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ORIGINAL PAPER

Efficacy of encapsulated sodium nitrite as a new tool for feral pig management

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Abstract Worldwide feral pigs threaten native biodiversity, agricultural production and pose a risk to biosecurity as potential disease vectors. In New Zealand, the management of feral pigs has long been restricted to hunting, trapping, fencing and limited poisoning with 1080, warfarin and phosphorus. Sodium nitrite ($NaNO_2$) is commonly used at very low concentrations in the food industry. At high doses, $NaNO_2$ induces methaemoglobinaemia in mammals restricting the transport of oxygen by the red blood cell and in toxic doses leads to central nervous system anoxia, lethargy and death. Pen and field trials with pigs have been undertaken with an encapsulated formulation of $NaNO_2$, designed to overcome the bitter taste of $NaNO_2$ and mixed into a palatable paste bait. In pen trials, eight out of nine pigs consumed a lethal dose of paste bait. The average time to death was 59.5 min (±23.96 SD); symptoms lasted an average of 42.13 min (±19.12 SD) and included pale extremities, lethargy and ataxia. In a field trial, 12 radio-collared feral pigs were baited with the toxic paste bait formulation in prototype bait stations, where 11 of the 12 pigs consumed a lethal dose. Encapsulated $NaNO_2$ has potential as an additional tool for the management of feral pigs, particularly when shooting and hunting is not practical or possible. Data in these studies were used to register this bait as a vertebrate toxic agent for feral pig management in New Zealand. This represents the first known registration of $NaNO_2$ worldwide for use as a vertebrate toxic agent.

Keywords Sodium nitrite · $NaNO_2$ · Vertebrate pesticide · Feral pigs · Methaemoglobinaemia

Key message

- Feral pigs are a threat to biodiversity and biosecurity worldwide and management options are limited.
- A common food ingredient, sodium nitrite ($NaNO_2$), has been encapsulated and a paste bait containing this formulation shown to be palatable and lethal to feral pigs.
- Encapsulation has improved the palatability and effectiveness of the paste bait compared to results with the raw form of $NaNO_2$.
- $NaNO_2$ has an antidote, low risk of secondary poisoning and can be used without an operator's in New Zealand, and therefore should be considered a viable tool for feral pig management in New Zealand.

Introduction

In New Zealand, the broad-scale control of vertebrate pest species has been substantially reliant on anticoagulants and sodium fluoroacetate (1080) (Eason et al. 2006). Alternatives to 1080, used for field control of pest species, such as second-generation anticoagulant rodenticides, have

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resulted in concerns regarding wildlife contamination and bioaccumulation worldwide (Young and de Lai 1997; Stone et al. 1999; US EPA 2004, 2008; Eason et al. 2010a), and that these compounds are not considered humane (Littin et al. 2000, 2002), particularly for larger animal pest species like feral pigs (*Sus scrofa*). In New Zealand, feral pigs have previously been poisoned with 1080, phosphorus and warfarin (NPCA 2008), although these are not registered for feral pig management, and phosphorus and warfarin have been deemed inhumane for feral pig management (Cowled and O'Connor 2004; Sharp and Saunders 2004).

Feral pigs are listed in the Global Invasive Species Database as one of the worst alien invasive species (Lowe et al. 2000). They have been described as impacting primary production through the predation of livestock (Choquenot et al. 1997; Seward et al. 2004) and through damage to pasture and crops (Choquenot et al. 1996; Schley and Roper 2003; McLeod 2004; West et al. 2009). Being omnivorous, feral pigs cause serious damage to native flora and fauna and are considered a serious agent of decline in many countries (Choquenot et al. 1996; Seward et al. 2004; Brescia et al. 2008; Lapidge et al. 2012). They are also regularly cited as a risk to biosecurity due to being a vector or potential vector for diseases including foot and mouth disease, African swine fever, brucellosis and tuberculosis (Davis 1998; McLeod 2004; Seward et al. 2004; Hutton et al. 2006; Cozzens et al. 2010).

In New Zealand, feral pigs negatively impact native ecosystems through disturbing natural restoration of native forest, preying on ground nesting birds, their chicks, eggs and digging up their burrows, and impacting pastoral production (Batema and Meddens 2006; NPCA 2008; Krull et al. 2013). Whilst the economic and biodiversity impacts of feral pigs in New Zealand have not been widely quantified, their economic impacts have been estimated overseas in dollar values per annum as approx. $106.5 million in Australia (McLeod 2004) and $800.5 million in the USA (Pimentel et al. 2005). Worldwide they have also been responsible for the extinction of various species of small mammals (Pimentel et al. 2001). Up until the completion of the research reported here, the management of feral pigs in New Zealand for agricultural and biodiversity gains as well as for any biosecurity response to a potential disease outbreak was limited to culling wild populations through hunting.

In New Zealand, it has been deemed necessary to develop alternative toxins (ERMA 2007) for controlling pest species that are effective and humane, have an antidote and are less persistent than second-generation anticoagulants. In response to this need, a new class of compounds are now emerging that have been termed "red blood cell toxins" (Eason et al. 2010a), based on their mode of action. *para*-aminopropiophenone (PAPP) represents the first compound in this class and is a potent and selective toxin for stoat (*Mustela erminea*) and feral cat (*Felis catus*) control (Murphy et al. 2007). It is toxic to carnivores, whilst birds, rodents, brushtail possums (*Trichosurus vulpecula*) and other mammals including humans are less sensitive (Savarie et al. 1983; Fisher et al. 2005, 2008; Eason et al. 2010b). The onset of symptoms is rapid, and stoats and feral cats are usually unconscious quickly, after eating baits (Eason et al. 2010b). PAPP poisoning causes abnormally elevated levels of methaemoglobin, referred to as methaemoglobinaemia.

Methaemoglobin is a form of haemoglobin which cannot transport oxygen and its levels in the blood are normally around 1 % of total haemoglobin (Bradberry 2011). Levels of methaemoglobin in the blood above 70 % are usually fatal, creating a lethal deficit of oxygen in cardiac muscle and the brain, and the resulting rapid lack of oxygen to the brain and other vital organs quickly leads to death from respiratory failure (Wright et al. 1999; Eason et al. 2010a). Common signs of severe methaemoglobinaemia include shortness of breath, cyanosis, lethargy, loss of consciousness and bluish colouring of the skin especially in areas of high blood supply like lips, gums, hands/paws and nose (Kennedy et al. 1997; Wright et al. 1999; Eason et al. 2014).

Sodium nitrite ($NaNO_2$) represents the second compound in this class of red blood cell toxins being developed and was first identified as a potential toxin for feral pig management by Australian researchers in 1985 (Sullivan 1985), and this research was further developed in 2008 (Cowled et al. 2008). Although chemically distinct from PAPP, its toxic effects are mediated through the same mode of action. At high doses, $NaNO_2$ causes severe methaemoglobinaemia, central nervous system anoxia, lethargy and death. Animals and humans both widely consume nitrite in their diets (Cockburn et al. 2013), and $NaNO_2$ is commonly used at low concentrations as a colour fixative and preservative in meats and fish (Binkerd and Kolari 1975; Epley et al. 1992).

The acute toxicity of $NaNO_2$ is well characterised with numerous observations of accidental poisoning of livestock (Bouchet and Bouchet 1938; Robinson 1942; Winks et al. 1950; Counter et al. 1975) as well as researchers delivering doses of $NaNO_2$ directly to pigs to better understand the dose response and its potential as a management tool (Winks et al. 1950; London et al. 1967; Sullivan 1985; Cowled et al. 2008; Eason et al. 2009; Shapiro et al. 2009; Foster 2011; Lapidge et al. 2012).

A study by Cowled et al. (2008) aimed at identifying potential toxins for feral pig management looked to identify any unique physiological or metabolic weaknesses.

The researchers identified the susceptibility of pigs to methaemoglobin-forming compounds, which is one of the attributes of $NaNO_2$. They commented on previous research that showed pigs have very low levels of methaemoglobin reductase which is the enzyme that reverses methaemoglobin formation. As well as this metabolic weakness, it was noted that $NaNO_2$ induces a humane death, when compared with 1080 (Cowled et al. 2008), and that, like PAPP, it has an antidote in the form of methylene blue (Eason et al. 2010b).

The LD50 for pigs orally gavaged with $NaNO_2$ solution has previously been reported as approx. 90 mg/kg (Winks et al. 1950; Deeb and Sloan 1975). Captive trials by Cowled et al. (2008) found that $NaNO_2$ delivered to pigs via gavage with >90 mg/kg and via free feeding with >400 mg/kg resulted in lethal doses. Further pen trials in the USA since (Foster 2011) reported similar findings with the minimum lethal gavage dose for feral pigs reported as 113 mg/kg.

The potential use of $NaNO_2$ as a vertebrate toxic agent is limited by poor stability and low palatability due to its incredibly bitter taste (Cowled et al. 2008; Shapiro et al. 2009). Several different research groups in Australia, New Zealand and the USA have been working on taste masking and various methods of encapsulating $NaNO_2$ with the aim of improving stability and taste (Lapidge et al. 2009; Eason et al. 2010a; Lapidge et al. 2012). Although worldwide, the main interest in $NaNO_2$ as a pesticide is for poisoning pigs, research by the Invasive Animals Cooperative Research Centre (IA-CRC, Australia) has investigated other potential target species including rodents (Eason et al. 2010a), and in New Zealand, research has focused on encapsulating $NaNO_2$ for the management of feral pigs and brushtail possums.

Initial studies conducted by Connovation Ltd in New Zealand (Shapiro et al. 2009) with technical grade raw $NaNO_2$ that attempted to poison pigs and possums were unsuccessful. Animals either rejected baits containing $NaNO_2$ or ate too little to achieve toxic effects and subsequent research by Connovation Ltd identified an effective method of encapsulating $NaNO_2$. The purpose of the below studies was to determine the effectiveness of encapsulated $NaNO_2$, in a paste matrix bait, manufactured in New Zealand by Connovation Ltd—for the management of feral pigs. These data, as well as efficacy data generated for brushtail possums, were used to register this bait as a vertebrate toxic agent for the management of feral pigs and brushtail possums in New Zealand. This represents the first registration of $NaNO_2$ worldwide for use as a vertebrate toxic agent and the only toxin currently registered for feral pig management in New Zealand.

Materials and methods

Pen trial

Nine domestic pigs of the large white breed, five females and four males, were housed outside in three groups of three pigs in pens at the Lincoln University Farm Animal Facility. Each group of three pigs were in a pen constructed from wire mesh measuring 5 × 4 m, hay was provided for bedding and water was available ad libitum. Pigs were fed a standard grain maintenance diet in a concrete trough for the acclimatisation period of the first 7 days. After the acclimatisation period, on day eight, the concrete troughs were removed and two prototype bait stations were placed in each of the three pens. These consisted of wooden boxes made from *pinus radiata*—measuring 800-mm long, 450-mm wide and 190-mm deep—each with a hinged lid that had a 5-cm lip protruding from the front face to allow pigs to open the lid with their snouts (Fig. 1a, b). A single metal stake was driven into the ground at the four corners of each bait station, and wire was used to secure the bait stations to the stakes. The standard grain maintenance diet was removed, and each bait station was loaded with three balls of non-toxic paste bait each weighing approx. 250 g. The lids of all of the bait stations were wired open to enable pigs to feed readily from them. The non-toxic paste bait consisted of a mixture of peanut butter (35 %), kibbled wheat (20 %), ground maize (15 %), margarine (15 %) and sugar (15 %). The non-toxic paste is thick enough that it holds its own shape and can be rolled into balls. On day nine the same amount of non-toxic bait (as day eight) was placed in each station and the lids were closed to determine whether pigs could readily open the lids to access the bait. Pre-feeding out of the bait stations continued daily for a total of 8 days. The toxic trial was run on day 16 and three balls of toxic bait, each weighing 250 g, were placed in each of the bait stations. Each 250 g ball of toxic paste bait contained 25 g (10 %) $NaNO_2$, 1.31 g (0.5 %) encapsulant and 223.69 g (89.5 %) non-toxic paste bait. Pigs were closely observed to record symptoms of methaemoglobinaemia, and the time to the onset of these symptoms, the duration of these symptoms and the time to death were recorded. The amount of bait consumed in each of the three pens was also recorded. A veterinarian was present for the toxic component of the trial to observe the symptoms of poisoning and to allow an initial welfare assessment to be made.

Field trial

The trial site for this study consisted of a 10-hectare-fenced block of land at the Robert Wicks Research Facility 20 km

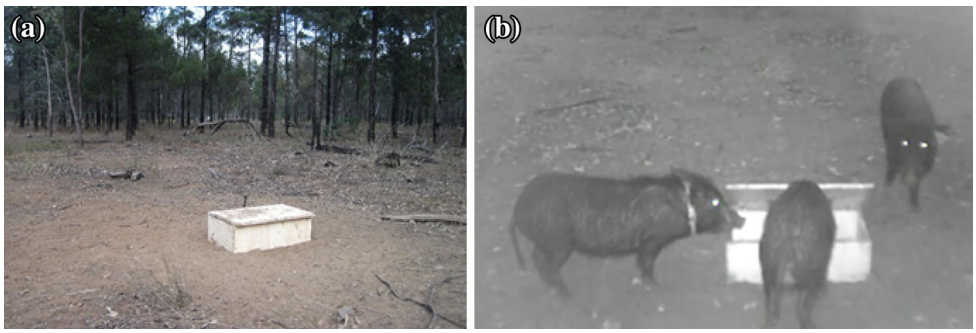

Fig. 1 Photo of a prototype bait station (**a**) and pigs accessing this bait station to consume toxic bait (**b**) during the field trial

north of Inglewood, Queensland, Australia. The vegetation in the block consisted predominantly of several species of the native Australian Cypress-pine (*Callitris* genus). Twelve wild-caught feral pigs (five males and seven females) were captured, on a nearby property, using a live capture trap and then transported in a covered trailer to the research facility. Pigs were anesthetised with a combination of Xylazine (2.2 mg/kg) and Zoletil (4 mg/kg), and sedatives were administered with a pole syringe. Once sedated, each pig was fitted with a VHF transmitter collar and an ear tag and then released into the fenced site 2 weeks prior to the start of the trial to acclimatise.

All bait placement, toxic and non-toxic, was carried out using the same bait box design used in pen trials. Boxes were secured to the ground using the same technique as the pen trial. In addition, a single 80-cm-metal stake was driven into the ground on a 45° angle on the back side of each box to stop the lid opening beyond 45° and this caused the lid to close when pigs finished feeding and limit non-target species access. Three bait boxes were used to lay baits and these were set out in a triangle formation with a single box at each point of the triangle and approx. 10 m between each bait box.

A single Reconyx Hyperfire^{TM} infrared motion detecting video camera was set up at each of the three bait boxes; each camera was attached to a single metal stake that was driven into the ground approx. 3 m from the bait box. Cameras were used to confirm individual pigs were readily accessing the bait boxes and that non-target species were excluded. Non-target species at the trial site included Eastern Grey kangaroos (*Macropus giganteus*), Apostlebirds (*Struthidea cinerea*), Australian Ravens (*Corvus coronoides*) and Australian Magpies (*Cracticus tibicen*).

Boxes were each baited with four non-toxic bait balls, these weighed approx. 250 g each. The bait formulation was identical to that used in the pig pen trials. Boxes were baited with non-toxic bait balls on nights one and two, and then boxes were left empty on night three. Bait box lids were left closed and relied on pigs opening them to access bait unlike cage trials where they were initially wired open.

On night four each box was baited with nine toxic baits giving a total of 27 baits, and these baits were the same formulation as used in pig pen trials. Toxic baiting continued on night five and six with two 250 g baits per box on each of these nights. The amount of bait that was eaten and footage from the cameras were checked at sunrise each morning. Pig carcasses were located using telemetry, and signals were received on a TR4 receiver and a hand-held, 3-element yagi directional aerial. All pig carcasses were disposed of by burning in a furnace. Non-toxic paste bait and toxic bait containing encapsulated $NaNO_2$ were prepared on site immediately prior to the pre-feeding and toxic sections of the trial. The encapsulant material and the paste bait matrix add a level of stability to the $NaNO_2$, however, the toxic bait still needs to be used soon after manufacture. The commercially available registered toxic bait is sold as a pre-mixed paste with a shelf life of 1 month.

Samples of the encapsulated $NaNO_2$ active and of the encapsulated $NaNO_2$ in paste bait were analysed by Flinders Cook Ltd (Technical Services) to confirm the concentration of $NaNO_2$ active prior to each trial. Samples of the encapsulated $NaNO_2$ contained 95 % w/w $NaNO_2$ active and 5 % encapsulant material. Samples of the encapsulated $NaNO_2$ in paste bait contained 10.00 ± 0.30 % $NaNO_2$. The method of analysis was based on an internationally recognised analytical method described in Vogel (1979).

Results

Pen trial

In the pen trial, eight out of nine pigs (88.9 %; 95 % binomial CI 51.8–99.7 %) consumed a lethal dose of paste bait containing encapsulated $NaNO_2$ (Table 1). One survivor did not consume a lethal dose of paste bait. Pigs that consumed a lethal dose died on average 59.5 min (±23.96 SD) after ingesting the toxic bait. For the eight pigs that consumed a lethal dose of toxic bait, the average time to clinical signs first appearing was 17.38 min (±6.84 SD),

Table 1 Key times for clinical symptoms appearing in pigs (pen trial) after consuming paste baits containing $NaNO_2$

Group	Pig	Sex	Weight (kg)	First appearance clinical symptoms (mins)	Clinical symptoms duration (mins)	Time to death (mins)
1	1	F	32.8	14	25	39
1	2	M	32.0	25	68	93
1	3	F	32.4	26	30	56
2	4	M	35.4	10	32	42
2	5	M	36.8	15	39	54
2	6	M	36.6	10	38	48
3	7	F	26.4	25	76	101
3	8	M	32.4	14	29	43
3	9	F	33.5	36	52[a]	Recovered

[a] After this time the pig no longer showed any symptoms of methaemoglobinaemia

and the average duration of symptoms was 42.13 min (±19.12 SD). In groups one and two, where all three pigs consumed a lethal dose of paste bait, the entire 1500 g of paste bait presented to each group was consumed. In the third group, where only two of the three pigs consumed a lethal dose, 670 g of the total 1500 g presented was consumed. All deaths were unremarkable and involved some or all of the following symptoms observed in chronological order: pale nose and extremities, blue tongues, vomiting (in two pigs), lethargy, ataxia, slight tremors collapse and death. The veterinarian who attended the toxic component of the trial to observe poisoning symptoms concluded that "the sodium nitrite caused a rapid death with little distress signs evident from the pigs".

Field trial

In the pig field trial on the first night of toxic baiting, 11/12 pigs (91.7 %; 95 % binomial CI 61.5–99.8 %) consumed a lethal dose of the toxic paste bait. A total of 26/27 of the toxic baits (6500 g out of the total 6750 g of bait) were consumed and video camera footage showed all pigs consumed well in excess of a lethal dose. On night four the only pig that did not consume any toxic bait was observed as being excluded from the bait stations by other pigs. There was no bait take on night five or six, and the one surviving pig was seen to approach within 5 m of bait stations but did not access any of them. The carcasses of the pigs that consumed the toxic bait were located by telemetry on the morning after they consumed toxic bait and were located on average 148 m (±74 SD) from the nearest bait station. No non-target species were seen accessing the bait stations or baits although four Eastern grey kangaroos were observed on the cameras within 5 m of bait stations on several occasions.

Discussion

An encapsulated form of $NaNO_2$ has been developed which has been shown to be palatable and effective for the management of feral pigs when presented as a paste bait in a purpose built bait station. The encapsulation of $NaNO_2$ prior to dispersion through the paste matrix improved the palatability of the paste bait, resulting in increased effectiveness and mortality in pigs compared with results from previous trials with the raw form of $NaNO_2$ (Shapiro et al. 2009).

In the time since the pen trials reported here were undertaken further trials have been completed with this formulation of encapsulated $NaNO_2$. In 2014, pen trials were carried out by the Texas Parks and Wildlife Department (TPWD) on feral pigs using encapsulated $NaNO_2$ produced by Connovation Ltd in an almost identical paste matrix bait. The trial killed 19/21 pigs over two nights of toxic baiting in pen trials at the Kerr Wildlife Management Area, Hunt, Texas, USA (Pers. Comm. Justin Foster, TPWD).

Current research in New Zealand, Australia and the USA is focusing on the efficacy and delivery of $NaNO_2$ for managing feral pigs in different habitats, and as suggested by Cowled et al. (2008), researchers are looking at the potential of a concentrate formulation that can be applied to different bait matrices regionally (Pers. Comm. Linton Staples, ACTA). Thus making use of grains or other food types that pigs are already accessing and therefore removing the need to try and get pigs feeding on an unfamiliar food type. This concentrate formulation would consist of the encapsulated $NaNO_2$ in an oil slurry that could then be applied to different bait matrices at the same concentration of 10 % active ingredient as per the currently registered paste bait.

Sufficient quantity of $NaNO_2$ needs to be ingested quickly to induce fatal methaemoglobinaemia and death. $NaNO_2$ at high doses appears to induce a humane death in pigs, and times to death were relatively rapid compared with other toxins used for feral pig management worldwide (e.g. Warfarin, phosphorus and 1080) but comparable to those observed for stoats and feral cats poisoned with PAPP (Eason et al. 2010b). Times to death for pigs reported here were quicker than those from a previous trial that also involved pigs freely consuming baits containing $NaNO_2$, and pigs died on average after 141 min (±49 SD) (Cowled et al. 2008).

The sequence of behavioural changes in pigs that consumed the paste bait containing $NaNO_2$ is consistent

with our understanding of the toxicology of $NaNO_2$, namely that it is rapidly absorbed and quickly induces methaemoglobinaemia. In this regard, it is noteworthy that the encapsulant we have chosen, which is added to improve stability and to overcome taste aversion, does not appear to alter the anticipated toxicity of $NaNO_2$ or impact negatively on welfare. In fact, in terms of welfare, the taste masking ability of the encapsulant allows pigs to eat a substantial amount of bait in a short space of time and to succumb quickly to the effects of methaemoglobinaemia.

Observations regarding the welfare of pigs poisoned with paste baits containing $NaNO_2$, made by the veterinarian present during the pen trial, were consistent with an independent assessment of the humaneness of $NaNO_2$ for killing pigs conducted at the Institute of Medical and Veterinary Sciences in Adelaide in 2008. Domestic pigs that consumed a lethal dose of bait containing $NaNO_2$ died within three hours, and the authors concluded that "the symptoms would suggest that sodium nitrite satisfies a general understanding of what a humane poison would be" (Institute of Medical and Veterinary Science 2010).

Baits containing encapsulated $NaNO_2$ have an antidote in the form of methylene blue, it can be used safely in bait stations to limit non-target access, and it has the advantage over other acutely acting vertebrate toxic agents in that it can be used without an operator's licence in New Zealand. In the context of animal pest management in New Zealand, encapsulated $NaNO_2$ has potential as an additional tool for the management of feral pigs, particularly when shooting and hunting are not practical or possible. The data generated from the trials reported in this paper as well as efficacy data generated for brushtail possums were added to registration dossiers, and in November 2013, a paste bait formulation containing encapsulated $NaNO_2$ was registered in New Zealand under the trade name *BAIT-RITE* paste for the management of feral pigs and brushtail possums.

Author contribution statement

LS, SH and CE conceived and designed the experiments. PA developed the $NaNO_2$ encapsulation technique. PA and CB refined the technique. LS and SH ran the trials. LS wrote the manuscript. All authors read and approved the manuscript.

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