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Reducing Induction Stress in the Australian Feedlot System

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Executive summary

Induction stress and subsequent adverse health impacts are well documented in the beef cattle feedlot sector. Despite the best efforts of feedlot managers, stress and associated health problems remain as a major economic cost in the industry. Bovine respiratory disease is the most common animal health problem in the feedlot environment, costing the Australian beef feedlot industry approximately 40 million dollars per annum. Increased levels of stress during transition into the feedlot have been associated with an increased likelihood of the disease. Despite management strategies including backgrounding, low-stress handling, transition diets, and vaccination schedules, the prevalence of bovine respiratory disease remains a significant problem in Australian feedlots.

Bromide has been recognised in recent years as a potential therapeutic agent for ruminant livestock systems, with international patents awarded for its use as a therapeutic agent for tremorergic intoxications and for stress. Bromide, as a known anxiolytic and pan-neuronal inhibitor, has been demonstrated to have GABAergic activity and to reduce activity in the central amygdala; both important mechanisms for mitigation of the stress response. Additionally, a long elimination phase and wide therapeutic window make bromide a suitable candidate for minimising stress during and after many management and husbandry practices on farms and feedlots, and in transport settings. Previous research has investigated the use of bromide as a sedative in cattle (Genicot *et al.* 1991), and it has been widely used as a calmative agent in horses (Ho *et al.* 2010). Our research has previously focussed on its use in sheep, with pharmacokinetic and efficacy studies being undertaken, outcomes of which have supported the use of bromide in other ruminant livestock systems (Quast *et al.* 2015b; Combs *et al.* 2019).

Although both species are ruminants, pharmacokinetic profiles are expected to differ between sheep and cattle, as is the case between sheep and horses (Raidal and Edwards 2008), and similar to other pharmacological agents frequently used in production livestock such as lidocaine and macrocyclic lactones. For this reason establishing the pharmacokinetic parameters of bromide in cattle is required before further applied research can be undertaken. Serum concentrations of bromide have previously been reported in cattle at varying dietary bromide inclusion rates (Knight and Costner 1977), but many pharmacokinetic parameters of importance were omitted. The long elimination phase of bromide makes it a potential candidate for use in feedlots where induction stress is high, but interventions are kept to a minimum. It was anticipated that stress amelioration during feedlot induction and the transition phase will reduce the likelihood of morbidity and mortality in feedlot cattle and therefore increase productivity as an indirect measure of effect. Also, as production measures are rarely applicable for product registration purposes, impacts on mortality and indicence of disease were investigated as the necessary measures for registration of a pharmaceutical product, particularly in Australia and the United States.

To investigate the efficacy of use of bromide to mitigate feedlot induction stress in cattle, the following project objectives were determined:

1. To define the regulatory landscape for bromide in animal production industries;
2. To determine the pharmacokinetic profile, including quantitation of residues, for bromide in cattle; and,
3. To identify health and production outcomes for cattle treated with bromide at feedlot induction.

To determine the pharmacokinetic profiles of bromide, twenty-one steers were randomly assigned to the treatment groups and given as an oral dose containing 100 mg/kg, 300 mg/kg, or 500 mg/kg

LW of bromide as the potassium salt. Serial blood sampling was undertaken at 15 time points, 0–72 hours after treatment. Serum bromide concentrations were determined by colourimetric spectrophotometry. Three steers were assigned to a control group to observe baseline (endogenous) serum and tissue bromide concentrations. Steers were euthanased at 7, 21, or 42 days, and tissue bromide concentrations were determined by ion chromatography. In this trial, bromide exhibited first order pharmacokinetics, with a terminal elimination half-life ($t_{1/2}$) in serum of 7.75, 7.06 and 6.52 days for 100, 300 and 500 mg/kg doses, respectively. After oral dosing with bromide, return to baseline was approximately 28, 34 and 42 days for 100, 300 and 500 mg/kg dose rates respectively. Tissue concentrations of bromide after oral administration were measured at days 7, 21 and 42 in fat, kidney, liver and muscle tissues. Return to baseline values for bromide in tissues was dependent on dose and varied between 7–42 days.

An applied study was undertaken in an Australian feedlot to determine the merit of bromide as a therapeutic agent for stress mitigation on feedlot induction, measuring health and production outcomes. Cattle (n = 1936) were recruited in August and September 2019 as part of the feedlot's normal intake. All animals were placed on 120 day feeding regimens and underwent typical husbandry during their time on feed. Recruited cattle were drafted into six sets of replicates that comprised of one treatment pen and one control pen. Cattle were allocated to treatment or control pens by order of presentation at the induction chute. Treatment cattle were given a low dose of bromide orally and both cohorts was followed to slaughter.

Bromide salts are highly soluble in water (the notable exception being silver bromide) and are therefore fully disassociated in solution. Consequently, the pharmacokinetics and pharmacodynamics of the bromide anion (Br^-, referred to as Br or bromide in this report) are independent of the salt counter-cation. As such, the Australian Pesticides and Veterinary Medicines Authority (APVMA) approved a research permit for a feedlot efficacy study using bromide, based on the pharmacokinetics and residue depletion data.

Major findings

An in-house review identified an opportunity to register bromide salts in multiple countries. Although both a production claim and therapeutic claim are feasible, determining the desired indication will be critical for regulatory approval. Both human food safety and target animal safety will require various studies but no major issues are anticipated.

This study identified that oral treatment with bromide at a low variable dose rate weight (LW) at feedlot entry did not have a significant effect on animal performance (P = 0.09; 'deads and salvage culls in' average daily gain) when all animals were considered across the full duration of time on feed (120 days). No difference was observed at slaughter in meat quality scores. Statistical significance was not reached due to the relatively low number of deaths experienced during the study, there was, however, evidence that treatment with bromide reduced mortality with untreated animals found to be 2.8 times more likely to be registered as notifiable deaths (mortalities and salvage culls) than their untreated counterparts. Also, treatment with bromide showed a trend towards reduced mortalities by up to 50% (P = 0.081).

When hospitalisations within the first 28 days on feed were considered, the active therapeutic window, a greater number of control cattle were hospitalised for BRD-related illness compared to

their untreated counterparts (*P* = 0.014). This data suggests that bromide may assist in mitigating BRD-related presentations in the first 28 days in the feedlot commensurate with the time of effect after a single oral dose at induction. Specifically, calculated odds ratios suggested that control group cattle were twice as likely to be treated for suspected respiratory disease in the first 28 days on feed compared to treated cattle. This represents a significant disease reduction in bromide treated steers in this study during the early phase on feed.

Together, the outcomes of this project support bromide as a potential candidate as an anxiolytic agent with a pharmacokinetic profile that suits use in intensive beef operations. This was futher indicated by the results of the applied trial in which differences were observed for mortality, morbidity for the first 28 days on feed, and without adverse effects on meat quality.

Recommendations

Opportunities for the registration of bromide as a therapeutic agent for management of stress-related disorders in feedlot cattle should be pursued for the benefit of Australian intensive beef production systems.

Table of contents

1 Background

The cattle feedlot is a unique situation in the production chain as large numbers of naive animals are introduced at one time, often in a randomized manner, with the subsequent need for those animals to establish new feeding patterns, transition onto novel feed, establish new hierarchical structures as well as maintaining their normal growth profile during a high stress period.

Investigations into the effects of disease and the resultant cost to industry for the feedlot sector showed that mortality rates were highest in those cattle on feed for the shortest periods of time (<85 days) Perkins (2010) suggesting that induction is a suitable stop / go decision point to improve productivity in those animals entering the system. Of all diseases of feedlot cattle, Bovine Respiratory Disease (BRD) has been identified as the major cause of morbidity and mortality with 18.2% of inducted cattle treated for BRD within the first 50 days (Barnes 2015). In 2015, total cost to the industry at the time was estimated to be $50M per annum. However, these costs are likely to be an under-representation as they do not take into account the cost of feeding animals which will be pulled and treated, or die during this critical window. Bovine respiratory disease particularly is known to be associated with reduced immune status, caused by stress at feedlot induction. Therefore, reducing stress at feedlot entry is likely to reduce prevalence of diseases resulting in reduced productivity, morbidity and mortality in the induction period, with resulting economic benefits to the producer.

In addition to disease mitigation, a number of management approaches attempt to address the problem of feedlot induction stress. Research to date has mainly focussed around direct approaches for treatment or prevention of disease or mitigation of management stressors by low stress handling and improved facility design. Some try to minimize the number of changes an animal will experience coincidently in their environment by, for example, only introducing animals from the same home mob, sourcing only locally bred stock which are familiar with the local environmental conditions, or providing smaller home pens to minimize inter-animal conflict during establishment of new hierarchies. Backgrounding cattle in an environment co-located to the feedlot is also becoming an increasingly common practice. However, in a number of management situations, or very large commercial operations, these mitigation strategies are impractical, difficult, costly, or simply impossible to implement under certain conditions or seasons.

Current practices generally work to treat conditions resulting from feedlot induction stress, such as respiratory or gastrointestinal disease as well as injuries caused by dominance and aggression (also known as 'buller syndrome'), rather than minimizing the stress that may give rise to a higher incidence of these conditions. Feedlot induction therefore presents a significant welfare checkpoint that presents an opportunity for a therapeutic intervention that could minimize stress-induced inappetance and ill-health in feedlot cattle prior to the onset of significant clinical signs. Mitigating stress and improving productivity in this critical window will improve commercial outcomes for the beef feedlot sector in Australia.

Bromide offers considerable potential as a stress-mitigating therapeutic agent in livestock. Bromide has a long history of use in human clinical medicine due to its antiepileptiform activity (Meierkord *et al.* 2000; Okuda *et al.* 2000; Pearce 2002) although more recently falling out of favour as newer and more targeted anti-seizure medications have come to market (Friedlander 2000). It is, however, still widely used in veterinary medicine for seizure control in dogs (Chandler 2006; Chang *et al.* 2006) and cats (Boothe *et al.* 2002) due to its pan neuronal inhibitory activity. Bromide is thought to have multimodal effects on stress pathways in the CNS with GABAergic activity and an inhibitory effect on the stress response in the central amygdala (Balcar V.J. 1987; Combs 2020b). Bromide has historically been used widely for anxiety in humans and as a calmative agent in horses(Everly Jr G.S.

1981). Our research has shown that lambs with experimentally induced perennial ryegrass toxicoses show increased concentrations of faecal cortisol, which are reduced by treatment with bromide (Combs *et al.* 2018; Combs *et al.* 2019) and that bromide has high oral bioavailablity, a long half life and a wide safety margin when utilised as a therapeutic agent in production species (Raidal and Edwards 2008; Quast *et al.* 2015a; Combs *et al.* 2019; Combs 2020a). This research thus opened the possibility of bromide also being used for stress mitigation in a wide range of production applications.

To achieve this aim, this report presents a suite of three studies that together evaluated the efficacy of a new treatment for mitigation of production loss caused by feedlot induction-related stress.

2 Project objectives

2.1 Defining the regulatory landscape for bromide in production animal industries.

Currently the regulatory landscape, at an international level, for registration of bromide as a therapeutic agent in the production industries is unclear. As bromide is a naturally occurring substance, which can be ingested at relatively high concentrations in some products such as irrigated vegetables and seafood, there are few regulations currently identifiable around maximum residue limits (MRL) in food products for human consumption. A review of the current regulatory requirement for product registration for bromide and its close counterparts (iodide, chloride) will be carried out in-house at Elanco. This will involve consultation with regulatory bodies in a number of global jurisdictions including North and South America, Europe, China and Australia. This analysis will identify the key regulatory requirements for registration of bromide as a treatment for feedlot induction stress worldwide.

2.2 Quantitation of residues of potassium bromide in tissues of cattle treated with oral potassium bromide at one of three treatment levels.

This analysis will determine bromide residues in meat and offal from treated animals compared to their untreated counterparts. Data from this study will be used to support an application for registration through the Australian Pesticides and Veterinary Medicines Authority (APVMA) and other registration bodies. The maximum dose that can be administered to give therapeutic concentrations for the required treatment timeframe (<3 weeks) and potential withholding periods will be determined as part of this analysis.

2.3 Identification of production outcomes for cattle treated with a bromide salt at feedlot induction.

This study will determine the production benefits of administration of a single oral dose of bromide at feedlot induction using the optimal treatment level identified in Part 1. Briefly, a minimum of 1100 animals will be treated with a single oral dose of bromide at induction, and their production and health status compared to untreated counterparts. Animals will be grown out for 120 days as per standard feedlot practice. Weight gain will be monitored on entry to the trial, at <28 days where possible and on exit from the feedlot. Health and behavioural monitoring will be carried out weekly by observation and by reporting of adverse health effects by feedlot staff. Sick or injured animals will be removed as per normal practices. This analysis will determine efficacy of the therapeutic agent in reducing the effects of feedlot induction stress in an authentic setting.

Once these three areas of research have been conducted, the efficacy and suitability of bromide as a mitigation strategy for feedlot induction stress in cattle, and the path to registration in various international jurisdictions will be better understood. This is a key outcome of this project and the necessary next step to commercialisation of this product for the production animal industries.

3 Methodology

3.1 Freedom to operate

Currently the regulatory landscape, at an international level, for registration of bromide as a therapeutic agent in the production industries is unclear. Bromide is a naturally occurring substance which can be naturally ingested at relatively high concentrations in some products such as irrigated vegetables and seafood. As such, there are few regulations currently identifiable around maximum residue limits in food products for human consumption. A review of the current regulatory requirement for product registration for bromide and its close counterparts (iodide, chloride) was carried out in-house at Elanco. Regulatory bodies were consulted in a number of global jurisdictions including North and South America, Europe, China and Australia. In addition, this regulatory evaluation assessed the regulatory requirements associated with drug safety and effectiveness and not include any assessment of potential formulation, manufacturing, or intellectual property.

3.2 Determination of bromide pharmacokinetics administered orally to cattle

3.2.1 Animals

This trial was undertaken at Charles Sturt University in the New South Wales Department of Primary Industries Large Animal Nutrition Unit, and at the Charles Sturt Veterinary Diagnostic Laboratory. All procedures in this study were carried out with the approval of the Charles Sturt University Animal Care and Ethics Committee (Protocol A17-065). Twenty-one Aberdeen Angus beef steers with an average weight of 318 kg were purchased from a local supplier. Of these animals, 18 were selected for inclusion in the pharmacokinetics study, with groups equivalent and stratified by entry date and body weight. Three animals were selected as 'controls' to determine endogenous residue concentrations in tissues and serum. On entry to the trial, all animals underwent a full health check, were treated with a broad spectrum anthelminthic preparation (IVOMEC-Plus, Merial) and a tail vein blood sample was taken for full ruminant health screening. Animals were housed in a large, dirt cattle yard, with free access to fresh water and fed a mix of lucerne and oaten hay *ad libitum* until removal to treatment pens.

3.2.2 Acclimation and allocation to treatment groups

Study animals were acclimated to the yards for seven days prior to any interventions. After this initial period, all steers were trained to be accustomed to being led and halter tied and moved through the race daily and weighed weekly using a walk-on weigh crate (TSi, Gallagher Systems). Scales were calibrated prior to each weighing using a known calibration weight (75kg). Weights were recorded down to two decimal places. After initial training, animals were allocated to cohorts (control (n = 3), 1 (n = 9, 3 per treatment group) or 2 (n = 9, 3 per treatment group)) based on temperament and trainability, and weight stratification, with those animals most responsive to training being in the earlier cohorts (control and 1), and those requiring more handling being in the latter cohort (2).

Animals in the first cohorts (control and 1) were rotated in and out of the animal house to acclimate them to crates similar in size and design to the metabolic crates to be used for the experiment. After seven days acclimation to the animal facility, the first cohort was inducted into the experiment with

cohort 2 following at the completion of cohort 1. No more than nine animals were housed in metabolic crates at any one time.

3.2.3 Preparation of animals and collection of control samples

To determine endogenous concentrations of bromide in serum and tissues, three animals were selected as controls. These animals were trained as described above prior to entry to the animal house. On entry to the trial, animals were subjected to tail vein venipuncture with collection of a minimum volume of 50 ml whole blood in coagulation tubes. After blood collection, animals were fitted with a sham collection collar (see Figure 1) without cannulation. Animals were then removed for post mortem as per protocol below.

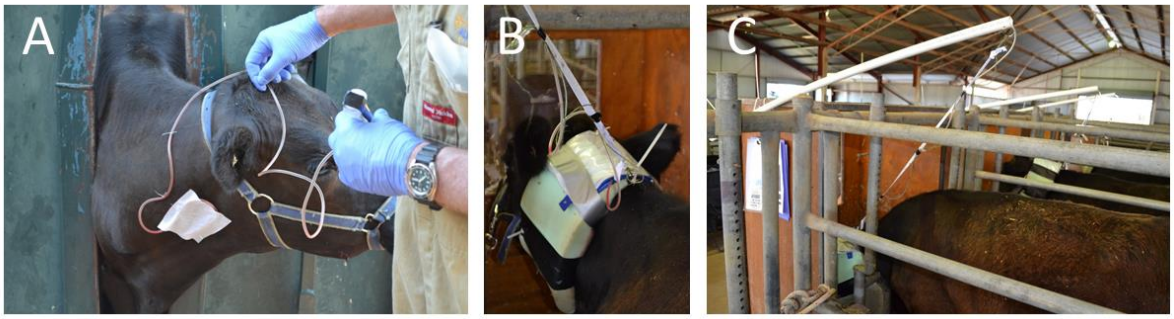

Figure 1. Fabricated collar designed to ensure catheter security and remote venous sampling. A) 'S' bend extension and suture location. B. Steer fitted with foam neck collar to protect the cannulation site and extension set. C. Giving set to allow remote sampling for venous blood sample collection. The collection port of the extension set sits outside the metabolic crate at the end of the whole poly-pipe tubing, the giving set allows the animal to eat, drink and lie down without compromising the cannulation site or extension line.

3.2.4 Treatments

On entry to the trial, animals in the treatment cohorts (1 & 2) were yarded overnight without access to food or water to achieve a shrunk body weight for dose calculation. Animals were randomly assigned to treatment groups after stratification by body weight. After weighing on induction, steers were restrained in the crush and a jugular catheter (Angiocath, 14 g x 13.3 cm) placed aseptically and sutured to the skin (Figure 1). Catheters were flushed with heparinised saline then a connection-set (3 mm ID x 150 cm, Codan GmbH, Lensahn, Germany), primed with heparinised saline, and head collars were fitted. Collars were made from foam rubber (4 cm thick, 19 cm wide, with an internal diameter of 38 cm) held by contact adhesive (suitable for foam rubber) and two cable ties. The collars were attached on each side of the animal's head collar using cable ties and the extension set, primed with heparinised saline, was inserted through the foam collar at a dorsal mid-point, with a Buhner needle. The connection set was then pulled through the material of the collar to exit externally at the lateral midpoint, ipsilateral to the jugular. The catheter was then re-inserted just below that external exit point to continue through foam collar to re-exit internally in the middle of the ventral quadrant (Figure 1). A connection was made to the jugular catheter and the connection joint attached to the skin of the animal with acrylic adhesive. A loop was made in the connection set between the jugular connecting joint and the collar (to allow some 'give') and sutured to the skin to prevent movement of the extension line under the foam collar.

Collars were placed over the steer's head and adjustment made to the connection-set to allow sufficient 'give' under the collar for movement of the head, but not so much that excessive length could catch on rails, gate-hooks etc. The remainder of connection set was pulled through the collar and rolled up on the dorsal aspect of the collar, then placed in a small plastic bag attached to the collar by contact adhesive and duct-tape. This allowed 65-75 cm of 'free' line for researchers to utilise during sample collection (Figure 1). Extension lines were suspended above the animal using a novel device which allowed the animals to undertake normal movement whilst not impeding the extension set for blood collection. The collection part of the extension set was then secured outside the metabolic crate allowing blood collection to occur without contact with the subject.

A pre-treatment (T0) blood sample was collected via the extension set prior to dosing with KBr by gastric intubation. Dose rates were calculated on shrunk body weight at 100 mg/kg, 300 mg/kg and 500 mg/kg. A stock solution of potassium bromide (400 g/L bromide) was prepared by dissolving the potassium salt in water and the required volume calculated for each individual animal. A gastric tube was inserted into the oesophagus, ensuring entry to the rumen, and the required oral dose was administered. This was followed with 5L of water to ensure that all sample was flushed from the gastric tubing.

After dosing, the animals were removed to metabolic crates, and tethered by a lead-rope connected to the head-collar at a low point adjacent to the feed trough to allow free access to food and water, and to enable animals to stand up and lie down but without the ability to turn around.

3.2.5 Sample collection

Venous blood samples (50 ml) were collected by remote sampling at 0, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 30, 60 and 72 hours with a minimum volume of 50 ml whole blood collected. Blood samples were allowed to rest for a minimum of one hour prior to separation of serum by centrifugation at 12000 rpm for 20 minutes. Serum was removed from each sample tube and pooled giving a total volume of serum collected of between 20-30 ml for each animal at each time point. Serum samples were stored at 4 ^{o}C before removal to the laboratory for storage at -20 ^{o}C for bromide extraction and analysis.

Urine pools were collected at 6, 12, 18, 24, 30, 50 and 72 hours post treatment with volume recorded. A 50 ml sample of urine was retained from each time-point and the remainder discarded. Fresh faecal samples were collected at 24, 48 and 72 hours post treatment. All samples were retained at 4 ^{o}C before storage at -20 ^{o}C for processing.

At the end of the sampling period, collars and catheters were removed and all animals were returned to the cattle yards until date of necropsy.

3.2.6 Necropsy

Animals were randomly assigned to a cull date of either 7, 21 or 42 days within each treatment group with two animals at each cull date, one from each cohort (n = 2 per timepoint, one from each treatment cohort and treatment dose – 100mg / kg; 300mg / kg and 500mg / kg LW). At the required date after treatment, animals were removed from the mob, restrained in the crush, and a whole blood sample of 50 ml in volume was collected from the jugular vein using a 21 g vacutainer needle. Serum retained as previously described. After blood collection, animals were removed by cattle

truck to the Veterinary Diagnostic Laboratory, Charles Sturt University for full necropsy and tissue sample collection.

On entry to the necropsy facility, the animals were restrained in a hydraulic crush and euthanised by captive bolt prior to exsanguination via severing of both carotid arteries. The animal was then attached via shackles and partially lifted using a mechanical hoist, allowing postural drainage to achieve optimal exsanguination of the carcass. The spinal cord was also severed at this same time to ensure neurological death had occurred. Once death was confirmed and exsanguination was complete, the carcass was processed according to standard abattoir procedures. 100–250 g of the following tissues were transferred to a 70 ml sample pot to be retained for analysis: rump, sirloin, subcutaneous fat, renal fat, kidney and liver. All samples were removed immediately for storage at -20 ^{o}C prior to processing and extraction of bromide. A faecal sample was also collected from the colon of each animal and stored at -20 ^{o}C for later analysis.

3.2.7 Bromide extraction from tissues and serum

3.2.8 Colourimetric quantitation of bromide concentration in serum

Plasma bromide concentrations of each sample were determined by colourimetric spectrophotometry as previously described (Tietz 1976), with minor modification. Once thawed, 0.35 mL of serum was added to 3.15 ml of 10% trichloroacetic acid (TCA), Sigma-Aldrich, T6399-250G (TCA) in a 10 ml centrifuge tube and vortexed for 30 seconds. After being left to stand for 10 minutes, the serum and TCA were centrifuged for 15 minutes at 3000 x G. Two and a half millilitres of supernatant was then mixed with 0.25 ml of gold chloride, Sigma-Aldrich, G4022-1G, Gold (III) chloride trihydrate and left to stand for 30 min. Absorbance was then measured with a UV spectrophotometer at 440 nm. Standards were run at the time of sample analysis and bromide concentrations later extrapolated from the standard curve. The limit of quantification of the assay was determined to be 25 µg/ml.

3.2.9 Ion chromatography quantitation of bromide concentration in tissues

Seventy millilitre sample pots of each tissue (muscle, fat, kidney and liver) were thawed and chopped in a kitchen food processor (Mini Wizz, Breville, Botany, NSW) for 30 seconds, except for kidney which was processed for 1 min . Subsamples (2 g) were weighed into a 15 ml centrifuge tube. Five ml of de-ionised water and 6 ml of hexane was then added to the sample and vortexed for 30 seconds. This mixture was then decanted into a homogeniser (Omni Mixer, Sorvelle, USA) and processed at maximum speed for 1 min, then decanted back into the same centrifuge tube. Centrifugation (Allegra X-30R, Beckman Coulter) at 4000 x G for 10 minutes was done to ensure complete phase separation. The lower aqueous phase was then aspirated with a glass pipette into a 5 ml syringe barrel then passed through a 0.22 µm filter into a 5 ml Poly Vial (Thermo Scientific). This was then capped for subsequent analysis using ion-chromatography.

Standards made of the appropriate control tissue spiked extracts, as processed above, were analysed first as each tissue matrix is different and method adjustments were accordingly done to account for changes in bromide elution time and the impact of matrix suppression on chromatography. Tissue extract standards were compared with spiked aqueous extracts to establish extraction efficiency and for verification of chromatography peaks and elution times.

Instrumentation: DIONEX IC-2000 ion-chromatography unit, DIONEX IonPac AS19 4 x 250 mm IC column, DIONEX IonPac AG19 4 x 50 mm IC Guard Column, Dionex AERS 500 4 mm suppressor and DIONEX ASM-3 autosampler. Limit of detection was 0.5 mg/L. The Br concentration was normalized to the mass of tissue processed and presented as mean ± standard deviation.

3.3 Bromide for the mitigation of bovine respiratory disease and improved health outcomes in beef feedlots

3.3.1 Cattle

All procedures in this study were carried out with the approval of the Charles Sturt University Animal Care and Ethics Committee (Protocol A18-088). Data from the pharmacokinetic and residue depletion studies were used to support an application for an APVMA research permit to conduct this efficacy trial. Angus cattle (n = 1936) were recruited between 17-07-2019 and 26-09-2019 as part of the feedlot's normal feedlot intake and were inducted into the feedlot. All animals were placed on 120 day feeding regimens. Inducted cattle comprised of 1836 steers and 100 heifers. At induction cattle weights varied between 285–558 kg. Mean and s.e.m. induction weights for each treatment group are shown in Table 2 below. These animals were destined for EU markets after 120 days on feed, were Angus Hormone Growth Promotant (HGP)-free, with a target weight of 320 kg HSCW. Numbers of animals per pen varied between 120-180 head dependent on arrivals but were balanced within replicates. Feedlot management constraints and varying induction dates saw cattle exits managed in contemporal replicates. No period greater than 24 hours was recorded between exit and slaughter. Distance to slaughter was approximately 600km with no substantial lairage time.

3.3.2 Treatment

Cattle were designated to treatment pens at the induction crush by alternating treated and untreated animals by order of presentation. Cohorts were selected by availability and presentation at induction at the facility. Animals were not backgrounded prior to induction and were inducted directly on arrival. All animals were destined for EU markets and therefore did not contain hormone implants. At induction, treatment steers were dosed with bromide orally using a Genesis LPG Power Doser™ (Genesis Industries, Australia).

The study cattle were drafted into six sets of paired pens (replicates). Pens were selected within rows and were co-located. No edge pens were used. Access to water, shade and bunk space was identical for all pens included in the study. Paired pens were comprised of cattle from the same induction session that were allocated to either treatment or control groups at induction by order of presentation at the crush.

Between treatment groups, induction weights were normally distributed, and were not significantly different. Due to natural variability in cattle at point of supply, mean induction weights varied between pen replicates and therefore replicate was treated as a block for the purposes of this study. This variation in induction weight was expected due to the purchase of cattle from various origins.

At induction, cattle received an ear tag, anthelmintic drench (Oxfen LV®, Virbac, Australia) and intranasal vaccine against bovine herpesvirus 1 (Rhinogard®, Zoetis, Australia) as per the feedlot's standard induction protocol. Cattle allocated to a treatment pen were treated with bromide by oral dosing. Cattle allocated to the control group received no treatment. The control cattle were untreated. Once inducted, cattle entered the feedlot system and were managed under standard feedlot practices. Pen riders remained blinded to treatments. Standard feedlot practices regarding pulls and hospitalisation were followed. Animals removed for hospitalisation, found dead, or removed from the trial as salvage culls were noted and analysed as part of the feedlot data records.

3.3.3 Performance and meat quality parameters

Feedlot performance data including weight gain, feed consumption per pen, morbidity (with subsequent treatment protocols) and mortality were collected from feedlot records. All steers were weighed at time of slaughter (shrunk body weight) and data correlated to ID data for individual animal reporting. Dry matter was applied from the diet formulation for the determination of DMI

and FCR. Routine necropsy was not performed. Therefore records of cause of death are predicated on recording by feedlot staff. At slaughter, production and MSA and AUSMEAT meat quality data (defined in Table 1) were collected including meat colour, marbling score as recorded by the processor.

Table 1. Description of MSA and AUSMEAT grading metrics

Metric	Description
AUSMEAT fat colour	Categorical fat colour scoring system ranging from 0 (white) to 7 (yellow).
AUSMEAT marbling	Qualitative marbling score ranging from 0 (nil) to 9 (abundant). Assessed based upon the amount of marbling present in the eye muscle.
AUSMEAT meat colour	Categorical meat colour scoring system ranging from 1a (light) to 7 (dark).
EMA	Surface area of the eye muscles. Measured in square cm using an AUS-MEAT grid
HSCW	Hot standard carcase weight
Hump height	An indicator of tropical breed content. Measured in gradients of 5 mm
MSA Index	The MSA Index is a single number and standard national measure of the predicted eating quality and potential merit of a carcase. The MSA Index is a number between 30 to 80, expressed to two decimal places (ie 54.62), to represent the eating quality potential of a whole carcase.
MSA marbling	MSA marbling scores are used to provide a finer scale than the AUS-MEAT scores. It is assessed based upon amount as well as distribution of the marbling within the eye muscle. Each MSA marbling score is divided into tenths for grading, creating a score range from 100 to 1,190 in increments of 10.
Ossification	Ossification score is a measure of physiological maturity of the beef carcase through bone development in the vertebrae.
Rib fat cold	Rib fat thickness is the measured depth of subcutaneous fat over the quartered rib site between the 5th and 13th ribs.

3.3.4 Statistical Analysis

Feedlot performance data were analysed with culls omitted to identify the standalone performance effect of treatment and with culls included to assess the true applied outcomes of the treatment. Tabulated and visual representations of the recorded raw data were presented to support model fitting. Linear modelling was employed to examine the effects of treatment on liveweight gain (presented throughout as average daily gain), dry matter intake, and feed conversion ratio. A priori knowledge of feedlot performance assisted model fitting, and Akaike Information Criterion was calculated to support the final model selection. Replicates were included in the analysis as a fixed effect. Analysis of variance was employed to determine if differences were present between treatment and control groups. Salvage cull and mortality count data were subjected to generalised linear mixed modelling using a binomial distribution to assess the influence of fixed effects including treatment and replicate. The resulting model was subjected to chi-square tests to assess the effect of predictors on morbidity and mortality. Hospitalisation (health management) was assessed using the same methodology. All analyses were conducted in the statistical package 'R' (R Core Team 2020). Graphics were produced using ggplot2 (Wickham 2016).

3.3.5 Cost Benefit analysis

Parameters for cost benefit analysis were identified from variables with a *P-value* ≤ 0.10).

4 Results

4.1 Freedom to operate

4.1.1 Potential indications and effectiveness assessment

A therapeutic and/or production indication could be pursued for bromide and both indication options present various challenges. The production claim of increased rate of weight gain is based on the hypothesis that administration of bromide would reduce stress in newly grouped cattle due to its action as a pan-neuronal inhibitor with anxiolytic activity at doses lower than those used for sedation . This could result in quicker resumption of a normal rate of feed intake and subsequently greater rate of gain than in 'stressed animals'. It is likely that this increased rate of gain would only last a finite period and non-treated animals might eventually reach similar weight gains due to adaptation to stress and compensatory growth. Hence, the duration of the production claim would likely be confined to a short period during and immediately after the stress event and not during the entire production period. Although this may not affect product registration, it could reduce the potential market. Numerous market blocks, such as the European Union and many Asia-Pacific countries, do not permit registration of products specifically for a production indication in the absence of a therapeutic need. This would further limit the available market for bromide globally.

A therapeutic indication would maximise the potential market for bromide. The indication of 'reduction of stress' or some similar derivation would be challenging but not impossible to obtain in beef cattle. Most regulatory agencies require a therapeutic claim to be directly linked to a clinical disease that can be diagnosed by a veterinarian or producer. While generally recognised as a deleterious condition to animal well-being, establishing clinical signs of stress could be difficult. Conversations and alignment, where possible, with regulatory agencies will be needed, as there is no universally recognized clinical sign of stress. The only food animal drug with a similar claim is Stresnil (azaperone), indicated for the control of aggressiveness when mixing or regrouping weanling or feeder pigs. This indication was obtained by evaluating the number of pigs fighting per pen and the duration of each fight. In companion animals, various drugs exist for reduction in anxiety in dogs. These indications were obtained by establishing a behavioural assessment of anxiety/stress. To obtain a therapeutic indication for bromide for reduction of stress, would require establishing a behavioural indicator of stress, and a measure of its reduction in cattle. This 'measure' would need to be recognized and accepted by regulatory agencies. Specific and consistent biomarkers for stress are limited and in general, regulatory agencies do not currently accept biomarkers as an indicator of clinical signs of stress. Certain markets may accept a biomarker approach if an exact biomarker, only expressed in stressed animals, could be established. This biomarker would have to be robust and its suitability negotiated with regulatory agencies prior to study implementation.

No major issues are expected when demonstrating target animal safety. Published literature in other species would suggest that bromide has a large margin of safety. In dogs, few serious adverse events have been reported. A complete margin of safety in the target species would be required for registration. User safety could be a concern in certain markets. Bromide has known effects in humans at high doses and may require special shipping, handling, or storage. Such factors may affect the cost of production.

The acute and chronic toxicity profiles of bromide have been well established and available in the published literature. Therefore, an argument could be made with most regulatory agencies that additional toxicology work for this molecule is not required. Maximum residue limits (MRL) are currently not required for bromide, however, with the expanded use of this molecule in animal agriculture, the need to establish a maximum residue limit (MRL) for some markets may be required. Due to the infrequent use of bromide in the European Union no MRL is required. However, it may be

pertinent to request scientific advice as to the need to reevaluate the necessity of an MRL. To that extent, acceptable daily intake (ADI) is set in the European Union (0.4 mg/kg BW; 24 mg/person) and Australia (1.0 mg/kg BW; 70 mg/adult, 40 mg/child). For registration, a complete human food safety package would need to be conducted. This would include complete residue assessment (hot and cold depletion), comparative metabolism, pharmacokinetic (ADME) assessment, and analytical method validation.

No major issues are expected during the assessment of environmental safety due to the ubiquitous status of bromide. The ecotoxicity effects of bromide are known and it is unlikely to be classed as persistent or bioaccumulative. Nevertheless, a full environmental assessment would need to be conducted once the specific dose and duration are established.

4.1.2 Summary of findings

There is an opportunity to register KBr, MgBr, or other bromide salts, in multiple countries. Determining the desired indication will be critical. Although both a production claim and therapeutic claim are feasible, both have challenges. Both human food safety and target animal safety will require various studies but no major issues are expected.

4.2 Pharmacokinetics of bromide in cattle

4.2.1 Serum

In steers, bromide exhibits first order pharmacokinetics, with a terminal elimination half-life in serum ($t_{1/2}$) of 7.75, 7.06 and 6.52 days for the 100, 300 and 500 mg/kg dose rates, respectively (Figure 2).

After oral dosing with bromide (as the potassium salt), return to baseline serum concentrations occurred at approximately 28, 34 and 42 days for 100, 300 and 500 mg/kg dose rates respectively (Figure 2).

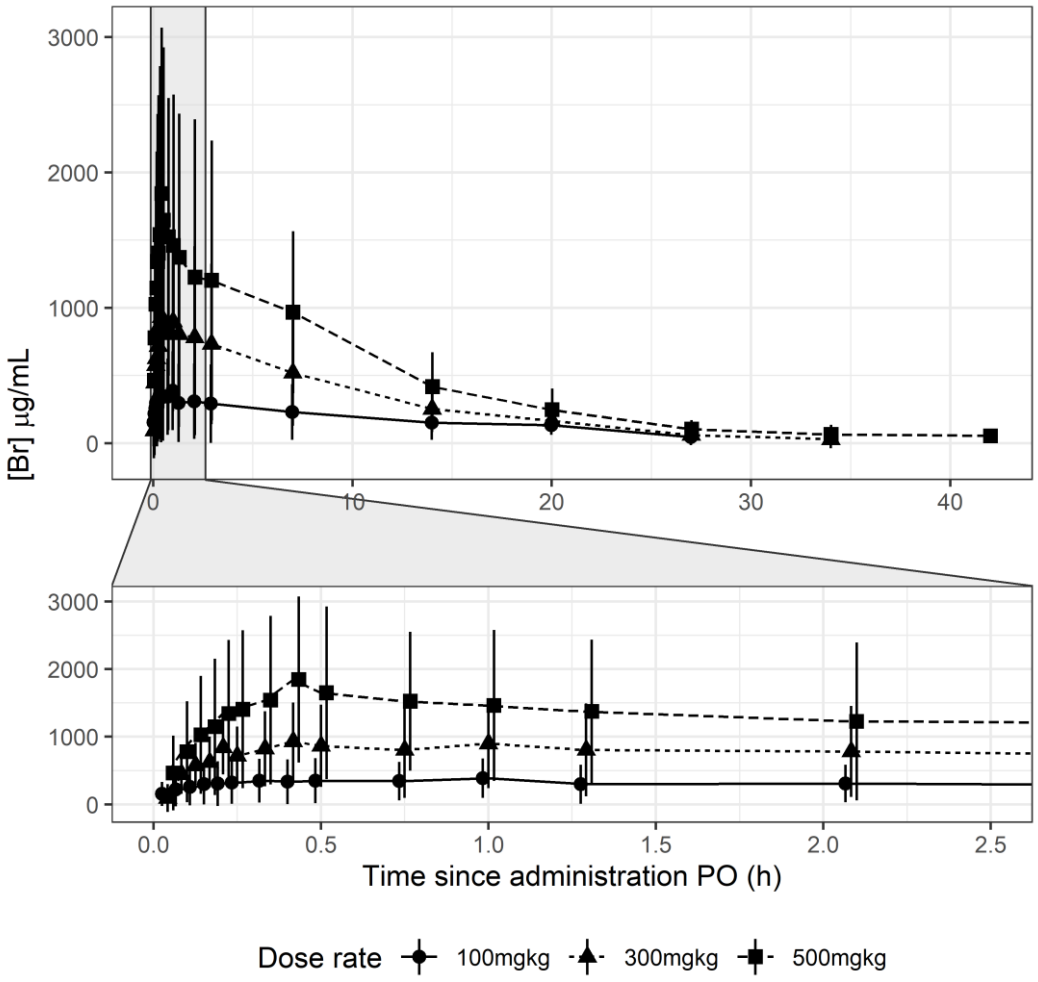

Figure 2. Serum concentrations of bromide over time (days) in animals dosed with 100 mg/kg, 300 mg/kg and 500 mg/kg oral potassium bromide. Expanded section highlights concentrations between 0–2.5 hours.

4.2.2 Tissue depletion of bromide in tissues

Tissue bromide concentrations varied between tissues (Figure 3). Muscle bromide concentrations in dosed animals were observed to be less than 10% of serum concentrations at the same time point. Baseline bromide in meat was < 6μg/g in untreated controls. Bromide concentrations in fat were found to be lower than in meat, likely reflecting the reduced vascularity of this tissue compared to muscle (meat). Thus, the baseline values in fat were also lower than meat tissues from the same animals (baseline fat [Br] < 4 μg/g). The concentrations of bromide in liver were found to be similar to those observed in meat with a similar baseline (baseline liver [Br] < 5 μg/g). These values are consistent with a single compartment pharmacokinetic model. Concentrations in kidney tissue were higher than values observed in other tissues analysed. Baseline values for bromide in kidney were observed to be threefold higher than any other tissue measured [Br] < 15 μg/g.

Together, these data show that animals dosed at 100 mg/kg, bromide concentrations in muscle returned to baseline by day 7, in fat by day 21 and in liver and kidney by day 42. In animals dosed at 300 mg/kg, bromide concentrations in muscle returned to baseline by day 7, in fat, liver and kidney by day 42. In animals dosed at 500 mg/kg dose, bromide concentrations in muscle, fat and kidney returned to baseline by day 42. Bromide concentrations in liver were still above baseline at 42 days. The population normal range for baseline concentrations of bromide in the various tissues still needs to be established. The baseline bromide values noted above are based on concentrations measured in three untreated control animals for each tissue.

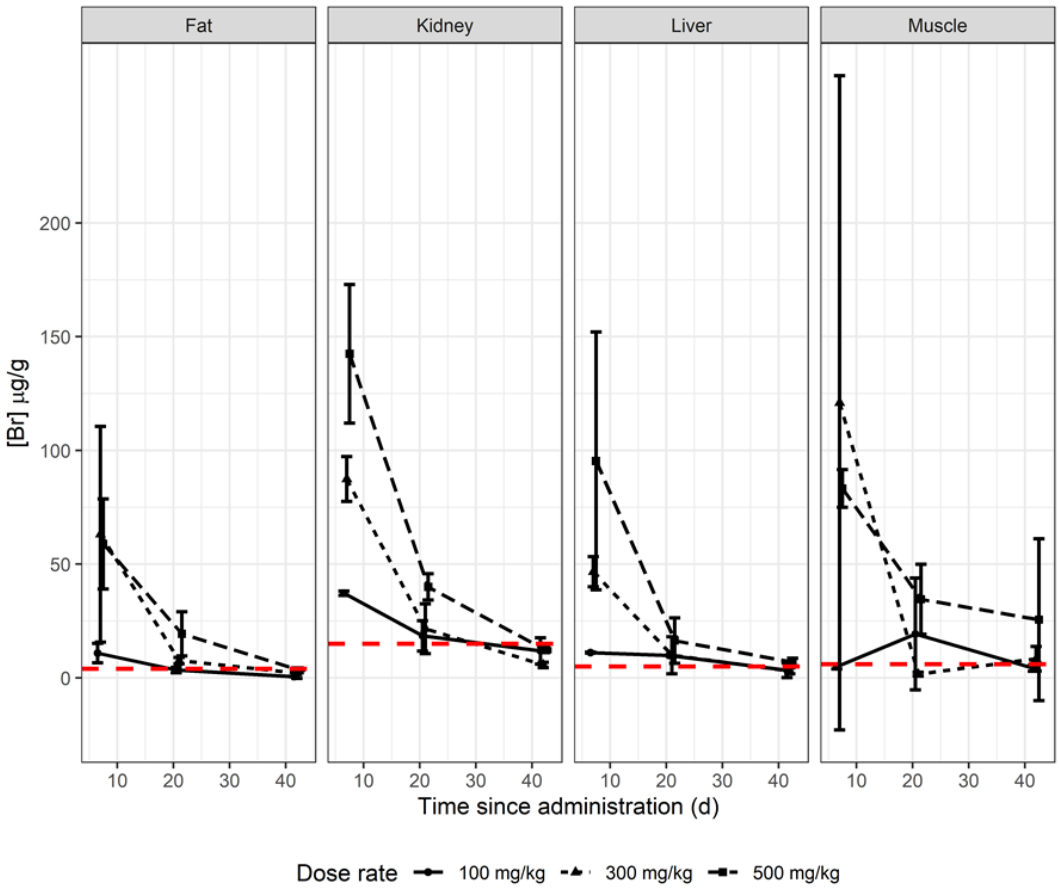

Figure 3. Mean concentration of bromide over time in kidney, liver, muscle and fat collected from animals dosed with 100 mg/kg, 300 mg/kg and 500 mg/kg oral potassium bromide respectively (n = 2 at each dose x timepoint). Time is denoted in days (d) post oral administration. Values were determined by ion chromatography. Red dashed line denotes established endogenous baselines from untreated control animals (n = 3).

4.3 Use of bromide in feedlot for mitigation of induction stress – impacts on feedlot performance

4.3.1 Feedlot performance

Whole population production performance was examined. Average daily gain (ADG) was calculated for cull animals that were removed from the trial due to mortality or significant morbidity leading to salvage slaughter. Average daily gain values for deaths and salvage cull cattle in addition, or separately to, cattle that completed time on feed were coalesced, resulting in a whole population datasets. Cattle that died during the trial were assigned an exit weight of 0 kg, to capture the effect of this loss on product output. Average daily gain values for cattle that were culled from the trial due to significant morbidity were calculated using the individual animal's induction weight and last recorded hospital weight or draft weight. In all instances the last recorded hospital or draft weight was within two days of feedlot exit. Four individuals that were culled due to morbidity registered negative ADG values. These values were considered accurate and included in the analysis. Calculated ADG values were compared between treatments (Table 2).

When deads and salvage cull cattle were included in the performance data, an increased growth rate was observed in the bromide treated group compared with the untreated control group (Bromide: 1.86 kg/head/d, n = 970; controls: 1.76 kg/animal/d, n = 966; *P* = 0.097). Variation from the mean was greater in the control group (SD = 1.53) compared with the bromide treated group (SD = 0.99), supporting evidence of an increased number of sub-performing animals in the control group (Figure 4). When deads and salvage culls were omitted from the analyses, no difference was observed between the treatment and control groups (*P* = 0.892).

Table 2. Summary of statistics for average daily gain (ADG). Data is shown as mean ± SD for both population groups: A) where deads and salvage culls are included, or B) excluded from the analysis.

LWG, live weight gain; DOF, days on feed; ADG, average daily gain; DMI, dry matter intake F:G; feed intake to livewight gain ratio; HSCW, hot standard carcase weight.

Item	Deads and savlage culls included			Deads and savlage culls omitted		
	Bromide	Control	*P* value	Bromide	Control	*P* value
Induction Weight	418.72 ± 45.73	417.76 ± 45.44	0.635	419.20 ± 45.27	418.36 ± 44.71	0.668
Exit Weight	661.91 ± 83.80	657.16 ± 103.02	0.112	669.35 ± 57.42	670.50 ± 57.39	0.650
LWG	243.19 ± 69.4	239.40 ± 92.47	0.072	250.14 ± 37.19	252.14 ± 39.03	0.239
DOF	128 ± 11	128 ± 15	0.117	129 ± 5	130 ± 6	0.732
ADG	1.86 ± 0.99	1.76 ± 1.53	0.097	1.95 ± 0.32	1.94 ± 0.34	0.892
DMI	13.1 ± 1.76	13.3 ± 1.67	<0.001	–	–	–
F:G	5.6 ± 0.17	5.8 ± 0.29	0.38	–	–	–
HSCW	–	–	–	367.12 ± 32.49	368.67 ± 32.45	0.281

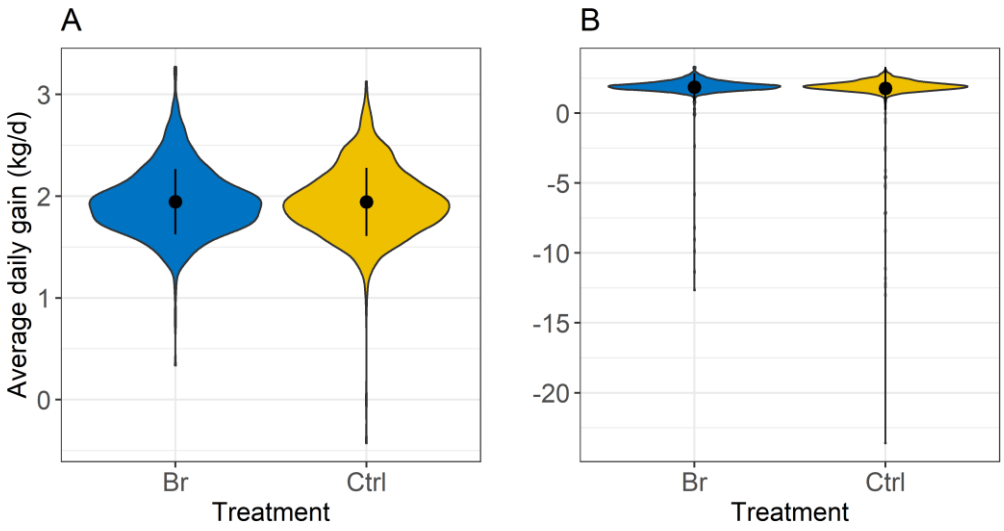

Figure 4. Average daily gain by treatment over whole of life (~120 days) (bromide treatment, blue; control, yellow), including population distribution when: A) culls and deads were omitted, and B) culls and deads were included. Point range denotes median and standard deviation. One data point was omitted from the final graphic frame (Ctrl group; ADG = -24 kg/d).

In order to determine if pen / location effects were influencing performance outcomes, paired pen replicates were compared to assess impact of time of induction and location on feedlot performance (Figure 5). This trial occurred in a commercial feedlot, intakes were limited by availability of source cattle and intakes could include cattle from multiple vendors. As such, some pen difference was expected, and subsequently observed (Figure 5). To accommodate this, all modelling has included replicate (block) as a fixed effect.

A significant difference was observed in ADG between paired pen replicates ($P < 0.001$). Subsequent *post-hoc* analysis indicated replicate 1 had the highest ADG, replicate 3 had the lowest ADG, and all other replicates were not significantly different to each other. When salvage cull and dead cattle were omitted from the data set, the effect of replicate was still present ($P < 0.001$) with replicate 1 having the highest ADG, followed by replicate 5. Replicates 2,4 and 6 recorded the lowest ADG Figure 6). Together, this data indicated that variability in cohort intake is a stochastic effect of the production system, and was not a significant factor in the overall response to treatment.

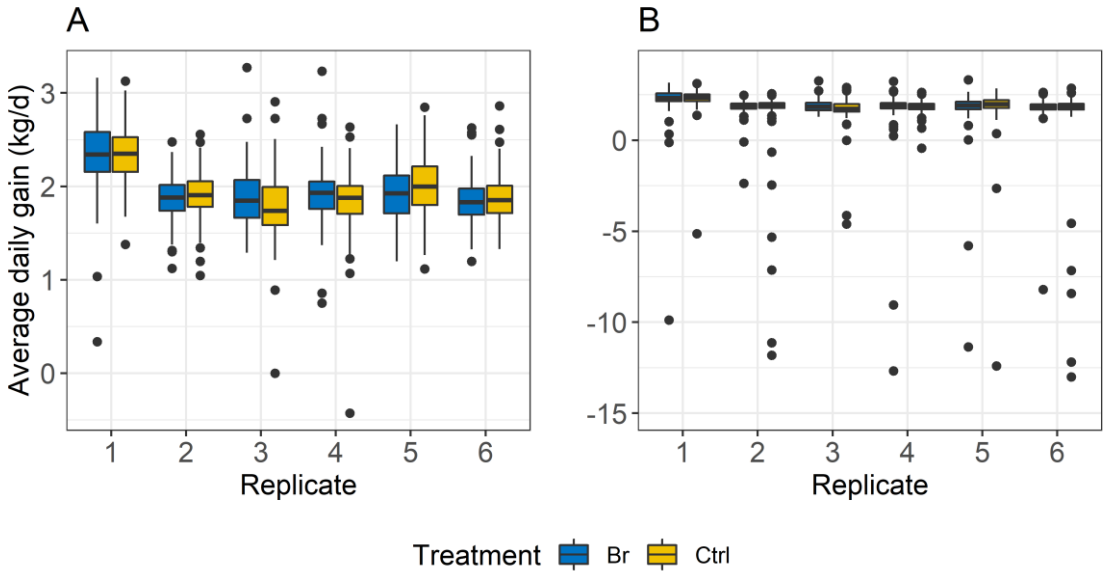

Figure 5. A) Average daily gain (kg/d) by paired pen replicate and treatment for all intakes including deads and salvage culls. B) ADG % variation from mean performance by treatment and replicate indicating greater negative variation from the mean in untreated replicates. Bromide treatment n = 970 (blue); control, n = 966 (yellow).

4.3.2 Feed Intake

To determine if feed intake and feed efficiency were affected by treatment with bromide at induction, pen feed intake was modelled for all treatment and replicate groups over time. Pen feed intake was significantly greater in control cattle compared to bromide cattle ($P < 0.001$; Figure 6). Estimated marginal means indicated bromide treated pens consumed 0.2 kg/head/day less feed on a dry mater basis compared to control pens ($P < 0.001$). Feed intake also differed between replicates ($P < 0.001$). Feed intake in the first thirty days suggested bromide treated cattle went onto feed faster in the first week but consumed less feed between days 7–28 ($P = 0.096$)(Figure 7). Feed conversion over the total time in the feedlot was not significantly different between bromide and control groups ($P = 0.381$), averaging 5.6 and 5.8 kg dry matter/kg body weight gain respectively.

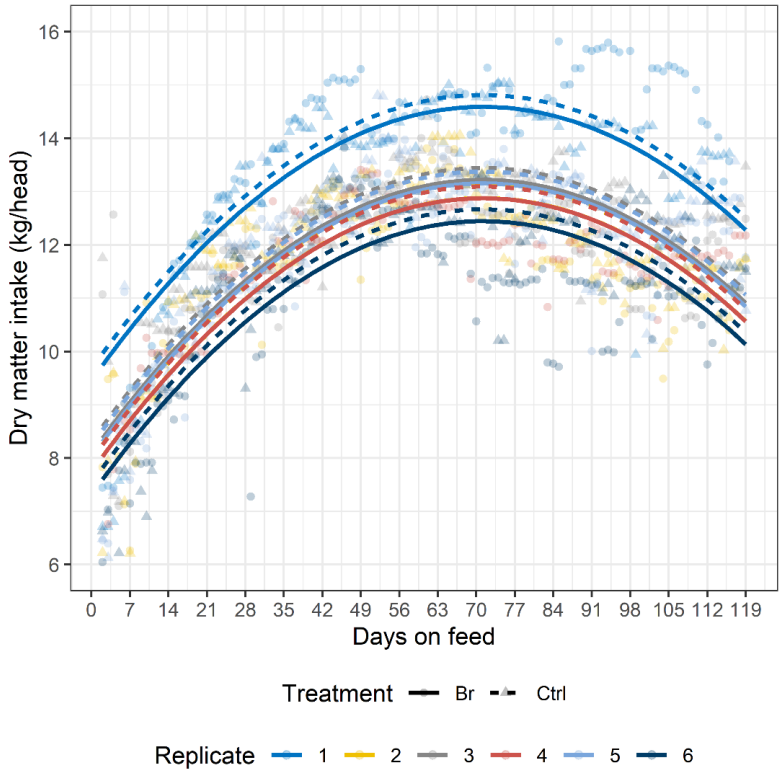

Figure 6. Average 'as fed' feed intake over lifetime in feedlot by treatment and replicate, including deads and salvage culls.

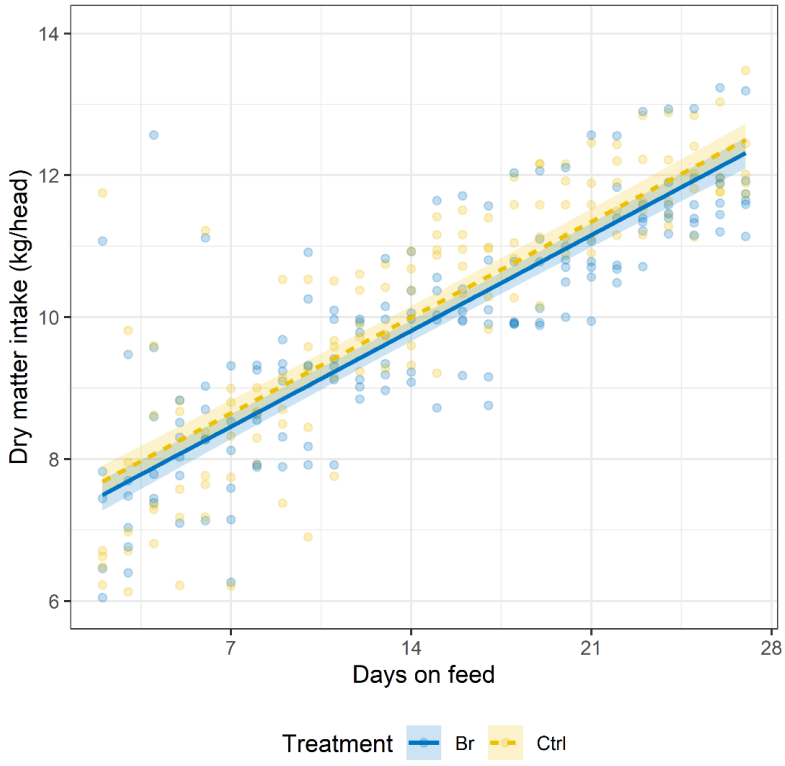

Figure 7. 'As fed' feed intake by treatment during the first 28 DOF by treatment, including deads and salvage culls

4.3.3 Mortality and salvage culls

Mortality has a significant impact on feedlot profitability. Death resulting from bovine respiratory disease is estimated to cost a feedlot up to $1647.53 per head (Blakebrough-Hall, McMeniman, & Gonzalez, 2020). In addition to mortality, substantial morbidity that leads to the removal of an animal from the feedlot (known as a "salvage cull") also affects overall profitability. Although some costs can be recouped from sale or slaughter of culled cattle, these animals still represent a loss within the feedlot system.

In this study, 54 animals prematurely left the feedlot due to death or salvage culls. A greater number of animals died in the control group (n = 15, 68.2%) compared to the bromide treatment group (n = 7, 31.8% of death loss) (Table 4), suggesting a positive effect of treatment on health outcomes overall (*P* = 0.081). Calculated odds ratios supported this observation, with control group animals being 2.86 (CI: 0.91,5.71; *P* < 0.001) times more likely to die during the trial than their untreated counterparts. No difference in mortality was observed between replicates (*P* = 0.417) indicating that this was not a pen effect. Deaths (as specified by feedlot records) were predominantly due to BRD or determined as unknown (Table 4).

Table 3. Incidence of mortality and salvage culls between treatment and control groups across 120 days by treatment and replicate.

Outcome	Predictor	Level	Occurrence (% (N))	Odds Ratio (CI)	P > | z |
Deads	Treatment	Br	0.7 (970)	1	< 0.001
		Ctrl	1.6 (966)	2.86 (0.91, 5.71)	< 0.001
	Replicate	R1	0.8 (241)	1	< 0.001
		R2	1.5 (326)	1.86 (0.40, 13.08)	0.461
		R3	0.6 (358)	0.671 (0.08, 5.63)	0.692
		R4	0.6 (327)	0.735 (0.09, 6.17)	0.759
		R5	1.2 (345)	1.402 (0.27, 10.18)	0.698
		R6	2.1 (339)	2.517 (0.60, 17.01)	0.253
Salvage Culls	Treatment	Br	1.4 (970)	1	< 0.001
		Ctrl	1.9 (66)	1.3 (0.64, 2.68)	0.466
	Replicate	R1	0.4 (241)	1	< 0.001
		R2	1.2 (326)	2.98 (0.44, 58.50)	0.330
		R3	2.2 (358)	5.488 (1.00, 102.158)	0.110
		R4	2.1 (327)	5.251 (0.93, 98.50)	0.122
		R5	2.9 (345)	7.166 (1.36, 131.96)	0.061
		R6	0.6 (339)	1.423 (0.14, 30.78)	0.774

Table 4. Proportion of mortality during time on feed by cause of death and treatment group.

Reason for death	% Bromide (n)	% Control (n)
Bovine respiratory disease	0.2 (2)	0.5 (5)
Cast	0.0 (0)	0.2 (2)
Severe lameness	0.2 (2)	0.3 (3)
Prolapsed rectum	0.0 (0)	0.1 (1)
Tracheitis	0.1 (1)	0.0 (0)
Unknown	0.2 (2)	0.4 (4)

No difference was observed in the occurrence of salvage culls between the bromide and control groups (*P* = 0.468; Table 3), or between replicates (*P* = 0.060). There was, however, a tendency towards increased salvage culls in replicate 5 in both bromide and control groups compared to other replicates. The reason for the high number of salvage culls in this pen is unknown but is supported by the calculated odds ratio (OR) of 7.166 (CI 1.36, 131.96) compared to replicate 1. Between replicates, some level of variation was expected due to livestock history and differences in weather conditions due to staggered induction dates. By this analysis, bromide appears to reduce mortality in feedlot cattle, but not the occurrence of salvage culls compared to controls.

4.3.4 Health management outcomes

One proposed claim for bromide as a treatment for induction stress in feedlot cattle is to reduce the incidence of bovine respiratory disease via an indirect mechanism. To examine this claim, hospitalisations were investigated for both treatment and control groups during their time on feed.

During the trial, 199 animals were pulled from their home pen and moved into the hospital pen. Of these, 134 animals were treated for respiratory disease by identification of the use of the antibiotic Draxxin (tulathromycin). Generalised linear mixed modelling showed no significant difference in hospitalisation rates for BRD or conditions other than BRD between control and bromide treated cattle (50.7%, n = 68 versus 49.0%, n = 66; *P* = 0.833; and 58.8%, n = 38 versus 41.5%, n = 27; *P* = 0.161; Table 5). Non-BRD related pulls were classified as any that were not treated with Draxxin® (tulathromycin) and therefore represented a variety of other health conditions including lameness and necrotic laryngitis. Further investigation of hospitalisation by disease suggested no single health issue was causative of the difference between treatments as increased interventions were present across most disease classifications over the entire feeding period (Table 6).

As the duration of the effect of bromide in this study was likely to be limited to <35 days post treatment by our pharmacokinetic analysis, hospitalisations during the first 28 days on feed were considered (Table 5). A greater number of control cattle were hospitalised for BRD-related treatment compared with bromide treated cattle (65.0% of hospitalised cattle, control n = 37 versus 35.0% of hospitalised cattle, bromide n = 20; *P* = 0.014) during the first 28 days on feed suggesting that bromide may have contributed to a reduction in BRD-related presentations during the active treatment period in this trial. Odds ratios showed control group cattle were 1.97 (CI 1.14,3.49; *P* = 0.017) times more likely to be treated for BRD in the first 28 days compared to treatment cattle. Conversely, non-BRD related hospitalisation was not significantly different between treatment and control groups at 28 days (*P* = 0.086).This represents a significantly improved health outcome specifically related to BRD in the early time on feed after induction for bromide treated animals, due to the reduced numbers of BRD hospitalisations in this group.

Table 5. Proportion of treatment groups requiring hospitalisation during 120 days on feed (DOF by treatment group.

DOF	**Disease**	**Treatment**	**Occurrence (% (N))**	**Odds Ratio (95% CI)**	**P > | z |**
120 days	BRD	Br	6.8% (970)	1	< 0.001
		Ctrl	7.0% (966)	1.04 (0.73, 1.48)	0.307
	Non-BRD	Br	2.8% (970)	1	< 0.001
		Ctrl	3.9% (966)	1.43 (0.87, 2.36)	0.461
28 days	BRD	Br	2.1% (970)	1	< 0.001
		Ctrl	3.9% (966)	1.97 (1.14, 3.49)	0.017
	Non-BRD	Br	1.0% (970)	1	< 0.001
		Ctrl	2.0% (966)	1.94 (0.91, 4.13)	0.087

Table 6. Proportion of total feedlot steers requiring hospitalisation during time on feed by disease and treatment group. Disease definitions were derived from the recorded treatment regimen for each animal. Where multiple treatments were given, priority was given to bovine respiratory disease (BRD).

Disease	% Bromide (n)	% Control (n)
BRD	6.8 (66)	7.04 (68)
Disease Unknown	0.41 (4)	0.83 (8)
Eye injury	0.1 (1)	0.21 (2)
Footrot	0.21 (2)	0.41 (4)
High DOF lameness or open wound	0.1 (1)	0.1 (1)
Lameness	1.75 (17)	1.76 (17)
Non-eater	0 (0)	0.1 (1)
Tracheitis	0.21 (2)	0.52 (5)
Total	9.59 (93)	10.97 (106)

4.3.5 Slaughter data – MSA grading

Carcase data for each animal were identified from the MSA database and concatenated with feedlot data by an alignment of RFID tag. Using this method, 1,873 matches were recorded. Nine RFID tags failed to read or be reported at the abattoir by staff. There were no significant differences ($P > 0.05$) in meat quality parameters between the control and treatment groups, with the exception of rib fat, which was greater in the control cohort ($P = 0.015$)(Tables 7 and 8). A small number of cattle in each group graded poorly for meat colour ('dark cutters' - grades 4– 6), with no apparent difference between treatments ($P > 0.05$). Calculated odds ratios supported this result.

Table 7. Effects of bromide treatment on carcase characteristics and MSA grading measurements of feedlot cattle (continuous response variables).

Item	Bromide	Control	*P* value
Hot standard carcase weight (kg)	367 ± 1.10	369 ± 1.10	0.281
Hump height (mm)	68.8 ± 0.22	69.0 ± 0.20	0.856
EMA (cm^2)	76.7 ± 0.17	76.8 ± 0.16	0.642
Rib fat (mm)	10.5 ± 0.13	10.7 ± 01.12	0.015
pH	5.5 ± 0.00	5.5 ± 0.00	0.673
MSA Index	63.3 ± 0.04	63.26 ± 0.04	0.182
MSA Marbling	367 ± 1.97	364 ± 1.84	0.811

Table 8. Effects of bromide treatment on carcase characteristics and MSA grading measurements of feedlot cattle (categorical response variables).

Item	Classifier	Odds Ratio (95% CI)	Pr >|z|	*P* value
Ossification	Ctrl:110	1		0.177
	120	0.95 (0.83, 1.08)	0.426005	
	130	0.99 (0.86, 1.14)	0.885794	
	140	1.03 (0.88, 1.2)	0.715073	
	150	0.98 (0.74, 1.3)	0.886731	
	160	0.75 (0.52, 1.08)	0.117325	
	170	0.54 (0.31, 0.95)	0.033551	
	180	0.95 (0.42, 2.16)	0.905607	
	190	–	–	
	200	0.89 (0.22, 3.59)	0.867589	
AUSMEAT Marbling	Ctrl:0	1		0.447
	1	0.93 (0.53, 1.63)	0.802	
	2	0.87 (0.49, 1.54)	0.632	
	3	1.17 (0.63, 2.18)	0.615	
	4	0.79 (0.31, 2.02)	0.629	
	5	0.92 (0.21, 4.1)	0.911	
Meat colour	Ctrl:1C	1		0.077
	2	0.93 (0.84, 1.03)	0.179	
	3	0.81 (0.67, 0.98)	0.032	
	4	0.94 (0.23, 3.77)	0.929	
	5	2.51 (0.88, 7.19)	0.086	
	6	0.95 (0.35, 2.54)	0.916	
	1B	–	0.964	
Fat colour	Ctrl	1		0.198
	Bromide	1.54 (0.78, 3.03)	0.217	

4.3.6 Cost Benefit analysis

A detailed cost benefit analysis was undertaken to determine the financial benefit to the operation based on an intervention time plus cost of $2 per head. As treatment was included in the standard induction process the time cost was nominal in real terms but for the purposes of cost calculation was estimated as as additional 20 minutes / 100 head inducted at labour cost of $25/h. Total cost of treatment / head was $2.08.

Assuming a death loss of 0.72% in the Bromide treatment, and 1.55% in the control treatment; hot standard carcase weight of 368 kg; and carcase price of $7.00/kg, then an extra 0.83 head per 100 head inducted reach a saleable carcase weight (305.44 kg per 100 head inducted; or $2138.08 per 100 head inducted). Given the total cost of bromide is $208 per 100 head, the net profit from using

the product translates to $19.30 per head inducted. At a reduced HCW price of $5.00/kg the net profit is $13.19/head inducted.

5 Discussion

5.1 Review of the regulatory landscape

There is a precedent to register bromide salts for use in ruminants in multiple markets globally dependent on the indication. Although both a production claim and therapeutic claim are feasible for this product, both have challenges and / or advantages. A use claim for production may not be applicable in certain jurisdictions, whilst a therapeutic claim would rely on an indirect mechanisim for the improvement of health outcomes. Evidence from this study suggests that both are possible. Whilst improvements in morbidity during the early feeding period were identified in this study, the potential for an increase in growth rate and feed efficiency warrants further trials. Both human food safety and target animal safety will require various studies but no major issues would be expected given the ubiquitous nature of bromide in nature and low risk of toxicity at the dose examined.

5.2 Pharmacokinetic profile of bromide in cattle

Bromide was absorbed rapidly following oral administration, with C_{max} occurring at 25 minutes despite potential delays due to dilution in the rumen. Similar C_{max} values have been reported in sheep and horses, when similar dose rates were given *per os*; 120 mg/kg bromide in sheep and horses compared to 100 mg/kg in the current study (Raidal and Edwards 2008; Quast *et al.* 2015b). Rumen volume is significantly larger in cattle compared to sheep, but despite increased rates of dilution following oral administration, similar C_{max} values were observed. Ruminal epithelial cells express large conductance channels permeable to chloride (Stumpff *et al.* 2009) and the rumen has a high chloride absorptive capacity, even against the net electrochemical gradient for the ion (Dobson and Phillipson 1958; Scott 1970). It is likely that these high conductance channels are also responsible for overcoming a relatively lower initial bromide concentration in the ruminal/gastric fluid compared with monogastric species. The difference in rumen volume between sheep (10-20 L) and cattle (150-200 L) does, by our analysis, not appear to effect the rate of Br absorption.

The $t_{½}$ of bromide in cattle, of approximately seven days does not align with values previously reported in other species. The $t_{½}$ of Br is longer in sheep (14 days), and shorter in the horse (3 days). In dogs ($t_{½}$ = 12-24 days), bromide is subject to extensive reabsorption, the net result being continual recycling of the anion, leading to a long elimination phase (Trepanier and Babish 1995). Ruminal reabsorption has been reported in sheep with secondary peaks observed in pharmacokinetic profiles of sheep given bromide *per os* (120 mg/kg)(Quast *et al.* 2015b), but does not appear to occur in cattle at a similar dose rate. Possible reasons for the absence of secondary peaks is the increased rate of elimination observed in cattle, reducing opportunity for reabsorption and the likelihood of redistribution to the rumen and omasum through chloride channels. Bromide is not metabolised, so changes in concentration are limited to absorption, distribution and elimination. The relatively short $t_{½}$ of bromide in cattle compared with sheep, therefore, suggests reduced elimination times via increased renal activity and urine output. Changes in urine output have previously been reported in sheep and cattle fed sodium chloride and potassium chloride (Dewhurst *et al.* 1968; Spek *et al.* 2012; Constable *et al.* 2014), but no comparative studies between the two species have been published to support differences in the renal response of cattle leading to a reduction in Br $t_{1/2}$. In addition, Dewhurst *et al.* (1968) also reported an increase in renal activity in sheep treated with KCl when compared to NaCl, suggesting the chloride salt selected for therapeutic application may be important to elimination time.

The reported pharmacokinetic profile of bromide supports it use in beef cattle feedlots for the mitigation of stress and subsequent stress-related health problems. The described long elimination phase supports the use of bromide at induction, and a therapeutic window that covers the greatest risk period for morbidity and mortality in feedlot cattle.

5.3 Bromide for treatment of induction stress

This study inducted a total of 1,936 animals into a South Australian feedlot, during the period July – September 2019 of which 1,882 completed 120 days on feed. Animals were randomly stratified and allocated to treatment pens at induction with every second animal treated with oral bromide in simple solution using a standard industry power-doser. This study identified that oral treatment with bromide at feedlot entry resulted in a trend towards decreased mortality (P = 0.081*)* A trend towards greater average daily gain (P = 0.097) when deads and salvage culls were included in the analysis was also observed.

Anxiolytic therapeutics such as bromide have been shown to increase appetite and feed intake in some species (Baird-Heinz *et al.* 2012). Conversely, in cattle, a decrease in stress behaviours could result in an increase in feed coversion in the absence of an increase in feed intake. In future research, feed intake and feed conversion ratios post-induction and treatment with bromide may be better assessed utilising individual feed intake data management systems, available at several commercial feedlots

Due to the relatively low occurrence of mortality in the feedlot engaged with this study, only a trend for mortality (P = 0.081) was identified by this analysis. A larger sample size could confer a significant difference in response to bromide treatment using frequentist methods. Retrospective power analysis suggests a study including 6000 head would resolve a significant outcome in 90% of instances. A trial using this number of cattle is feasible in many Australian feedlots. No difference was observed at slaughter in meat quality scores suggesting that meat quality is not negatively affected by treatment with oral bromide at feedlot induction.

Although overall morbidity did not differ, positive effects of a single dose of bromide on animal health during the first 28 days in the feedlot. Futher investigation in high risk cattle is required as morbidity rates were low at the host feedlot site during this project. Repeat treatment at 14 day or 28 days could increase the window of efficacy and further reduce rates of morbidity and potentially, mortality, with commensurate increase in profitability for the producer.

6 Conclusions/recommendations

6.1 Bromide confers positive impacts on feedlot cattle health and production

Data generated in this report indicate that a single dose of oral bromide, delivered at feedlot induction, resulted in a trend for reduced mortality during the early feeding period and therapeutic window. The utility of bromide as an oral treatment shows high utility due to low cost, ready availability and high safety margin in cattle. Its ubiquitous presence as a naturally occurring substance, with high environmental stability, is also indicative of its utility in this setting. Data reported in this single cohort study suggests that treatment of feedlot cattle with bromide could reduce health and mortality costs in feedlot cattle sufficiently to significantly increase profit margins. We recommend further investment in registration of a therapeutic product and development of a suitable oral product for the Australian feedlot sector through a commercial provider with a distribution network capable of delivering a product to Australia and worldwide.

7 Key messages

7.1 Stress mitigation by treatment with bromide at feedlot induction will improve welfare outcomes and increase profitability for Australian grain-fed beef producers

This study has shown that a single oral dose of bromide at feedlot induction can potentially reduce morbidity from respiratory disease in Australian feedlot steers and mortality during early days on feed after treatment at feedlot induction. These findings indicate a unique opportunity for Australian producers to maximise profit margins by administering a single, low cost non-antibiotic therapeutic at feedlot induction to mitigate stress, reduce disease risk, potentially increase feed conversion and production performance. Development of a therapeutic for the Australian feedlot sector would benefit grain-fed cattle producers as well as meeting increased consumer demands for improved animal health and welfare outcomes in conjunction with reduced antibiotic use in intensive livestock systems in Australia and worldwide.

Extrapolation of potential net profit gains by treatment with bromide at feedlot induction suggests that development of a commercial bromide therapeutic agent could significantly increase profit margins in Australian grain-fed production systems. Further research could confirm benefits on feed conversion as well as confirming morbidity and mortality outcomes identified in this study. The potential for bromide to be utilised as a therapeutic for mitigation of other production stressors such as weaning, road transport and live-export should also be investigated with further studies. If effective, this product would allow Australian intensive beef producers to meet demands for improved welfare outcomes for cattle in Australian livestock systems.

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