Animal 17 (2023) 100685

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Drinking water chlorination in dairy beef fattening bulls: water quality, potential hazards, apparent total tract digestibility, and growth performance

L. Llonch [a], M. Verdú [b], S. Martí [a], C. Medinyà [c], J. Riera [d], J. Cucurull [b], M. Devant [a,*]

[a] Ruminant Production Program, Institut de Recerca i Tecnologia Agroalimentàries, Torre Marimon, 08140 Caldes de Montbui, Barcelona, Spain
[b] Animal Nutrition and Feed Industry, bonÀrea Agrupa, 25210 Guissona, Lleida, Spain
[c] Nutrición Animal S.L., 08650 Sallent, Barcelona, Spain
[d] Nanta S.A., 28760 Tres Cantos, Madrid, Spain

ARTICLE INFO

Article history:
Received 19 May 2022
Revised 11 November 2022
Accepted 14 November 2022
Available online 21 November 2022

Keywords:
Finishing cattle
Water disinfection
Performance
Animal health
Total tract digestion

ABSTRACT

The first study aimed to evaluate the effect of drinking water disinfection (chlorination: NaClO 15%) and conditioning (acidification: H_3PO_4 diluted 1:5 in water) on water quality, water and feed consumption, apparent total tract digestibility, and its potential hazardous effects on Holstein bulls fed high-concentrate diets. Twenty-four animals (221 ± 20.9 kg of BW, and 184 ± 9.9 days of age) were individually assigned to one of four treatments according to a 2 × 2 factorial arrangement: conditioning (with or without acidification) and disinfection (with or without chlorination). The entire study lasted 210 days. Physicochemical and microbiological water quality, water and feed consumption, haematological and biochemical blood parameters, and apparent total tract digestibility were measured; data were analysed via a mixed-effects model. Chlorination and acidification increased ($P = 0.02$) free residual chlorine in water, and chlorination reduced ($P = 0.01$) total coliform and *Clostridium perfringens* counts in water. Treatment did not affect water consumption, total DM intake, or blood parameters. At the beginning of the study, NDF digestibility decreased ($P = 0.04$) with acidification, however, this was restored at the end of the study. The second study evaluated the potential benefit of drinking water chlorination and acidification on the performance of crossbred Holstein bulls fed high-concentrate diets under commercial conditions. Ninety-six animals (322 ± 35.0 kg of BW, and 220 ± 14.2 days of age) were allocated into six pens assigned to one of the two treatments: untreated drinking water or drinking water treated with chlorination and acidification for a total of 112 days. Physicochemical and microbiological water quality, water and concentrate consumption, eating behaviour, growth performance, and carcass quality were analysed via a mixed-effects model. Water conditioning and disinfection increased ($P = 0.01$) free residual chlorine concentration and reduced ($P = 0.04$) total coliform count in water. Although water consumption and eating behaviour were similar between treatments, water conditioning and disinfection increased average daily weight gain ($P = 0.03$), BW before slaughter ($P = 0.01$), and hot carcass weight ($P = 0.01$). In conclusion, drinking water chlorination and acidification in fattening dairy beef bulls is recommended as it improves growth performance without any detrimental side effects on health or nutrient digestibility.

Implications

It is foreseen that water quality will decline in the coming decades and, therefore, methods to improve it need to be evaluated. The present study aimed to determine if drinking water disinfection (chlorination) combined or not with conditioning (acidification) was beneficial in fattening cattle production. The water disinfection methods evaluated not only reduced the microbial load in water but also had a positive impact on animal performance. Thus, disinfecting drinking water using chlorination together with acidification is recommended in fattening cattle as it will improve animal welfare, productivity, and sustainability.

* Corresponding author.
E-mail address: maria.devant@irta.cat (M. Devant).

https://doi.org/10.1016/j.animal.2022.100685
1751-7311/

Introduction

Water is an essential nutrient; limiting access to drinking water impairs animal welfare and may also impair feed consumption and animal productivity (Utley et al., 1970; Murphy, 1992; Grout et al., 2006; Devant et al., 2019). Moreover, water is a limited resource, and it is foreseen that its quality will decline in the coming decades (World Health Organization (WHO), 2022). Drinking water treatments to improve quality must be designed to target potential contaminants. Microbial hazards and drinking water safety continue to be the primary concern in both developing and developed countries. In general terms, the greatest microbial risks are associated with the ingestion of water contaminated with faeces, which can be a source of pathogenic bacteria, viruses, protozoa, and helminths (WHO, 2022). Animals have behavioural and physiological mechanisms to avoid toxic, contaminated, or spoiled foods. Aversive tastes, such as bitterness related to plant toxins or sourness related to spoiled microbe-contaminated feeds, are often associated with toxic compounds (Munger, 2016). Some studies have shown that ruminants have the capacity to avoid drinking water contaminated with faeces (Willms et al., 2002); alternatively, various studies found that ruminants can tolerate high bacterial loads in drinking water (Beede and Myers, 2000; Jemison and Jones, 2002). However, animals that consumed faeces-contaminated water reduced their productivity (Willms et al., 2002; Lardner et al., 2005). In addition, water troughs, under specific conditions, may become reservoirs for Campylobacter (Savill et al., 2003) and Cryptosporidium (Willms et al., 2002), pathogens that can cause foodborne diseases.

Chlorination is the most common water disinfection treatment; however, its effectiveness is limited by water pH and, therefore, water acidification may be necessary. Even though supplying safe drinking water is of unquestionable importance, there is no published evidence regarding the effects of chlorination alone or combined with acidification and its effects on improving water palatability, consumption, and performance, or its potential hazardous effects on animal health. In the present study, we hypothesised that drinking water treated with H_3PO_4 and NaClO 15% m/v would increase animal productivity by increasing water quality and consumption without any negative effects on the animal's health. Two studies were designed to address this hypothesis: the first study, which was conducted in a reduced number of animals, aimed to evaluate the effect of drinking water conditioning (acidification) and/or disinfection (chlorination) on physicochemical and microbiological water quality, its potential hazardous effects on animal health, water and feed consumption, and apparent total tract digestibility in Holstein bulls fed a high-concentrate diet. Subsequently, the objective of a second study, which was conducted in a larger number of animals in a commercial dairy beef feedlot, was to evaluate the effect of drinking water acidification and chlorination on growth performance and carcass quality of crossbreed Holstein bulls fed a high-concentrate diet. Verdú et al. (2021) published an abstract reporting the preliminary results of the first study.

Material and methods

First study

Animals, housing, experimental design, and treatments

Twenty-four Holstein bulls (221 ± 20.9 kg of BW, and 184 ± 9.9 days of age) were kept in individual concrete slatted floor pens (1.9 × 3.3 m) at the Nial experimental farm of the Corporación Alimentaria Guissona, S.A. – bonÀrea Agrupa (Guissona, Lleida, Spain) between February and September 2020. The experimental barn was naturally ventilated and illuminated, and pens were equipped with two feeders and a water trough. The study had a randomised balanced design with covariance adjustment, using a 2 × 2 factorial arrangement of treatments; the factors were drinking water conditioning (with or without acidification) and drinking water disinfection (with or without chlorination). Animals were randomly assigned to one of four treatments: **CTR** (n = 6), drinking water without acidification or chlorination; **AC** (n = 6), drinking water with only an acidification treatment; **CHLO** (n = 6), drinking water with only a chlorination treatment; **ACCHLO** (n = 6), drinking water with acidification and chlorination treatments. The entire study lasted 210 days divided into 15 14-day periods, and the water used in all treatments was collected from the Segarra-Garrigues waterway irrigation system. Water acidification was achieved with H_3PO_4 (Tashia, S.L., Artesa de Segre, Lleida, Spain), which was diluted 1:5 in water (236 g/L), with adjustments according to weekly on-farm pH determinations, adding 0.25 and 0.65 mL/L in AC and ACCHLO treatments, respectively, to the water tank via an automatic dosing system (Grundfos Holding A/S, Bjerringbro, Denmark), according to the supplier's recommendations (Tashia, S.L., Artesa de Segre, Lleida, Spain). The water acidification aimed to achieve a pH between 6.5 and 7, which is considered optimal to ensure effective chlorination. For the chlorination treatment, NaClO 15% m/v (Tashia, S.L., Artesa de Segre, Lleida, Spain) was diluted 1:5 in water (36 g/L), which was added to the water tank via an automatic dosing system (Grundfos Holding A/S, Bjerringbro, Denmark) at a dose of 0.15 mL/L, according to weekly on-farm free residual chlorine determinations. Contact time between water and NaClO should be at least 30 min within a pH range of 6.5–7 to achieve a free residual chlorine concentration $\geq$0.30 mg/L, thus assuring the disinfection efficacy of the water chlorination treatment according to the supplier's recommendations (Tashia, S.L., Artesa de Segre, Lleida, Spain); and, as required by the Water Safety Royal Decree (RD 140/2003), the free residual chlorine concentration should be $\leq$1 mg/L.

Animals were fed, *ad libitum*, a commercial concentrate in pellet form formulated to cover their nutritional requirements (Fundación Española para el Desarrollo de la Nutrición Animal, FEDNA, 2008). The ingredient composition of the concentrate was as follows: 42.0% corn, 14.5% wheat middling, 12.0% corn gluten feed, 10.0% wheat, 6.14% barley, 4.94% sunflower expeller, 2.89% palm oil, 2.07% calcium carbonate, 1.75% beet pulp, 1.50% cane molasses, 0.80% urea, 0.50% sodium bicarbonate, 0.25% salt, and 0.10% ammonium chloride. The nutrient composition of the concentrate (on DM basis) was as follows: 3.18 Mcal/kg of metabolisable energy, 12.1% CP, 4.96% ether extract (**EE**), 4.91% ash, 14.8% NDF, and 41.3% non-fibre carbohydrates. Throughout the study, animals had *ad libitum* access to wheat straw (5.9% CP, 2.6% EE, 76.6% NDF, and 6.7% ash) and water.

Water quality measurements

Physicochemical and microbiological water quality were periodically assessed. To monitor and ensure the correct application of the chemical products to achieve the parameters for the treatments designed in this study, water samples from the central taps of each treatment, at the start of the water line supply, were collected every week. Water samples were collected with 10 mL glass flasks and mixed with the corresponding tablet kit (Tashia, S.L., Artesa de Segre, Lleida, Spain) for photometer readouts (Grundfos Holding A/S, Bjerringbro, Denmark) for on-farm pH and free residual chlorine concentration determinations. In addition, every 28 days, water samples from the central taps of each treatment were collected for physicochemical analysis (conductivity, pH, hardness, water dry residue, calcium, magnesium, chloride, sulphate, nitrate, nitrite, free residual chlorine, combined residual chlorine, residual chlorine, and turbidity) and microbiological

analysis (total coliform, *Escherichia coli*, *Faecal enterococci*, and *Clostridium perfringens*). Samples for physicochemical analysis were collected with a 1–1.5 L non-sterile unspecific bottle. Samples for microbiological analysis were collected with a 350 mL sterile bottle containing sodium thiosulfate (VWR, Part of Avantor, Radnor, PA). Samples for physicochemical and microbiological analysis were refrigerated at 4 °C and sent for analysis within the next 24 h to the bonÀrea Agrupa analytical laboratory (Guissona, Lleida, Spain).

Water and feed consumption

Water consumption was recorded automatically by a water trough measurement system (iPERL sensor, SENSUS, Morrisville, NC) using a water meter that recorded volume (m^3) and speed (L/h) installed in the water pipeline supplying each water trough. This water measurement system recorded L of water consumed per pen in 1-h intervals, which was summarised as daily consumption per animal. Feed (concentrate and straw, separately) offered and refusals were weighed on a scale (GRAM Group, Hospitalet del Llobregat, Barcelona, Spain). Feed offered was recorded daily, and feed refusals were recorded every 14 days throughout the experimental period. Animal BW was recorded on a scale (MOBBA INDUSTRIAL CATALUNYA, S.A., Badalona, Barcelona, Spain) every 14 days until slaughter.

Apparent total tract digestibility

Apparent total tract digestibility was sampled at two time points, from day 35 to day 42, and from day 140 to day 147. Chromium oxide (1 g/kg DM) was added to the concentrate as an indigestible marker for nutrient digestibility determination. During each 7-day period, samples of concentrate offered and refusals from each animal were collected. Faecal grab samples were collected from the rectum on the last 3 days throughout each week, dried at 100 °C for 48 h, and composited by animal and period on an equal DM basis.

Potential hazardous effect measurements

Health status (presence of coughing, visible discharge in nose or eyes, droopy ears, head tilt, diarrhoea, bloat, and fever) was recorded daily. According to the European Food Safety Authority guidelines for tolerance studies in animal feeding (EFSA, 2011), blood samples were collected from each animal on days 0, 42, 112 and 210 for routine haematological and biochemical analysis (white blood cell count, red blood cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration, red cell distribution width, haemoglobin distribution width, absolute segmented neutrophil count, absolute lymphocyte count, absolute monocyte count, absolute eosinophil count, absolute large lymphocyte count, absolute basophil count, platelets, mean platelet volume, mean platelet concentration, platelet distribution width, albumin, alanine aminotransferase, amylase, aspartate aminotransferase, total bilirubin, calcium, creatine kinase, chloride, cholesterol, creatinine, alkaline phosphatase, phosphorous, γ-glutamyl transpeptidase, glucose, lactate dehydrogenase, magnesium, potassium, total protein, sodium, urea, and haptoglobin). Bulls were moved into a squeeze chute (Priefert Ranch Equipment, S01-Model 91, Austin, TX) and their heads were caught. Blood samples were taken via jugular venipuncture using a vacutainer and an 18 G needle. For haematological analysis, 4 mL of blood was collected in ethylenediaminetetraacetic acid (**EDTA**) vacutainer tubes (BD, Franklin Lakes, NJ), inverted, and stored at 5 °C until analysis. For biochemical analysis, 10 mL of blood was collected in spray-dried clot activator vacutainer tubes (BD, Franklin Lakes, NJ). For glucose analysis, 4 mL of blood was collected in sodium fluoride and potassium oxalate vacutainer tubes (BD, Franklin Lakes, NJ). The vacutainer tubes for biochemical and glucose analysis were then centrifuged at 1 500*g* at 4 °C for 15 min, and the serum from each tube was divided equally between three Eppendorf tubes. Samples were frozen at −20 °C for further analyses.

Chemical analyses

Physicochemical and microbiological water quality were analysed in the bonÀrea Agrupa analytical laboratory (Guissona, Lleida, Spain). The physiochemical water parameters analysed and analytical methodology used were as follows: conductivity, measured by a conductometer (Crison GLP 32, Hach Lange Spain S.L.U., Barcelona, Spain); pH, measured by a pH meter (Titrando 836, Metrohm AG, Herisau, Switzerland); calcium, magnesium, and chloride, measured by volumetric measurement (Titrando 836, Metrohm AG, Herisau, Switzerland); hardness, calculated by the calcium and magnesium equation [(Cax2.49) + (Mgx4.11)] using TiamoTM software (Titrando 836, Metrohm AG, Herisau, Switzerland); sulphate, measured by colorimetric test (VISOCOLOR Macherey-Nagel, Düren, Germany); nitrate and nitrite, measured by colorimetric test with 10–150 mg/l NO_3^- and 0.025–0.5 mg/l NO_2^-, respectively (Merck KGaA (Darmstadt, Germany); free and combined residual chlorine, measured by colorimetric test with 0.1–2.0 mg/L Cl_2 (Merck KGaA, Darmstadt, Germany), residual chlorine was the sum of free and combined residual chlorine; and turbidity, measured by a spectrophotometer (Varian Cary 50, Varian Inc., Palo Alto, CA). The microbiological water parameters analysed and analytical methodology used were as follows: total coliform and *Escherichia coli*, measured as established in Ministerial Order SCO/778/2009; and faecal enterococcus (UNE-EN ISO 7899-2) and *C. perfringens* (UNE-EN ISO 14189), measured as established in RD 140/2003.

Concentrate and straw sampling were conducted monthly for nutrient composition determination. Samples were dried for 24 h at 103 °C for DM determination (method number 925.04; Association of Official Analytical Chemists, AOAC, 1995), and for 4 h at 550 °C for ash determination (method number 642.05; AOAC, 1995). The Kjeldahl method was used on samples for CP determination (method number 988.05; AOAC, 1995). Neutral detergent fibre determination was based on the method of Van Soest et al. (1991) specifically procedure A with the addition of sodium sulphite. Ether extract content was determined using a Soxhlet apparatus following acid hydrolysis preparation (method number 942.05; AOAC, 1995). Total starch content was analysed using the polarimetric method according to EU Regulation No. 152/2009 for feed analyses.

Regarding apparent total tract digestibility, each sample was subdivided into duplicates weighing 0.5 g prior to chromium oxide digestion. Chromium oxide digestion was divided into two parts, the first digestion step mixed the sample with 4 mL of HNO_3 at 220 °C for 15 min in a microwave oven (Ultrawave model, Milestone, Sorisole, Italy), resulting in colourless solutions with a green solid at the bottom of the digestion tube. This solid is attributed to Cr_2O_3(s). In the second step, 3 mL of H_2SO_4, 0.5 mL of $HClO_4$, and 2 mL of hydrofluoric acid were added to the same digestion tube, starting a new digestion procedure at 260 °C for 15 min. Finally, the Cr concentration was measured by inductively coupled plasma optical emission spectrometry (model Optima 4300D, Perking-Elmer, Shelton, CT). The recovery rate of chromium oxide was not measured in the present study as 100% recovery was assumed in agreement with Cr_2O_3 recovery rates measured by Titgemeyer et al. (2001).

Calculations and statistical analysis

Apparent total tract digestibility data were calculated using total faecal output, which was further estimated as the ratio of

chromium intake in relation to chromium concentration in the faeces.

Each animal within the pen was considered an experimental unit. The univariate procedure of SAS® software 9.4 (Statistical Analysis Systems, Cary, NC, USA) verified that all data were normally distributed except for microbial counts, which were transformed to a log scale to achieve a normal distribution before statistical analysis. The values presented herein corresponded to non-transformed means of the raw data, but SEM and *P*-values corresponded to the ANOVA of log-transformed data.

Data, including water quality, water and feed consumption, apparent total tract digestibility, and blood parameters, were analysed using a mixed-effects model with repeated measures using SAS® software 9.4 (Statistical Analysis Systems, Cary, NC, USA). The model included initial BW as a covariate and acidification, chlorination, period, and the interactions between them as fixed effects; initial BW was not included as a covariate for the statistical analysis of water quality data, and the statistical analysis of apparent total tract digestibility did not include period as a fixed effect. Analysis of water and feed consumption, apparent total tract digestibility, and blood parameters included pen as a random effect. Period was considered a repeated measure in the analysis of water quality, water and feed consumption, and blood parameters. Central tap, in water quality analysis, or pen, in water and feed consumption, and blood parameters, were subjected to four variance–covariance structures, which included compound symmetry, variance components, autoregressive order one, and heterogeneous autoregressive order one. The variance–covariance structure that minimised Schwarz's Bayesian criteria was considered the most suitable analysis. For all analyses, significant differences were accepted if $P \leq 0.05$ and tendencies were discussed at $0.05 < P \leq 0.10$.

Second study

Animals, housing, experimental design, and treatments

Ninety-six crossbred Holstein bulls (322 ± 35.0 kg of BW, and 220 ± 14.2 days of age) were fattened in a commercial farm (Agromont, Montgai, Lleida, Spain) for a total of 112 days, divided into eight 14-day periods. The animals were allocated to an outdoor roofed concrete floor barn with one concrete wall and straw as bedding, between May and September 2021, with an ambient temperature of 22.1 ± 3.46 °C and an environmental humidity of 62.5 ± 8.01% on average (Vallfogona de Balaguer weather station, Servei Meteorològic de Catalunya). The study had a randomised balanced complete block design with covariance adjustment. Animal BW was stratified from heaviest to lightest to allow assignment to weight blocks, afterwards, they were randomly allocated to six pens (15–17 animals per 72 m^2 pen) separated by metal fences within each of the three weight blocks. Animals were assigned to one of two treatments (three pens per treatment): CTR (n = 49), drinking water without disinfection treatment, and ACCHLO (n = 47), drinking water with acidification and chlorination. Water used for the study was collected from the Segarra-Garrigues waterway irrigation system. Water acidification and chlorination treatments were like those previously described in the first study, except for H_3PO_4 dosage, which was 0.33 mL/L, and NaClO dosage, which was 0.23 mL/L. Each pen measured 12 m × 6 m (72 m^2 per pen) and was equipped with a concentrate feeder with one feeding space, a straw feeder with seven feeding spaces, and a water trough (20 cm × 20 cm, no pressure was required). Pens were deeply bedded with straw that was replaced every 14 days. An antenna located at the concentrate feeder detected each animal's visit to the computerised feeder (GEA WestfaliaSurge, Germany) via a transponder placed in the left ear of each bull, as described by Devant et al. (2012). Water troughs were cleaned weekly.

Animals were fed a commercial concentrate in pellet form, formulated to cover their nutritional requirements (FEDNA, 2008). The ingredient composition of the concentrate was as follows: 33.0% corn, 15.7% barley, 15.0% hominy feed, 14.6% soybean hulls, 14.0% corn dried distiller's grains, 3.47% wheat middling, 1.88% palm oil, 1.22% calcium carbonate, 0.55% urea, 0.30% salt, and 0.20% premix. The nutrient composition of the concentrate (DM basis) was as follows: 3.31 Mcal/kg of metabolisable energy, 11.3% CP, 5.00% EE, 3.68% ash, 18.2% NDF, and 49.2% non-fibre carbohydrates. Throughout the study, animals had *ad libitum* access to wheat straw (5.9% CP, 2.6% EE, 76.6% NDF, and 6.7% ash; DM basis) and water.

Water quality measurements

Physicochemical and microbiological water quality were periodically assessed. To monitor and ensure the correct application of the chemical products to achieve the parameters for the treatments designed in this study, water samples from the central taps of each treatment were collected weekly, and water samples from the water trough in each pen were collected every 14 days for on-farm pH and free residual chlorine concentration determinations with a photometer as described in the first study. Every 28 days, water samples from the central taps of each treatment were collected for physicochemical and microbiological analysis. Water sampling methods were the same as those described in the first study.

Performance, feed intake, and eating behaviour

BW was recorded every 14 days, until bulls were transported to the slaughterhouse, with a scale (FX1 model, TEXAS TRADING GmbH, Windach, Germany). Water consumption was recorded by the automatic water trough system (iPERL sensor, SENSUS, Morrisville, NC) described in the first study. This water measurement system recorded L of water consumed per pen in 5-min intervals, which was summarised as daily consumption and divided by animals per pen. Daily water consumption was also manually recorded due to the potential failure of the automatic water trough system. Straw was offered *ad libitum*, and the total number of straw bales offered during the study was recorded to estimate total straw consumption. The straw consumption calculated was considered an estimation given that straw was also used for bedding in this study. Data regarding eating behaviour and concentrate intake were averaged for each 14-day period following Devant et al. (2012). Meal criteria (maximum amount of time between visits to the feed troughs to consider a visit as a part of the same meal) were calculated using a model described by Bach et al. (2006) for each bull and each 14-day period. Subsequently, visits to the automatic feeders were separated into meals and meal duration, size, intermeal duration, and eating rate were calculated.

Carcass quality

On day 135 after initiation of the study, bulls were weighed prior to transport by lorry to a commercial slaughterhouse (La Closa, bonÀrea Agrupa, Guissona, Spain). Transport distance was less than 35 km and the time spent waiting before slaughter was less than 6 h. Animals were slaughtered by commercial practices and following EU Regulation No. 1099/2009 on the protection of animals at the time of killing or slaughtering. After slaughtering, hot carcass weight (**HCW**) was registered for every animal. Dressing percentage was calculated by dividing HCW by the BW recorded before slaughter. Following the (S)EUROP categories described by EU Regulations No. 1208/81 and No. 1026/91, carcass conformation was classified, where "E" corresponded to an

excellent conformation, "U" to a very good conformation, "R" to a good conformation, "O" to a fair conformation, and "P" to a poor conformation. The fat cover was classified according to EU Regulations No. 1208/81, which utilises a classification system by numbers (1, 2, 3, 4, 5), where 5 corresponds to a very high degree of covering fat and heavy fat deposits in the thoracic cavity and 1 is classified as a low degree, with no fat cover.

Chemical analyses

The water parameters and concentrate nutrient determinations were the same as in the first study.

Calculations and statistical analysis

Only the pen was considered the experimental unit, and the animals within the pen were considered sampling units for some parameters. The univariate procedure of SAS® software 9.4 (Statistical Analysis Systems, Cary, NC, USA) verified that all data were normally distributed except for microbial counts, which were transformed to a log scale to achieve a normal distribution before statistical analysis. The values presented herein corresponded to non-transformed means of the raw data, but SEM and *P*-values corresponded to the ANOVA of log-transformed data.

Data were analysed using a mixed-effects model with repeated measures using SAS® software 9.4 (Statistical Analysis Systems, Cary, NC, USA). Water quality data included the treatment and period as fixed effects. The model of water and concentrate consumption, BW, average daily gain (**ADG**), feed efficiency, and eating behaviour data included the initial BW as a covariate, and treatment, period, and their interaction as fixed effects. The interaction between treatment and pen was included as a random effect. The period was considered a repeated measure, and the animal nested within the interaction between treatment and pen (the error term) was subjected to four variance–covariance structures, which were compound symmetry, variance components, autoregressive order one, and heterogeneous autoregressive order one. The variance–covariance structure that minimised Schwarz's Bayesian criteria was considered the most suitable analysis. Data of initial BW, initial and final age, final BW and BW before slaughter, HCW, and dressing percentage were analysed with the same model without the period effect (initial BW, initial and final age without initial BW as covariates). The interaction between treatment and pen was included as a random effect with the animal as the subject. Categorical variables (carcass classification) were analysed by a chi-square test. Differences were declared significant at $P \leq 0.05$, and trends were discussed at $0.05 < P \leq 0.10$ for all models.

Results

First study

One animal from the CHLO treatment group was removed from the analysis due to illness; all data corresponding to this animal were removed prior to statistical analysis.

Water quality

Regarding main tap water parameters measured on farm with the photometer, the pH measured was lesser in acidified treatments than in non-acidified (6.69 vs 7.49 ± 0.119, $P = 0.01$, respectively), and free residual chlorine concentration was greater in chlorinated treatments than in non-chlorinated (0.40 vs 0.00 ± 0.063 mg/L, $P = 0.01$, respectively), as expected. Free residual chlorine also tended to be greater in acidified treatments than in non-acidified (0.25 vs 0.15 ± 0.061 mg/L, $P = 0.10$, respectively).

Regarding the physiochemical water quality analysed in the laboratory (Table 1), the most critical parameters included the significant acidification by chlorination interaction ($P = 0.01$) observed for pH, with CTR and CHLO being greater than AC and ACCHLO, and ACCHLO lesser than AC. Also, significant acidification by chlorination interaction ($P = 0.02$) was observed for free residual chlorine and residual chlorine, with CHLO and ACCHLO being greater than CTR and AC, and ACCHLO greater than CHLO; combined residual chlorine was greater in chlorinated treatments than in non-chlorinated (0.11 vs 0.00 mg/L, $P = 0.01$, respectively).

Regarding microbiological water quality (Table 1), total coliform and *C. perfringens* counts were greater in non-chlorinated treatments than in chlorinated (total coliform: 346 vs 13 colony-forming units [**CFU**]/100 mL, $P = 0.01$, respectively; and *C. perfringens*: 9 vs 1 CFU/100 mL, $P = 0.01$, respectively). Other microorganism counts analysed (*E. coli* and faecal enterococcus) showed they were inexistent in all treatments.

Water and feed consumption

Water, concentrate, and straw intake (Table 2) were not affected by acidification or chlorination.

Haematological and biochemical blood parameters

Haematological data are presented in Table 3. Absolute large lymphocyte concentration was lesser in non-acidified treatments than in acidified (0.025 vs 0.034 K cells/μL, $P = 0.05$, respectively). Platelet distribution width was greater in non-chlorinated treatments than in chlorinated treatments (7.37 vs 6.98 g/dL, $P = 0.03$, respectively). Mean platelet concentration tended to be lesser in chlorinated treatments than in non-chlorinated (22.0 vs 20.9 g/dL, $P = 0.10$, respectively).In addition, significant acidification by chlorination interaction ($P = 0.01$) was observed for the absolute segmented neutrophil count, with AC and CHLO being greater than ACCHLO, and CTR lesser than AC. Tendencies in the interaction between acidification and chlorination were observed in red blood cell distribution width, which tended to be greater ($P = 0.06$) in ACCHLO than in AC and CHLO; mean corpuscular volume, which tended to be greater ($P = 0.08$) in CTR and ACCHLO than in CHLO; and absolute eosinophil concentration, which tended to be greater ($P = 0.08$) in CHLO than in the other treatments.

Biochemical data are described in Table 4. Total bilirubin (0.13 vs 0.15 mg/dL, $P = 0.04$, respectively), γ-glutamyl transpeptidase (23.6 vs 32.5 U/L, $P = 0.01$, respectively), and lactate dehydrogenase (2 561 vs 3 011 U/L, $P = 0.01$, respectively) were lesser in non-acidified treatments than in acidified. Potassium tended to be greater in non-acidified treatments than in acidified (4.78 vs 4.64 mg/dL, $P = 0.10$, respectively). Urea tended to be greater in non-chlorinated treatments than in chlorinated (24.8 vs 22.4 mg/dL, $P = 0.09$, respectively). An acidification by chlorination interaction tendency ($P = 0.07$) was observed for aspartate aminotransferase, with ACCHLO being greater than the other treatments.

Apparent total tract digestibility

At the beginning of the study, apparent total tract digestibility of DM, organic matter (OM), CP, and EE were similar between treatments (Table 5), except for starch, which tended to be lesser in non-chlorinated treatments than in chlorinated (97.9 vs 98.1%, $P = 0.10$, respectively), and for NDF, which was greater in non-acidified treatments than in acidified (38.8 vs 27.6%, $P = 0.04$, respectively). At the end of the study, the apparent total tract digestibility of DM, OM, CP, EE, and NDF were similar between treatments (Table 5), except for the apparent total tract digestibility of starch, which tended to be lesser in non-chlorinated treatments than in chlorinated (97.7 vs 98.2%, $P = 0.07$, respectively).

Table 1
Physicochemical and microbiological water quality during the 210 days of the first study on drinking water chlorination in fattening Holstein bulls.

	Treatment[1]					*P*		
Water quality parameters	CTR	AC	CHLO	ACCHLO	SEM	A	C	A × C
n	7	7	7	7				
Physicochemical								
Conductivity (µs/cm)	258^{z}	258^{z}	275^{x}	270^{y}	1.7	0.05	0.01	0.09
pH[2]	7.82^{a}	7.38^{b}	7.88^{a}	6.82^{c}	0.082	0.01	0.02	0.01
Hardness (°)	4.67^{a}	4.96^{a}	4.54^{a}	2.46^{b}	0.541	0.06	0.01	0.02
Water dry residue (mg/L)	164^{b}	154^{b}	156^{b}	179^{a}	5.7	0.16	0.10	0.01
Calcium (mg/L)	40.6^{a}	35.4^{a}	40.5^{a}	19.6^{b}	4.42	0.01	0.04	0.05
Magnesium (mg/L)	9.77	14.3	6.80	13.4	3.522	0.20	0.76	0.44
Chloride (mg/L)	14.7	15.5	18.0	16.6	1.25	0.75	0.05	0.26
Sulphate (mg/L)	26.4	25.7	27.1	25.0	1.27	0.16	1.00	0.46
Nitrate (mg/L)	0.83	0.00	0.00	0.83	1.179	1.00	1.00	0.36
Nitrite (mg/L)	0.00	0.00	0.00	0.00	–	–	–	–
Free residual chlorine[3] (mg/L)	0.00^{c}	0.00^{c}	0.27^{b}	0.44^{a}	0.031	0.16	0.01	0.02
Combined residual chlorine[3] (mg/L)	0.00	0.00	0.10	0.13	0.010	0.18	0.01	0.18
Residual chlorine (mg/L)	0.00^{c}	0.00^{c}	0.37^{b}	0.57^{a}	0.061	0.10	0.01	0.02
Turbidity (NTU)	0.11	0.10	0.03	0.00	0.010	0.02	0.01	0.36
Microbiological (CFU/100 mL)								
Total coliform[3]	377	315	5	20	0.1	0.99	0.01	0.86
Escherichia coli	0	0	0	0	–	–	–	–
Faecal enterococci	0	0	0	0	–	–	–	–
Clostridium perfringens	10	8	1	1	0.2	0.44	0.01	0.44

Abbreviations: CTR = without treatment; AC = acidification; CHLO = chlorination; ACCHLO = acidification and chlorination; A = acidification effect; C = chlorination effect; A × C = interaction effect between acidification and chlorination; NTU = nephelometric turbidity unit; CFU = colony-forming units.
[a–c]Values within a row with different superscripts differ significantly at $P \leq 0.05$.
[x–z]Values within a row with different superscripts differ significantly at $0.05 > P \leq 0.10$.
[1] Type of drinking water treatment applied.
[2] Period: $P \leq 0.10$.
[3] Period: $P \leq 0.05$.

Table 2
Feed intake of fattening Holstein bulls during the 210 days of the first study on drinking water chlorination.

	Treatment[1]					*P*		
Item	CTR	AC	CHLO	ACCHLO	SEM	A	C	A × C
n	6	6	5	6				
Intake (kg DM/day)								
Concentrate[2]	7.81	7.94	7.79	8.26	0.233	0.18	0.50	0.43
Straw[2]	0.59	0.59	0.63	0.57	0.036	0.46	0.76	0.45
Total[2]	8.40	8.53	8.41	8.84	0.265	0.22	0.47	0.50
Water consumption[2] (L/day)	37.0	36.0	33.4	35.9	2.44	0.74	0.42	0.46

Abbreviations: CTR = without treatment; AC = acidification (conditioning); CHLO = chlorination (disinfection); ACCHLO = acidification and chlorination (conditioning and disinfection); A = water acidification effect; C = water chlorination effect; A × C = interaction effect between water acidification and chlorination.
[1] Type of drinking water treatment applied.
[2] Period: $P \leq 0.01$.

Second study

One animal from the CTR treatment group was removed from the study due to severe lameness; all data corresponding to this animal were removed prior to the statistical analysis.

Water quality

Regarding parameters measured on farm with the photometer, main tap water pH was lesser in ACCHLO than in CTR (7.10 vs 7.85 ± 0.100, $P = 0.01$, respectively), and free residual chlorine was greater in ACCHLO than in CTR (0.30 vs 0.08 ± 0.059 mg/L, $P = 0.01$, respectively), as expected. Measurements in water collected from pen water troughs showed that pH was lesser in ACCHLO than in CTR (6.86 vs 7.68 ± 0.038, $P = 0.01$, respectively), and a significant treatment by period interaction was observed for free residual chlorine (0.13 vs 0.08 ± 0.013 mg/L, $P = 0.01$, mean ACCHLO vs mean CTR), with ACCHLO being greater than CTR in periods 1, 2, 4 and 7.

Regarding the physicochemical parameters of water analysed in the laboratory (Table 6), all parameters were similar between treatments except for pH, which was lesser ($P = 0.01$) in ACCHLO than in CTR. The free residual chlorine analysed in the laboratory was similar between treatments; however, effects were observed for the microbiological parameters of water, also analysed in the laboratory (Table 6). Total coliform counts were lesser ($P = 0.04$) in ACCHLO than in CTR, and those of *E. coli* and *C. perfringens* tended to be lesser ($P = 0.08$ and $P = 0.10$, respectively) in ACCHLO than in CTR.

Water and feed consumption, performance, and eating behaviour

Performance and eating behaviour data are presented in Table 7. Acidification and chlorination of drinking water (ACCHLO) increased final BW ($P = 0.01$) and ADG ($P = 0.03$) compared with bulls that drank untreated water (CTR). A significant treatment by period interaction ($P = 0.01$) was observed for mean BW as bulls under ACCHLO treatment had greater mean BW than with CTR

Table 3
Haematological parameters of fattening Holstein bulls during the 210 days of the first study on drinking water chlorination.

	Treatment[1]					*P*		
Item	CTR	AC	CHLO	ACCHLO	SEM	A	C	A × C
N	6	6	5	6				
WBC[2] (K cells/μL)	8.59	10.25	8.58	8.50	0.591	0.18	0.13	0.13
RBC[2] ($\times 10^6$ cells/μL)	8.70	8.54	9.07	8.47	0.239	0.11	0.52	0.36
HGB[3] (g/dL)	12.6	12.0	12.1	12.1	0.30	0.32	0.53	0.38
HCT (%)	33.0	31.4	31.8	31.9	0.79	0.36	0.68	0.26
MCV[2] (fL)	38.2^{y}	37.0^{yz}	35.5^{z}	38.1^{y}	1.06	0.51	0.45	0.08
MCHC[2] (g/dL)	38.1	38.3	38.1	37.9	0.23	0.98	0.44	0.46
RDW[3] (%)	22.9^{yz}	22.4^{z}	22.6^{z}	23.6^{y}	0.41	0.53	0.25	0.06
HDW[2] (g/dL)	2.18	2.16	2.17	2.16	0.029	0.67	0.72	0.92
NEU (K cells/μL)	2.16^{bc}	3.28^{a}	2.83^{ab}	1.93^{c}	0.334	0.74	0.29	0.01
LYM[2] (K cells/μL)	5.67	6.17	4.95	5.77	0.512	0.20	0.27	0.75
MONO[2] (K cells/μL)	0.42	0.38	0.33	0.35	0.047	0.74	0.19	0.57
EOS[2] (K cells/μL)	0.23^{z}	0.29^{z}	0.49^{y}	0.33^{z}	0.062	0.44	0.02	0.08
L-LYM[2] (K cells/μL)	0.025	0.038	0.024	0.030	0.0048	0.05	0.32	0.45
BASO[2] (K cells/μL)	0.090	0.086	0.088	0.092	0.0088	0.98	0.84	0.63
PLT[2] (K cells/μL)	412	326	343	410	48.3	0.84	0.87	0.11
MPV[2] (fL)	6.70	7.27	8.09	7.55	0.571	0.98	0.14	0.32
MPC[2] (g/dL)	22.2	21.8	20.1	21.6	0.68	0.40	0.10	0.15
PDW[2] (g/dL)	7.28	7.46	7.00	6.96	0.182	0.69	0.03	0.52

Abbreviations: CTR = without treatment; AC = acidification; CHLO = chlorination; ACCHLO = acidification and chlorination; A = acidification effect; C = chlorination effect; A × C = interaction effect between acidification and chlorination; WBC = white blood cell; RBC = red blood cell; HGB = haemoglobin; HCT = haematocrit; MCV = mean corpuscular volume; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; HDW = haemoglobin distribution width; NEU = absolute neutrophil; LYM = absolute lymphocyte; MONO = absolute monocyte; EOS = absolute eosinophil; L-LYM = absolute large lymphocyte; BASO = absolute basophil; PLT = platelets; MPV = mean platelet volume; MPC = mean platelet concentration; PDW = platelet distribution width.
[a–c]Values within a row with different superscripts differ significantly at $P \leq 0.05$.
[y–z]Values within a row with different superscripts differ significantly at $0.05 > P \leq 0.10$.
[1] Type of drinking water treatment applied.
[2] Period: $P \leq 0.05$.
[3] Period: $P \leq 0.10$.

Table 4
Biochemical parameters of fattening Holstein bulls during the 210 days of the first study on drinking water chlorination.

	Treatment[1]					*P*		
Item	CTR	AC	CHLO	ACCHLO	SEM	A	C	A × C
n	6	6	5	6				
Albumin[2] (g/dL)	3.43	3.25	3.24	3.17	0.105	0.23	0.20	0.61
ALT[2] (U/L)	17.3	17.6	17.0	18.7	0.86	0.26	0.62	0.39
Amylase[2] (U/L)	151	123	130	131	16.1	0.41	0.68	0.37
AST[2] (U/L)	80.6^{z}	81.0^{z}	80.3^{z}	108.6^{y}	7.99	0.07	0.08	0.07
Total bilirubin[2] (mg/dL)	0.13	0.14	0.13	0.15	0.008	0.04	0.76	0.77
Calcium[2] (mg/dL)	10.8	10.6	10.6	10.6	0.09	0.15	0.24	0.25
Creatine kinase (U/L)	189	223	258	258	59.9	0.77	0.36	0.77
Chloride[2] (mmol/L)	97.2	97.4	97.7	96.7	0.51	0.38	0.90	0.26
Cholesterol[2] (mg/dL)	123	124	106	117	8.8	0.47	0.16	0.58
Creatinine[2] (mg/dL)	0.90	0.89	0.88	0.87	0.047	0.86	0.64	0.99
ALP[2] (U/L)	275	210	222	237	26.3	0.34	0.62	0.13
Phosphorous[2] (mg/dL)	9.4	9.2	9.4	9.3	0.29	0.64	0.97	0.88
GGTP[2] (U/L)	25.7	30.5	21.4	34.5	3.54	0.01	0.96	0.23
Glucose[2] (mg/dL)	101	99	100	97	2.8	0.32	0.56	0.90
LDH[2] (U/L)	2 617	2 840	2 504	3 181	150.8	0.01	0.44	0.12
Magnesium[2] (mg/dL)	1.40	1.25	1.30	1.28	0.067	0.18	0.59	0.30
Potassium[2] (mg/dL)	4.72	4.66	4.84	4.62	0.086	0.10	0.62	0.34
Total protein[2] (g/dL)	6.73	6.91	7.11	7.00	0.189	0.85	0.22	0.43
Sodium[2] (mmol/L)	142.1	141.2	142.4	142.0	0.59	0.26	0.36	0.59
Urea nitrogen[2] (mg/dL)	24.1	25.5	21.3	23.4	1.49	0.24	0.09	0.80
Haptoglobin (mg/mL)	0.14	0.27	0.17	0.26	0.065	0.11	0.88	0.78

Abbreviations: CTR = without treatment; AC = acidification; CHLO = chlorination; ACCHLO = acidification and chlorination; A = acidification effect; C = chlorination effect; A × C = interaction effect between acidification and chlorination; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; GGTP = γ-glutamyl transpeptidase; LDH = lactate dehydrogenase.
[y–z]Values within a row with different superscripts differ significantly at $0.05 > P \leq 0.10$.
[1] Type of drinking water treatment applied.
[2] Period: $P \leq 0.05$.

treatment from period 3 to the end of the study (period 8). Also, a significant treatment by period interaction ($P = 0.04$) was observed for concentrate intake, where bulls under ACCHLO treatment had greater concentrate intake than those on the CTR treatment in all periods, except 1 and 5. Feed efficiency was similar between treatments. Regarding eating behaviour parameters, significant

Table 5
Apparent total tract digestibility of fattening Holstein bulls for two different weeks during the 210 days of the first study on drinking water chlorination.

	Treatment[1]					*P*		
Item	CTR	AC	CHLO	ACCHLO	SEM	A	C	A × C
N	6	6	5	6				
From day 35 to day 42								
DM (%)	69.5	68.0	66.2	66.4	2.04	0.32	0.49	0.68
OM (%)	70.9	69.2	67.9	67.7	1.88	0.24	0.62	0.71
Starch (%)	98.2	97.5	98.4	97.8	0.38	0.43	0.10	0.88
CP (%)	66.4	67.2	62.5	65.6	2.73	0.17	0.92	0.98
EE (%)	69.0	69.7	59.9	60.4	6.43	0.24	0.62	0.71
NDF (%)	36.8	33.2	28.7	22.0	4.46	0.04	0.26	0.73
From day 140 to day 147								
DM (%)	79.3	79.9	77.9	80.2	0.87	0.53	0.13	0.39
OM (%)	80.4	80.8	72.2	81.3	0.91	0.72	0.19	0.39
Starch (%)	97.2	98.2	98.1	98.2	0.28	0.12	0.07	0.11
CP (%)	81.4	77.8	76.3	79.8	2.06	0.48	0.97	0.11
EE (%)	77.6	79.1	75.9	81.2	2.60	0.92	0.22	0.48
NDF (%)	58.6	58.2	54.1	58.0	2.39	0.36	0.49	0.38

Abbreviations: CTR = without treatment; AC = acidification; CHLO = chlorination; ACCHLO = acidification and chlorination; A = acidification effect; C = chlorination effect; A × C = interaction effect between acidification and chlorination; OM = organic matter; EE = ether extract.
[1] Type of drinking water treatment applied.

Table 6
Physicochemical and microbiological water quality during the 112 days of the second study on drinking water chlorination in fattening Holstein bulls.

	Treatment[1]			*P*	
Water quality parameters	CTR	ACCHLO	SEM	T	P
n	4	4			
Physicochemical					
Conductivity (µs/cm)	292	295	5.1	0.59	0.04
pH	8.02	6.88	0.065	0.01	0.01
Hardness (°)	7.18	6.38	1.068	0.50	0.53
Water dry residue (mg/L)	205	132	79.3	0.41	0.33
Calcium (mg/L)	40.0	35.3	7.98	0.59	0.91
Magnesium (mg/L)	7.92	7.06	0.652	0.26	0.01
Chloride (mg/L)	24.2	19.9	7.04	0.57	0.55
Sulphate (mg/L)	34.2	37.4	2.76	0.31	0.45
Nitrate (mg/L)	1.84	2.30	1.386	0.76	0.68
Nitrite (mg/L)	0.01	0.00	0.005	0.37	0.50
Free residual chlorine (mg/L)	0.21	0.30	0.059	0.18	0.07
Combined residual chlorine (mg/L)	0.20	0.23	0.099	0.79	0.66
Residual chlorine (mg/L)	0.41	0.53	0.080	0.20	0.22
Turbidity (NTU)	1.95	2.03	0.528	0.90	0.02
Microbiological (CFU/100 mL)					
Total coliform	1 200	228	4.3	0.04	0.57
Escherichia coli	21	0	2.4	0.08	0.50
Faecal enterococci	96	7	2.8	0.11	0.18
Clostridium perfringens	17	2	1.9	0.10	0.13

Abbreviations: CTR = without treatment; ACCHLO = acidification and chlorination; T = treatment effect; P = period effect; NTU = nephelometric turbidity unit; CFU = colony-forming units.
[1] Type of drinking water treatment applied.

treatment by period interactions was observed for the number of daily meals ($P = 0.01$), meal duration ($P = 0.04$) and intermeal duration ($P = 0.01$). Specifically, meal duration was greater in ACCHLO than in CTR in periods 4 and 5, but the number of daily meals and intermeal duration presented erratic patterns. A tendency in the interaction between treatment and period was observed for eating rate ($P = 0.08$) with no clear pattern between treatments in any period. Finally, a significant treatment by period interaction was observed for water consumption ($P = 0.02$), with no clear pattern between treatments in any period.

Carcass quality

Regarding carcass quality (Table 8), bulls drinking water with an acidification and chlorination treatment had greater BW before slaughter ($P = 0.01$) and HCW ($P = 0.01$) than bulls drinking untreated water. The carcass quality parameters assessed were similar between treatments.

Discussion

Effectiveness of drinking water disinfection treatment: chlorination with or without acidification

Data from the present studies support that chlorination is effective against most common waterborne pathogens; chlorine-based chemicals provide sufficient residual disinfectant levels to prevent microbial re-growth and help maintain this condition throughout the water distribution system. Bacteria of the coliform group (e.g., *E. coli*), associated with environmental and faecal contamination of the water, have been proven to be useful indicators of enteric bacterial pathogens in water disinfection models, but poor indicators of non-bacterial pathogens (National Research Council (NRC), 1980). Faecal thermo-tolerant coliform indicates faecal contamination; however, they do not directly relate to water counts of known bacteria harmful to human health. *Clostridium perfringens* is

Table 7
Intake, performance, eating behaviour, and water consumption of fattening Holstein bulls during the 112 days of the second study on drinking water chlorination.

	Treatment[1]			*P*		
Item	CTR	ACCHLO	SEM	T	P	T × P
n	48	47				
Initial age (day)	220	220	2.9	0.86	–	–
Final age (day)	333	332	2.9	0.83	–	–
Initial BW (kg)	322	323	7.2	0.85	–	–
Final BW (kg)	517	541	5.0	0.01	–	–
Mean BW (kg)	439	452	3.1	0.01	0.01	0.01
Average daily gain (kg/day)	1.73	1.95	0.064	0.03	0.01	0.38
Concentrate intake (kg DM/day)	7.67	8.19	0.191	0.05	0.01	0.04
Water consumption (L/day)	39.4	43.5	3.02	0.25	0.01	0.02
Number of daily meals	8.55	8.71	0.535	0.78	0.01	0.01
Meal size (kg DM/meal)	0.94	0.98	0.083	0.69	0.01	0.93
Meal duration (min/meal)	8.44	8.64	0.233	0.44	0.01	0.04
Intermeal duration (min/intermeal)	168	162	12.0	0.67	0.01	0.01
Eating rate (g DM/min)	147	152	10.0	0.62	0.01	0.08
Feed efficiency (kg/kg DM)	0.24	0.24	0.010	0.52	0.01	0.85

Abbreviations: CTR = without treatment; ACCHLO = acidification and chlorination; T = treatment effect; P = period effect; T × P = interaction effect between treatment and period.
[1] Type of drinking water treatment applied.

Table 8
Carcass quality of fattening Holstein bulls during the 112 days of the second study on drinking water chlorination.

	Treatment[1]			*P*
Item	CTR	ACCHLO	SEM	T
n	48	47		
Age before slaughter (day)	359	358	3.0	0.66
BW before slaughter (kg)	541	566	6.5	0.01
Hot carcass weight (kg)	305	321	4.4	0.01
Dressing percentage (%)	56	57	0.5	0.51
Fatness[2] (% of animals)				
1	2	0	–	0.30
2	94	89		
3	4	11		
Conformation[3] (% of animals)				
U	2	11	–	0.11
R	90	87		
O	8	2		

Abbreviations: CTR = without treatment; ACCHLO = acidification and chlorination; T = treatment effect.
[1] Type of drinking water treatment applied.
[2] The fat cover was classified according to EU Regulations No. 1208/81, which utilises a classification system by numbers (1, 2, 3, 4, 5), 5 corresponds to a very high degree of covering fat and heavy fat deposits in the thoracic cavity and 1 is classified as a low degree, with no fat cover.
[3] The conformation of carcasses was classified according to EU Regulations No. 1208/81 and No. 1026/91, which utilises the (S)EUROP categories, where "E" corresponded to an excellent conformation, "U" to a very good conformation, "R" to a good conformation, "O" to a fair conformation, and "P" to a poor conformation.

a gram-positive bacterium, also associated with the intestinal tract, but it is not considered the best microbiological quality indicator for water because this bacterium produces resistant spores even if water chlorination treatment has been applied. Regarding the microbiological water quality in the first study, lower total coliform and *C. perfringens* counts in chlorinated treatments (13 and 1 CFU/100 mL, respectively) compared with higher counts in non-chlorinated water (346 and 9 CFU/100 mL, respectively) indicated the effectiveness of the chlorination treatment, although microbial load did not achieve the levels established by RD 140/2003 for human water consumption (0 CFU/100 mL of total coliform and *C. perfringens*). Other microorganism counts analysed (*E. coli* and faecal enterococcus) were zero in all treatments from the first study and complied with the limits established by RD 140/2003. Therefore, based on microbial counts, water treated with chlorination and acidification did not show benefits compared with water treated with chlorination without acidification in reducing microbial counts in water.

Chlorination can be added to drinking water as elemental chlorine (chlorine gas), sodium hypochlorite solution, or dry calcium hypochlorite. When adding chlorine to water, two chemical species, known together as "free residual chlorine", are formed: hypochlorous acid (HOCl, electrically neutral) and hypochlorite ion (OCl^-, electrically negative). Hypochlorous acid is not only more reactive than the hypochlorite ion but is also a stronger disinfectant and oxidant. At low pH (6.5–7), hypochlorous acid dominates while at high pH (>8), the hypochlorite ion is predominant (NRC, 1980). Thus, water acidification via the addition of acids is recommended to improve the speed and efficacy of chlorine disinfection. In the first study, the physicochemical parameters of the water were below the thresholds established by RD 140/2003 in all treatments. Specifically, free residual chlorine in both chlorinated treatments was lower than the limit established by RD 140/2003 (<1.0 mg/l), but free residual chlorine differed between chlorinated treatments. Treating drinking water with chlorination and acidification is recommended because free residual chlorine concentrations were almost double compared with chlorination without acidification and using the same dose of NaClO 15% m/v (0.44 vs 0.27 mg/l, respectively). This indicated that acidification can enhance the effectiveness of chlorination and should be recommended as a measure to improve the free residual chlorine concentration in water. Therefore, water chlorination with acidification allowed a higher free residual chlorine concentration, suggesting that the disinfectant action of chlorine would persist longer, improving the water disinfection treatment.

In the second study, the physicochemical water parameters were also below the thresholds established by RD 140/2003 in all treatments. Despite a lower free residual chlorine concentration in the chlorination and acidification treatment in the second study than in the first study (0.30 vs 0.44 mg/l, respectively), the total coliform count in water of the second study was also reduced, from 1 200 to 228 CFU/100 mL for CTR and ACCHLO, respectively, however, still exceeding the limits established by RD 140/2003 for human water consumption (0 CFU/100 mL total coliform count). A potential explanation for the higher microbial load observed in the second study compared with the first could be due to the level of difficulty experienced when up-scaling the water treatment methodology and controlling the environmental factors that affect water quality in commercial farm settings versus an experimental

farm, where smaller water systems could facilitate water disinfection monitoring and management.

Effects of drinking water chlorination treatment on water and feed consumption

Cattle are sensitive to water palatability and avoid drinking contaminated water, e.g., containing salt, nitrate, or faecal microorganisms, with faecal contamination being the most predictable factor reducing water palatability (Schütz, 2012; Willms et al., 2002; Wright, 2007). The positive effects of drinking clean water seem to cause an increase in palatability and water consumption, which consequently lead to increased feed intake and improved animal performance (Holechek, 1980; Willms et al., 2002; Lardner et al., 2005). Although the specific compounds responsible for reducing water palatability are unknown, Dohi et al. (1999) identified organic compounds in cattle faeces that appeared to be responsible for causing avoidance. Water disinfection by-products could also affect water palatability, but there is no information available about these potential effects.

In the first study, water consumption was not affected by the treatments, even though non-chlorinated treatments had a lower microbiological water quality. Water consumption in the second study was also not statistically different per water treatment; however, on average, bulls consuming chlorinated and acidified water numerically drank 4 L/day more than bulls consuming untreated water. The total coliform count in disinfected water was reduced by 334 CFU/100 mL in the first study, and by 972 CFU/100 mL in the second study, indicating that total coliform in the second study were reduced by a factor of three compared with the first study. These results might suggest an improvement in water palatability in the second study due to a greater reduction of microbial growth in disinfected water, with a total coliform count of almost 1 000 CFU/100 mL less than in untreated water. Furthermore, concentrate intake increased from day 28 until the end of the second study in bulls consuming disinfected water, among improvements in other performance parameters discussed below. Like water pH, free residual chlorine and other physiochemical water parameters were numerically similar between studies. Thus, it seems that total coliform count in water might explain why there were no differences in water consumption in the first study, while in the second, the consumption of untreated water was numerically reduced due to potentially lower water palatability related to water microbial contamination.

Effects of drinking water chlorination treatment on apparent total tract digestibility

After a short period of consuming acidified water, NDF digestibility decreased, on average, by 9%, in accordance with statements that water with a pH outside the preferred range (between 6 and 9) may cause symptoms of digestive upset (NRC, 2001; Looper and Waldner, 2002). Although decreases in NDF digestibility were not pronounced, one explanation could be that H_3PO_4 or reduced pH in the rumen may reduce or damage fibrolytic rumen microbiota, which is responsible for fibre degradation (Calsamiglia et al., 2002). The short-term effect on NDF digestibility disappeared in the long term, suggesting that the ruminal environment requires a short adaptation to acidification by-products or reduced pH to recover NDF digestibility. The long-term effect of water chlorination had been observed in starch digestibility, though the impact was low (0.5% reduction). The digestibility of other nutrients, such as CP and EE, were not significantly affected by water disinfection suggesting that long-term consumption of these water disinfection treatments would not disturb the cattle's ruminal and total tract digestion.

Potential hazardous effects of drinking water chlorination treatment

Water chlorination improves the microbiological quality of water, and therefore, the risk of contracting infectious diseases through water could be reduced. Additionally, chlorination may be potentially hazardous due to a reaction of by-products with organic compounds resulting in carcinogenic complexes, such as total trihalomethanes, five haloacetic acids, bromate, and chlorite (NRC, 1980; Anderson et al., 1982). In the first study, some haematological and biochemical parameters were affected by acidification and/or chlorination, however, all values were within the reference range by age for cattle (Doornenball et al., 1988; Joerling and Doll, 2019). Supporting the haematological and biochemical results, no adverse clinical signs, such as intoxication or diarrhoea, were observed in bulls drinking water treated with acidification and/or chlorination. Accordingly, water chlorination, with or without acidification, presented no adverse effects on animal health in fattening dairy beef cattle.

Effect of drinking water chlorination and acidification on growth performance

Although the first study was not designed to assess growth performance, an observed feed intake numerical increase of 0.45 kg DM/day, on average, by bulls consuming chlorinated and acidified water supported the idea that improving water quality could have benefits on animal performance. The second study was designed to test the effects of water disinfection on performance after observing these differences in the bulls' growth performance. In this second study, improvements in animal performance were observed for concentrate intake in bulls consuming chlorinated and acidified water from period 2 (28 days of study) until the end of the study. Consequently, these bulls consuming chlorinated and acidified water gained 0.20 kg/day, their BW was greater from period 3 (42 days of study) until the end of the study, and BW before slaughter and HCW increased 25 and 16 kg, respectively, in comparison with cattle drinking untreated water. As previously discussed, water consumption was statistically similar between treatments, however, bulls consuming chlorinated and acidified water numerically consumed 4 L/day more than bulls consuming untreated water. This considerable increase in water consumption of bulls drinking chlorinated and acidified water could be associated with their increase in concentrate intake and, consequently, improvements in growth performance. Nevertheless, mechanisms by which water chlorination and acidification improved performance parameters are not truly understood and probably cannot only be attributed to an increase in water and concentrate consumption.

In conclusion, disinfection (chlorination: NaClO 15%) and conditioning (acidification: H_3PO_4) of drinking water improves its microbiological quality without any detrimental effects on water and feed consumption, ruminal digestibility, and the health of dairy beef cattle. Moreover, treating drinking water with chlorination and acidification improves the performance and carcass quality of fattening bulls.

Ethics approval

All calves used were managed following the principles and guidelines of the Animal Care Committee of the Institut de Recerca i Tecnologia Agroalimentàries (RD 53/2013; project number: 10478).

Data and model availability statement

None of the data were deposited in an official repository. The data/models that support the study findings are available from the authors upon request and after authorisation by all authors.

Author ORCIDs

L. Llonch: https://orcid.org/0000-0002-9828-9505
M. Verdú: https://orcid.org/0000-0003-4055-5804
S. Martí: https://orcid.org/0000-0002-8115-0582
J. Cucurull: https://orcid.org/0000-0003-1029-7453
M. Devant: https://orcid.org/0000-0003-1310-2623

Author contributions

L. Llonch: Data curation resources, Writing – Review & Editing.
M. Verdú: Conceptualization, Methodology, Investigation, Data curation resources, Writing – Review & Editing.
S. Martí: Conceptualization, Methodology, Investigation. Writing – Review & Editing.
C. Medinyà: Conceptualization, Methodology, Investigation.
J. Riera: Conceptualization, Methodology, Investigation.
J. Cucurull: Conceptualization, Methodology, Investigation.
M. Devant: Conceptualization, Methodology, Investigation, Data curation resources, Writing – Review & Editing, Project administration, Funding acquisition.

Declaration of interest

None.

Acknowledgements

The authors thank the collaboration of the personnel of the Nial experimental farm from bonÀrea Agrupa, and of La Riba commercial farm from Agromont S. L.

Financial support statement

This work was supported by DARP (Generalitat de Catalunya) and by FEADER (European Union) - Operational Group "Guia per a la optimització de l'ús i el tractament de l'aigua de beguda en vedells d'engreix" (grant number 56 21 026 2017 5A). IRTA also thanks the support of L'Associació Catalana de Criadors de Boví de Carn de Catalunya (ASOPROVAC), Ramaders d'Alcarràs, bonÀrea Agrupa, NANTA S.A., and Soluciones Integrales para la Nutrición Animal (SINUAL).

References

Anderson, A.C., Reimers, R.S., DeKernion, P., 1982. A brief review of the current status of alternatives to chlorine disinfection of water. American Journal of Public Health 72, 1290–1293. https://doi.org/10.2105/ajph.72.11.1290.
Association of Official Analytical Chemists (AOAC), 1995. Official Methods of Analysis. AOAC, Arlington, VA, USA.
Bach, A., Iglesias, C., Devant, M., Ràfols, N., 2006. Performance and feeding behavior of primiparous cows loose housed alone or together with multiparous cows. Journal of Dairy Science 89, 337–342. https://doi.org/10.3168/jds.S0022-0302(06)72099-9.
Beede, D.K., Myers, Z.H., 2000. Water nourishment of dairy cattle. Proceedings of the 24th Quebec Symposium on Dairy Nutrition, 19 October 2000, Saint-Hyacinthe, Quebec, Canada, pp. 1–28.
Calsamiglia, S., Ferret, A., Devant, M., 2002. Effects of pH and pH fluctuations on microbial fermentation and nutrient flow from a dual-flow continuous culture system. Journal of Dairy Science 85, 574–579. https://doi.org/10.3168/jds.S0022-0302(02)74111-8.
Council Regulation (EEC) No. 1208/81 of April 28, 1981. Determining the Community scale for the classification of carcasses of adult bovine animals. Official Journal of the European Union, L 123, 1–6. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:01981R1208-20060824&from=en.
Council Regulation (EEC) No. 1026/91 of April 22, 1991. Amending Regulation (EEC) No. 1208/81 determining the Community scale for the classification of carcasses of adult bovine animal. Official Journal of the European Union, L 106, 2–3. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:1991:106:FULL&from=EN.
Council Regulation (EC) No. 1099/2009 of September 24, 2009. Protection of animals at the time of killing. Official Journal of the European Union, L 303, 1–30. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2009:303:FULL&from=BG.
Commission Regulation (EC) No. 152/2009 of January 27, 2009. Laying down the methods of sampling and analysis for the official control of feed. Official Journal of the European Union, L 54, 1–130. http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:054:0001:0130:EN:PDF.
Devant, M., Marti, S., Bach, A., 2012. Effects of castration on eating pattern and physical activity of Holstein bulls fed high concentrate rations under commercial conditions. Journal of Animal Science 90, 4505–4513. https://doi.org/10.2527/jas.2011-4929.
Devant, M., Verdú, M., Medinyà, C., Riera, J., Marti, S., 2019. PSXII-10 Voluntary water intake of calves and fattening Holstein bulls fed high-concentrate diets. Journal of Animal Science 97, 410–411. https://doi.org/10.1093/jas/skz258.814.
Dohi, H., Ogura, S., Kosako, T., Hayashi, Y., Yamada, A., Shioya, S., 1999. Separation of deterrents to ingestive behavior of cattle from cattle feces. Journal of Animal Science 77, 756–761. https://doi.org/10.2527/1999.773756x.
Doornenball, H., Tong, A.K., Murray, N.L., 1988. Reference values of blood parameters in beef cattle of different ages and stages of lactation. Canadian Journal of Veterinary Research 52, 99–105.
European Food Safety Authority (EFSA), 2011. Technical guidance: Tolerance and efficacy studies in target animals. EFSA Journal 9, 2175. https://doi.org/10.2903/j.efsa.2011.2175.
Fundación Española para el Desarrollo de la Nutrición Animal (FEDNA), 2008. Necesidades Nutricionales Para Rumiantes de Cebo: Normas FEDNA. FEDNA, Madrid, Spain.
Grout, A.S., Veira, D.M., Weary, D.M., Von Keyserlingk, M.A.G., Fraser, D., 2006. Differential effects of sodium and magnesium sulfate on water consumption by beef cattle. Journal of Animal Science 84, 1252–1258. https://doi.org/10.2527/2006.8451252x.
Holechek, J.L., 1980. The effects of vegetation type and grazing system on the performance, diet and intake of yearling cattle PhD Thesis. Oregon State University, Corvallis, OR, USA.
Jemison, J.M., Jones, C., 2002. Watering Systems for Livestock. Water Quality Bulletin #7129, University of Maine Cooperative Extension, Orono, ME, USA.
Joerling, J., Doll, K., 2019. Monitoring of iron deficiency in calves by determination of serum ferritin in comparison with serum iron: A preliminary study. Open Veterinary Journal 9, 177–184. https://doi.org/10.4314/ovj.v9i2.14.
Lardner, H.A., Kirychuk, B.D., Braul, L., Willms, W.D., Yarotski, J., 2005. The effect of water quality on cattle performance on pasture. Australian Journal of Agricultural Research 56, 1–8. https://doi.org/10.1071/AR04086.
Looper, M.L., Waldner, D.H., 2002. Water for Dairy Cattle: Guide D-107. Cooperative Extension Service, New Mexico State University, Las Cruces, NM, USA.
Ministerial Order SCO/778/2009, of March 17, 2009. Alternative methods for the microbiological analysis of water for human consumption. State official bulletin of Spain government, BOE-A-2009-5316, 78, 30417–30420. https://www.boe.es/eli/es/o/2009/03/17/sco778.
Munger, S.D., 2016. Chapter 16 - The mechanisms of salty and sour taste. In Chemosensory transduction: The detection of odors, tastes, and other chemostimuli (Zufall, F., Munger, S.D.). Elsevier, Academic Press, London, UK, pp. 287–297. doi: 10.1016/C2014-0-00871-4.
Murphy, M.R., 1992. Water metabolism of dairy cattle. Journal of Dairy Science 75, 326–333. https://doi.org/10.3168/jds.S0022-0302(92)77768-6.
National Research Council (NRC), 1980. Drinking water and health, Volume 2. Safe drinking water committee, Board on toxicology and environmental health hazard. National Academy Press, Washington, DC, USA.
National Research Council (NRC), 2001. Nutrient Requirements of Dairy Cattle. 7th edition. The National Academies Press, Washington, DC, USA. doi: 10.17226/9825.
Royal Decree (RD) 140/2003, of February 7, 2003. The sanitary criteria for the quality of water for human consumption. State official bulletin of Spain government, BOE-A-2003-3596, 45, 1–40. https://www.boe.es/eli/es/rd/2003/02/07/140/con.
Savill, M., Hudson, A., Devane, M., Garrett, N., Gilpin, B., Ball, A., 2003. Elucidation of potential transmission of *Campylobacter* in New Zealand. Water Science and Technology 47, 33–38.
Schütz, K., 2012. Effects of providing clean water on the health and productivity of cattle. Retrieved on 26 October 2022 from https://envirolink.govt.nz/assets/Envirolink/1051-NLRC142-Effects-of-providing-clean-water-on-the-health-and-productivity-of-cattle.pdf.
Titgemeyer, E.C., Armendariz, C.K., Bindel, D.J., Greenwood, R.H., Löest, C.A., 2001. Evaluation of titanium dioxide as a digestibility marker for cattle. Journal of Animal Science 79, 1059–1063. https://doi.org/10.2527/2001.7941059x.
Utley, P.R., Bradley, N.W., Boling, J.A., 1970. Effect of restricted water intake on feed intake, nutrient digestibility and nitrogen metabolism in steers. Journal of Animal Science 31, 130–135. https://doi.org/10.2527/jas1970.311130x.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Verdú, M., Marti, S., Riera, J., Medinya, C., Cucurull, J., Devant, M., 2021. PSIX-8 Drinking water acidification and chlorination improves microbiological water quality without detrimental effects on performance or hazard side-effects in bulls fed high-concentrate diets. Journal of Animal Science 99, 438–439. https://doi.org/10.1093/jas/skab235.785.

Willms, W.D., Kenzie, O.R., McAllister, T.A., Colwell, D., Veira, D., Wilmshurst, J.F., Entz, T., Olson, M.E., 2002. Effects of water quality on cattle performance. Journal of Range Management 55, 452–460. https://doi.org/10.2307/4003222.

World Health Organization (WHO), 2022. Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda [Cataloguing-in-publication data available at]. WHO Graphics, Geneva, Switzerland.

Wright, C.L., 2007. Management of water quality for beef cattle. Veterinary Clinics of North America: Food Animal Practice 23, 91–103. https://doi.org/10.1016/j.cvfa.2006.12.002.