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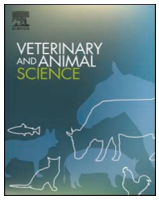

The quality and nutritional value of beef from Angus steers fed different levels of humate (K Humate S100R)

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ABSTRACT

This study compared the effect of four levels of K Humate S100R (potassium humate) supplementation on the quality, shelf-life, and nutritional properties of beef. Angus steers ($n = 40$) were individually housed and fed either 0, 35, 70, or 140 g K Humate S100R/animal/day for 100 days, following a 30 day adjustment period. The steers were slaughtered at the completion of the feeding study. The left m. *longissimus lumborum* (LL) was collected at 24 h *post-mortem* and aged for either 2 or 6 weeks before analysis. K Humate S100R supplementation did not affect beef drip loss, cooking loss, shear force, sarcomere length, ultimate pH, intramuscular fat content, or total volatile basic nitrogen concentrations ($P > 0.05$). Steers supplemented with 70 g/day K Humate S100R produced beef with higher a^* values on Days 1 and 3 of retail display ($P < 0.05$). Beef mineral composition was unchanged by K Humate S100R supplementation ($P > 0.05$), but there were minor changes to the fatty acid profile. Specifically, the ratio of omega-6 to omega-3 ($P < 0.05$) and C20:2n-6 concentrations ($P < 0.05$) increased with supplementation level. Together, these results demonstrate no detrimental effects on beef quality and shelf-life as a result of K Humate S100R supplementation.

1. Introduction

Humate is a biologically active compound that is derived from decaying organic material (e.g., peat deposits) and contains different fractions of humus, humic acid, fulvic acid, ulmic acid, and mineral elements (Livens, 1991; Peña-Méndez et al., 2005). There has been substantial interest in the application of humate as a feed additive for livestock production, because of its potential health and growth promoting properties and potential effects on meat quality (Esenbuğa et al., 2008; Kim et al., 2019; Lacková et al., 2022; Mišta et al., 2012). The literature, however, reveals variability in livestock responses to humate supplementation.

McMurphy et al. (2009), for example, reported that feed intakes, growth rates, and ruminal pH of steers were unaffected by feeding humate, although rumen ammonia concentrations decreased when humate supplementation was provided at 1 % total dry matter (DM). Zralý and Písaříková (2010) found that piglets fed 1 % sodium humate for 21 days had reduced concentrations of selenium in their muscle tissues and increased concentrations of calcium and iron in their blood. Steers supplemented with potassium humate (5.8 g/kg of feed) produced beef that was less tender, had lower pH values, and contained higher concentrations of intramuscular fat (IMF) and saturated fatty acids (SFA) as compared to non-supplemented steers (Mokotedi et al., 2018). Brown et al. (2007) found that steers supplemented with 1 % humate for 94 days produced beef with higher concentrations of sodium, sulphur, and zinc to that found in non-supplemented steers. Wang et al. (2020b) concluded that sodium humate supplementation had no effects on lamb growth performance or nutrient digestibility and therefore, was of no value as a feed additive. In addition, Ozturk et al. (2012) reported a dose dependent effect of humate supplementation on broiler thigh and breast meat colour and water holding capacity as well as affecting the amounts of phosphorous and high- and low-density lipids in the plasma.

These studies proposed that the mineral components of humate will participate in the maintenance of homeostasis, supporting enzyme activity (including those associated with metabolism), and thereby effect meat quality (Deng et al., 2020). Alternatively, they propose that associated effects of humate on immune function, digestion, and animal health, via its potential antioxidant, anti-inflammatory, and antibiotic properties, may infer secondary effects on meat quality (Wang et al., 2021). Yet, it is apparent from these examples that humate supplementation effectiveness may be a function of the livestock species and/or supplementation level. The literature also shows that humate

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Table 1
Proximate and mineral composition of K Humate S100R, K Humate S100R pellets, and the basal diet.[1,2].

Proximate	K Humate S100R	Humate pellet	Control pellet
Acid detergent fibre,%	< 2.0	12.0	12.0
Crude fibre,%	2.5	3.4	3.3
Crude protein,%	3.6	16.0	16.7
Dry matter,%	93.2	90.7	91.0
Metabolisable energy, MJ/kg DM)	6.9	12.1	12.4
Neutral detergent fibre,%	< 10.0	27.0	29.0
Minerals			
Calcium,%DM	0.14	.	.
Iron, ppm DM	2700	.	.
Magnesium,%DM	0.31	.	.
Phosphorous,%DM	0.04	.	.
Potassium,%DM	18.0	.	.
Selenium, ppm DM	3.0	.	.
Sodium,%DM	0.21	.	.
Sulphur,%DM	0.40	.	.

[1] Abbreviations include dry matter (DM), and parts per million (ppm).
[2] Cattle were fed control pellets ad libitum as the basal diet, only after all the daily assignment of humate pellets had been *consumed.* Humate and control pellets were both made using the same base components that included cereal chaff, barley, millmix (wheat bran and pollard), wheat, lucerne hay, soybean meal, canola oil, acid buffer, salt, and vitamin/mineral (ALPS) standard premix. K Humate S100R was included within the humate pellet formulation at 35 g/kg and excluded from the control pellet formulation.

effects on animal performance are not always reflected in the nutritional value and quality of the meat – albeit these studies investigated humate effects on the meat from rabbits, pigs, fish, and poultry (Esenbuğa et al., 2008; Kim et al., 2019; Lacková et al., 2022; Mišta et al., 2012). Comparatively, there has been little research of humate effects on the nutritional value and quality of beef.

Ataollahi et al. (2024) recently found that K Humate S100R supplementation at 70 g/day resulted in Angus steers having higher feed intake and feed efficiency to non-supplemented steers, however weight gain and carcass parameters were comparable between the four levels of K Humate S100R supplementation that were investigated. The current study aimed to build upon the findings of Ataollahi et al. (2024) and compare the effect of four levels of K Humate S100R supplementation on the quality, shelf-life, and nutritional properties of aged beef from feedlot finished Angus steers.

2. Materials and methods

This study was approved by the Animal Ethics Committee of the NSW Department of Primary Industries (ORA20/23/025). All of the experimental procedures adhered to the Australian Government guidelines for 'Best Practice Methodology in the Use of Animals for Scientific Purposes'.

2.1. Study design, animal slaughter, and sampling

Details for the experimental procedures, dietary treatments, as well as steer feed intake, growth rates, and carcass parameters have been previously described (Ataollahi et al., 2024). In summary, 40 Angus steers were stratified by liveweight and randomly allocated to ten blocks of four pens, wherein they were individually housed and fed one of four levels of K Humate S100R supplementation (0, 35, 70, and 140 g humate/animal/day). K Humate S100R supplementation was provided using two different pellets (Quayle Milling Ltd., AUS) that were formulated with (35 g/kg; humate pellet) or without (0 g/kg; control pellet) potassium humate (K Humate S100R, Omnia Specialities Australia, AUS; Table 1). Humate and control pellets were both made using the same base components that included cereal chaff, barley, millmix (wheat bran and pollard), wheat, lucerne hay, soybean meal, canola oil, acid buffer, salt, and vitamin/mineral (ALPS) standard premix. To achieve the different levels of supplementation, steers assigned to 35 g of K Humate S100R were fed daily with 1 kg of humate pellets; those assigned to 70 g of K Humate S100R were fed with 2 kg of humate pellets; and those assigned to 140 g of K Humate S100R were fed with 4 kg of humate pellets. The remainder of the feed requirements were exclusively delivered as control pellets, which were provided ad libitum, as the basal diet, and only after all the humate pellets had been consumed. The feeding study continued for 100 days and followed on from an adjustment period of 30 days, during which pellets were gradually introduced into the diets to reduce the risk of acidosis.

At the completion of the feeding study, cattle were transported as a single herd to a commercial Australian abattoir (Wagga Wagga, AUS) where they were slaughtered, eviscerated, and dressed in accordance with industry norms. Carcasses were chilled at 3–4 °C for ~ 24 h before the left m. *longissimus lumborum* (LL) (HAM: 5150 (AUS-MEAT, 2020)) was removed and collected from the boning room. The LL were vacuum packaged and transported to the Centre for Red Meat and Sheep Development (Cowra, AUS), where they were held together and wet aged. At the conclusion of the initial 2 week ageing period, two steaks of ~ 400 g (thickness: 10–12 cm) were removed from the caudal end of each LL. The first steak was vacuum packaged and aged for another 4 weeks (6 weeks in total) and the second steak was sectioned for analysis of quality, shelf-life, and nutritional parameters. The mean ± standard deviation temperature under which samples were wet aged was 1.6 ± 0.4 °C. Sample dissection was standard across both steaks, with subsections were prepared from the same location and then held frozen at −25 °C until their analysis, except for those analyses where unfrozen samples were necessary (colour stability and drip loss). For the samples wet aged for 6 weeks, only the drip loss, cooking loss, shear force, total volatile basic nitrogen, and ultimate pH variables were measured. While sample blocks were prepared from the same location within the steaks, cooking loss and shear force analyses were completed independently for the 2 or 6 week aged samples. Specifically, the sampled blocks from 2 week aged samples were cooked, weighed, and analysed on a separate day to the 6 week aged samples. This means that, even though the same methods were applied, the potential effects of day (batch) cannot be differentiated from the potential effects of ageing period.

2.2. Determination of meat quality and shelf-life

2.2.1. Cooking loss and shear force

Frozen and vacuum packaged sample blocks (86.3 ± 11.9 g, one sample block per wet ageing period per steer) were submerged in a 71 °C water bath for 35 min Holman et al. (2017). They were then removed and placed into iced water for 30 min, whereafter packaging removed and the samples were patted dry with paper towelling. Cooking loss (CL) was calculated as the percentage change in sample weight before and after cooking. Samples were placed into resealable plastic bags and refrigerated overnight, to standardise their temperatures to 3–4 °C. Six cuboidal strips (cross-sectional area: 1 cm^2) were prepared from each sample and tested using a texture analyser (model LRX, Lloyd Instruments, UK) with attached Warner-Bratzler blade (crosshead speed: 20 cm/min) (Holman et al., 2015). Using a cutting line perpendicular to the muscle fibre direction, the peak shear force required to sever each sample strip was recorded and the average reported in Newtons.

2.2.2. Drip loss

Fresh sample cores (diameter: 2.5 cm, one sample core per wet ageing period per steer) were placed into EZ-DripLoss tubes (Danish Meat Research Institute, DEN) and held at 3–4 °C for 72 h (Kilgannon et al., 2018). The change in sample weight before and after storage was used to calculate as the drip loss (%).

2.2.3. Sarcomere length

Thin sample slices (thickness: < 1 mm) were analysed using laser-diffraction and the average of five technical replicates reported as the sarcomere length (μm) (Bouton et al., 1973).

2.2.4. Ultimate pH

Samples of 1 g were homogenised with 6 mL of buffer solution (150 mM KCl, 5 mM iodoacetate; pH 7.0 U at 4 °C) and incubated to 20 °C in a water bath (Dransfield, 1994). A pH meter (smartCHEM-CP, TPS Pty Ltd, AUS) fitted with a spear-type gel electrode was first calibrated using 6.8 and 4.0 U standard buffers at 20 °C. This was used to record duplicate measures for ultimate pH (pHu).

2.2.5. Total volatile basic nitrogen

Samples of 10 g were homogenised with 100 mL of distilled water and held at room temperature for 30 min, being agitated intermittently during this period. Homogenates were filtered and held at 3–4 °C overnight. From each sample, a 10 mL aliquot was added to a glass distillation tube and steam distillation was performed using a Kjeldahl automated distillation unit (Kjeltec 8400, FOSS, DEN) (Holman et al., 2021). Total volatile basic nitrogen (TVB-N) values were calculated according to the sample's consumption of hydrochloric acid (0.1 mol/L) and recorded as mg/100 g fresh weight.

2.2.6. Total moisture and intramuscular fat

Samples of ~ 25 g were lyophilised at −50 °C and then ground. The change in sample weight before and after freeze-drying was used to calculate total moisture (%). Within individual glass vials, these samples were allowed to equilibrate to room temperature (for 2 h) and then analysed using a NIR analyser (Bruker, AUS) to determine the intramuscular fat concentration (IMF) of fresh weight (Bailes et al., 2022).

2.2.7. Retail colour

Samples (thickness: 3–4 cm, one sample slice per steer) were placed onto individual Styrofoam trays and overwrapped with PVC food film. These were allowed to bloom, for 30–45 min at 3–4 °C, before making the initial colour measurements (Day 0). Measurements were made using a calibrated spectrophotometer (model 45/0-L, HunterLab Inc. PRC) with a 25 mm aperture and using Illuminant D65 and 10° standard observer settings (Holman et al., 2015). The muscle fibre orientation was perpendicular on the measured surface, with duplicate readings made while avoiding fatty deposits and connective tissue. Samples were displayed under refrigeration and continuous lighting (NEC 58 W tubes that delivered ~ 1000 lx to the sample surface) to simulate retail conditions. At daily intervals, three additional colour measurements were made (Day 1–3) using the same method as for Day 0. The CIE colorimetrics (L*, a* and b*) were recorded and used to calculate the hue and chroma values (AMSA, 2012; CIE, 1977).

2.3. Determination of meat fatty acid and mineral concentrations

2.3.1. Fatty acids

The fatty acid concentrations of the freeze-dried samples were determined using the modified one-step method of Clayton et al. (2012). First, 10 mg of freeze-dried and ground sample were combined with 2 mL of methanol:toluene (4:1 v/v) and 10 μg/mL of each internal standard (C13:0 and C19:0). Fatty acids were methylated with the addition of 200 μL of acetyl chloride and subsequently incubated at 100 °C for 60 min. Once cooled, 5 mL of 6 % potassium carbonate solution was added to each sample. Centrifugation was then used to separate the upper toluene supernatant phase, which was transferred into a 2 mL glass vial fitted with a Teflon lined screw-cap lid.

Individual fatty acid methyl esters (FAME) were quantified using an Agilent 7890A GC fitted with dual BPX70 capillary columns (length: 30 m, internal diameter: 0.25 mm, film thickness: 0.25 μm, SGE Analytical Science, AUS) and dual flame ionisation detectors (FIDs). Helium was used as the carrier gas having a split ratio of 10:1, total flow rate of 12.4 mL/min, and a column flow rate of 0.9 mL/min. The inlet temperature was 250 °C, pressure was 107.8 kPa, and injection volume was 2.5 μL into a focus inlet liner (internal diameter: 4 mm, no. 092002, SGE Analytical Science, AUS). The oven temperature was held at 150 °C for 30 s, before being increased by 10 °C/min up to 180 °C; then by 1.5 °C/min up to 220 °C; and lastly by 30 °C/min up to 260 °C. This final temperature was held for 5 min to result in a total run time of 36.5 min. FID temperature was 280 °C, its gas flow rate for helium was 35 mL/min, instrument air was 350 mL/min, and nitrogen make-up gas was 30 mL/min. FAME peaks were identified against the retention times of commercial standards and published data (Clayton et al., 2012; Or-Rashid et al., 2010). FAME concentrations were calculated against a 3-point standard curve. Cis- and trans-double bond geometries, and conjugated linoleic acids (CLA) are described, where concentrations of t11–18:1 and t10–18:1 are reported as co-elution. Data were transformed and reported as mg/100 g fresh weight.

Table 2
The quality and shelf-life parameters for beef from steers supplemented with four different levels of K Humate S100R. Where appropriate, the wet ageing period is shown in parenthesis. Means ± standard error and level of significance (*P*-values) are included.[1].

Quality and shelf-life	Dietary treatment (K Humate S100R supplementation level, g/day)				*P*-values
	Control	35	70	140	
Cooking loss (2 weeks),%	17.2 ± 0.50	17.2 ± 0.33	17.3 ± 0.33	17.3 ± 0.51	0.933
Cooking loss (6 weeks),%	19.2 ± 0.82	19.6 ± 0.53	20.1 ± 0.54	20.6 ± 0.83	0.298
Drip loss (2 weeks),%	0.3 ± 0.09	0.3 ± 0.06	0.3 ± 0.06	0.4 ± 0.09	0.439
Drip loss (6 weeks),%	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.02	0.505
Intramuscular fat,%	4.3 ± 0.49	4.6 ± 0.32	5.0 ± 0.32	5.4 ± 0.49	0.160
Sarcomere length, μm	1.6 ± 0.05	1.5 ± 0.03	1.5 ± 0.03	1.5 ± 0.05	0.675
Shear force (2 weeks), N	44.7 ± 3.29	46.8 ± 2.14	48.9 ± 2.16	51.0 ± 3.32	0.241
Shear force (6 weeks), N	59.9 ± 3.96	62.1 ± 2.58	64.3 ± 2.61	66.6 ± 4.01	0.296
Total moisture,%	26.9 ± 0.81	27.3 ± 0.53	27.7 ± 0.53	28.1 ± 0.82	0.378
TVB-N (2 weeks), mg/100 g	5.4 ± 0.10	5.4 ± 0.07	5.5 ± 0.07	5.5 ± 0.10	0.665
TVB-N (6 weeks), mg/100 g	6.2 ± 0.14	6.2 ± 0.09	6.2 ± 0.09	6.2 ± 0.14	0.980
Ultimate pH (2 weeks)	5.6 ± 0.02	5.6 ± 0.01	5.6 ± 0.01	5.6 ± 0.02	0.467
Ultimate pH (6 weeks)	5.6 ± 0.15	5.7 ± 0.10	5.8 ± 0.10	5.9 ± 0.15	0.230

[1] Abbreviations include total volatile basic nitrogen (TVB-N).

2.3.2. Minerals

Microwave digestion and inductively coupled plasma-optical emission spectroscopy or inductively coupled plasma mass spectroscopy were used to determine the mineral profile of each lyophilised sample (Carrilho et al., 2002). These analyses were undertaken at the commercial laboratories of the Wollongbar Primary Industries Institute (Wollongbar, AUS). Mineral concentrations were reported as mg/100 g fresh weight.

2.4. Statistical analysis

Data were first evaluated for obvious outliers and errors. Using Stata (Version 7, www.stata.com), the meat quality, nutritional, and colour variables were analysed using multiple linear mixed models (REML). The base model fitted K Humate S100R supplementation level as a fixed

term. To this base model block, pen, animal and kill order were tested for significance as random terms, but ultimately omitted from all models ($P > 0.05$). The fixed effects of wet ageing period were confounded by the effects of batch and therefore were omitted from all models. The initial weight of the shear force sample blocks was included as a covariate term for the analysis of shear force and cooking loss data. Display period and its interaction with K Humate S100R supplementation level were included as additional fixed terms for the analysis of colour parameters. Least significant difference (LSD) were used to determine which means were significantly different from each other, applying $P < 0.05$ as indicative of significance.

3. Results and discussion

3.1. Meat quality and shelf-life

There were no significant effects of K Humate S100R supplementation level on the beef quality parameters ($P > 0.05$; Table 2). This is a novel observation, offering insight into the effect of K Humate S100R supplementation level on beef quality measures for tenderness, juiciness, overall liking, retail potential, and spoilage.

The amount of water that is lost when beef is placed under retail display or cooked represents a decline in yield, as this fraction cannot be sold or consumed. The point of water loss will dictate whether this cost is incurred by the retailer (drip loss) or consumer (cook loss). High amounts of water loss can also detract from the retail potential for beef, with consumer's willingness to purchase impacted by visible water or purge inside the packaging (Warner, 2023). The comparable drip and cooking loss values for all steaks, within each wet ageing period, confirms that K Humate S100R supplementation is not detrimental to associated beef steak yield and retail appearance characteristics. The absence of a humate supplementation effect on drip loss was also reported for bovine, poultry and pig meat (Domínguez-Negrete et al., 2019; Mokotedi et al., 2018), with authors suggesting that humate reduces peroxidation and this has a positive association to water holding capacity metrics (Bai et al., 2013). The absence of any humate supplementation effects on total moisture ($P > 0.05$) acts to affirm the insignificant relationship between dietary humate and the water holding capacity of beef.

Several studies have demonstrated IMF to be associated with consumer satisfaction with beef eating quality, including tenderness, juiciness, flavour, and overall liking (Corbin et al., 2015; Holman & Hopkins, 2021; Holman et al., 2021; Luchak et al., 1998; O'Quinn et al., 2012). The observed IMF concentrations were ~ 5 % and therefore, within the reported limits for beef satisfactory for juiciness and flavour attributes (Thompson, 2004). This percentage was lower than scores previously reported in other studies of Angus beef (Baud et al., 1997; Frank et al., 2016; Torres-Vázquez et al., 2018), but the age at slaughter and the hot carcass weight of the cattle in this current study were less than industry norms (Ataollahi et al., 2024) and these factors can impact on the IMF content (Hopkins et al., 2006). These factors may further address the different IMF results reported by Mokotedi et al. (2018), wherein cattle supplemented with humate were reported to produce beef with higher IMF compared to that produced by non-supplemented cattle. Although there were other between study experimental differences, such as basal diet and cattle breed, that could have likely contributed to the disparate IMF findings. Consequently, humate supplementation may have little independent influence on fat biosynthesis and regulation of IMF in Angus steers.

The sarcomere length for all beef steaks were comparable ($P = 0.675$). This outcome confirms the absence of any abattoir-chiller effect (i.e., cold- or heat-shortening of the muscle fibres as a result of aberrant muscle pH decline rates) on the tenderness of the product (Ataollahi et al., 2024; Smulders et al., 1990). The observed sarcomere lengths are within the expected range reported in the literature for beef (Holman et al., 2020; Hughes et al., 2014; Purchas, 1990) and suggest that the tenderness of the beef steaks is unaffected by K Humate S100R supplementation. This was clarified with further analysis of shear force and IMF data, as sarcomere length accounts for a minor fraction of the total variation in a consumer score for beef tenderness (Holman et al., 2020; Smulders et al., 1990).

Shear force is closely related to the consumer's perception of beef tenderness and overall eating quality (Silva et al., 2018). Yet, there was no effect of K Humate S100R supplementation level observed on the shear force for beef aged 2 or 6 weeks ($P > 0.05$). In addition, it was observed that all the beef in this study would be considered to be tough by the majority of consumers. This finding is based on consumer thresholds that define beef as tough when shear force is > 42.6 N (Holman et al., 2020) and > 41.4 N (Liang et al., 2016). A standardised cooking method and level of doneness were applied to determine shear force and these same parameters are used for the sensory panels against which thresholds are defined. However, it is notable that shear force quantifies tenderness in isolation to other sensory properties (such as taste and aroma) and the other foods that would normally be served in combination with a beef steak (Holman & Hopkins, 2021). This finding shows that K Humate S100R supplementation had no detrimental effect on beef shear force. Research with pork confirms these results, with the authors proposing the comparable pH in meat from each supplementation level as contributing to this outcome (Bai et al., 2013). The same could be true for the current study, especially as past research with beef has shown humate supplementation to decrease shear force (Mokotedi et al., 2018). This study did find pH differences between humate supplementation levels and proposed that this modulated the proteolytic activities during rigor and ageing periods, potentially via oxidative (Mokotedi et al., 2018). The variation in shear force protocols between this and the current study may have contributed to this discrepancy (Holman et al., 2016). Yet, it was also observed that K Humate S100R supplementation level had no effect on TVB-N concentrations in beef aged for 2 or 6 weeks ($P = 0.665$ and $P = 0.980$, respectively). TVB-N concentrations increase as meat protein structures degrade as a result of enzymatic and microbial proteolytic activities (Bekhit et al., 2021). This result suggests that K Humate S100R supplementation does not modulate beef proteolysis, albeit a study of myofibrillar fragmentation index, desmin, and calpain activity would confirm this observation.

TVB-N is used by different authorities to quantify the freshness or spoilage of red meat products, with concentrations of 15–20 mg/100 g often stipulated as the upper limit for fresh, unspoilt meat (Bekhit et al., 2021; FAO, 1986; Korean Ministry of Agriculture & Forestry, 2015; National Standard of the People's Republic of China, 2016). All the beef, regardless of ageing period, can therefore be classified as being fresh and unspoilt. Some nations (e.g., Australia) use total viable microbial counts (TVC) and a threshold of log 7 cfu/g as an indicator of beef freshness or spoilage (CSIRO, 1995; Kim et al., 2018). A recent study has shown that beef with a TVB-N concentration of 5.1 mg/100 g as the equivalent of it having a TVC of log 7 cfu/g (Holman et al., 2021). Based on TVB-N and its confidence intervals, it could be said that all the 6 week aged beef steaks were spoilt. There is, however, variance in the application of TVB-N to determine freshness-spoilage as its findings do not always align with other indicators of spoilage (e.g., microbial population, peroxidation, sensory attributes) (Bekhit et al., 2021). Nonetheless, this TVB-N result is likely due to the interaction between storage time and the temperature under which the samples were aged, with higher temperatures associated with higher rates of spoilage (Giannuzzi et al., 1998; Han et al., 2024). The ultimate pH was comparable across the levels of K Humate S100R supplementation, after 2 or 6 weeks of ageing ($P = 0.467$ and $P = 0.230$, respectively). Ultimate pH is impacted by the microbial population of vacuum packaged beef, with higher levels often associated with lactic acid bacterium proliferation (Borch et al., 1996; Gill & Newton, 1978). The absence of a K Humate S100R supplementation level effect on ultimate pH suggests that its use as a feed additive does not reduce or extend the shelf-life of beef. This finding is supported by the comparable pH at 24 hour results that have been reported for

Table 3
The colour parameters for beef from steers supplemented with four different levels of K Humate S100R (diet) and placed on retail display for a total of 3 days (display). Means ± standard error and level of significance (*P*-values) are included.[1].

Colour parameter	Dietary treatment (K Humate S100R supplementation level, g/day)				*P*-values		
	Control	35	70	140	diet	display	interaction
*L**	43.3 ± 0.31	42.2 ± 0.3	41.9 ± 0.3	42.1 ± 0.3	0.347	0.667	NS
*a**	22.9 ± 0.27	23.1 ± 0.3	23.9 ± 0.3	23.1 ± 0.3	0.078	0.185	<0.001
*b**	20.2 ± 0.22	20.1 ± 0.2	20.3 ± 0.2	19.8 ± 0.2	0.247	0.332	NS
Hue	0.7 ± <0.01	0.7 ± <0.01	0.7 ± <0.01	0.7 ± <0.01	0.314	0.557	<0.001
Chroma	30.5 ± 0.3	30.6 ± 0.3	33.4 ± 0.3	30.5 ± 0.3	0.109	0.212	<0.001

[1] Abbreviations include not significant ($P > 0.05$, NS). Hue and chroma were calculated as per AMSA (2012).

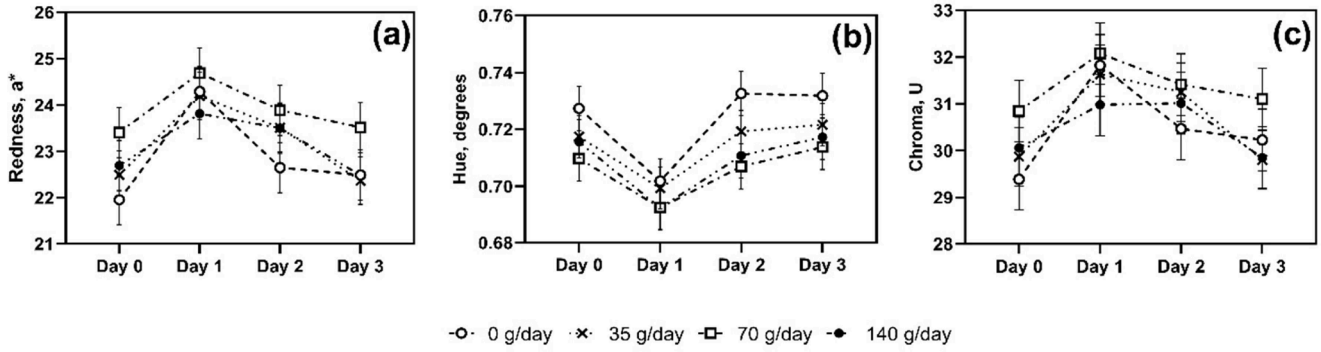

Fig. 1. The effect of K Humate S100R supplementation level by display period (days) interaction ($P < 0.001$) on beef (**a**) redness (a^*); (**b**) colour intensity (hue); and (**c**) colour brightness (chroma). Means are plotted with standard error bars.

Table 4
The mineral concentrations for beef from steers supplemented with four different levels of K Humate S100R. Means ± standard error and level of significance (*P*-values) are included.[1].

Minerals, mg/100 g fresh	RDI [2]	Dietary treatment (K Humate S100R supplementation level, g/day)				*P*-values
		Control	35	70	140	
Calcium	100–130	7.4 ± 0.8	7.6 ± 0.5	7.7 ± 0.5	7.8 ± 0.8	0.759
Copper	1.2–1.7	0.05 ± <0.01	0.06 ± <0.01	0.06 ± <0.01	0.06 ± <0.01	0.382
Iron	8–18	1.35 ± 0.06	1.36 ± 0.04	1.37 ± 0.04	1.37 ± 0.06	0.807
Magnesium	310–420	25.3 ± 0.8	25.4 ± 0.5	25.4 ± 0.5	25.5 ± 0.8	0.900
Phosphorous	1000	217.4 ± 6.3	217.5 ± 4.1	217.5 ± 4.1	217.5 ± 6.3	0.995
Potassium	2800–3800	373.2 ± 10.0	370.0 ± 6.5	366.9 ± 6.6	363.7 ± 10.1	0.559
Sodium	460–920	41.8 ± 1.2	21.2 ± 0.8	42.6 ± 0.8	43.0 ± 1.2	0.538
Sulphur	–	218.8 ± 6.6	219.4 ± 4.3	219.9 ± 4.4	220.4 ± 6.7	0.881
Zinc	8–14	3.42 ± 0.15	3.40 ± 0.10	3.39 ± 0.10	3.38 ± 0.15	0.862

[1] The other minerals tested for, but not detected (within the limits of reporting, 0.05 DM%), included aluminium, arsenic, boron, cadmium, cobalt, chromium, manganese, molybdenum, nickel, led, and selenium.

[2] Recommended daily intakes (RDI) are for adults (aged 19–50 years old) and were derived from NHMRC (2019).

cattle supplemented with different levels of humate (Ataollahi et al., 2024; Brown et al., 2007).

There was no independent effect of K Humate S100R supplementation level or retail display on beef colour parameters ($P > 0.05$; Table 3). However, there was a significant second-order interaction between K Humate S100R supplementation level and retail display period for beef a^* (redness), hue (intensity) and chroma (brightness) ($P < 0.001$; Fig. 1).

From the interaction between K Humate S100R supplementation level and display period, it was observed that cattle supplemented with 70 g/day K Humate S100R produced beef that was more red (higher a^* values) than observed for the control samples at Day 1 of retail display, and more red (higher a^* value) than all other diets at Day 3 of retail display ($P < 0.05$; Fig. 1). Beef redness is a function of the oxidative status of the myoglobin in meat – with different concentrations having different effects on beef appearance. For example, oxymyoglobin is associated with redness, deoxymyoglobin with purpleness, and metmyoglobin with discolouration or brownness (Suman et al., 2014). With this in mind, it seems that the supplementation of K Humate S100R at 70 g/day may have contributed to the synthesis of myoglobin, as suggested by poultry and pork studies (Disetlhe et al., 2019; Esenbuğa et al., 2008; Kim et al., 2019), and preservation of oxymyoglobin in the beef. The latter antioxidant effect of humate supplementation on myoglobin redox status across retail display is the most likely pathway of action, given the comparable iron concentrations found for these same samples (Table 4). Further, it seems that this antioxidant effect of humate (i.e., from mineral elements, such as selenium and iron (Cusack, 2008)) is dose dependent, as this same advantage was not observed when K Humate S100R was supplemented at 140 g/day. This may be the result of humate effects on total antioxidant capacity and malondialdehyde concentrations in the blood of supplemented calves and pigs (Wang et al., 2021, 2020a). The redness (a^*) of beef is associated with consumer satisfaction with the appearance of a beef product and has been used to define the threshold whereby beef with a^* values < 14.5 is unacceptable to 95 % of consumers (Holman et al., 2017). Consequently, if we apply this threshold to a^* value results (Table 3), we can observe that dietary treatment had no practical effect on the acceptability of beef colour to the consumer. This is because all of the beef steaks had a^* values > 14.5, irrespective of display period and K Humate S100R supplementation level treatment. It is likely that the effects of dietary treatment by display period interaction on hue and chroma are the result of the aforementioned variation to a^* (redness). This is because a^* is used to calculate (*post hoc*) both hue and chroma (AMSA, 2012). Studies of broilers supplemented with humate confirm these outcomes, with meat a^*, hue, and

Table 5
The fatty acid concentrations (mg/100 g) for beef from steers supplemented with four different levels of K Humate S100R. Means ± standard error and level of significance (*P*-values) are included.[1].

Fatty acids	Dietary treatment (K Humate S100R supplementation level, g/day)				*P*-values
	Control	35	70	140	
∑SFA	2170.9 ± 252.0	2313.3 ± 164.4	2455.6 ± 165.9	2598.0 ± 255.0	0.295
C12:0	6.1 ± 0.7	6.3 ± 0.5	6.6 ± 0.5	6.8 ± 0.7	0.533
C14:0	175.3 ± 24.1	187.0 ± 15.7	198.6 ± 15.9	210.3 ± 24.4	0.370
C15:0	25.2 ± 3.2	26.9 ± 2.1	28.5 ± 2.1	30.2 ± 3.3	0.341
C16:0	1271.0 ± 143.8	1339.3 ± 93.8	1407.7 ± 94.6	1476.0 ± 145.5	0.379
C17:0	50.4 ± 5.9	54.0 ± 3.8	57.6 ± 3.9	61.3 ± 5.9	0.252
C18:0	579.4 ± 71.2	631.6 ± 46.5	683.8 ± 46.9	736.1 ± 72.1	0.175
C20:0	3.9 ± 0.5	4.4 ± 0.3	4.8 ± 0.3	5.3 ± 0.5	0.085
C22:0	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	0.588
C24:0	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	0.878
anteisoC15:0	11.5 ± 1.4	12.2 ± 0.9	13.0 ± 0.9	13.8 ± 1.5	0.315
isoC15:0	7.2 ± 1.1	7.9 ± 0.7	8.6 ± 0.7	9.3 ± 1.1	0.209
anteisoC17:0	36.3 ± 4.3	39.0 ± 2.8	41.6 ± 2.8	44.2 ± 4.4	0.262
∑MUFA	1906.5 ± 212.4	2040.7 ± 138.5	2175.0 ± 139.8	2309.3 ± 214.9	0.241
C14:1n-5	44.7 ± 5.7	46.9 ± 3.7	49.1 ± 3.7	51.3 ± 5.8	0.472
C17:1n-7	12.9 ± 1.0	13.5 ± 0.7	14.0 ± 0.7	14.6 ± 1.0	0.307
C18:1n-7	71.1 ± 13.2	63.6 ± 8.6	56.2 ± 8.7	48.7 ± 13.4	0.296
C18:1n-7t	73.3 ± 7.7	80.0 ± 5.0	86.7 ± 5.1	93.4 ± 7.8	0.107
C18:1n-9	1573.6 ± 180.5	1704.8 ± 117.7	1836.1 ± 118.8	1967.3 ± 182.6	0.178
C18:1n-9t	13.1 ± 2.5	14.3 ± 1.6	15.5 ± 1.6	16.6 ± 2.5	0.371
C20:1n-9	6.3 ± 0.7	6.7 ± 0.5	7.2 ± 0.5	7.7 ± 0.7	0.229
C20:1n-15	1.4 ± 0.2	1.6 ± 0.1	1.7 ± 0.1	1.9 ± 0.2	0.057
C22:1n-9	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	0.661
C24:1n-9	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.389
∑PUFA	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	0.686
C16:3n-4	0.2 ± < 0.1	0.2 ± < 0.1	0.2 ± < 0.1	0.2 ± < 0.1	0.577
C20:3n-9	2.6 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	0.727
∑n-3	66.7 ± 3.9	68.2 ± 2.6	69.7 ± 2.6	71.2 ± 4.0	0.478
C18:3n-3	29.7 ± 2.6	31.5 ± 1.7	33.4 ± 1.7	35.3 ± 2.6	0.184
C20:3n-3	0.7 ± 0.1	0.7 ± < 0.1	0.7 ± < 0.1	0.7 ± 0.1	0.984
C20:4n-3	0.3 ± < 0.1	0.3 ± < 0.1	0.3 ± < 0.1	0.3 ± < 0.1	0.140
C20:5n-3	12.0 ± 0.9	11.8 ± 0.6	11.6 ± 0.6	11.4 ± 0.9	0.703
C22:5n-3	21.2 ± 1.1	21.0 ± 0.7	20.9 ± 0.7	20.7 ± 1.1	0.779
C22:6n-3	2.9 ± 0.2	2.9 ± 0.1	2.9 ± 0.1	2.8 ± 0.2	0.700
∑n-6	187.3 ± 12.4	197.6 ± 8.1	208.0 ± 8.2	218.3 ± 12.6	0.122
C18:2n-6	135.1 ± 10.0	144.4 ± 6.5	153.8 ± 6.6	163.1 ± 10.1	0.084
C18:3n-6	0.4 ± < 0.01	0.4 ± < 0.1	0.5 ± < 0.1	0.5 ± < 0.1	0.272
C20:2n-6	1.2 ± 0.1^{b}	1.4 ± 0.1^{ab}	1.5 ± 0.1^{a}	1.6 ± 0.1^{a}	0.031
C20:3n-6	10.2 ± 0.7	10.9 ± 0.4	11.6 ± 0.5	12.3 ± 0.7	0.067
C20:4n-6	36.8 ± 2.4	36.9 ± 1.5	37.0 ± 1.6	37.1 ± 2.4	0.925
C22:4n-6	3.2 ± 0.2	3.2 ± 0.1	3.3 ± 0.1	3.4 ± 0.2	0.593
C22:5n-6	0.4 ± < 0.1	0.4 ± < 0.1	0.4 ± < 0.1	0.4 ± < 0.1	0.344
n-6:n-3	2.8 ± 0.1^{b}	2.9 ± 0.1^{ab}	3.0 ± 0.1^{a}	3.1 ± 0.1^{a}	0.028
∑CLA	7.1 ± 0.9	7.5 ± 0.6	7.9 ± 0.6	8.3 ± 0.9	0.422
c9t11CLA	5.7 ± 0.7	6.0 ± 0.5	6.3 ± 0.5	6.6 ± 0.8	0.474
t10c12CLA	1.3 ± 0.2	1.4 ± 0.1	1.5 ± 0.1	1.7 ± 0.2	0.265
∑EPA+DHA	14.9 ± 1.1	14.7 ± 0.7	14.4 ± 0.7	14.2 ± 1.1	0.691

[1] Abbreviations include the sum of C18:3n-3, C20:3n-3, C20:4n-3, C20:5n-3, C20:5n-3 and C22:6n-3 (∑n-3); sum of C18:2n-6, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, C22:4n-6 and C22:5n-6 (∑n-6); sum of C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0 and C24:0 (∑SFA); sum of C14:1n-5, C15:1n-5, C16:1n-7t, C16:1n-7, C17:1n-7, C18:1n-9t, C18:1n-7t, C18:1n-9, C18:1n-7, C20:1n-15, C20:1n-9, C22:1n-9 and C24:1n-9 (∑MUFA); sum of C20:3n-9, ∑n-3 and ∑n-6 (∑PUFA); sum of c9,t11CLA and t10,c12CLA (∑CLA); ratio of ∑n-6 to ∑n-3 (n-6:n-3); and sum of C20:5n-3 and C22:6n-3 (∑EPA+DHA). Means within rows with different superscripts were significantly ($P < 0.05$) different.

chroma values reported to increase with supplementation level (Esenbuğa et al., 2008; Ozturk et al., 2012).

3.2. Fatty acid and mineral concentrations

There were no significant effects from K Humate S100R supplementation level on the mineral composition of beef ($P > 0.05$; Table 4). To allow comparisons to be made to nutritional guidelines, it is important that we assess the effect of humate supplement on beef mineral concentrations as g per 100 g serve (fresh) weight. All the beef steaks, irrespective of K Humate S100R supplementation level, were shown to have comparable concentrations of calcium, copper, iron, magnesium, phosphorous, potassium, sodium, sulphur, and zinc. These concentrations were found to align with those reported previously for beef (Williams, 2007), but diverge from the results presented in the literature for livestock supplemented with humate (Brown et al., 2007; Zralý & Písaříková, 2010). The source and processing methods applied to humate will impact its chemistry and mineral profile (Livens, 1991), and thereby influence the transfer of mineral elements from the rumen into the small intestine. Specifically, the colloidal characteristics of a humate product will affect its capacity to form chelates (complexes) with metal ions, which can impact their bioavailability (Zralý & Písaříková, 2010). This has been the proposed mechanism for comparable mineral concentrations in the blood of rabbits and broilers fed different levels of humate (Lacková et al., 2022; Rath et al., 2006). The interactions between the humate type and the basal diet could, therefore, contribute to the observation of humate supplementation effects on the mineral composition of meat. Irrespective, these results help to affirm beef as a rich source of dietary phosphorous, iron and zinc – with a single serving of beef contributing to the daily recommended intake for each of these nutrients (NHMRC, 2019).

K Humate S100R supplementation level had minor effects on beef fatty acid concentrations (Table 5). Specifically, the concentration of C20:2n-6 and the ratio of omega-6 to omega-3 fatty acids (n-6:n-3) were observed to increase with increased levels of K Humate S100R supplementation ($P < 0.05$; Table 5). The n-6:n-3 is used to define the 'healthiness' of red meat products, with a lower ratio preferable because of the potential for biochemical competition and/or inhibition between the functionalities of these fatty acids (Clayton, 2014; Savoini et al., 2016). Based on this premise, it could be suggested that higher levels of humate supplementation will result in less healthy beef – results that may arise from the proposed humic acid influences on biohydrogenation, namely the microbially mediated enzymatic desaturation and elongation processes for fatty acids within the rumen (Disetlhe et al., 2019). Research of pigs supplemented with different levels of fulvic acid (a constituent of humate) found there was a quadratic relationship between fulvic acid and malonaldehyde concentration in the meat, a result indicative of an antioxidant effect and the preservation of unsaturated fatty acids (Bai et al., 2013). Wang et al. (2021) also observed an increase in the total oxidative capacity of blood from weaner cattle supplemented with humate. These peroxidation factors were associated with the proportional increase in polyunsaturated fatty acids, as well as an increase in the omega-3 and omega-6 fatty acids concentrations in the meat of broilers fed humic acid (a constituent of humate) (Disetlhe et al., 2019). This could be the mechanism by which C20:2n-6 and n-6:n-3 values were impacted by K Humate S100R supplementation level in the current study. Although analysis of peroxidation factors in the beef samples would be necessary to affirm this pathway. Furthermore, the practicality of this finding should be considered within the context of the concentration of 'health claimable' fatty acids and reports from the FAO (FAO, 2010) that 'provided omega-3 and omega-6 intakes adhere to their individual guidelines, there is no rationale to support a recommended ratio for human health'.

4. Conclusions

This study demonstrates there were no detrimental effects on beef quality and shelf-life as a result of K Humate S100R supplementation. This included measures of beef tenderness, juiciness, yield, and freshness. Cattle supplemented with 70 g/day K Humate S100R produced beef that was more red (highest a^* value) in colour than beef from cattle fed the other supplementation levels. Beef redness is associated with consumer satisfaction with its appearance. It is noted that irrespective to K Humate S100R supplementation level, all of the beef steaks were within an acceptable range for consumer satisfaction with colour (a^* values > 14.5). K Humate S100R supplementation did not have an impact on beef mineral composition and its status as a rich source of dietary phosphorous, iron, and zinc. K Humate S100R supplementation increased the ratio of omega-6 to omega-3 fatty acids. The practical significance of this effect was marginal and when combined with the absence of further effects on fatty acid concentrations, these results suggest that all of the beef steaks (regardless of humate supplementation level) were of comparable nutritional value to the consumers. In conclusion, the effects of K Humate S100R supplementation at any level (0, 35, 70, or 140 g/day) on beef quality, nutritional value, and retail potential were of minor practical importance. A limitation to the current study is the type of humate that was supplemented to steers. Specifically, alternative humate products have different chemical composition and therefore may have alternative effects on meat quality than were observed herein. The current study investigated 140 g/day as the maximum level of K Humate S100R supplementation and therefore, the effects of higher levels of supplementation were not confirmed. These paucities require additional research.

Ethical statement

The animal study protocol was approved by the Animal Ethics Committee of the NSW Department of Primary Industries (ORA20/23/025). All of the experimental procedures adhered to the Australian Government guidelines for 'Best Practice Methodology in the Use of Animals for Scientific Purposes'.

CRediT authorship contribution statement

Forough Ataollahi: Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **John W. Piltz:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Geoff R. Casburn:** Writing – review & editing, Methodology, Investigation. **Benjamin W.B. Holman:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis.

Declaration of competing interest

The authors declare no conflict of interest.

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