Final report

Evaluation of powdered Calcium Peroxide on enteric methane emissions and productivity of feedlot cattle in Australia

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Abstract

This project examined the potential of an oxidising agent in powdered form [Calcium Peroxide (70-80% wt)/calcium hydroxide (18-28% wt)/water (0-2% wt)] to theoretically decrease methane and increase cattle performance. A feedlot productivity study of 450 head, testing groups of cattle with two doses of powdered Calcium Peroxide and a control was commenced at Tullimba feedlot, but was terminated after 5 days due to persistent cattle refusal of the diets containing Calcium Peroxide. Handling of Calcium Peroxide in powdered form also presented significant risks to workplace health and safety, and accordingly the experiment deviated from the original planned methodology for incorporating Calcium Peroxide into the diets. It was concluded that Calcium Peroxide in powdered form was not suitable for direct addition to feedlot diets.

Executive summary

Background

This project examined the potential of an oxidising agent in powdered form [Calcium Peroxide (70-80% wt)/calcium hydroxide (18-28% wt)/water (0-2% wt)] to theoretically decrease methane and increase cattle performance. Recent international research and patents have reported that oxidising agents may mitigate methane.

One of the promising compounds listed in global patents include Calcium Peroxide. Calcium is typically included in feedlot diets through supplemental limestone, and replacing this calcium source with Calcium Peroxide may be possible, whilst decreasing feedlot methane production. The studies to quantify the effect of Calcium Peroxide on greenhouse gas emissions, rumen fermentation and acid base balance of beef cattle were designed to be conducted in respiratory chambers at the University of New England. In addition, a feedlot productivity study was designed to test the effect on cattle productivity and carcase grading.

Objectives

The objectives of this project were:

1) Delivery of a 70 day feedlot cattle (domestic short fed category) study using up to 30 cattle with respiration calorimetry chambers to measure the greenhouse gas emissions from cattle fed up to four dose of powdered Calcium Peroxide and one untreated control group.
   a) Determine the effect of dietary concentration of powdered Calcium Peroxide on greenhouse gas emissions, rumen fermentation and acid-base balance of short fed cattle during the up to 70 day feeding period.
   b) Determine the effect of powdered Calcium Peroxide on rumen, liver and kidney pathology at slaughter.

2) Delivery of a 450 head (45 x 10 head pens), 100 day feedlot productivity study to evaluate the effect of two doses of powdered Calcium Peroxide and a control group on feedlot performance (DMI, F:G, ADG) and carcase characteristics (HSCW, MSA grading data).

A feedlot productivity study of 450 head, testing groups of cattle with two doses of powdered Calcium Peroxide and a control was commenced at Tullimba feedlot, but was terminated after 5 days. The productivity study occurred prior to the respiratory calorimetry study. A decision to terminate the experiment was made as the intake of the Calcium Peroxide treatment groups was unacceptably low (40 to 60% compared to control) impacting the ability to be slaughtered at the same time as the Control group and cattle welfare. The respiration chamber experiment was not commenced.

Results/key findings

- The Calcium Peroxide source was found to contain acceptable contents of macro and trace elements.
- Feeding Calcium Peroxide in powdered form is not recommended for direct addition to feedlot diets, due to fine dust upon mixing which required significant workplace health and safety mitigation measures.
- There was a high coefficient of variation after mixing for 7 minutes.

- Inclusion of Calcium Peroxide at 1.28 to 1.72 % DM resulted in depression of intake by 40 – 60 % by cattle on a starter diet. This was likely due to the use of raw, powdered Calcium Peroxide which would have reacted with moisture and organic matter in the mouth of the cattle causing heat and fizzing. The feedlot experiment was not continued past the starter phase.

Future research and recommendations

Further research on incorporating Calcium Peroxide into a coated pellet is recommended to overcome handling risks as well as reactivity in the mouth before reaching the rumen.

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1. Background

Powdered Calcium Peroxide is a novel and promising feed additive that has been theorised to mitigate methane in several international patents.

In other methane inhibitor studies globally there has been the observed occurrence of potentially negative effects of inhibiting methane significantly which is hypothesized to be due to the accumulation of hydrogen in the rumen. Methane production in ruminants is driven by an evolutionary need to absorb and eliminate excess hydrogen that forms in the rumen as a result of the fermentation process in the rumen. Compared to other methane inhibiting products MLA has worked with, the powdered Calcium Peroxide may have possible effects on rumen hydrogen utilisation.

This project was designed to evaluate ability of Calcium Peroxide to suppress methane and hydrogen in feedlot cattle through inclusion of different levels of calcium peroxide in the diet of cattle. The studies to quantify effect of calcium peroxide on greenhouse gas emissions, rumen fermentation and acid base balance of beef cattle were designed to be conducted in respiratory chambers at the University of New England. In addition, a feedlot productivity study was designed to test the effect on cattle productivity and carcase weight and quality parameters.

2. Objectives

The objectives of this project were:

3) Delivery of a 70 day feedlot cattle (domestic short fed category) study using up to 30 cattle with respiration calorimetry chambers to measure the greenhouse gas emissions from cattle fed up to four dose of Calcium Peroxide one untreated control group.
   a) Determine the effect of dietary concentration of Calcium Peroxide on greenhouse gas emissions, rumen fermentation and acid-base balance of short fed cattle during the up to 70 day feeding period.
   b) Determine the effect of Calcium Peroxide on rumen, liver and kidney pathology at slaughter.

4) Delivery of a 450 head (45 x 10 head pens), 100 day feedlot productivity study to evaluate the effect of two doses and a control group of Calcium Peroxide on feedlot performance (DMI, F:G, ADG) and carcase characteristics (HSCW, MSA grading data).

A feedlot productivity study of 450 head, testing 2 doses of Calcium Peroxide and a control was commenced at Tullimba feedlot, but was terminated after 5 days. The decision to terminate the experiment was made as the intake of the two treatment groups had persistently been unacceptably low from the perspective of the cattle's wellbeing, and ability to be slaughtered at the same time as the Control group. As a result, the experimental objectives of the study had by day 5 been compromised to such an extent, that further compromise to cattle wellbeing was not justified.
Because of the palatability problems with the Calcium Peroxide, the decision was made with MLA to not commence the respiration calorimetry chamber experiment.

3. Methodology

Evaluation of Calcium Peroxide in a feedlot productivity study

Experimental design and measurements

A randomised complete block experiment was designed and commenced at Tullimba Feedlot, with three treatments:

1) A control treatment consisting of a normal short-fed feedlot diet containing 0.7 % (DM basis) total dietary calcium from calcium carbonate (limestone).
2) A low Calcium Peroxide (CaO_2) treatment group fed a normal short-fed feedlot diet, supplemented with CaO_2 such that total Ca from CaO_2 is equal to that in the control (basal) diet (i.e. 0.7 % Ca DM basis).
3) A High Calcium Peroxide (CaO_2) treatment group fed a normal short-fed feedlot diet, but supplemented with CaO_2 such that total Ca from CaO_2 is equal to 0.95 % Ca (DM basis)

All procedures were approved by the University of New England Animal Ethics Committee (Approval number ARA23-041). Three-weeks prior to commencement of the experiment, 500 *Bos taurus* steers were weighed on a static scale (Gallagher, Hamilton, NZ) calibrated with 600 kg of certified weights, and treated with a pour-on broad-spectrum endectocide (Dectomax® Pour-On, Zoetis, Parsippany, NJ), an oral flukicide (Exifluke 240®, Bayer, Leverkusen, Germany), clostridial vaccine (Ultravac® 5in1, Zoetis, Parsippany, NJ), Bovine Respiratory Disease vaccine (Bovilis® MH + IBR, Coopers Animal Health, Macquarie Park, NSW, Australia), then backgrounded on pasture and sorghum silage until introduction to the feedlot pens. No hormonal growth promotant was applied. This induction weight was used to select 450 steers with a narrow liveweight range, which were then stratified into 15 liveweight blocks of 30 steers each.

Within each liveweight block, steers were randomly assigned to 1 of the 3 treatments, resulting in 15 replicate pen groupings per treatment (N = 45 pens). Twelve days prior to the commencement of the experiment, all steers were drafted among 45 pens of 10 head each. The 15 blocks of matched Control, Low and High Calcium Peroxide treatment pens were randomly located within a contiguous group of pens in the feedlot, and each contiguous group of pens was randomly located along a single row of pens in the feedlot. Blocks 1 to 8 were considered cohort 1 and Blocks 9 to 15 were considered cohort 2, such that from this point onwards, all diet transitions and measurement activities were conducted on cohort 2 one calendar day after cohort 1. The steers were fed a common pre-starter diet for 5 days, followed by a common starter ration (containing calcium from limestone in a liquid supplement) for 7 days. At day zero, commencement of the experimental period, each cohort was weighed for an initial liveweight (mean ± s.d.: Control 357.7 ± 24.20 kg; Low Calcium Peroxide 357.4 ± 24.63 kg, High Calcium Peroxide 357.5 ± 24.53 kg), then retained on the common starter diet until the Calcium Peroxide treatments were introduced on day 5.

The treatment diets were first fed to the cattle using a starter diet on day 5 of the experimental period, respectively for each cohort, designed to be fed for 3 days before transitioning to the Intermediate I diet.

Diets, mixing and intake recording

All cattle were fed a tempered barley feedlot ration in a concrete feedbunk fitted to each pen. Diets (Appendix 1.1) were mixed in a wagon mixer (274-12 Feed Mixer, Rotomix, Dodge City, KS). The ingredients were added in the following load order: first, grain; second, millrun; third whole cottonseed; fourth, hay; fifth, water; last dry supplement premix and calcium source (limestone or Calcium Peroxide) while the mixer was rotating. There was a deviation in final experimental methodology and the proposed methodology in relation to addition of Calcium source to the Ration, which was added directly to the finished feed and not pre-mixed in a supplement and added to finished feed. Rations were mixed for 7 minutes, to be adjusted on results of homogeneity of calcium within a batch. On day 9, the diets were reformulated to remove additional water from the diet (Appendix 1.2, starter only).

A bunk-scoring protocol (Lawrence 1998) was implemented, aiming for minimal orts. Each feed bunk was scored for feed refusals (orts) 60 minutes before feeding, after which that day's feed intake was calculated, recorded and delivered.

Feed analysis

A mixer test for homogeneity of Ca in the TMR was conducted whereby 10 grab samples were collected across the entire mixer feedout of each experimental starter diet after mixing for 7 minutes. Each sample was bagged separately, dried at 80 °C for 48 h, ground and then analysed for Ca content by ICP-MS.

Sixty three of the 20 kg bags of Calcium Peroxide were sampled across the 7 pallets, and pooled sequentially into 21 samples which were analysed for heavy metals content by ICP-AES. Minimum, maximum and mean dietary content (DM-basis) of each element, considering the maximum inclusion of Calcium Peroxide in the High finisher diet, was compared to published tolerances of cattle (Klasing *et al.* 2005).

4. Results

Heavy metal and calcium contents of Calcium Peroxide

The heavy metals content of the Calcium Peroxide were compared against expected intake in the High Calcium Peroxide diet (1.75 % DM inclusion), and none exceeded published tolerances of cattle (Table 1).

Table 1. Results of heavy metal testing of Calcium Peroxide

	Calcium Peroxide Sample (DM-basis)		Expected diet content at 1.75% DM inclusion		Maximum tolerance of cattle[1]
Element	Mean	Max	Mean	Max	
Boron (B) (mg/kg)	13.99	27.20	0.20	0.38	150 ppm
Calcium (Ca) (mg/kg)	511,112.10	533,111.00	7,155.57	7,463.55	15,000
Potassium (K) (mg/kg)	ND	ND	ND	ND	2,000
Magnesium (Mg) (mg/kg)	4,220.53	4,660.93	73.86	81.57	600
Sodium (Na) (mg/kg)	ND	ND	ND	ND	NA
Phosphorus (P) (mg/kg)	1,188.04	1,453.15	20.79	25.43	700
Sulphur (S) (mg/kg)	1,235.05	2,154.00	21.61	37.70	300
Silicon (Si) (mg/kg)	1,291.85	1,466.20	22.61	25.66	700
Silver (mg/kg)	0.07	0.20	0.00	0.00	NA
Aluminium (mg/kg)	829.52	900.00	14.52	15.75	1000
Arsenic (mg/kg)	2.73	3.30	0.05	0.06	NA
Beryllium (mg/kg)	0.22	0.37	0.00	0.01	NA
Cadmium (mg/kg)	0.25	0.28	0.00	0.00	NA
Cobalt (mg/kg)	0.16	0.18	0.00	0.00	NA
Chromium (mg/kg)	15.52	17.00	0.27	0.30	100
Copper (mg/kg)	8.11	13.00	0.14	0.23	40
Iron (mg/kg)	772.90	4,381.00	13.53	76.67	50
Mercury (mg/kg)	0.28	0.36	0.00	0.01	2
Manganese (mg/kg)	26.67	33.00	0.47	0.58	2000
Molybdenum (mg/kg)	0.34	0.68	0.01	0.01	5
Nickel (mg/kg)	2.87	3.20	0.05	0.06	100
Lead (mg/kg)	0.49	0.56	0.01	0.01	100
Antimony (mg/kg)	1.12	1.40	0.02	0.02	NA
Selenium (mg/kg)	0.10	0.16	0.00	0.00	5
Tin (mg/kg)	6.44	7.50	0.11	0.13	100
Strontium (mg/kg)	204.29	240.00	3.58	4.20	2000
Uranium (mg/kg)	2.00	2.10	0.04	0.04	NA
Barium (mg/kg)	4.35	5.00	0.08	0.09	NA
Vanadium (mg/kg)	6.03	6.60	0.11	0.12	500
Zinc (mg/kg)	22.69	260.00	0.40	4.55	500

[1] *From: Klasing, K. C., et al. "Mineral tolerance of animals." Mineral Tolerance of Animals (2005)*

Photos and description of the raw product as received

As received, the Calcium Peroxide appears as a very light, off-white powder, with similar particle size and behaviour as cornstarch (Figure 1). It was highly prone to dust formation and clouding.

Figure 1. Calcium Peroxide appears as a very soft, fine, off-white powder

Work, Health & Safety and handling of Calcium Peroxide

A deviation from the planned experimental methodology occurred during the project after a risk assessment. Following the MSDS of Calcium Peroxide, a risk assessment was formulated (Appendix 8.2). The propensity of the product to dust cloud required full PPE for staff handling the product, including P2 mask, safety goggles, and disposable coveralls Type 4/5/6. Despite the measures and precautions taken, research team members and staff reported adverse events (stinging sensation in eyes and skin) after handling the product.

Initially, the dry supplement and Calcium Peroxide were pre-mixed together into a single, dry supplement. After undertaking the risk assessment, this method of incorporation of Calcium peroxide into the ration was not able to be utilised. This was due to formation of dust clouds containing calcium peroxide upon opening and emptying of the mixer, leading to lengthy suspension in the air, and eventual settling of Calcium Peroxide dust throughout the feedmill where it posed a touch-hazard. To reduce risks associated with mixing the product in an indoor space, as part of the risk assessment development, the combined dry supplement was mixed outside in a sealed concrete mixer, but the clouding issue upon unsealing the mixer remained (Figure 2). Therefore, the risk assessment determined that the dry supplement and Calcium Peroxide should be added separately to the feed mixer wagon (Figure 3). To prevent residual accumulation of Calcium Peroxide dust on mixer, tractor and loader, this was performed in an open air environment.

Figure 2. Photo of Calcium Peroxide clouding out from stationary cement mixer after mixing with dry supplement (in a sealed mixer) was finished, mixture was allowed to settle, and lid was removed.

Figure 3. Cloud of Calcium Peroxide emerging from mixer wagon on addition to feed

Coefficient of variation on mixing

A mixer test for the starter diet was completed after mixing for 7 minutes. This showed an unacceptably high CV for calcium in dry matter (47.6 % for Low Calcium Peroxide and 16.0 for High Calcium Peroxide diets), including for Control (38.4 %). Considering that the starter diet is a high-roughage formulation, and that the main calcium sources (limestone in Control and Calcium Peroxide in Low and High diets) were added as dry powders, directly into the finished feed and not pre-mixed into a supplement. This was a deviation from the planned methodology, and it occurred because of the risk assessment, mentioned above. mixing time would need to be substantially increased for high-roughage diets to achieve homogeneity. Mixing time required for homogeneity would be expected to be lower in higher grain formulations. Tullimba SOPs set ration mixing time as 5 minutes for a starter diet and 3 minutes for Transition and Finisher diets, which is an industry standard timing.

Overall summary of the study

Immediately after introduction of the treatment diets, pen intakes (as-fed) of the Low and High Calcium Peroxide treated cattle fell significantly (8.43 ± 0.30 kg DM/head.day pre-treatment starter intake *cf*. 6.37 ± 1.20 kg DM/head.day treatment starter intake for Low Calcium Peroxide cattle; and 8.37 ± 0.26 kg DM/head.day pre-treatment starter intake *cf*. 5.52 ± 1.59 kg DM/head.day treatment starter intake for High Calcium Peroxide cattle), while Control pen cattle intakes remained unchanged (8.61 ± 0.34 kg DM/head.day pre-treatment starter intake *cf*. 9.03 ± 0.16 kg DM/head.day treatment starter intake, Figure 4). On the scheduled day of transition to the Intermediate I diet for cohort 1 (day 8 of the experimental period), the Control pens were eating well (increased from pre-experimental starter intake) and they were ready to transition. Meanwhile, intake of the Calcium Peroxide groups were still depressed, and not sufficiently high to transition the steers to a higher grain ration. The decision was made to keep all pens and treatment groups on the starter diets and transition them together.

On day 10 of the study, five days after introduction of Calcium Peroxide in the treatment diets for cohort 1, intakes of the Calcium Peroxide groups were still persistently low, and they were still unable to transition to Intermediate I diets, meanwhile Control cattle were overdue for transition. By day 10, the intakes of the Calcium Peroxide treatment groups had been below maintenance for 4 days, and cattle welfare was becoming compromised. The divergence in intake, growth trajectory and ability to transition to Intermediate I and eventually finisher diets meant that the experimental aims were severely compromised. Considering the ability to deliver on the experimental aims and the risks posed to animal welfare, it was not possible to justify continuing to feed the Calcium Peroxide diets to the animals. While intake had plateaued in the Calcium Peroxide groups by days 8 and 9, the research team judged that the cattle would not be able to reach Control levels of intake quickly enough to restore the cattle to an uncompromised welfare state, while the Calcium Peroxide remained in their diets. Considered in terms of the experimental aims, even if intakes had recovered quickly, and the experiment continued through the full feeding period, the overall growth, feed efficiency and post-slaughter results of the Calcium Peroxide groups would have been determined by the first 5 days of the treatment period. This raised serious questions about the value of persisting with the experiment, considering the risks to cattle welfare. The decision to terminate the experiment was made on day 9, after 5 days on the experimental diets. All cattle were reverted to the common Tullimba diet. Intakes of the Calcium Peroxide groups increased immediately and these pens of steers were readily stepped up to realign with capped increments of feed on offer.

Feed intake

The first day that the experimental diets were introduced to each cohort (day 5), there was a very small (~5 kg average/pen) decline in pen intakes of the two Calcium Peroxide treatments (Figure 4). However, for the next 3 days, feed intakes fell by 40 % in the Low Calcium Peroxide group and by up to 60 % in the High Calcium Peroxide group, such that overall for the treatment period cattle consumption was 9.00 ± 0.84, 6.38 ± 1.48 and 5.48 ± 2.08 kg DM/head.day, for Control, Low Calcium Peroxide and High Calcium Peroxide groups, respectively. For the manufacture of the ration on day 9, the additional water was removed from the ration formulation in case there was some sort of reaction between the water in diet and the Calcium Peroxide was causing the depressed intakes. Despite the very low intakes of the 3 previous days, intakes of the Calcium Peroxide groups increased only modestly with this change, and the pens still did not eat all the feed offered, which suggested a poor prognosis for normalised intakes during the transition diet phases of the feeding period, and subsequent finisher period. There appeared to be a dose-response to Calcium Peroxide inclusion in feed intake, such that intake depression was more severe in the High Calcium Peroxide group than the Low Calcium Peroxide group (an additional reduction of 0.90 kg/head.day, over the whole treatment period.

It is interesting to note that on the first day of being offered the Calcium Peroxide diets, the pens in those treatments consumed almost all the feed offered, and aversion to the diet did not arise until the following day. This suggests that neophobia was not the cause of the reduced intake, but rather depressed intake was a behavioural adaptation to the cattle's experience of consuming the feed on day 5. What that experience consisted of is not able to be answered at present.

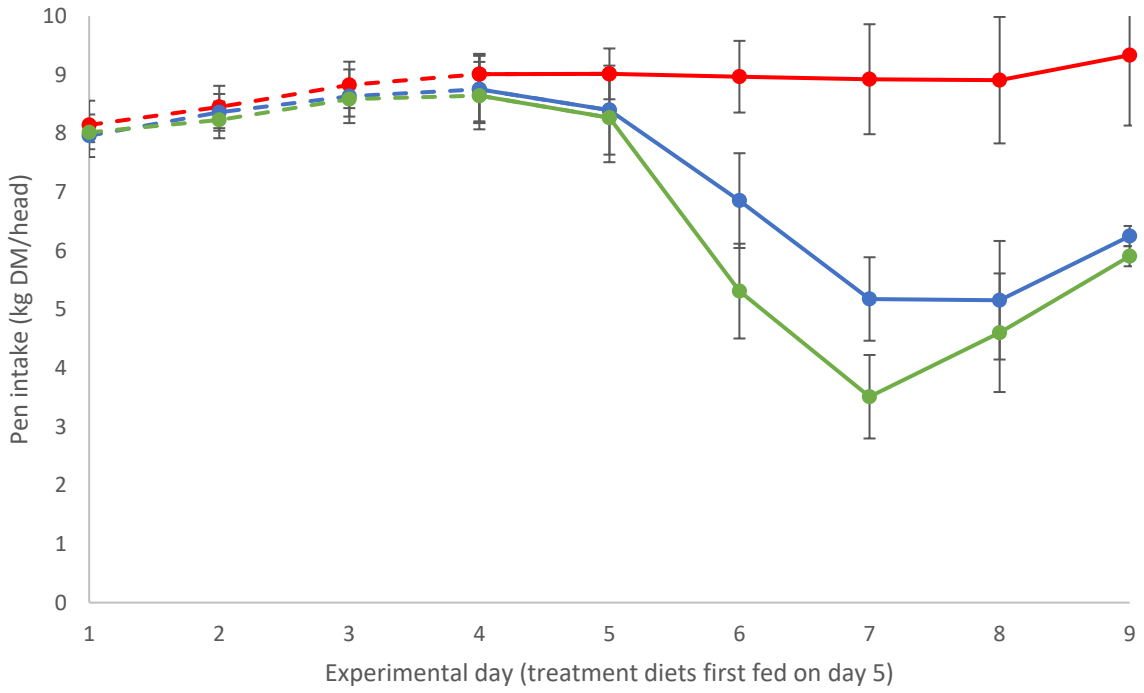

Figure 4. Mean pen intake (kg DM/head.day). Common adaptation diet, dashed lines. Experimental diets, solid lines. Red, Control; Blue, Low Calcium Peroxide; Green, High Calcium Peroxide.

Table 2. Mean pen intake (kg DM/head.day ± s.d.) during the treatment period, d 5 – 9. Inclusive.

Control		Low Calcium Peroxide		High Calcium Peroxide	
Pen	Intake (kg, as-fed)	Pen	Intake (kg, as-fed)	Pen	Intake (kg, as-fed)
A11b	9.85 ± 0.401	A11a	6.71 ± 1.676	A10b	5.02 ± 3.419
A12b	9.55 ± 0.602	A13a	6.11 ± 1.854	A12a	4.42 ± 3.330
A13b	10.11 ± 0.531	A14b	6.58 ± 2.142	A14a	4.35 ± 3.062
A15a	9.43 ± 0.161	A16a	6.16 ± 2.401	A15b	5.66 ± 2.213
A16b	9.13 ± 0.349	A17a	6.95 ± 1.293	A17b	5.50 ± 1.771
A18B	8.74 ± 0.305	A19A	6.29 ± 1.321	A18A	6.68 ± 1.312
A20A	7.42 ± 0.622	A19B	6.81 ± 1.134	A20B	5.40 ± 1.904
A21A	8.35 ± 0.072	A22A	5.90 ± 1.534	A21B	5.44 ± 1.904
A22B	8.29 ± 0.844	A23B	5.87 ± 0.977	A23A	5.69 ± 1.095
A24A	9.52 ± 0.174	A25A	6.41 ± 0.987	A24B	5.06 ± 1.837
A25B	8.83 ± 0.553	A26B	6.63 ± 0.788	A26A	5.69 ± 1.991
A27A	8.53 ± 0.653	A27B	5.89 ± 1.361	A28A	5.87 ± 1.921
A28B	9.76 ± 0.384	A29B	6.45 ± 1.670	A29A	6.41 ± 1.770
A30A	8.14 ± 0.294	A31A	6.31 ± 1.893	A30B	5.16 ± 1.863
A32B	9.17 ± 0.101	A32A	6.49 ± 2.097	A31B	6.21 ± 1.611

5. Discussion and Conclusion

Previous work (Patent No. PCT/EP2022/071462) demonstrated the ability of an oxidising agent similar to Calcium Peroxide (Urea Peroxide) to inhibit methanogenesis in ruminants when administered orally as either a bolus or as pellets comprising of (i) urea bydrogen peroxide or magnesium peroxide or a combination thereof, (ii) food components, and (iii) a coating able to protect the active ingredients from saliva during oral administration. In this present experiment, Calcium Peroxide was administered in-feed in powdered form, without any protective coating. Like Urea Peroxide, Calcium Peroxide hydrolyses to produce Hydrogen Peroxide in aqueous solutions with organic matter, which further decomposes to water and oxygen (Demeyer 1982). When Hydrogen Peroxide from either source decomposes to oxygen, the oxidative-reductive potential (ORP) of the rumen fluid is elevated, and this is the suspected mode of action of Calcium Peroxide. Under normal, anaerobic conditions of rumen metabolism, Acetyl CoA produced by the hydrolysis of complex feed substrates is converted into a variety of volatile fatty acids and other products of fermentation. Hydrogen produced in these pathways is utilised by methanogenic archaea and methane is produced as a by-product. When ORP is elevated through oxygenation of the rumen (microaerobic conditions), Acetyl CoA is instead directed to the tricarboxylic acid cycle, where it is completely oxidised to carbon dioxide, instead of CH_4 as an end-product (Magdalena *et al.* 2022). Previous *in vivo* research in sheep (Demeyer 1982) has demonstrated that unhydrolysed Calcium Peroxide which made its way into the rumen would be hydrolysed there, producing water and O_2. Administration of Calcium Peroxide to sheep has been demonstrated to reduce CH_4 concentration in respired air, and reduce protozoal counts in rumen fluid of sheep (protozoa are symbiotically associated with methanogenic archaea) (Demeyer 1982).

In the present experiment, the combination of uncoated Calcium Peroxide with water in the presence of organic substrates, both in the TMR and from saliva in the mouth, will result in an exothermic hydrolysis reaction that would likely be unpleasant in the mouth and create a fizzing sensation from the production of O_2 (Demeyer 1982). The sensation of this reaction at point of consumption is likely sufficiently unpleasant to cause the reduction in feed intake observed in the present trial, although other metabolic impacts in the rumen can't be ruled out.

A consideration when seeking explanation for the reduced intake of Calcium Peroxide-treated groups in the present trial is the impact of additional calcium (Ca) and of dietary cation-anion difference (DCAD) in the diet. In the High Calcium Peroxide diet, Ca was provided in excess of requirement (Ca:Phosphorus = 3.2:1). In feedlot diets urolithiasis is rarely due to excess Ca, and more commonly, uroliths are formed of phosphorus, which is high in grain-based diets. Rush and Groteluesch en (1979) recommend Ca:P rations of < 6:1 to prevent the formation of phosphatic calculi, and High CaO_2 diet will provide Ca in excess of requirements (ratio up as high as 2.7:1), which poses a low risk of predisposing some cattle to urolithiasis (urinary crystals/stones or calculi/uroliths). Despite their name, urinary calculi in ruminants, in particular, feedlot cattle, are most commonly formed of phosphorus (P), not Ca, due to the high P content of grain. Both calcium and phosphorus are normally maintained in balance due to the ability to mobilise or sequester Ca and P from the skeletal stores. However, when P levels become high relative to Ca, such as in grain-based diets, supplementation with Ca is required, to maintain ratios of Ca:P less than 6:1 (Rush & Groteluschen 1979), to prevent the formation of phosphatic urinary calculi. Ca:P ratios of up to 7:1 have been shown to have no effect on cattle performance (Fluharty n.d.). Calcium is a weak contributor to DCAD, as it is not a strong ion. The DCADs of the starter diets in the present experiment ranged from 4.2 – 18.9, from 4.0 – 19.0, and from 4.2 – 23.5 mEq/100 g DM for the Control, Low Calcium Peroxide and High Calcium Peroxide starter diets, respectively, depending on the equation used. No diet was strongly negative in DCAD nor high in Chloride (all diets ~ 6.6 g Cl/kg DM), and so DCAD is not suspected as a cause of low intake in the Calcium Peroxide groups in this experiment.

Overall, it is concluded that the powdered form of Calcium Peroxide appears unsuitable for application in the Australian feedlot diet due to WHS and handling issues, and severe reduction of intake by cattle when included in a TMR. However, successful inclusion of peroxide sources as coated pellets in beef and dairy rations overseas, with inhibition of methane emissions and improvement in animal performance suggest that it is worthwhile considering ways to overcome these barriers to persist with research with this product, for example with pelleting.

6. Future research and recommendations

The observation that intake reduction did not occur until the second day of feeding suggests that further investigation of the experience of ingestion and digestion and subsequent feedback from this product are worthy of further investigation. The cost-benefit of preparing a coated pellet containing Calcium Peroxide should be investigated, as this is a way to overcome WHS issues and will likely overcome palatability problems with raw Calcium Peroxide oxidising in the mouth of the animals, albeit with a potentially substantial increase in cost of the product.

7. References

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8. Appendix

8.1 Diets

Table 3. Formulated composition of experimental starter diets

	Dry matter basis			As-fed basis		
Ingredient, %	**Control**	**Low**	**High**	**Control**	**Low**	**High**
Barley Tullimba Tempered	45.86	45.88	45.89	46.53	46.56	46.56
Hay oaten	24.24	24.25	24.25	20.89	20.90	20.90
Mill Run	14.13	14.58	14.14	12.73	13.13	12.73
Cottonseed whole	9.81	9.81	9.82	8.55	8.55	8.55
Molafos Gold[1]	2.94	2.94	2.94	3.80	3.80	3.80
Limestone	1.74	-	-	1.42	-	-
DSM 0.75% Premix	1.28	1.25	1.24	1.04	1.03	1.02
Calcium Peroxide		1.28	1.72	-	1.05	1.41
Water	-	-	-	5.03	4.99	5.03
Nutrient						
NEm Beef, Mcal/kg	1.63	1.64	1.63	1.31	1.32	1.31
Dry Matter, %	100.00	100.00	100.00	80.65	80.65	80.64
Moisture, %				19.35	19.35	19.36
NEg Beef, Mcal/kg	1.04	1.05	1.04	0.84	0.85	0.84
Grain DM, %	45.58	45.60	45.61	36.76	36.78	36.78
ME Beef, MJ/kg	11.55	11.61	11.56	9.32	9.36	9.32
Concentrate DM, %	64.74	65.22	64.78	52.21	52.60	52.24
Roughage DM %	28.93	28.94	28.95	23.33	23.34	23.34
Roughage NDF %	17.49	17.49	17.50	14.10	14.11	14.11
RUP % DM %	3.79	3.81	3.79	3.05	3.07	3.06
Crude Protein , %	13.85	13.93	13.85	11.17	11.24	11.17
Total NDF, %	33.02	33.19	33.04	26.63	26.76	26.64
Total ADF, %	19.59	19.65	19.60	15.80	15.84	15.80
Premix, %	3.05	2.54	2.97	2.46	2.05	2.40
Crude fat, %	3.47	3.49	3.47	2.80	2.81	2.80
Crude Fibre, %	13.70	13.74	13.71	11.05	11.08	11.06
Starch, %	45.02	45.21	45.05	36.31	36.46	36.33
DMD, %	77.30	77.70	77.35	62.34	62.66	62.37
DOMD, %	76.57	76.97	76.62	61.76	62.07	61.79
Ash, %	6.25	6.25	6.22	5.04	5.04	5.02
Calcium Peroxide, %		1.28	1.73		1.03	1.39
Calcium from $CaCO_3$, %	0.70			0.56		
Calcium from Calcium Peroxide, %		0.71	0.95		0.57	0.77
Cobalt, ppm	256.71	252.17	249.85	207.03	203.37	201.48
Copper, ppm	17,100	16,797	16,643	13,790	13,546	13,421
Calcium, total, %	0.87	0.88	1.12	0.70	0.71	0.91
Phosphorus total, %	0.43	0.43	0.43	0.34	0.35	0.34
Sodium, %	0.23	0.23	0.23	0.19	0.18	0.18
Potassium, %	1.00	1.01	1.00	0.80	0.81	0.80
Chloride, %	0.50	0.49	0.49	0.40	0.40	0.40
Salt, %	0.43	0.42	0.42	0.34	0.34	0.34
Magnesium, %	0.33	0.33	0.33	0.26	0.26	0.26
Sulfur, %	0.27	0.27	0.27	0.22	0.22	0.22
Iodine, ppm	846.37	831.38	823.74	682.58	670.48	664.27
Selenium, ppm	170.98	167.95	166.41	137.89	135.45	134.19
Zinc, ppm	137.47	136.12	134.75	110.87	109.78	108.67
DCAD, mEq	47.69	50.41	48.97	38.46	40.65	39.49
Ca:P,	0.88	0.88	0.88	0.71	0.71	0.71
VItamin A, IU	2,256,950	2,216,978	2,196,608	1,820,182	1,787,937	1,771,370
Vitamin D, IU	282.60	277.61	275.06	227.91	223.88	221.81
Vitamin E, IU	31.84	31.43	31.22	25.68	25.35	25.18
Monensin, ppm	25.65	25.19	24.96	20.68	20.32	20.13

[1]Molafos Gold

Table 4. Formulated composition of experimental Intermediate I diets

	Dry matter basis			As-fed basis		
Ingredient, %	**Control**	**Low**	**High**	**Control**	**Low**	**High**
Barley Tullimba Tempered	57.98	58.26	58.21	58.88	59.17	59.10
Hay oaten	18.35	18.44	18.32	15.83	15.91	15.80
Mill Run	10.27	10.32	10.23	9.26	9.30	9.22
Cottonseed whole	7.97	8.01	7.80	6.95	6.98	6.80
Molafos Gold	2.44	2.45	2.47	3.15	3.17	3.20
DSM 0.75% Premix	1.24	1.25	1.25	1.01	1.02	1.02
Limestone	1.76	-	-	1.44	-	-
Calcium Peroxide	-	1.27	1.72	-	1.04	1.41
Water	-	-	-	3.48	3.40	3.45
Nutrient						
NEm Beef, Mcal/kg	1.70	1.71	1.70	1.37	1.37	1.37
Dry Matter, %	100.00	100.00	100.00	80.63	80.63	80.61
Moisture, %				19.37	19.37	19.39
NEg Beef, Mcal/kg	1.10	1.11	1.10	0.89	0.89	0.89
Grain DM, %	57.69	57.98	57.92	46.52	46.74	46.69
ME Beef, MJ/kg	11.93	11.99	11.94	9.62	9.67	9.62
Concentrate DM, %	72.07	72.42	72.17	58.11	58.39	58.18
Roughage DM %	22.18	22.29	22.07	17.89	17.97	17.79
Roughage NDF %	13.37	13.44	13.32	10.78	10.84	10.73
RUP % DM %	3.93	3.94	3.92	3.17	3.18	3.16
Crude Protein , %	13.72	13.79	13.71	11.06	11.12	11.05
Total NDF, %	29.29	29.44	29.22	23.62	23.74	23.56
Total ADF, %	17.07	17.15	17.01	13.76	13.83	13.71
Premix, %	3.03	2.53	2.98	2.44	2.04	2.41
Crude fat, %	3.20	3.22	3.18	2.58	2.59	2.56
Crude Fibre, %	11.87	11.93	11.83	9.57	9.62	9.54
Starch, %	53.55	53.82	53.73	43.18	43.39	43.31
DMD, %	83.19	83.60	83.28	67.07	67.40	67.13
DOMD, %	82.34	82.75	82.44	66.39	66.72	66.45
Ash, %	6.20	6.23	6.21	5.00	5.03	5.01
Lysine, , %	0.06	0.06	0.06	0.02	1.83E-02	1.81E-02
Calcium Peroxide, %		1.28	1.73	0.57	-	-
Calcium from $CaCO_3$, %	0.71			-	0.57	0.77
Calcium from Calcium Peroxide, %		0.70	0.95	200.89	202.39	202.17
Cobalt, ppm	249.15	251.03	250.79			
Copper, ppm	16,594	16,719	16,703	13,380	13,480	13,465
Calcium, total, %	0.85	0.85	1.10	0.69	0.69	0.89
Phosphorus total, %	0.40	0.41	0.40	0.33	0.33	0.32
Sodium, %	0.21	0.22	0.22	0.17	0.17	0.17
Potassium, %	0.89	0.89	0.88	0.71	0.72	0.71
Chloride, %	0.43	0.43	0.43	0.35	0.35	0.35
Salt, %	0.41	0.42	0.42	0.33	0.34	0.34
Magnesium, %	0.30	0.30	0.30	0.24	0.24	0.24
Sulfur, %	0.26	0.26	0.26	0.21	0.21	0.21
Iodine, ppm	821.36	827.54	826.76	662.26	667.22	666.48
Selenium, ppm	165.93	167.18	167.02	133.79	134.79	134.64
Zinc, ppm	128.77	129.67	129.34	103.83	104.55	104.27
DCAD, mEq	36.44	36.51	35.63	29.38	29.44	28.72
Ca:P,	0.74	0.74	0.75	0.60	0.60	0.60
VItamin A, IU	2,190,279	2,206,748	,204,679	1,766,012	1,779,235	1,777,257
Vitamin D, IU	274.15	276.21	275.95	221.05	222.70	222.45
Vitamin E, IU	33.41	33.64	33.60	26.94	27.12	27.09
Monensin, ppm	24.89	25.08	25.05	20.07	20.22	20.20

Table 5. Formulated composition of experimental Intermediate II diets

	Dry matter basis			As-fed basis		
Ingredient, %	**Control**	**Low**	**High**	**Control**	**Low**	**High**
Barley Tullimba Tempered	70.13	70.48	70.11	71.26	71.56	71.26
Hay oaten	12.12	12.06	12.11	10.46	10.40	10.46
Cottonseed whole	6.54	6.66	6.54	5.90	6.00	5.90
Mill Run	5.75	5.80	5.81	5.01	5.06	5.07
Molafos Gold	2.48	2.47	2.48	3.21	3.20	3.21
DSM 0.75% Premix	1.25	1.25	1.24	1.02	1.02	1.02
Limestone	1.74	-	-	1.43	-	-
Calcium Peroxide	-	1.28	1.71	-	1.05	1.40
Water	-	-	-	1.72	1.71	1.69
					-	-
Nutrient						
NEm Beef, Mcal/kg	1.76	1.77	1.76	1.42	1.43	1.42
Dry Matter, %	100.00	100.00	100.00	80.65	80.59	80.68
Moisture, %				19.35	19.41	19.32
NEg Beef, Mcal/kg	1.16	1.16	1.16	0.93	0.94	0.93
Grain DM, %	69.80	70.14	69.78	56.29	56.53	56.29
ME Beef, MJ/kg	12.34	12.40	12.34	9.95	9.99	9.96
Concentrate DM, %	78.84	79.31	78.88	63.59	63.92	63.64
Roughage DM %	15.37	15.37	15.36	12.39	12.38	12.39
Roughage NDF %	9.17	9.16	9.17	7.40	7.38	7.40
RUP % DM %	4.06	4.09	4.06	3.28	3.29	3.28
Crude Protein , %	13.60	13.68	13.60	10.97	11.02	10.98
Total NDF, %	25.33	25.43	25.34	20.43	20.50	20.45
Total ADF, %	14.52	14.58	14.52	11.71	11.75	11.72
Premix, %	3.02	2.54	2.97	2.44	2.05	2.40
Crude fat, %	2.98	3.00	2.98	2.40	2.42	2.40
Crude Fibre, %	9.99	10.03	10.00	8.06	8.08	8.06
Starch, %	61.74	62.06	61.75	49.80	50.01	49.82
DMD, %	88.55	89.01	88.58	71.42	71.74	71.46
DOMD, %	87.60	88.06	87.63	70.65	70.97	70.69
Ash, %	6.16	6.18	6.16	4.97	4.98	4.97
Calcium Peroxide, %	-	1.29	1.72		1.04	1.39
Calcium from $CaCO_3$, %	0.70	-	-	0.56		
Calcium from Calcium Peroxide, %	-	0.71	0.94		0.57	0.76
Cobalt, ppm	251.21	250.86	250.61	202.61	202.18	202.18
Copper, ppm	16,730	16,707	16,690	13,493	13,465	13,465
Calcium, total, %	0.82	0.83	1.07	0.66	0.67	0.86
Phosphorus total, %	0.38	0.38	0.38	0.30	0.30	0.30
Sodium, %	0.21	0.20	0.20	0.17	0.16	0.17
Potassium, %	0.76	0.77	0.76	0.61	0.62	0.62
Chloride, %	0.37	0.37	0.37	0.30	0.30	0.30
Salt, %	0.42	0.42	0.42	0.34	0.34	0.34
Magnesium, %	0.27	0.27	0.27	0.22	0.22	0.22
Sulfur, %	0.26	0.26	0.26	0.21	0.21	0.21
Iodine, ppm	828.10	826.94	826.12	667.88	666.47	666.48
Selenium, ppm	167.29	167.06	166.90	134.93	134.64	134.64
Zinc, ppm	123.11	123.09	122.93	99.29	99.20	99.17
DCAD, mEq	17.22	17.88	17.58	13.89	14.41	14.18
Ca:P,	0.67	0.67	0.67	0.54	0.54	0.54
VItamin A, IU	2,208,263	2,205,176	2,202,987	1,780,995	1,777,254	1,777,272
Vitamin D, IU	276.27	275.89	275.62	222.82	222.35	222.35
Vitamin E, IU	35.86	35.90	35.80	28.92	28.93	28.88
Monensin, ppm	25.09	25.06	25.03	20.24	20.20	20.20

Table 6. Formulated composition of experimental Finisher diets

	Dry matter basis			As-fed basis		
Ingredient, %	**Control**	**Low**	**High**	**Control**	**Low**	**High**
Barley Tullimba Tempered	82.50	82.91	82.53	83.66	84.00	83.69
Cottonseed whole	6.60	6.63	6.60	5.94	5.96	5.94
Hay oaten	5.38	5.41	5.39	4.69	4.71	4.69
Molafos Gold	2.53	2.54	2.53	3.27	3.28	3.27
DSM 0.75% Premix YG18801001	1.24	1.25	1.25	1.02	1.02	1.02
Limestone	1.75	-	-	1.43	-	-
Calcium Peroxide	-	1.26	1.70	-	1.03	1.39
Nutrient						
NEm Beef, Mcal/kg	1.8	1.8	1.8	1.3	1.3	1.3
Dry Matter, %	100.0	100.0	100.0	80.6	80.6	80.6
Moisture, %	-	-	-	19.4	19.4	19.4
NEg Beef, Mcal/kg	1.2	1.2	1.2	0.8	0.8	0.8
Grain DM, %	82.0	82.4	82.0	36.8	36.8	36.8
ME Beef, MJ/kg	12.8	12.8	12.8	9.3	9.4	9.3
Concentrate DM, %	85.5	85.9	85.5	52.2	52.6	52.2
Roughage DM %	8.7	8.7	8.7	23.3	23.3	23.3
Roughage NDF %	5.0	5.0	5.0	14.1	14.1	14.1
RUP % DM %	4.2	4.3	4.2	3.1	3.1	3.1
Crude Protein, %	13.6	13.7	13.6	11.2	11.2	11.2
Total NDF, %	21.4	21.5	21.4	26.6	26.8	26.6
Total ADF, %	12.1	12.2	12.1	15.8	15.8	15.8
Premix, %	3.0	2.5	3.0	2.5	2.1	2.4
Crude fat, %	2.9	2.9	2.9	2.8	2.8	2.8
Crude Fibre, %	8.2	8.2	8.2	11.1	11.1	11.1
Starch, %	69.7	70.0	69.7	36.3	36.5	36.3
DMD, %	93.8	94.3	93.8	62.3	62.7	62.4
DOMD, %	92.8	93.2	92.8	61.8	62.1	61.8
Ash, %	6.1	6.1	6.1	5.0	5.0	5.0
Urea, %	-	-	-	-	-	-
Calcium Peroxide, %		1.3	1.7	-	1.0	1.4
Calcium from $CaCO_3$, %	0.7	-	-	0.6	-	-
Calcium from Calcium Peroxide, %	-	0.7	0.9	-	0.6	0.8
Cobalt, ppm	249.6	250.8	250.7	207.0	203.4	201.5
Copper, ppm	16620	16702	16696	13,790	13,546	13,421
Calcium, total, %	0.8	0.8	1.0	0.7	0.7	0.9
Phosphorus total, %	0.3	0.3	0.3	0.3	0.3	0.3
Sodium, %	0.2	0.2	0.2	0.2	0.2	0.2
Potassium, %	0.6	0.6	0.6	0.8	0.8	0.8
Chloride, %	0.3	0.3	0.3	0.4	0.4	0.4
Salt, %	0.4	0.4	0.4	0.3	0.3	0.3
Magnesium, %	0.2	0.2	0.2	0.3	0.3	0.3
Sulfur, %	0.3	0.3	0.3	0.2	0.2	0.2
Iodine, ppm	822.6	826.7	826.4	682.6	670.5	664.3
Selenium, ppm	166.2	167.0	167.0	137.9	135.4	134.2
Zinc, ppm	115.4	115.9	115.8	110.9	109.8	108.7
DCAD, mEq	-3.4	-3.4	-3.6	38.5	40.7	39.5
Ca:P,	0.6	0.6	0.6	0.7	0.7	0.7
VItamin A, IU	2193675	2204557	2203738	1,820,182	1,787,937	1,771,370
Vitamin D, IU	274.3	275.7	275.6	227.9	223.9	221.8
Vitamin E, IU	38.0	38.2	38.1	25.7	25.3	25.2
Monensin, ppm	24.9	25.1	25.0	20.7	20.3	20.1