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Replacing sodium bicarbonate with half amount of calcareous marine algae in the diet of beef cattle

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ABSTRACT - This study evaluated the effects of feeding calcareous marine algae or sodium bicarbonate as rumen buffer on the performance, behaviour, *in vitro* diet digestibility, and meat quality of beef cattle. A total of 180 Charolaise bullocks (536±38 kg; 14±1 months of age) were divided into two homogeneous groups and fed a diet with a mineral mix containing 40% sodium bicarbonate or 20% calcareous marine algae (CMA) for the entire fattening period (130 days). Of the *in vivo* and *in vitro* parameters evaluated, CMA supplementation improved average daily gain and feed conversion ratio and reduced the prevalence of bloat and lameness. Bullocks fed CMA tended to exhibit a calmer behaviour while in the pen. Supplementation with CMA improved rumen pH and *in vitro* digestion. Meat from bullocks fed CMA showed a lower pH and higher lightness and tenderness. The results suggest that CMA is more effective than sodium bicarbonate in buffering beef cattle, with a positive impact on growth performance, feed efficiency, health, and meat quality.

Keywords: acidosis, buffer, behavior, digestibility, lithothamnium, meat quality

Introduction

Acute and subacute rumen acidosis frequently affect intensively reared beef cattle, which are fed high-energy diets rich in highly fermentable carbohydrates. Acidosis reduces feed intake, growth performance, damages the gastrointestinal tissue, leads to inflammation due to lipopolysaccharides released from the death of gram-negative bacteria and predisposes cattle to diseases such as liver abscess and laminitis (Sgoifo Rossi and Compiani, 2016). Typical signs are rumen bloat, due to a high production and low absorption of volatile fatty acids (VFA), and loose manure due to a hyperosmotic environment in the gut.

Sodium bicarbonate (BIC) is commonly supplemented to buffer rumen pH, although its buffering activity is reduced in condition of low rumen pH (5.5-5.8), as it has a pKa of 6.25 (Russell, 1998). In addition, BIC rapidly solubilizes into rumen fluid, with a fast but not long-lasting buffering activity. Because of this, a high amount is required to buffer the rumen pH of cattle fed high-energy diets (0.7-1% of DM) (Krause, 2008). Giving a high amount of BIC in diets rich in phosphorous as those offered to beef cattle can increase urine pH above 6.8, increasing the risk of developing urolithiasis.

Calcareous marine algae (CMA), the skeletal of seaweed *Lithothamnium calcareum* harvested in the North Sea, could represent an alternative to BIC. Nearly 25% of CMA consists of calcium, in the form of calcium carbonate, a powerful buffer: bicarbonate ion (HCO_3^-) can react with one proton, while carbonate ion (Co_3^{--}) can react with two protons. Compared with limestone (100% calcite), CMA has a specific mineral

composition (65% calcite, 12% vaterite, and 23% aragonite; Celtic Sea Minerals Ltd.), making it soluble at rumen pH (Cruywagen et al., 2015). Cruywagen et al. (2004) found an improvement in rumen pH by supplementing an increasing dose of CMA, from 0.125 to 1.2% dietary DM. Cruywagen et al. (2015) found that half a dose of CMA (0.4% DM) was more effective than BIC (0.8% DM) in buffering the rumen pH of dairy cows fed a high-energy diet. For beef cattle, supplemental calcium is beneficial to balance the calcium:phosphorous ratio and can improve meat tenderness by increasing muscle calcium, as calpain activity is calcium-dependent (e.g. Wheeler et al., 1997).

The objective of this study was to evaluate the effect of replacing BIC with half amount of CMA on performance, health, behaviour, rumen fermentation, *in vitro* digestibility, and meat quality of beef cattle.

Material and Methods

Procedures involving animals were carried out in compliance with EC Council Directive 2010/63. The study was carried out in a feedlot (latitude 45.6974, longitude 9.1376, and altitude 288 m) in northern Italy. It involved 180 newly received Charolaise male cattle imported from France (536±38 kg; 14±1 months of age), which were monitored throughout the fattening period (130 days).

At the arrival, cattle were weighed, and conformation was evaluated on a five-point scale (McKiernan, 2007). Cattle were treated for endo- and ectoparasites (Ivomec plus®, Merial Italia SpA, MI, Italy) and vaccinated against respiratory disease viruses (Cattlemaster 4®, Zoetis Italia S.r.l., Rome, Italy) on arrival and 21 days later as a booster. Cattle were blocked by weight and conformation and randomly allotted to slatted floor pens, each containing 10 animals, with a space allowance of 3.5 m^2/head, to obtain two homogeneous groups fed diets differing for the buffer included in the mineral-vitamin premix: BIC CMA. Animals were inspected twice a day by the farm veterinarian to evaluate the prevalence of diseases related to nutritional disorders – lameness and bloat. Bloat was defined as evident flank distention (score 1 to 3 of Min et al., 2005), while cattle were considered affected by lameness in the case of swollen joints and/or with deviation from normal walking (score 1 to 5 of Spercher et al., 1997) not caused by traumatic events.

Cattle were fed *ad libitum* a high-energy total mixed ration diet (Table 1) daily in the morning at 07.00 h and the feed pushed-up in the bunk at 12.00 and at 17.00 h, with an estimated orts of 5-10% based on farm data and previous experiments carried out in the same farm (Sgoifo Rossi et al., 2012). The amount of feed administered was recorded daily and once a week, before fresh feeding administration, and residual feed in the bunk was collected and weighed. The BIC and CMA diets of the two groups differed only in terms of the composition of mineral and vitamin mix.

The BIC mineral mix contained 400 g·kg^{-1} of sodium bicarbonate, which was replaced with 200 g·kg^{-1} of CMA (AcidBuf®, Celtic Sea Minerals, Cork, Ireland) and adjusted in terms of the calcium and magnesium content (Table 2).

Animals were fed an adaptation diet for 25 days and then gradually adapted to the finisher diet (three days with 2/3 adaptation diet and 1/3 finisher diet, three days with 50% adaptation diet and 50% finisher diet, and three days with 1/3 adaptation diet and 2/3 finisher diet). Cattle were individually weighed at days 0, 21, 100, and 130, and the average daily gain (ADG) was calculated.

Behaviour during weighing was evaluated at the same intervals. A temperament score was assigned whilst cattle were restrained in the chute (1 = calm, no movement; 2 = slightly restless; 3 = squirming, occasional shaking of chute; 4 = continuous vigorous movement of chute; 5 = rearing, twisting, or violently struggling; Grandin, 1993), and exit (1 = walk, 2 = trot, 3 = run, and 4 = jump; Lanier and Grandin, 2002). Behaviour was also evaluated in 2-h observation sessions at days 30, 60, 90, and 120, recording the number of mounting and fighting events (Cozzi et al., 2013).

Feed samples (200 g) were collected weekly and pooled for dry matter, crude protein, ether extract, starch, and ash determination, according to AOAC (1990); neutral-detergent fibre was determined according to Van Soest et al. (1991); the net energy content of the diet, as metabolisable energy, was calculated using the reference values for all feed ingredients reported by NRC (2000); and calcium,

phosphorous, magnesium, and sodium were determined using ICP-MS methods (Agilent 7500cx). As previously reported, orts were collected once a week to determine dry matter.

Faeces samples were collected from the rectus of one cattle/pen on days 21 and 100 to determine dry matter, crude protein, crude fat, ashes, starch (AOAC, 1990), and fibre fractions (Van Soest et al., 1991). At the same time, blood samples were collected from the jugular vein to determine plasma glucose, lactic acid, and serum urea. Plasma glucose was assessed by the glucose oxidase/peroxidase method (ILAB 300 plus, Instrumentation Laboratory, Milan). Plasma was obtained by centrifuging blood collected with EDTA tubes at 3500 rpm for 10 min (Venosafe®, Terumo). Plasma lactate was determined using a commercial kit (Lactate dry fast – Sentinel Diagnostics, Milan, Italy), while plasma urea concentration was determined by colorimetric assay (Technicon AAII Autoanalyser). From the same animals, 200 mL of rumen fluid was collected at the slaughterhouse and transported refrigerated to the laboratory for pH (Hanna Instruments, HI5522), N-NH_3, and lactic, acetic, propionic, and butyric acid determination. Gas were determined with high-performance liquid chromatography (Shimatzu, Kyoto, Japan).

To conduct a preliminary test on the applicability of rumen bolus for pH monitoring, a bolus fitted with a pH probe (Smaxtec, Graz, Austria) was inserted into the rumen of three cattle per group, assigned to different pens, at day 21, four days before the diet change, and the pH was monitored every 10 min for 35 days (data not statistically analysed; Figure 1). Particle size analysis of faeces was performed pooling

Table 1 - Experimental diets

Feed ingredient (g·kg^{-1} DM)	Adaptation diet		Fattening diet	
	BIC	CMA	BIC	CMA
Mineral-vitamin with sodium bicarbonate (BIC)	18.2	-	15.5	-
Mineral-vitamin with calcareous marine algae (CMA)	-	18.2	-	15.5
Copra expeller	25.9	25.9	22.1	22.1
Sunflower meal 28% CP	25.9	25.9	22.1	22.1
Rape cake	17.3	17.3	14.7	14.7
Urea	1.8	1.8	2.3	2.3
Slow-release non-protein nitrogen (Optigen®)	2.7	2.7	3.9	3.9
Corn meal	250.5	250.5	413.7	413.7
Rice bran	86.4	86.4	125.0	125.0
Wheat straw	129.6	129.6	73.5	73.5
Corn silage, 35% DM	369.6	369.6	134.4	134.4
Fresh brewer grains, 25% DM	72.0	72.0	108.0	108.0
Nutritional values				
Dry matter (g·kg^{-1})	503.3	503.6	623.6	624.0
Metabolisable energy (MJ·kg^{-1} DM)[1]	10.18	10.18	11.71	11.76
Crude protein (g·kg^{-1} DM)	126.5	127.1	145.5	14.63
Neutral detergent fibre (g·kg^{-1} DM)	361.5	362.7	281.3	282.3
Starch (g·kg^{-1} DM)	321.7	322.2	424.5	425.2
Ca (g·kg^{-1} DM)	7.3	7.6	7.7	7.8
P (g·kg^{-1} DM)	4.5	4.5	5.4	5.4
Mg (g·kg^{-1} DM)	2.9	2.9	2.4	2.4

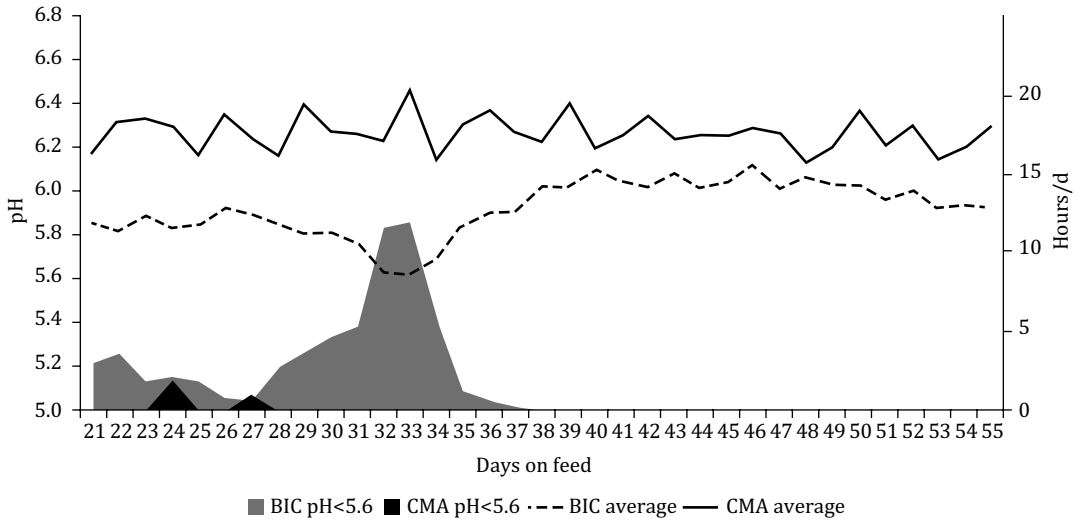
[1] Estimated from the reference values reported by NRC (2000).

the faeces collected from the rectus from all the cattle in the pen using a Nasco Digestion Analyser (Nasco Fort Atkinson, WI, USA), comprised of three metal screens with a pore size of 4.76, 2.38, and 1.59 mm, respectively. Approximately 500 g of faeces were collected and gently washed through the sieves until the water was clean. Orts was collected and dried for dry matter determination (AOAC, 1990), and distribution between sieves (% of overall weight) was calculated. Given the impossibility of measuring individual feed intake, data were not statistically analysed.

Assuming an *in vivo* average dry matter intake of 10 kg and a daily administration of 200 g/head of the studied mineral-vitamin premix containing CMA (20% DM), the mineral-vitamin premix was pharmaceutically diluted in a representative sample of the basal diet at a concentration of 2%, equal to a proportion of 0.5% of CMA. A similar dilution was performed for the BIC mineral-vitamin premix containing calcium carbonate (53.5%) and sodium bicarbonate (40.0%). *In vitro* neutral detergent fibre (NDF) digestibility (NDFD) was measured as detailed in Comino et al. (2014). Briefly,

Table 2 - Mineral and vitamin premixes

Ingredient (g·kg^{-1} as fed)	BIC	CMA
Calcium carbonate	535.0	400.0
Calcareous marine algae (CMA; Acid Buf®)	-	200.0
Sodium chloride	-	100.0
Sodium bicarbonate (BIC)	400.0	-
Magnesium oxide	20.0	-
Mycotoxin binder	20.0	20.0
Corn gluten feed	15.0	270.0
Vitamins	5.0	5.0
Micro minerals	4.0	4.0
Flavour	0.5	0.5
Live yeast	0.5	0.5
Ca (g·kg^{-1} DM)	224.0	223.3
Mg (g·kg^{-1} DM)	14.5	14.4
Na (g·kg^{-1} DM)	82.7	40.8
P (g·kg^{-1} DM)	0.1	1.9



BIC - sodium bicarbonate; CMA - calcareous marine algae.

Figure 1 - Rumen pH monitored continuously with a bolus across diet transition (from day 25 to day 33; nine days) in three animals per group fed BIC or CMA diet.

CMA and BIC total mixed ration subsamples were weighed (0.5 g) in 100-mL flasks (Schott Duran, Wertheim/Main, Germany).

To test the *in vitro* effect of CMA on fibre and dry matter digestibility (DMD), a medium, composed of a buffer, macromineral solution, micromineral solution, and resazurin as the redox state indicator of the system, was introduced into the flasks. A reducing solution was added to create an anaerobic environment. The flasks were then placed into a water bath and purged with CO_2 to obtain complete anaerobiosis. Rumen fluid was collected from a dry cow fed 2 kg of concentrate per day and given *ad libitum* access to grass/alfalfa mixed hay (55% NDF; 14% CP). Rumen fluid was then stirred, filtered through four layers of cheesecloth, and inoculated into each flask to start the fermentation process.

Subsamples were incubated for intervals of 4, 8, 24, and 48 h at 39.5 °C. Two analytical replicates were incubated per treatment for each incubation time. After incubation, each subsample was filtered through crucibles (Robu Glass Filter-ROBU H3, Borosilicate 3.3, 30 mL-Por. 2, Hattert, Germany), rinsed three times with boiling water, dried overnight at 105 °C, and weighed for DMD determination. The liquid derived from the first filtration process was collected and analysed for volatile fatty acids and lactic acid content by high-performance liquid chromatography (HPLC). Amylase-treated NDF (aNDF) was then determined on each subsample DM residue using a raw fibre extractor (FIWE, VELP Scientifica, Usmate Velate, Italy).

Each crucible containing the DM residue was connected with the raw fibre extractor tubes, and the residue was boiled for 1 h in a neutral detergent solution that included heat-stable amylase (Number A3306; Sigma Chemical Co.) and filtered through the same crucibles. The neutral detergent residuals were then rinsed three times with boiling water, dried overnight at 103 °C, and weighed to calculate NDFD at 4, 8, 24, and 48 h. Acid detergent fibre (ADF) was sequentially determined on NDF residues obtained at the different incubation times for the determination of ADF digestibility (ADFD) (Van Soest, 1991).

Analyses of HPLC were performed in duplicate for each treatment and interval. The lactic and monocarboxylic acids (acetic, propionic, and butyric acids) were determined by HPLC in the acid extract from rumen fluid using an Aminex HPX-87H strong cation exchange resin column at 41 °C, 0.0025M H_2SO_4 mobile phase, and ultraviolet detector at 210 nm as described in Canale et al. (1984).

Cold carcass weight (as 98% of hot carcass weight) was recorded, and dressing percentage calculated. SEUROP conformation (converted on a six-point scale: S = 6, E = 5, U = 4, R = 3, O = 2, P = 1) and fattening score (1-5) were assessed by a professional assessor (EU, 2006. Council Regulation EC 1183/2006).

At 24 h post-mortem, carcass pH was measured on three cattle per pen on *longissimus dorsi* between the 5th and 6th ribs (HI 98150, HANNA Instruments Inc., Woonsocket, RI, USA), and 5 cm-thick *longissimus dorsi* samples were then taken in the same position. Before being vacuum-packaged, two thin slices were removed perpendicular to the muscle fibres (nearly 10 g) for calcium titration and chemical composition (dry matter, ether extract, crude protein, and ash).

Muscle calcium was determined with inductive coupled plasma-optical emission spectrometry (ICP-OES), while chemical composition was determined on samples trimmed from external fat and connective tissue and homogenized for 30 s according to AOAC (1990). Samples were then weighed, vacuum-packaged, and stored at 2±1 °C for 6 days.

At the end of the established ageing period, samples were unpackaged, blotted dry, and reweighed to evaluate purge loss. At the opening, a thin slice perpendicular to the muscle fibre was removed to create a fresh cut surface, which was allowed to bloom for 1 h at refrigerated temperature in a dark room. The instrumental colour was then assessed with a chromameter (Minolta Camera, Co., Osaka, Japan) calibrated on the CIE L* a*b * colour space (Calibration Plate 21533131 Y 93.4 x 0.3456 y 0.3321, Minolta Cameras Co., Osaka, Japan), illuminant D65 and view angle 10. Samples were blotted dry before being weighed. After colour measurement, 3 cm thick samples were weighed, cooked in a water bath at 75 °C core temperature (monitored with a temperature meter Hanna Instruments HI98840 temperature meter) and refrigerated overnight. On the subsequent day, samples were weighed again to measure

cooking loss. After cooking loss determination, six cylindrical cores, 1.27 cm in diameter, parallel to the fibre orientation, were obtained and used for shear force evaluation, using a Warner-Bratzler shear force texture analyser (model 4466; Instron Corp., Canton, MA). Peak force ($kg \cdot cm^{2\ -1}$) was recorded.

Statistical analysis was performed with SAS software (Statistical Analysis System, version 9.4) with the REML procedure (PROC MIXED). For body weight, ADG, ADFI, FCR, behaviour in pen (average mounting/fighting events per pen), and faeces particle size, pen was considered as the experimental unit, and the model accounted for the fixed effects of dietary treatment and time (not for faeces particle size) and the random effect of the pen within treatment and time. The model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + c_k + \alpha_i\beta_j + e_{ijk},$$

in which Y_{ijk} = observation of the pen *k* given treatment *i* at period *j*, α_i = fixed effect of the *i*-th treatment, β_j = fixed effect of the *j*-th time (when relevant), c_k = the random effect of the *k*-th pen, $\alpha_i\beta_j$ = interaction between *i*-th treatment at *j*-th time, and e_{ijk} = residual error of the model.

For temperament parameters (average temperament score during restraining and exiting the chute), blood (glucose, urea, and lactic acid), and faeces data (dry matter, crude protein, starch, NDF, ADF, acid detergent lignin (ADL), cellulose, and hemicellulose), the animal was considered the experimental unit and the model accounted for the fixed effects of dietary treatment and time and random effect of animal within treatment and time. Repeated measures accounted for the effects of dietary treatment, sampling time, and their interaction. The model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + c_k + \alpha_i\beta_j + e_{ijk},$$

in which Y_{ijk} = observation of the animal *k* given treatment *i* at period *j*, α_i = fixed effect of the *i*-th treatment, β_j = fixed effect of the *j*-th time (when relevant), c_k = the random effect of the *k*-th animal, $\alpha_i\beta_j$ = interaction between *i*-th treatment at *j*-th time, and e_{ijk} = residual error of the model.

Meat quality data were analysed with one-way ANOVA, accounting for the fixed effect of dietary treatment and random effect of the animal. The model used was:

$$Y_{ij} = \mu + \alpha_i + \beta_k + e_{ik},$$

in which Y_{ij} = observation of the animal *k* given treatment *i*, α_i = fixed effect of the *i*-th treatment, β_k = the random effect of the *k*-th animal, and e_{ik} = residual error of the model.

Data from *in vitro* analyses were analysed with the REML procedure accounting for the fixed effect of dietary treatment and intervals of fluid collection and random effects of replicates. The model used was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i\beta_j + c_k + e_{ijk},$$

in which Y_{ijk} = observation of the replicate *k* given treatment *i* at interval *j*, α_i = fixed effect of the *i*-th treatment, β_j = fixed effect of the *j*-th interval, $\alpha_i\beta_j$ = interaction between *i*-th treatment at *j*-th interval, c_k = the random effect of the *k*-th replicate, and e_{ijk} = residual error of the model.

The significance of the differences in prevalence of bloat and lameness between groups was tested with the chi-square test. Significance was set for $P \leq 0.05$, while $P \leq 0.1$ was discussed as tendency.

Results

Considering the overall study period, CMA improved ADG (P = 0.02), although the differences in body weight were not significant. The average daily feed intake (ADFI) did not differ between groups. The higher ADG coupled with the similar ADFI improved FCR (P = 0.03) (Table 3).

Calcareous marine algae reduced the prevalence of acidosis-related diseases (P = 0.05; Table 4): 1 vs 0 bloat and 5 vs 1 lameness were recorded in the group fed BIC compared with the group fed CMA. On average, cattle of both groups were almost calm or slightly restless in the chute and exiting from it, walking or trotting, highlighting a good interaction with humans and with new circumstances (Table 4). Dietary treatment did not affect behaviour. Cattle tended to be quieter during restraining ($P<0.01$) and

exiting the chute (P = 0.06), approaching the end of the fattening cycle. Regarding pen behaviour, cattle that received CMA instead of BIC, overall tended to exhibit a lower number of fighting or mounting events (P = 0.09), while an interactive effect of time and treatment was not observed. Blood parameters were not affected by the dietary treatment (Table 5).

The group fed CMA showed higher fine particles of undigested fraction (P<0.01), suggesting a better fibre digestion. Lower faecal NDF (P = 0.09) and hemicellulose (P<0.01) and higher ADF (P<0.01) and ADL (P = 0.02) were recorded in the group fed CMA, while no differences were evident for DM, CP, and starch (Table 6).

Table 3 - Growth performance parameters (expressed as mean and standard error of the means) of cattle fed sodium bicarbonate (BIC) or calcareous marine algae (CMA) diet (n = 180)

	BIC	CMA	SEM	P diet	P day	P diet × day
			Weight (kg)			
Day 0	539	533	7.62			
Day 21	567	562	7.62	ns	<0.01	ns
Day 100	693	696	7.62			
Day 130	729	734	7.62			
			Average daily gain (kg/d)			
0-21	1.30	1.38	0.04			
0-100	1.53	1.63	0.04	0.02	<0.01	ns
0-130	1.46a	1.54b	0.03			
			Average daily feed intake (kg DM/d)			
0-21	10.32	10.31	0.10			
0-100	11.30	11.31	0.11	ns	<0.01	ns
0-130	11.51	11.52	0.11			
			Feed conversion ratio (kg DM/kg)			
0-21	8.48	7.65	0.25			
0-100	7.86	7.19	0.25	0.03	<0.01	ns
0-130	8.18a	7.66b	0.13			

a,b - P<0.05.

Table 4 - Health, temperament, and behaviour parameters (expressed as mean and standard error of the means) of beef cattle fed sodium bicarbonate (BIC) or calcareous marine algae (CMA) diet (n = 180)

	BIC	CMA	SEM	P diet	P day	P diet × day
Morbidity, % (n)	6.67 (6)	1.11 (1)		0.05		
			Average temperament score while restrained in the chute[1]			
Day 0	1.32	1.27	0.06			
Day 21	1.26	1.21	0.05	ns	<0.01	ns
Day 100	1.20	1.14	0.04			
Day 130	1.22	1.13	0.04			
			Average temperament score exiting the chute[2]			
Day 0	1.30	1.25	0.06			
Day 21	1.19	1.18	0.04	ns	0.06	ns
Day 100	1.20	1.19	0.04			
Day 130	1.27	1.21	0.05			
			Average mounting or fighting events per pen			
Day 30, % (n)	0.44	0.11	0.14			
Day 60, % (n)	0.56	0.11	0.19	0.09	ns	ns
Day 90, % (n)	0.67	0.22	0.31			
Day 120, % (n)	0.7	0.24	0.25			

[1] 1 = calm, no movement; 2 = slightly restless; 3 = squirming, occasional shaking of chute; 4 = continuous vigorous movement of chute; 5 = rearing, twisting, or violently struggling.
[2] 1 = walk; 2 = trot; 3 = run; 4 = jump.

Table 5 - Blood parameters (expressed as mean and standard error of the means) in beef cattle fed sodium bicarbonate (BIC) or calcareous marine algae (CMA) diet (n = 18)

	BIC	CMA	SEM	P diet	P day	P diet × day
			Blood glucose (mg·dL^{-1})			
Day 21	96.88	99.75	5.72	ns	<0.01	ns
Day 100	68.25	70.62	2.85			
			Serum urea (mg·dL^{-1})			
Day 21	31.38	32.50	2.14	ns	<0.01	ns
Day 100	42.00	43.38	2.68			
			Lactic acid (mg·dL^{-1})			
Day 21	32.45	34.75	3.52	ns	<0.01	ns
Day 100	22.96	24.90	0.96			

Table 6 - Faces composition (expressed as mean and standard error of the means) of cattle fed sodium bicarbonate (BIC) or calcareous marine algae (CMA) diet (n = 18)

	BIC	CMA	SEM	P diet	P day	P diet × day
			Dry matter (%)			
Day 21	17.94	17.80	0.42	ns	ns	ns
Day 100	17.55	17.95	0.38			
			Crude protein (% DM)			
Day 21	13.84	13.72	0.37	ns	<0.01	ns
Day 100	17.79	17.64	0.40			
			Starch (% DM)			
Day 21	9.57	9.28	0.33	ns	<0.01	ns
Day 100	13.74	14.17	0.51			
			Neutral detergent fibre (% DM)			
Day 21	74.42	73.93	0.52	0.09	<0.01	ns
Day 100	70.15	68.20	0.73			
			Acid detergent fibre (% DM)			
Day 21	23.42	24.53	0.51	<0.01	<0.01	0.01
Day 100	26.67A	28.47B	0.43			
			Acid detergent lignin (% DM)			
Day 21	4.71	4.37	0.52	0.02	<0.01	<0.01
Day 100	5.54A	7.92B	0.26			
			Cellulose (% DM)			
Day 21	18.71	20.16	0.78	ns	0.10	ns
Day 100	21.12	20.55	0.51			
			Hemicellulose (% DM)			
Day 21	50.99	49.40	0.82	<0.01	<0.01	ns
Day 100	43.49A	39.74B	0.52			
			Particle size (% by weight of residue in each sieve)			
Top sieve - 4.76 mm pore	3.30	2.00	0.33	<0.01	-	-
Middle sieve - 2.38 mm pore	21.00	4.00	0.46	<0.01	-	-
Bottom sieve - 1.59 mm pore	75.67	94.00	0.55	<0.01	-	-

A,B - P<0.01.

The results were in line with findings from *in vitro* data (Table 7). Overall, CMA administration improved NDF (P<0.01), ADF (P<0.01), and total dry matter (P = 0.03) *in vitro* digestion. As expected, fibre and total dry matter digestion improved with time (P<0.001), without an interactive effect between treatment and sampling time. Treatment did not affect *in vitro* VFA production, while there was an increase in VFA production during incubation for acetic, propionic, and butyric acids, with lactate showing the opposite trend (Table 8).

Regarding rumen fluid collected at slaughter (Table 9), the group fed CMA showed a higher pH (P<0.01) and butyric acid (P<0.01). Cattle fed CMA showed higher rumen pH and spend less time with rumen pH below 5.6. Indeed, in the first 15 days of diet change, monitored cattle in group fed BIC spent on average 4:09 h/day with pH<5.6, while those fed CMA spent only 00:12 h/day with pH <5.6.

No differences were detected for dressing percentage, carcass weight, conformation, and fat cover score (Table 10). Similarly, dietary treatment did not affect the chemical composition of the meat, muscle calcium, purge, and cooking loss. Conversely, meat from cattle fed CMA was characterised by a lower pH (P<0.05), higher lightness (P<0.05) and yellowness (P<0.05), and lower shear force (P<0.05). No abnormal pH, above 5.8, was recorded.

Table 7 - *In vitro* DMD, NDFD, and ADFD on ruminal fluid of cattle fed sodium bicarbonate (BIC) or calcareous marine (CMA) diet (expressed as mean and standard error of the means)

	Group	Interval (h)				SEM	P		
		4	8	24	48		Diet (D)	Interval (I)	D × I
NDFD (%NDF)	BIC	19.16a	23.16a	43.04b	62.04Ab	2.67	<0.01	<0.001	ns
	CMA	22.69a	27.57a	47.27b	64.83Bb				
ADFD (%ADF)	BIC	9.75Aa	10.05Aa	43.51b	51.74Ab	3.79	<0.01	<0.001	0.09
	CMA	19.56Ba	17.96Ba	38.45b	58.00Bc				
DMD (%DM)	BIC	5.00Aa	19.68b	60.12c	64.84Ac	8.40	0.03	<0.001	ns
	CMA	12.08Ba	22.43b	59.52c	67.21Bc				

NDFD - neutral detergent fibre digestibility; ADFD - acid detergent fibre digestibility; DMD - dry matter digestibility.
a,b,c - P<0.05.
A,B,C - P<0.01.

Table 8 - *In vitro* volatile fatty acid and lactate production of ruminal fluid of cattle fed sodium bicarbonate (BIC) or calcareous marine (CMA) diet (expressed as mean and standard error of the means)

VFA	Group	Interval (h)				SEM	P		
		4	8	24	48		Diet (D)	Interval (I)	D × I
Lactic acid (mg/dL)	BIC	54.79c	63.98c	20.70b	7.00a	5.97	ns	0.001	ns
	CMA	51.14c	32.41c	19.42b	6.28a				
Acetic acid (mg/dL)	BIC	175.04a	238.31ab	281.87b	305.87b	17.26	ns	<0.001	ns
	CMA	174.56a	229.49ab	307.99b	349.45b				
Propionic acid (mg/dL)	BIC	104.61a	128.95abc	155.52c	130.23b	6.43	ns	0.005	ns
	CMA	109.48a	102.52b	163.49b	142.89ab				
Butyric acid (mg/dL)	BIC	35.62a	99.58b	94.55b	95.26b	8.98	ns	0.002	ns
	CMA	48.59a	52.63a	114.52b	112.62b				

a,b,c - P<0.05.

Table 9 - Volatile fatty acid, lactic acid, and pH (expressed as mean and standard error of the means) of rumen fluid of cattle fed sodium bicarbonate (BIC) or calcareous marine (CMA) diet collected at slaughterhouse (n = 18)

	BIC	CMA	SEM	P
Lactic acid (mg/100 g)	1.91	1.26	0.42	ns
Acetic acid (mg/100 g)	67.33	69.28	15.40	ns
Propionic acid (mg/100 g)	17.50	17.56	1.23	ns
Butyric acid (mg/100 g)	12.45	10.88	0.75	<0.01
pH	5.62	6.25	0.06	<0.01

Table 10 - Carcass traits (n = 180) and meat quality (n = 54) of beef cattle fed sodium bicarbonate (BIC) or calcareous marine (CMA) diet (expressed as mean and standard error of the means)

	BIC	CMA	SEM	P
		Carcass trait		
Carcass weight (kg)	436	441	3.01	ns
Dressing percentage (%)	59.76	60.02	0.77	ns
Conformation E, % (n)	60.00 (54)	57.78 (52)	-	
Conformation U, % (n)	38.89 (35)	42.22 (38)	-	ns
Conformation R, % (n)	1.11 (1)	0.00 (0)	-	
Fat cover 2, % (n)	71.11 (64)	72.22 (65)	-	ns
Fat cover 3, % (n)	28.89 (26)	27.78 (25)	-	
		Colour		
L	44.20	47.62	0.85	<0.01
a*	19.92	21.34	0.87	ns
b*	11.97	13.29	0.39	0.02
Hue	31.20	32.12	0.77	ns
Chroma	23.40	25.43	0.95	ns
		Chemical composition		
Humidity (%)	73.01	73.28	0.15	ns
Fat (%)	2.89	2.69	0.09	ns
Protein (%)	23.12	23.06	0.13	ns
Ash (%)	0.97	0.96	0.01	ns
Calcium (mg/100 g)	4.18	4.23	0.73	ns
		Physical trait		
pH	5.68	5.62	0.02	0.02
Purge loss (%)	3.90	3.79	0.14	ns
Cooking loss (%)	23.37	23.44	0.35	ns
WBSF ($kg \cdot cm^{2-1}$)	3.18	2.97	0.07	0.03

WBSF - Warner-Bratzler shear force.

Discussion

The higher ADG, together with the similar ADFI, explains the improvement in FCR. The lack of effect of dietary treatment on cattle behaviour during weighing operations and the calmer behaviour approaching the end of the fattening cycle, highlight a progressive adaptation to handling. This confirms that temperament and sensitivity to stressful events are essentially related to inherent genetic factors, as demonstrated by the moderate to high temperament heritability (Haskell et al., 2014).

The calmer behaviour in the pen of cattle fed CMA could be explained by the better buffering effect. Although the study was conducted on weathers, care has to be taken to extend the data to beef cattle. Commun et al. (2012) reported a more agitate and aggressive behaviour after acidosis challenge. The present results can thus be explained by the higher capability of CMA to buffer rumen pH, highlighted from the results of rumen pH at slaughter and the lower frequency of diseases related to acidosis such as bloat and not-traumatic lameness.

Blood glucose and lactic acid were high at day 21, while at day 100, a reduction was evident, with values within or close to the reference range (Jackson and Cockcroft, 2008). This was unexpected, considering that starch and energy content of the diet were higher at day 100, but can be explained by the adaptation to the finisher diet. In fact, stocking cattle such as those involved in the present trial are usually fed a small amount of concentrate and a diet with lower energy and protein content before being collected and transferred to the finishing farm. Moreover, stocker cattle imported from abroad to Italy experience feed restriction, as they are undergone, in a short period of time, to the collection from native farms, mixing in the sale yard or collection centres, and to long transport (Sgoifo Rossi et al., 2013). Feed restriction markedly reduces rumen functionality and microbial population (Fluharty et al., 1996). The increase in blood urea, which is the end product of protein metabolism, can be explained by the increase in dietary protein or to a small imbalance between energy and protein in the diet.

Regarding the particle size of the undigested fraction, the higher fine residue found for CMA suggests an improvement in fibre degradation at the rumen level. The improvement in fibre and overall digestion, as highlighted from the faecal analysis and *in vitro* results, can be explained by the more effective buffer activity, as demonstrated by the higher rumen pH and reduction in pH fluctuation at the transition between the adaptation and finisher diet. However, the data related to rumen pH should just be taken as an indication due to the impossibility of determining the individual feed intake. Krajcarski-Hunt et al. (2002) found a decrease of *in situ* fibre digestion following sub-acute ruminal acidosis, likely due to the negative effect of the low pH on the microorganisms involved in fibre digestion. Mesgaran et al. (2010) reported a decrease in the *Fibrobacter succinogenes* population on *in vitro* models of acidotic diets, and even ciliate protozoa have been found to markedly decrease with rumen pH reduction (Nagaraja and Titgemeyer, 2007).

Regarding meat quality, pH is related to meat colour and texture traits. Indeed, a reduction in muscle pH increases the interaction between myofibrillar protein, improving light refraction and, therefore, determining a negative relationship between pH and meat colour (Abril et al., 2001). The difference in meat pH could be related to animal temperament. Fighting and competitive behaviours at the end of finishing can reduce muscle glycogen, thus limiting a decrease in pH after slaughter (Ponnampalam et al., 2017). Despite the known relationship between meat pH and tenderness, considering the narrow range of values found in the present study and the lack of abnormal pH, it seems that other factors determined the difference in meat tenderness. Agitated behaviour in the days before slaughter can increase beef toughness: release of epinephrine following stress responses improves calpastatin activity, which impairs calpain activity (Gruber et al., 2010).

Conclusions

In beef cattle, diets with sodium bicarbonate can be effectively replaced with calcareous marine algae at an inclusion rate of 50% of the original sodium bicarbonate. This strategy improves growth rate, feed conversion, and fibre and total dry matter digestion. It reduces acidosis and related diseases, also promoting a calmer behaviour with beneficial effects on meat quality. The outcomes thus confirm the higher effectiveness of calcareous marine algae as a rumen buffer compared with sodium bicarbonate.

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