain is one of the most important economic activities in the country. During the last 10 years, approximately 2 million cattle heads were slaughtered per annum, of which 38 % have been cull cows with 6 to 8 permanent incisors (INAC, 2018). Thus, the importance of cull cows is clear for the meat industry, but also for cattle farmers in terms of income, particularly in the cow-calf operations when cows are diagnosed as non-pregnant (Montossi et al., 2014).

Increasing energy intake in semi-extensive and extensive beef production systems has a positive effect on the carcass (Rodríguez et al., 2014) and meat quality (Luzardo et al., 2008), and also on the fatty acid profile of intramuscular fat (Pouzo et al., 2015). An increase in quantity and/or quality of forage or the inclusion of supplementation increase live weight gain (Poppi et al., 1987; Ramírez-Barboza et al., 2016), which in turn, improves carcass quality in terms of conformation and degree of finishing (Brito et al., 2008; Pouzo et al., 2015). Animal feeding systems with greater energy supply improve the consumer's acceptance in terms of lean and fat colour when compared to exclusively grass-fed animals (Realini et al., 2004; Ramírez-Barboza et al., 2016). According to Henschion et al. (2017), increasing the nutritive value of the diet of ruminants corresponds with the consumers' preferences overall, since this has important influences

on human health and wellbeing in terms of the fatty acid composition of meat.

Grass-fed beef production systems present a more beneficial omega6/omega3 ratio, and a greater polyunsaturated fatty acids / monounsaturated fatty acids (PUFA/MUFA) ratio in the intramuscular fat (IMF) compared to animals fattened in high-concentrate diets (Realini et al., 2004; Descalzo et al., 2005; Zea et al., 2007). Realini et al. (2009) reported that meat from grazing cattle supplemented with concentrates presented a greater acceptance from European consumers than those from exclusively grass-fed animals. According to a literature review carried out by Lagomarsino (2019), most of the research on the productivity and product quality of beef cull cows come from studies mainly associated with intensive feeding systems rather than grass-based diets.

We hypothesised that improvements in the nutrition of cull cows – either through greater forage allowances and/or with the restricted use of concentrate supplementation – enhance carcass and meat quality characteristics. The objective of this study was to evaluate the effect of different combinations of forage allowances of oat pasture and supplementation on animal performance, carcass, and meat quality of Hereford cull cows.

## Materials and Methods

The experiment was carried out according to the recommendations of the Animal Experimentation Honorary Committee of Uruguay (CHEA) and aligned with the EU directions on animal experimentation (2010/63/EU).

### Location and duration

This study was carried out during late autumn-winter for 130 days at "Glencoe" Experimental Station of the National Institute of Agricultural Research

(INIA Uruguay) - located at 32 ° 00' 24" S, 57 ° 08' 01" O and 124 m above sea level. After the fattening phase, cull cows were slaughtered in a commercial abattoir.

### Experimental design and treatments

The experiment was analysed as a completely randomized block design. Forty Hereford cull cows were assigned to four treatments ( $n = 10$  per treatment;  $n = 5$  per plot) according to body weight (BW). At the



beginning of the experiment the average BW was  $480.2 \pm 48.5$  kg and cows had mostly 6 permanent incisors. The treatments were generated by combining two forage allowances (FA; 2 and 4 % of BW) and three supplementation rates (0.8 and 1.6 % of BW) of whole rice bran (RB), as follows: T1 = FA 2 % + 0 % RB, T2 = FA 4 % + 0 % RB, T3 = FA 2 % + 0.8 % RB y T4 = FA 2 % + 1.6 % RB.

#### Pasture and supplementation

An annual forage crop of *Avena byzantina* (cv. INIA Halley) was used for direct grazing. Average forage biomass and height was 1608 kg DM/ha and 19.8 cm, respectively. The crude protein (CP) content of the forage crop was estimated according to AOAC (1990) (KJELTEC 2200 FOSS distiller), while acid detergent fibre (ADF) and neutral detergent fibre (NDF) were estimated according to the methodology described by Van Soest (1982) (ANKOM A 2000I). The nutritional value of the pre-grazing is presented on Table 1.

Table 1. Average nutritional value of the pre-grazing forage dry matter

Variable	%
Crude protein	12.8
Acid detergent fibre	25.3
Neutral detergent acid	45.1
Ash	10.8

Prior to the experiment, T3 and T4 animals had an adaptation period to RB supplementation for 10 days, in which the supplementation rate increased gradually until the target rate was reached (0.8 and 1.6 % BW, respectively). Rice bran supplementation was provided daily early in the morning by using one trough per supplemented group. Every 14 days the supplementation rate was adjusted according to the BW of each supplemented group. No leftovers of RB were observed throughout the experimental period. The nutritional value of RB was: 69.5 % DM digestibility, 17.3 % of CP, 11.1 % acid detergent fibre (ADF), 30.3 % NDF and 11.6 % ashes.

Animals from all treatments had access to fresh water and mineral blocks ad libitum. Mineral blocks presented the following composition: P: 5.9 %, Ca: 13.5 %, Mg: 1.0 %, NaCl: 47 %, Fe: 2500 mg/kg, Cu: 200 mg/kg, Co: 8 mg/kg, I: 40 mg/kg, Zn: 470 mg/kg, Se: 15 mg/kg, molasses: 5 %, vitamin A: 20000 IU/kg, vitamin D3: 2000 IU/kg, and vitamin E: 20 IU/kg.

#### Feeding treatments estimations

Total dry matter intake (DMI), animal requirements, CP and net energy (NE) balance were estimated for each group of cows considering forage biomass and

quality and using the assumption of an oat daily growth rate of 10 kg DM/ha/day (Millot, 1981), plus the addition of RB intake for T3 and T4. Based on the finishing beef cattle requirements from Nutritional Research Council (NRC, 2001), CP requirements (g/animal/day) were established, considering each group's average daily gain (ADG) and BW. As for NE requirements, the following equations were used (AFRC, 1993):

$$(1) \text{ NE basal maintenance (NE}_{bm}\text{)} (\text{Mcal/d}) = (0.53 \cdot (\text{BW}/1.08)^{0.67})/4.184$$

$$(2) \text{ NE weight gain (NE}_{g}\text{)} = \text{Energetic value of weight gain (EV}_{g}\text{, Mcal/kg)} \cdot \text{ADG (kg/d), where:}$$

$$\text{EV}_{g} = \text{C2} (4.1 + 0.0332\text{BW} - 0.000009\text{BW}^2) / (1 - \text{C3} \cdot 0.1475\text{ADG})$$

$$\text{C2: corrected by adult animal frame, breed and sex} = 1, \text{C3} = 1 (\text{ADG} > 0).$$

$$(3) \text{ EN grazing (EN}_{gz}\text{)} (\text{Mcal/d}) = \text{C} \cdot \text{DMI} \cdot (0.9 - \text{D}) + 0.05\text{T} / (\text{GMA} + 3) \cdot \text{BW} / 4.184, \text{ where:}$$

$$\text{C} = \text{constant } 0.006, \text{DMI} = \text{dry matter intake, D} = \text{pasture, dry matter digestibility, T} = \text{Ground topography (1, flat), and GMA} = \text{green dry matter availability.}$$

$$\text{o D (Dry matter digestibility, \%)} \text{ was estimated following Osítés et al. (2003) as } 88.9 - 0.779 \times \text{ADF \%}$$

#### Animal, carcass and meat quality determinations

Initial and final BW was recorded at the beginning of the trial, every 14 days. At the end of the experimental period, ADG was estimated for each treatment, as presented in Lagomarsino et al. (2020). During the slaughter process, carcasses were cut in halves, and hot carcass weight (HCW) was registered. After 48 hours, carcasses were ribbed between the 10<sup>th</sup> and 11<sup>th</sup> rib, and the ultimate pH was measured on the Longissimus thoracis muscle of each left half carcass using a pHmeter (Hanna 9125, Cluj-Napoca, Romania) previously calibrated. Next, half carcasses were quartered between the 10<sup>th</sup> and 11<sup>th</sup>, and the pistola cut from the left half-carcass was weighed and subsequently deboned. The weights of tenderloin, striploin, sirloin, outside round, inside, tri-tip, knuckle heel muscle, shank and flank on, fat and meat trimmings and bones were registered.

A 7-10 cm sample was taken from the Longissimus thoracis (from the 10<sup>th</sup> and 11<sup>th</sup> rib, following cranial direction) of each left half-carcass and which were vacuum-packaged and transported to the meat laboratory. Each meat sample was divided into two steaks of 2.54 cm thickness that were aged at 2-4 °C for 7 or 21 days, plus one cm steak was taken for fatty acid analysis. After aging, instrumental lean colour (CIE L\*: lightness, a\*: redness and b\*: yellowness) was

blooming with a Minolta chromameter CR-400 (Konica Minolta Sensing Inc., Japan) using a C illuminant, a 2° standard observer angle and 8 mm aperture size and calibrated with a white tile before use. Furthermore, according to the American Meat Science Association guidelines, Warner-Bratzler shear force (WBSF; model D2000- WB, G&R Electric Manufacturing Co Co, Manhattan, KS) was assessed (AMSA, 2016). Each steak was packed into polyethylene bags and cooked in a water bath until an internal temperature of 70 °C was reached. After cooking, six cores (1.27 cm diameter) were removed from each steak parallel to the longitudinal orientation of muscle fibers. Individual shear force (SF) values were averaged to assign a mean peak WBSF value to each sample.

Intramuscular fat (IMF) content was determined gravimetrically. Lipids were extracted using a mixture of chloroform-methanol according to Bligh and Dyer (1959) procedure. For the fatty acid determination, 0.03 g of fat were taken and dissolved with 2 mL of hexane, and afterwards 1 mL of a saturated solution of KOH in methanol was added and shaken for 2 min and then left to rest for 30 min (IUPAC, 1987). An aliquot was extracted from the upper layer for the subsequent determination of fatty acids. Fatty acid methyl esters were analyzed by gas chromatography coupled with a flame ionization detector (GC-FID; Konik HRGC 4000B, Konik Group, Barcelona, Spain) using a 90 % cyanopropyl polysilphenylene-siloxan capillary column (SGE BPX90 GC Column; 30 m, 0.25 mm i.d., and 0.25 µm film thickness; Trajan Scientific Australia Pty Ltd., Melbourne, Australia). Nitrogen was used as the gas carrier with 1 mL/min flow. The chromatographic conditions were: injection volume of 1 µL, the initial temperature of 80 °C for 0.5 min, increasing 3 °C/min until it reach 165 °C and held for 10 min, then increased at 10 °C/min to 180 °C and kept for 2 min, and finally increasing 15 °C/min to

reach 250 °C and held for 13 min. Identification of fatty acid methyl esters (FAME) was performed by comparing their retention times with those of the standards (Supelco® 37 Component FAME Mix). Fatty acids were expressed as a percentage of the total fatty acids identified.

#### Statistical analysis

The experiment was analysed as a completely randomized block design. Body weight and ADG were analysed using the PROC MIXED procedure from the Statistical Analysis System software (SAS Institute Inc., Cary, NC, USA, version 9.4) as a repeated measures analysis considering: feeding treatments, time and its interaction as fixed effects and the animal as a random effect. The best covariance structure was selected based on the Akaike Information Criterion (AIC).

Carcass traits (HCW, pistola cut and other cuts weights), pH WBSF and instrumental colour variables and fatty acid composition were analysed considering feeding treatment as the fixed effect using the GLM procedure. Normality of the data was formally tested for all variables using the Shapiro Wilks test. When data were not normally distributed, they were normalized by choosing the proper transformation method for normal distribution.

For the statical analyses of HCW, CCW and meat cuts, the covariates BW, HCW were used. After ANOVA, least squares means were calculated for treatment comparisons with a significance level of  $\alpha = 0.05$ , using the PDIFF option of LSMEANS adjusted by Tukey, when F-tests were significant ( $P < 0.05$ ). For the association analyses between WBSF (expressed in kgF, using a 4.5 kgF threshold) and the two maturing periods (7 and 21 days), contingency tables were used, and the contrasts were carried out using Pearsons' chi-square test.

## Results

The nutritional value of the post-grazing pasture is presented in Table 2.

Table 2. Average nutritional value of the post-grazing forage.

Variable	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	P-value
Dry matter biomass (kg/ha)	821.0 <sup>b</sup>	1009.7 <sup>a</sup>	964.2 <sup>ab</sup>	924.3 <sup>ab</sup>	0.0138
Height (cm)	6.2 <sup>c</sup>	8.1 <sup>a</sup>	6.9 <sup>b</sup>	6.7 <sup>b1</sup>	< 0.0001
Crude protein (%)	11.7	12.3	10.7	11.5	0.8245
Acid detergent fiber (%)	32.5	31.9	33.2	32.7	0.9429
Neutral detergent fiber (%)	56.6	56.5	58.1	59.1	0.6974
Ash (%)	14.4	13.6	13.2	13.5	0.7536

a, b, c: different letters within the same parameter indicated significant ( $P < 0.05$ ) differences among treatments.



Estimations of DMI, requirements, and balance of CP and NE for each treatment are presented in Table 3.

Table 3. Estimated dry matter intake, requirements, and balance of crude protein and net energy by feeding treatment.

Treatments		T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM <sup>6</sup>	P-Value
Estimated intake	CP <sup>1</sup>	1387.9 <sup>d</sup>	2267.9 <sup>b</sup>	1974.1 <sup>c</sup>	2643.3 <sup>a</sup>	23.2	< 0.0001
	NE <sup>2</sup>	27.8 <sup>d</sup>	43.8 <sup>b</sup>	38.4 <sup>c</sup>	50.6 <sup>a</sup>	0.4	< 0.0001
Requirements	CP <sup>1</sup>	941.4 <sup>b</sup>	1457.3 <sup>a</sup>	900.1 <sup>c</sup>	899.6 <sup>c</sup>	7.7	< 0.0001
	NE <sup>2</sup>	23.1 <sup>b</sup>	36.9 <sup>a</sup>	35.4 <sup>a</sup>	39.5 <sup>a</sup>	1.9	< 0.0001
	Nebm <sup>3</sup>	7.6	7.9	7.9	8.0	0.2	0.3023
	NEwg <sup>4</sup>	10.6 <sup>c</sup>	20.8 <sup>b</sup>	22.5 <sup>ab</sup>	26.4 <sup>a</sup>	1.7	< 0.0001
	NEg <sup>5</sup>	4.9 <sup>b</sup>	8.2 <sup>a</sup>	5.1 <sup>b</sup>	5.1 <sup>b</sup>	0.2	0.0008
Balance	PC <sup>1</sup>	446.4 <sup>d</sup>	810.6 <sup>c</sup>	1074.0 <sup>b</sup>	1743.7 <sup>a</sup>	26.9	< 0.0001
	NE <sup>2</sup>	4.6 <sup>b</sup>	6.9 <sup>ab</sup>	3.0 <sup>b</sup>	11.2 <sup>a</sup>	1.7	0.0100

<sup>1</sup>CP: crude protein (g/a/d); <sup>2</sup>NE: net energy (Mcal/a/d); <sup>3</sup>bm<sup>3</sup>: basal metabolism; <sup>4</sup>wg<sup>4</sup>: weight gain; <sup>5</sup>g<sup>5</sup>: grazing; <sup>6</sup>SEM: standard error of the means. <sup>a, b, c</sup>: different letters within the same parameter indicated significant (P < 0.05) differences among treatments.

Greatest NE and CP intakes (P < 0.05) were observed in those treatments with the greatest FA (T2) and supplementation rate (T4), while the opposite was observed in the treatment with the lowest FA and not

supplemented (T1). Requirements presented similar trends between treatments. Estimated intakes were above requirements for both CP and NE in all treatments, resulting in a positive balance.

Table 4. Initial and final body weight (BW) and average daily gain (ADG) by treatment.

Treatments	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM <sup>1</sup>	P-value
Initial BW (kg)	480.3	480.6	480.5	479.5	16.0	0.9999
Final BW (kg)	539.3 <sup>b</sup>	605.2 <sup>a</sup>	608.4 <sup>a</sup>	620.8 <sup>a</sup>	17.9	0.0131
ADG (kg/a/d)	0.455 <sup>b</sup>	0.958 <sup>a</sup>	0.984 <sup>a</sup>	1.087 <sup>a</sup>	0.074	< 0.0001

<sup>1</sup>SEM: standard error of the means. <sup>a, b, c</sup>: different letters within the same parameter indicated significant (P < 0.05) differences among treatments.

No significant differences (P > 0.05) were observed in the initial BW among treatments (Table 4; 480.2 ± 48.5 kg). However, the final BW was lower (P < 0.05) in T1 compared with the other three treatments (Table 4). Supplemented treatments (T3 and T4) presented 12 % and 15 % greater BW compared to T1 at the end of the experiment. Cows from T1 presented an ADG 52-58 % lower (P < 0.05) than the other three treatments, which did not differ among them.

T2, T3 and T4 presented a greater (P < 0.05) HCW and pistola cut weight compared to T1 (Table 5). In addition, cows from T1 had lower (P < 0.05) weights of the most valuable beef cuts represented by rump and loin, striploin and sirloin, and also the inside round, tri-tip, and flank. Nonetheless, these differences were canceled out when corrected by final BW as a co-variate, indicating that they were not explained by the effect of treatments (data not shown). The rest of the cuts, fat trimmings, and bones registered no differences among treatments (P > 0.05).

Ultimate pH (48 h post-mortem) and WBSF after aging for 7 and 21 days did not present (P > 0.05) significant differences among treatments (Table 6).

On the other hand, considering a 4.5 kgF value proposed by Miller et al. (2001) as threshold criteria for WBSF consumer's tenderness acceptance, 90 to 100 % of meat samples had values below 4.5 kgF in all treatments after 21 days of aging (Table 7).

Lean colour (CIE L\*, a\* and b\*) and WBSF are also presented by aging time over experimental treatments (Table 8).

No significant interaction (P > 0.05) was found between treatment and aging for WBSF and lean colour (data not shown). However, differences (P < 0.05) were found when comparing the two aging periods with lower values of WBSF when meat was aged for 21 d (Table 8). In addition, both L\* (lightness) and b\* (yellowness) values were greater (P < 0.05) for 21 d than 7 d, but no differences (P > 0.05) were found for a\* (redness) between both aging periods.

The IMF content and fatty acid composition by feeding treatment are shown in Table 9.

There were no significant differences (P < 0.05) in IMF content among treatments (4 % on average). The main fatty acids in all treatments were palmitic (16:0), stearic (18:0) and oleic (18:1), which represented

approximately 89 % (average across treatments) of total fatty acids identified. Their concentrations did not present significant differences ( $P > 0.05$ ) among treatments, and neither did myristoleic (C14:1), palmitoleic (C18:1), eicosadienoic (C20:2), eicosatrienoic (C20:3-n3) and dihomo- $\gamma$ -linolenic (C20:3-n6). Fatty acids linoleic (C18:2) and linolenic (C18:3) acids presented significant differences ( $P < 0.05$ ) among treatments, presenting the IMF from cows supplemented with RB (T3 and T4) with greater concentrations ( $P < 0.05$ ) of linoleic acid. In contrast, linolenic acid was present in greater proportion in the cows fed exclusively in grass (T1 and T2). Concentrations of arachidic (C20:0), arachidonic (C20:4), eicosapentaenoic - EPA (C20:5) and

docosapentaenoic - DPA (C22:5) acids were different among treatments with greater ( $P < 0.05$ ) concentrations in cows from T1. The CLA proportion was greater ( $P < 0.05$ ) in exclusively grass-fed animals (T1 and T2) compared to T4. Concentrations of SFA, MUFA and PUFA did not present differences among treatments. Feeding treatments resulted in greater ( $P < 0.05$ ) concentrations of n-6 fatty acids in cows from T1, T3 and T4 than T2, and n-3 concentration was greater ( $P < 0.05$ ) in T1 compared to the other three treatments. The PUFA:SFA ratio did not present significant differences ( $P > 0.05$ ) among treatments. Lastly, a greater ( $P < 0.05$ ) n-6/n-3 ratio was observed in T3 and T4 compared with T1 and T2.

Table 5. Carcass and cuts weights (kg) by feeding treatment.

Treatment	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM <sup>1</sup>	P-value
Hot carcass weight (kg)	266.2 <sup>b</sup>	292.0 <sup>ab</sup>	295.8 <sup>a</sup>	309.3 <sup>a</sup>	9.2	0.0172
Pistola cut (kg)	62.4 <sup>b</sup>	68.7 <sup>a</sup>	68.4 <sup>a</sup>	71.2 <sup>a</sup>	2.0	0.0318
Rump & loin (kg)	12.4 <sup>b</sup>	13.9 <sup>a</sup>	13.9 <sup>a</sup>	14.6 <sup>a</sup>	0.5	0.0239
Tenderloin (kg)	2.1	2.2	2.1	2.3	0.1	0.4720
Striploin (kg)	4.9 <sup>b</sup>	5.7 <sup>a</sup>	5.7 <sup>a</sup>	6.1 <sup>a</sup>	0.3	0.0239
Sirloin (kg)	5.4 <sup>b</sup>	6.1 <sup>a</sup>	6.1 <sup>a</sup>	6.3 <sup>a</sup>	0.2	0.0265
Inside round (kg)	7.7 <sup>b</sup>	8.1 <sup>a</sup>	8.2 <sup>a</sup>	8.6 <sup>a</sup>	0.3	0.0139
Outside round (kg)	7.1	7.7	7.6	7.9	0.3	0.1857
Knuckle (kg)	5.0	5.1	5.1	5.3	0.2	0.7786
Tri-tip (kg)	1.2 <sup>b</sup>	1.4 <sup>a</sup>	1.5 <sup>a</sup>	1.5 <sup>a</sup>	0.1	0.0087
Heel muscle (kg)	2.0	2.1	2.2	2.2	0.1	0.3256
Shank (kg)	1.8	1.9	2.0	2.0	0.1	0.2607
Flank on (kg)	3.9 <sup>b</sup>	5.1 <sup>a</sup>	4.8 <sup>ab</sup>	5.2 <sup>a</sup>	0.2	0.0015
Meat trimmings (kg)	3.5	3.5	3.6	4.1	0.2	0.1996
Fat trimmings (kg)	4.4	5.5	5.6	6.0	0.5	0.1020
Bones (kg)	12.1	12.9	12.8	13.1	0.4	0.2163
Meat trimmings (% 2)	1.3	1.2	1.2	1.3	0.1	0.5659
Fat trimmings (% 2)	8.9	11.4	11.6	12.1	1.0	0.1239
Bones (% 2)	9.5	9.2	9.0	8.9	0.2	0.0707

<sup>1</sup>SEM: standard means error. <sup>2</sup> Hot carcass weight = 100 %. <sup>a,b</sup>different letters within the same parameter indicated significant ( $P < 0.05$ ) differences between treatments.

Table 6. Meat quality characteristics by feeding treatment.

Treatments	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM <sup>1</sup>	P-value
Ultimate pH (units)	5.63	5.65	5.63	5.61	0.01	0.4658
L* (lightness) 7 d	33.93	34.40	34.78	34.69	0.53	0.6744
a* (redness) 7 d	17.27	17.52	17.28	17.91	0.44	0.7081
b* (yellowness) 7 d	9.16	9.42	9.57	9.48	0.28	0.7630
L* (lightness) 21 d	35.94	37.00	36.33	36.71	0.57	0.5835
a* (redness) 21 d	17.22	16.66	16.44	17.41	0.71	0.7427
b* (yellowness) 21 d	10.05	9.89	9.93	10.10	0.29	0.9459
WBSF2 7 d (kgF)	4.77	4.66	5.61	4.86	0.42	0.3440
WBSF2 21 d (kgF)	3.54	3.47	3.65	3.63	0.21	0.9101

<sup>1</sup>SEM: standard error of the means. <sup>2</sup>WBSF: Warner-Bratzler shear force

Table 7. Proportion (%) of meat samples with WBSF values lower than 4.5 kgF for each feeding treatment at two aging times (7 and 21 days).

Treatments	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	P-value
7 d	40	40	40	30	0.9562
21 d	100	100	90	90	0.4108

Table 8. Warner-Bratzler shear force and instrumental lean color (L\*, a\*, and b\*) by aging period (7 and 21 days) across treatments.

Ageing period (days)	7	21	SEM <sup>1</sup>	P-value
WBSF (kgF) <sup>2</sup>	5.0 <sup>b</sup>	3.6 <sup>a</sup>	0.2	< 0.0001
L* (lightness)	34.5 <sup>b</sup>	36.5 <sup>a</sup>	0.3	< 0.0001
a* (redness)	17.5	17.2	0.3	0.1773
b* (yellowness)	9.4 <sup>b</sup>	10.0 <sup>a</sup>	0.1	0.0036

<sup>1</sup>SEM: Standard error of the means. <sup>2</sup>Warner-Bratzler shear force. <sup>a, b</sup> different letters within the same parameter indicated significant ( $P < 0.05$ )

Table 9. Fatty acid composition (%) by feeding treatment.

Treatments	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM <sup>1</sup>	P-value
Intramuscular fat (%)	3.5	4.7	4.6	4.1	0.4	0.1380
C 14:0 (Myristic)	2.1 <sup>b</sup>	2.6 <sup>a</sup>	2.3 <sup>ab</sup>	2.1 <sup>b</sup>	0.1	0.0270
C 14:1 (Myristoleic)	0.35	0.40	0.40	0.30	0.04	0.1585
C 16:0 (Palmitic)	27.5	28.6	27.7	27.4	0.4	0.1166
C 16:1 (Palmitoleic)	3.8	4.0	4.0	3.4	0.2	0.1083
C 18:0 (Stearic)	16.2	15.7	15.7	17.1	0.6	0.2993
C 18:1n-9 (Oleic)	45.0	45.0	45.6	44.8	0.7	0.8678
C 18:2n-6 (Linoleic)	2.0 <sup>bc</sup>	1.7 <sup>c</sup>	2.3 <sup>ab</sup>	2.7 <sup>a</sup>	0.2	0.0006
C 18:3n-3 (Linolenic)	0.72 <sup>a</sup>	0.56 <sup>ab</sup>	0.42 <sup>b</sup>	0.41 <sup>b</sup>	0.05	0.0001
C 18:2 c9, t11	0.50 <sup>a</sup>	0.46 <sup>a</sup>	0.45 <sup>ab</sup>	0.37 <sup>b</sup>	0.03	0.0318
C 20:0 (Arachidic)	0.08 <sup>a</sup>	0.05 <sup>b</sup>	0.03 <sup>b</sup>	0.05 <sup>b</sup>	0.01	0.0183
C 20:2 n-6 (Eicosadienoic)	0.03	0.02	0.02	0.02	0.00	0.0892
C 20:3 n-3 (Eicosatrienoic)	0.14	0.08	0.13	0.14	0.02	0.0877
C 20:3 n-6 (Dihomo- $\gamma$ -linolenic)	0.05	0.04	0.03	0.05	0.01	0.2339
C 20:4 n-6 (Arachidonic)	0.76 <sup>a</sup>	0.41 <sup>b</sup>	0.47 <sup>b</sup>	0.56 <sup>ab</sup>	0.08	0.0248
C 20:5 n (Eicosapentaenoic)	0.27 <sup>a</sup>	0.19 <sup>b</sup>	0.12 <sup>b</sup>	0.13 <sup>b</sup>	0.03	0.0004
C 22:5 n-3 (Docosapentaenoic)	0.34 <sup>a</sup>	0.23 <sup>b</sup>	0.15 <sup>b</sup>	0.19 <sup>b</sup>	0.0	0.0019
C 22:6 n-3 (Docosahexaenoic)	0.09	0.08	0.06	0.05	0.01	0.0813
Saturated fatty acids (SFA)	46.0	46.9	45.8	46.7	0.7	0.5942
Monounsaturated fatty acids (MUFA)	49.1	49.3	50.0	48.6	0.7	0.5931
Polyunsaturated fatty acids (PUFA)	4.5	3.4	3.8	4.3	0.3	0.0557
n-6	2.9 <sup>a</sup>	2.2 <sup>b</sup>	2.9 <sup>a</sup>	3.4 <sup>a</sup>	0.2	0.0055
n-3	1.6 <sup>a</sup>	1.1 <sup>ab</sup>	0.9 <sup>b</sup>	0.9 <sup>b</sup>	0.1	0.0003
PUFA/SFA	0.10	0.07	0.08	0.09	0.01	0.0053
n-6/n-3	1.9 <sup>b</sup>	2.0 <sup>b</sup>	3.5 <sup>a</sup>	3.7 <sup>a</sup>	0.2	0.0001

<sup>1</sup>SEM: standard error of the means. <sup>a, b</sup>: Different letters within the same parameter indicated significant ( $P < 0.05$ ) differences between treatments.

## Discussion

Overall, diets with greater energy supply resulted in greater animal performance and, consequently, greater slaughter weight, carcass weight, and valuable cuts' weights. Additionally, meat quality was positively affected and modified the fatty acid profile, potentially benefiting human health.

As observed in our experiment, previous studies with cull cows (Restle et al., 2000; 2001; Aranha et al., 2018) and steers (Coppo et al., 2002; Beretta et al., 2006; Pouzo et al., 2015; Aranha et al., 2018) have reported that greater FA or the use of concentrate supplementation under grazing conditions on improved pastures or rangelands resulted in greater ADG and therefore, resulted in greater final BW. The carcass variables followed a similar response to the final BW. Greater final BW was achieved by cows from the greatest herbage allowance or supplemented rate used, which generated greater HCW, CCW, pistola cut weight and high-value individual cut weights. Similar findings were reported by del Campo et al. (2008) in steers, where the inclusion of concentrate supplementation or FA above 2 % of BW increased carcass weights compared to steers fed exclusively on pastures at 2 % of FA or not supplemented. Realini et al. (2004) also observed heavier carcasses on steers grazing improved pastures with additional use of

concentrate supplements when compared to animals fed exclusively on pastures. Other research studies on cull cows (Restle et al., 2000, 2001; Stelzleni et al., 2007), reported similar animal responses, specifically when considering individual cut weights within the pistola cut considered, indicating that as diet quantity and quality improves, so does slaughter BW, HCW and main high value cut weights.

In the present study, the final pH did not present differences among treatments with  $\text{pH} < 5.7$ , a threshold value below which the probability of the occurrence of dark, firm, and dry (DFD) cuts is reduced (McNally and Warriss, 1996). As for the values of NE of each feeding treatment, all of them presented a positive balance between dry matter intake and NE/CP requirements, which positively impacted on the final pH, reaching the desired range values (Immonen et al., 2000). The Uruguayan Meat Quality Audits have shown that an important proportion of the nationwide slaughter of cull cows (16.3 %) presented pH values above 5.8 (Correa and Brito, 2017). However, unlike the cows of the present experiment, these animals come from different finishing and management conditions, which could affect pH values. All animals were handled under a controlled nutritional status and good management practices during the experimental

period, preventing animals from being exposed to ante-mortem stress factors.

Tenderness is considered the most important palatability trait in meat, affecting consumer acceptance (Dikeman, 1987; Miller et al., 1995; Boleman et al., 1997). WBSF values did not differ among treatments for both aging periods (7 and 21 days) in this study. This finding agrees with Realini et al. (2004), Duckett et al. (2007) and Latimori et al. (2008) research studies, which did not find any differences in young steers on WBSF values when comparing different feeding treatments (concentrate and pasture). Schnell et al. (1997) evaluated different feeding periods using cull cows fed on high energy diets and did not find differences in meat shear force when comparing concentrate supply duration. Couvreur et al. (2019) observed that the shear force of cull cow's meat was not affected when comparing different types of finishing diets. Meat tenderness is mainly affected by the amount and solubility of connective tissue, the composition and contractile state of muscle fibers, and the extent of postmortem proteolysis (Joo et al., 2013) and depends on various factors such as breed, gender, age and slaughter and maturation conditions (Silva et al., 2010). As an animal matures, the stability of collagen cross-linking between molecules increases and partly explains the increase in meat toughness observed in old animals (Bailey, 1989; McCormick, 1994; Andreas et al., 1995). Several authors have established WBSF threshold values below which meat would be considered tender (Shackelford et al., 1995; Huffman et al., 1996; Miller et al., 2001; Platter et al., 2003; Rodas-González et al., 2009) which were not reached by any feeding treatment when meat was aged for 7 days. However, lengthening the meat aging period to 21 days allowed for extending the tenderization process, and WBSF values of all treatments were below 3.7 kgF. The decrease of WBSF values as the aging period increases agrees with the findings of Realini et al. (2004), working with Hereford steers and with that of Matulis et al. (1987), studying British breed 8-year-old cull cows finished in the high-concentrate diet. Similar results were observed by Kuss et al. (2005) working with Charolais and Nellore breeds animals and by Stelzleni et al. (2007) studying Angus x Brahman crossbreeds. In addition, Mandell et al. (2006) concluded that longer aging periods of 28 days were needed to reach acceptable WBSF values on meat from cull cows. The findings of the present study showed that 7 days of meat ageing are not enough to attain acceptable WBSF values in cull cows. However, Meilgaard et al. (1999) pointed out that some experts question the validity of using sensory "thresholds" values because they are ill-defined in theory but may

not reproduce results well, and may not even exist.

Lean color is one of the most relevant meat characteristics affecting consumer's purchase decisions (Faustman and Cassens, 1990). Usually, grass-fed beef is darker in appearance than cattle fed on high-concentrate diets (Muir et al., 1998; Vestergaard, et al., 2000; Realini et al., 2004; Couvreur et al., 2019). In our study,  $L^*$ ,  $a^*$  and  $b^*$  values did not differ among treatments in both aging times, indicating no effect of concentrate supplementation even at 1.6 % of BW. These results were similar to those reported by del Campo et al. (2008) and Luzardo et al. (2008) using steers in comparable feeding and management conditions as in this study, but also similar to those of the experiments carried out by Stelzleni et al. (2007) with concentrate-fed cull cows. On the other hand, Couvreur et al. (2019) found a trend in greater  $b^*$  for cows grazing pastures exclusively compared to similar groups of animals that were also being supplemented. Dark lean beef is often confused with dark-cutting beef due to glycogen depletion due to pre-slaughter stress (Tarrant, 1981).

The results of the present study showed a normal ultimate pH ( $pH \leq 5.65$ ) in all treatments which would indicate enough glycogen levels in muscle prior to slaughter. Darker meat colour from grass-fed cattle may be a consequence of more oxidative metabolism rather than a stress-related ante-mortem event, since greater myoglobin content and enzymes associated with oxidative metabolism have been observed on meat from grass-fed animals (Apaoblaza et al., 2020). If we extrapolate consumer benchmark values referred to meat colour lightness ( $L^*$ ) for lamb (Khlijji et al., 2010) to beef, the  $L^*$  values of all treatments would be very close to 34, the minimum value from which on average consumers would consider the meat lightness acceptable. However, Holman et al. (2017) reported that the redness ( $a^*$ ) provided the most simple and robust prediction of beef colour acceptability and when its value was equal to or above 14.5, where beef colour was considered acceptable (with 95 % acceptance) by consumers. In our study,  $a^*$  values in all treatments were above the mentioned threshold regardless of the aging period. Other authors also studied the relationship between instrumental and visual appraisal of colour (Goñi et al., 2008; Ripoll et al., 2012), however more research is needed to achieve conclusive findings.

Even though the diet has less influence in ruminants than in monogastric species on IMF fatty acid composition (Scollan et al., 2006), both feed and energetic intake affect ruminant carcass fat deposition and fatty acid composition of subcutaneous and



intramuscular fat (Mir et al., 2000). In our study, IMF content did not present differences among treatments. Similar results were observed in steers by Realini et al. (2004), Descalzo et al. (2005), Brito et al. (2008), Latimori et al. (2008). Grass-fed steers have shown greater n-3 PUFA concentrations on IMF, while grain-based systems increased the proportion of MUFA (French et al., 2003; Realini et al., 2004; Descalzo et al., 2005). However, scientific literature related to the effects of feeding systems on cull cow's meat fatty acid composition is scarce. Noci et al. (2005) found that PUFA and n-3 contents increased linearly in heifer's meat when the grazing period was longer (0, 40, 99, and 158 days) within the overall fattening process. The results observed for total n-6 and n-3 PUFA concentrations agree with those reported by Duckett et al. (2007) on steers, Noci et al. (2005) on heifers, and Stelzleni et al. (2008) on cull cows. It may be concluded that even within grass-based production systems, some differences may be found in fatty acids composition, depending on the level of supplementation, the type of supplement, genetics and animal age. Greater PUFA/SFA ratios were observed in both supplemented treatments and in the treatment with the lowest FA. Regardless of these differences, the ratio was always above the recommended threshold of 0.45 (UK Health Department, 1994).

The results of this experiment are consistent with studies in heifers in different grazing periods (Noci et al., 2005) and in finished steers in grazing regimes or in combinations of pastures with concentrates (Realini et al., 2004; Zea et al., 2007; Brito et al., 2008), who observed PUFA / SFA ratios below 0.45. In terms of n-

6/n-3 fatty acids ratio, despite those greater values being observed in the RB supplemented treatments (T3 and T4), all treatments presented values within the recommendations of the UK Health Department (1994). De la Fuente et al. (2009) compared the fatty acid profile of steers fed on grass from Uruguay with similar animals from the UK (grass based and supplement) and from Spain and Germany (feedlot) and observed that the Uruguayan samples presented greater omega 3 levels and better om6/om3 ratios, compared to the other 3 production systems. These findings showed the beneficial effects of meat consumption from grass-fed ruminants (Wood et al., 2004) on human health. A human diet with a high n-6:n-3 PUFA ratio promotes the pathogenesis of many chronic diseases, such as cardiovascular disease (Simopoulos, 2008). The optimal n-6:n-3 fatty acid ratio varies from 1:1 to 4:1 depending on the disease under consideration (Simopoulos, 2002).

The main source of conjugated linoleic acid (CLA) in the human diet is animal products such as dairy and ruminant meat (Chin et al., 1992). In the current experiment, CLA concentrations presented differences among treatments, being the lowest when the diet included the highest supplement level. The greater CLA production with pasture-based diets would be associated with the impact on the ruminal bacteria population and their biohydrogenation capacity, which would result in greater CLA concentrations in meat. Thus, our results agree with Camfield et al., 1999; French et al., 2003; Realini et al., 2004, and Scollan et al., 2006.

### Conclusions

The combination of greater forage allowances and/or supplementation resulted in heavier high-value cuts (e.g. rump and loin cuts). It is important to note that a 21 days-aging period was necessary to reach WBSF values for cull cows' meat to be considered acceptable in terms of tenderness. Despite

the scarcity of scientific literature available for cull cows, the results of the present study suggest that grass-based production systems with null or low use of supplements in British breeds produce acceptable carcasses, and meat quality traits and would associate with a healthy meat fatty acid profile.

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### Contributors' statement

Conceptualization: X. Lagomarsino and F. Montossi; Data curation: X. Lagomarsino and F. Cazzuli, Formal Analysis: X. Lagomarsino and F. Cazzuli; Funding acquisition: F. Montossi; Investigation and writing: X. Lagomarsino, F. Montossi, F. Cazzuli, S. Luzardo, M. Font; Methodology: X. Lagomarsino, F. Montossi; Project administration: F. Montossi.



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