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# Evaluation of Conditioned Taste Aversion as a Method to Reduce Predation of Wildlife Eggs by Feral Pig (*Sus scrofa*)

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## Abstract

Feral pigs are introduced predators to Australia and New Guinea. Predation by feral pigs on eggs and hatchlings of nationally protected sea turtles and other wildlife can cause significant mortality. Several control techniques are available to minimize pig predation but available techniques are expensive and of limited success. A potential method is to 'teach' feral pigs to avoid predating eggs by using Conditioned Taste Aversion (CTA). In this study, it was hypothesized that if eggs were treated with deterrent chemicals, feral pigs would develop an aversion to the eggs, and would stop predating them. Sixteen feral pigs were presented with eggs treated with the distasteful chemicals: TBZ and LiCl for 5 consecutive days following a pre-treatment phase during which they were fed untreated eggs. The percentage of eggs 'predated' during the treatment phase was lower than in the pre-treatment phase, though in most cases this difference was not statistically significant. Most eggs predated during the treatment phase were only partly eaten, suggesting that pigs were "taste-testing". In addition, pigs consumed similar proportion of untreated eggs and control eggs during the pre-treatment and post-treatment phases.

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Thus, taste aversion was not established. In a second experiment, external scent cues were applied to the eggshells. It was hypothesized that the pigs would only consume untreated eggs, and leaves the treated-scented eggs untouched. A new group of 16 feral pigs were presented with scented eggs treated with TBZ and LiCl for 10 consecutive days. The pigs still predated by partly consuming the scented-treated eggs. Similar percentages of untreated and scented-control eggs were predated during the pre-treatment and post-treatment phases. This study failed to provide support for CTA as an effective method of controlling feral pig predation of wildlife eggs.

***Keywords:*** Feral pigs; Conditioned Taste Aversion, TBZ, LiCl, pre-treatment, treatment, post-treatment, untreated, predated

## 1. Introduction

Throughout the world introduced animals pose problems for native fauna. This is especially true for Australia, whose fauna has developed in isolation from mammalian predators [1-6]. [7] claim that introduced vertebrate predators not only contribute to the decline and extinction of indigenous Australian species, but that they also prevent the successful re-establishment of populations of such species. Feral pigs (*Sus scrofa*) are one of these introduced predators. Introduced to Australia by early European settlers, feral pigs are now widespread throughout the country, and have become a major management concern in many important wildlife habitats.

Feral pigs, which are large bodied and aggressive omnivores, have a range of ecological impacts. They are known to cause general environmental degradation through damage to vegetation and soil. They are genuinely omnivorous, not only capable of denuding an area of important vegetative growth, but are also significant predators of numerous native animals. The carnivorous component of their diet includes earthworms, amphipods, centipedes, beetles, other arthropods, snails, frogs, lizards, snakes, and the eggs of freshwater crocodiles, sea turtles and ground nesting birds [8-10]. In particular, the ecological impacts of feral pig damage to sea turtle clutches is of great concern both in Australia and neighboring countries.

Feral pigs have opportunistic feeding habits, and they are able to exploit various food resources that vary enormously in abundance or availability depending on season, human disturbance and other factors. Wildlife eggs are one food resource that can be seasonally bountiful and make a significant contribution to the diet of feral pigs [9]. The destruction of egg clutches by feral pigs is reported to cause significant damage to wildlife populations by dramatically reducing recruitment into the next generation [10]. Because many of these animals, such as crocodiles and some sea turtles are themselves large bodied predators, this may have far reaching ecological consequences.

Thus, wildlife conservation initiatives identify the feral pig as a primary management concern. Choquenot *et al,* [11] reviewed control methods for mitigating the impacts of feral pig predation. The success of control methods depends on both the type and value of resources affected and the severity of damage caused by the pigs. Also important are practical concerns, such as the feasibility of reducing the damage in time to make the effort financially or biologically attractive, availability of funds, time, labor and equipment, the ability to coordinate management efforts, the people involved, and the ability to prevent re-invasion. The methods for controlling feral pigs to date have included shooting, trapping, hunting, habitat modification, fencing, and poisoning [11, 12]. The extent of investment in control techniques varies, generally in proportion to the economic loss caused by feral pigs. Generally, the choice of control techniques has put economic considerations ahead of ecological concerns. None of the above methods has been unconditionally successful in the long-term elimination of feral pigs in a given region. This is problematical for the control of egg predation, because it only takes a few pigs to inflict significant damage to egg clutches. Therefore, it is important to develop and test alternative techniques to control feral pig predation. The search for effective control of predation on eggs of native fauna by feral pigs is the focus of this study. One promising method is that of Conditioned Taste Aversion (CTA). This technique involves conditioning animals to avoid a certain type of food through the use of chemicals, the ingestion of which results in temporary sickness.

Aversive conditioning is a behavioral concept that is related to the evolutionary concept of Batesian mimicry, whereby otherwise palatable prey species mimic the behavior or appearance of unpalatable species [13]. Such methods have had some success in certain management situations [14]. This approach has been successfully used to reduce predation on eggs [15, 16] and to train livestock, including cattle, goats, horses ([17-19], and sheep ([20, 21] to avoid eating specific foods. This technique has had some success in reducing predation by coyotes, red foxes [22-25], and raccoons [16, 26], and red foxes. The mixed success of these studies prompted the current study of investigating the feasibility of CTA in feral pig to control predation of native wildlife eggs. CTA techniques have not been previously applied to feral pig egg predation. The aim of this study was to evaluate the applicability of CTA for the control of feral pig predation on wildlife eggs in general. This study examined whether the chemicals Thiabendazole (TBZ) and Lithium Chloride (LiCl) inserted into chicken eggs conditioned captive feral pigs to avoid consuming chicken eggs. It was expected that feral pigs would develop a behavioral modification after consuming treated eggs, generalize the aversion to the all chicken eggs and stop 'predating' all chicken eggs.

## 2. Materials and Methods

### Study Location

Research was conducted at the Robert Wicks Pest Animal Research Station (RWPARS), operated by the Queensland Department of Natural Resources (DNR) at Inglewood (300-km south-west of Brisbane). This station was chosen for the research because of the availability of a dedicated facility and trained staff.

### Experimental Animals

Feral pigs were captured in the Inglewood shire by professional pig hunters. Pigs were captured by trapping, using a panel trap with a top-hinged gate. This method was considered the most humane and environmentally friendly method available. The trapped pigs were encouraged to move from the traps into a transport cage. To avoid handling the animals, and to eliminate the stress caused by using tranquillizers and nooses, pigs were then released directly into separate group pens at the station. These group pens held up to seven pigs. When a sufficient number of pigs were gathered, the animals were relocated, via a series of races or gates into pens designed to house single animals (Figure 1).

**Figure 1:** Single pens

Each single pen consisted of an arena and a hutch. The weight, gender, colour and external specific characteristics of the experimental pigs were recorded.  Pigs participating in Experiment 1 were weighed using 100kg scales and their gender was determined from external sex morphology. The pigs were allowed to acclimatize to the pens for a one-week period.

Pigs maintained good level of health throughout the experimental period. To prevent pigs from overheating, automatic sprinklers sprayed waters over the single pens twice a day for two hours to limit the maximum temperature inside the hutches to approximately 30$^{o}$C.  At all times, drinking water was provided on demand from a drinker in each pen. The single pens were cleaned regularly. Females delivering piglets while in captivity were excluded from the research. Pigs were fed commercial pig feed.  During the experimental period, in order to motivate the pigs to consume the experimental chicken eggs, pigs were given 70% (700gr) of their recommended daily intake of commercial feed.

## Experimental Approach

The research was designed to evaluate the potential of Conditioned Taste Aversion (CTA) as a tool for reducing feral pig predation on the eggs of Australian wildlife.  Following commonly used protocols for CTA research [14, 16, 27], the experiments consisted of three research phases: pre-treatment, treatment and post-treatment.

This before-after design was chosen to allow an evaluation of the responses of individually penned feral pigs, because that provided a degree of experimental control not possible with free-ranging pigs. As a Before – After Control Impact (BACI) design, there were no controls within the pre-treatment and treatment phases, as the interest was in comparing the two. However, there was a control in the post-treatment phase.

### a. Experimental Design - Experiment 1

This experiment was carried out to test if it is possible to develop CTA in feral pigs, using chemicals that have proved useful for that purpose in other mammals. Two chemicals were tested: Thiabendazole (TBZ) and lithium chloride (LiCl) (Sigma Biochemicals and Reagents, Castle Hill, NSW). TBZ is characterized by rapid absorption [27], little taste and low toxicity [28], while LiCl is characterized by rapid absorption, a salty taste and high toxicity [29]. Both chemicals were tested at two dosages (low and high). Thus, there were two treatments (TBZ and LiCl), each with two dosages. The four categories are identified thus: TBZ low dose (TL), TBZ high dose (TH), LiCl low dose (LiL) and LiCl high dose (LiH). As each egg contained the same amount of TBZ or LiCl (see section preparation of eggs), dosage (low or high) was determined by the number of egg presented to pigs in the treatment phase. The actual dosage used was calculated by extrapolating figures from published works on other mammals, taking into account the different animal weights. The weight of feral pigs was reported to be between 30 –75 kg.

Pigs were randomly allocated to single pens. There were 16 pigs participating in Experiment 1. Four animals participated in each of the four treatments, in which treatments were allocated randomly to each pig. The experiment was conducted in three phases, with each phase lasting five days as outlined by the experimental protocol (Table 1). During days 1-5 (the pre-treatment phase), untreated eggs were fed to the pigs. On days 6-10 (the treatment phase), the appropriate number of treated eggs were offered. On days 11-15 (the post-treatment phase), untreated and control eggs (injected with distilled water, see section 2.4 for a full description), were offered to test if pigs are able to differentiate between treated/manipulated eggs and untreated eggs. Food consumption, including that of each egg, was recorded for each day, and the reaction of each pig to the treated eggs was observed and recorded (consumed/refused and symptoms - vomiting, diarrhea, listlessness- if consumed). Throughout the experiment, eggs which were cracked, partly eaten or fully eaten were considered ‘predated’, while eggs remaining intact were considered unpredated.

### b. Experimental Design - Experiment 2

This second experiment was designed after the results for Experiment 1 became known. Experiment 2 aimed to test whether it is possible to condition pigs to associate an external cue to determine whether an egg is edible or not. It was hypothesized that such an association would prevent predation of all eggs carrying the cue irrespective of contents. The external cue was one of two scents applied to the exterior of eggs. Scent 1 was creosote and Scent 2 was a common brand of aftershave (Brut brand). There were four treatments (Table 2). Treatments comprised of two treated eggs presented containing either TBZ or LiCl and scent on the eggshell.

**Table 1:** Experiment 1 protocol: Kinds and number of eggs presented during each phase

| **Phases** | **Pre-treatment** | **Treatment** | **Post-treatment** |
|---|---|---|---|
| **Period (days)** | 1 – 5 | 6 – 10 | 11 - 15 |
| **Kinds of eggs presented** | untreated | treated | untreated and control |
| **Number of eggs presented/day/pig** | 2 | | 1 untreated<br>1 control |
| **- TBZ low dosage** | | 2 | |
| **- TBZ high dosage** | | 4 | |
| **- LiCl low dosage** | | 2 | |
| **- LiCl high dosage** | | 4 | |

**Table 2:** Kinds of treatments for Experiment 2

| | | **Odor** | |
|---|---|---|---|
| | | **Creosote** | **Aftershave** |
| **Chemicals** | **TBZ** | Treatment 1<br>(Creosote + TBZ) | Treatment 3<br>(Aftershave + TBZ) |
| | **LiCl** | Treatment 2<br>(Creosote + LiCl) | Treatment 4<br>(Aftershave + LiCl) |

**Table 3:** Experiment 2 protocol: Kinds and number of eggs presented to each phase in Experiment 2

| | **Pre-treatment** | **Treatment** | **Post-treatment** |
|---|---|---|---|
| **Period (days)** | 1 - 7 | 8 - 17 | 18 - 23 |
| **Kinds of eggs presented** | untreated | scented-treated and untreated | scented-control and untreated |
| **Number of eggs presented/day/pig** | 2 | Each treatment was 2 scented-treated eggs and 2 Untreated eggs | 1 scented-control egg<br>1 untreated egg |
| - **TBZ + Creosote** | | | |
| - **LiCl + Creosote** | | | |
| - **TBZ + aftershave** | | | |
| - **LiCl + aftershave** | | | |

The experiment was conducted in three phases. The pre- and post-treatment phases each lasted for a week, the treatment phase lasted for ten days. The procedure during the pre-treatment and the post-treatment phases was similar to that of Experiment 1. However, in the treatment phase, both treated chicken eggs and the untreated eggs were presented (Table 3). To test if pigs were able to differentiate between treated/manipulated eggs and untreated eggs, in the post-treatment phase, pigs were presented with the both scented-control and untreated eggs.

**Preparation of eggs**

In both experiments, there were three types of eggs. In experiment one these were: untreated, treated and control, while in experiment two the types were: untreated, scented-treated, and scented-control eggs. Untreated eggs were normal un-manipulated eggs that were presented directly to the pigs. The treated eggs were manipulated eggs injected with chemicals, and the control eggs were manipulated eggs injected with distilled water. The scented-treated eggs were manipulated eggs injected with chemicals and appropriate scent applied to the shell. Control-scented eggs were manipulated eggs injected with distilled water and scent applied to shell.

Eggs were manipulated while wearing gloves to avoid contamination from the scent of the experimenter's hands. Both treated and control eggs were prepared by making a small hole, of a size appropriate for inserting a needle, in the shell with a fine drill (size 5/64-inch). The appropriate amount of egg content was then withdrawn with a hypodermic syringe (Terumo syringe eccentric tip 20cc/ml) and needle (16 gauge, 1.5 inch) and the predetermined amount of chemical was injected as an aqueous solution or suspension.

The effective dose for TBZ was 0.1g/kg of body weight, and for LiCl 0.2g/kg (Merck & Co., Inc). Thus, the total dosage for feral pigs with body weight between 30–75 kg was estimated as 3g for the low dose TBZ treatment and 6g for the high dose TBZ treatment. While for LiCl, it was estimated as 6g for the dosage and 12g for the high dose. The amount of TBZ and LiCl that could be inserted into each egg was limited by the solubility of the chemicals and the total amount of egg content that could be replaced with water without making eggs too 'runny'.

The solubility of chemicals differed. Solubility of LiCl in water is 76.92% (Merck & Co., Inc), making it easy to dissolve in water. However, solubility of TBZ is particularly low (3.84%) (Merck & Co., Inc). Thus, an agues suspension was prepared by shaking the fine TBZ powder with water in a 5 ml poly-propylene screw-cap centrifuge tube. In preliminary experiments, I established that the maximum volume of egg content that could be removed and replaced with water without rendering the egg (jumbo size ≈ 65ml) too 'runny' was 5–7ml. Moreover, it was found that maximum TBZ to be mixed with 5ml water is 1.5g, and maximum LiCl to be mixed in 7ml of water was approximately 3g. Therefore, each egg treated with TBZ was injected with a 5 ml suspension containing approximately 1.5g TBZ, while each egg treated with LiCl was injected with 7 ml of saturated solution containing approximately 3g LiCl. The dosages chosen for TBZ were 3g (low dose) inserting into two eggs and 6g (high dose) inserting into four eggs. The dosages chosen for LiCl were 6g (low dose) inserting into two eggs and 12g (high dose) inserting into four eggs.

The small holes in the eggs' shells were sealed with chemical free albumin, which was allowed to dry before the eggs were presented to the pigs. Control eggs presented during the post-treatment phase in Experiment 1 were manipulated eggs injected with distilled water and sealed, while in Experiment 2, control eggs were scent manipulated eggs (eggs injected with distilled water, sealed and scented).

In Experiment 1, all eggs were presented at 17:00 hours. In Experiment 2, all eggs were presented at 05:30 hours. This change of routine allowed the fate of eggs to be more clearly and frequently evaluated during daylight hours. Eggs were replaced daily during Experiment 2. The untreated eggs were placed on the left side of the pen when entering gate, while the scented-treated eggs were placed on the right side. The distance between the two locations was approximately 50–75 cm. The scented-treated eggs were differentiated from the untreated eggs by being previously marked with a pen. This facilitated scoring the type and number of eggs 'predated'.

## Statistical Analysis

Because this experiment involved taking repeated measures from individual pigs over several days, the most appropriate method of analysis is repeated measures ANOVA. Unfortunately, there were severe logistical limitations on replication, both in terms of space (available pens were limited) and pigs (only a limited number could be caught and maintained in captivity). Because replication was so low, distributions of the data failed to satisfy parametric assumptions of constant variance and normality. A repeated measure analysis is capable of accounting for both between subject variation due to the experimental treatments, and within subject (phases) variation, and testing for interactions between them. Thus the two components, treatment effects and between phases differences, had to be tested for using same tests, and therefore interaction could be tested for.

In experiment 1, treatment effect was used to test for differences in the consumption of low and high dosage TBZ and LiCl eggs, respectively. Data analyzed consisted of the total proportion of eggs eaten over the three phases. Differences between phases were tested for using the same test for several related samples. This was used to test for differences of the three treatment periods of experiments one and two. It was also used to test for differences between the three treatment periods, with data consisting of the total proportion of eaten eggs from each period. Because data from the three periods involves repeated measures from the same pig, the repeated measures ANOVA is the appropriate one to use.

## 3. Results

### Experiment 1

#### a. TBZ Treatment

There were significant differences in predation rates between the three experimental phases for both low and high dosages (Repeated Measures ANOVA, $F_{(1,6)} = 21.120$, $p<0.001$). The average proportion of eggs predated by pigs was similar in the pre-treatment and the post-treatment phases, regardless of whether the eggs were treated with low or high TBZ dosages (Figure 2). In the treatment phase, the trend for both dosages was the same, with the proportion of eggs predated, decreasing steadily from the first day to the third day then increasing during the last two days of the treatment phase.  The trend was same and the difference of proportion of eggs predated was consistent over all days for both treatments.

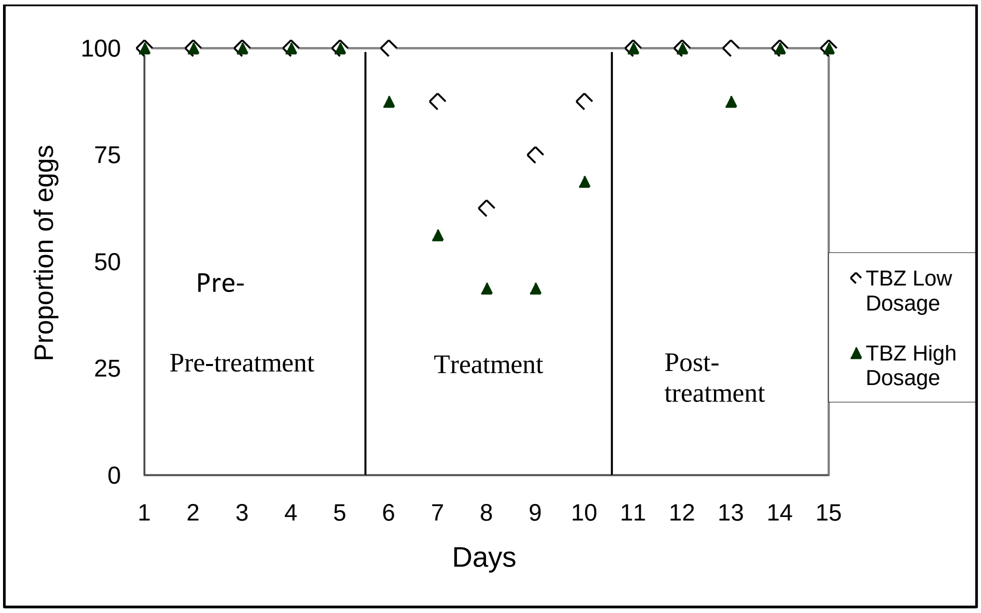

**Figure 2:** The proportion of eggs predated daily for TBZ low and high dosage treatment

During the pre-treatment phase, all 80 untreated eggs presented to the pigs were eaten.  During the treatment phase, 33 of 40 (82.5%) treated eggs presented in the low dosage treatment were predated, compared with 47 of 80 (58.75%) of TBZ treated eggs presented in the high dosage treatment (Table 4).  During the post treatment phase, pigs that had been exposed to TBZ low dosage eggs eaten all 20 untreated eggs and all 20 control eggs. Pigs that had been exposed to high TBZ dosage eggs eaten 19 of 20 untreated eggs and all control eggs during the post treatment phase.

**Table 4:** The number and proportion of eggs treated with TBZ predated during the treatment phase (sum over all days)

| **Treatment** | **TBZ low dosage** | | | | | **TBZ high dosage** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Pig Number** | 18 | 17 | 9 | 16 | | 7 | 15 | 8 | 2 | |
| **Number of eggs predated** | 9 | 7 | 8 | 9 | 33 | 18 | 12 | 8 | 9 | 47 |
| **Proportion of eggs predated** | 90 | 70 | 80 | 90 | 82.5 | 90 | 60 | 40 | 45 | 58.75 |

The proportion of eggs consumed in the TBZ low and high treatments did not differ significantly (Repeated Measures ANOVA, $F_{(2,12)}=4.48$, $p=0.08$). Overall, feral pigs predated a similar proportion of TBZ treated eggs in the low dosage treatment (i.e. 2 eggs/pig/day) and TBZ treated eggs over days in the high dosage treatment (4 eggs/pig/day) (Table 4). Moreover, there was no interaction between experimental phases and treatments (Repeated Measures ANOVA, $F_{(2,12)}$=3.26, *p*=0.07).

In the pre-treatment phase, eggs were fully consumed. However, during the treatment phase, the number of partly eaten eggs was much higher than that of fully eaten eggs (Table 5). During the post treatment phase, there was no significant difference between the number of control eggs and untreated eggs eaten by pigs in either the TBZ low or TBZ high treatments.

**Table 5:** The number of fully and partly predated eggs for TBZ low and high dosage during the treatment phase

| **Treatment** | | **Days** | | | | | **Total** |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | |
| **TBZ Low** | **Partly predated** | 7 | 7 | 5 | 6 | 7 | 32 |
| | **Fully predated** | 1 | 0 | 0 | 0 | 0 | 1 |
| **TBH High** | **Partly predated** | 12 | 9 | 7 | 7 | 11 | 46 |
| | **Fully predated** | 1 | 0 | 0 | 0 | 0 | 1 |

### b. LiCl Treatment

There were significant differences in predation rates between the three experimental phases for both low and high dosages (Repeated Measures ANOVA, $F_{(2,12)}$=11.32, *p*<0.05). The average proportion of eggs predated by pigs was similar in the pre-treatment and the post-treatment phases, regardless of whether the eggs were treated with low or high LiCl dosages (Figure 3).

During the pre-treatment phase, of the 80 untreated eggs presented to the pigs, 79 eggs were eaten. During the treatment phase, 33 of 40 eggs (82.5%) in the LiCl low dosage treatment were predated, compared with 68 of 80 (85%) of LiCl treated eggs presented in the high dosage treatment (Table 6). During the post-treatment phase, pigs that had been exposed to LiCl low dosage eggs eaten 19 of 20 untreated eggs and 18 of 20 control eggs. Pigs that had been exposed to LiCl high dosage eggs eaten 19 of 20 untreated eggs and all control eggs during the post treatment phase.

The proportion of eggs consumed in the LiCl low and high treatments did not differ significantly (Repeated Measures ANOVA, $F_{(1,6)}$=0.480, *p*=0.514). Overall, feral pigs predated a similar proportion of TBZ treated eggs in the low dosage treatment (i.e 2 eggs/pig/day) than LiCl treated eggs over days in the high dosage treatment (4 eggs/pig/day) (Table 6).

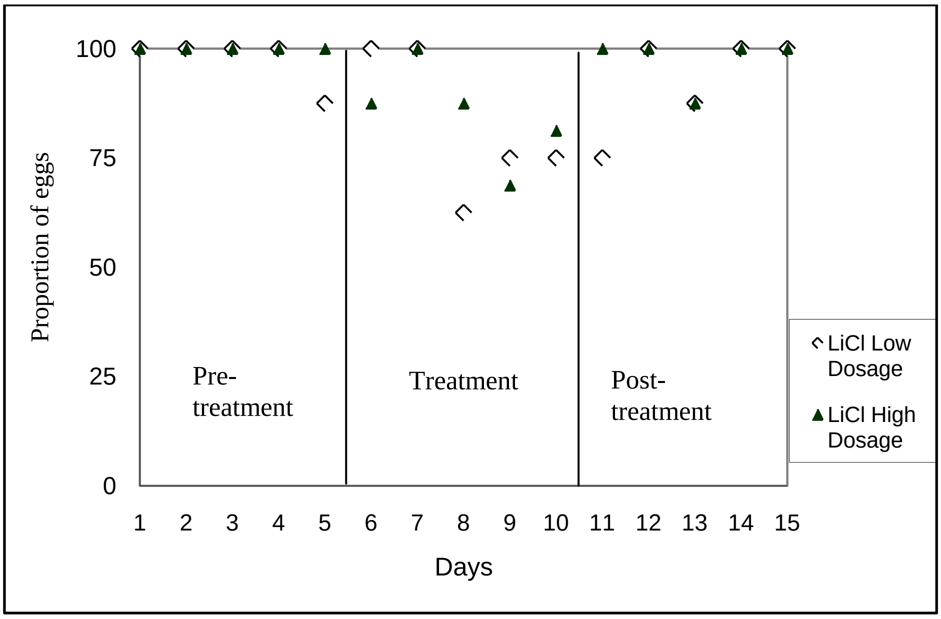

**Figure 3:** The proportion of eggs predated daily for LiCl low and high dosage treatment

**Table 6:** The number and proportion of eggs treated with LiCl predated during the treatment phase (sum over all days)

| **Treatment** | **LiCl low dosage** | | | | | **LiCl high dosage** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Pig Number** | 5 | 12 | 19 | 4 | | 10 | 1 | 20 | 6 | |
| **Number of eggs predated** | 7 | 10 | 8 | 8 | 33 | 14 | 16 | 19 | 19 | 68 |
| **Proportion of eggs predated** | 70 | 100 | 80 | 80 | 82.5 | 70 | 80 | 95 | 95 | 85 |

Moreover, there was no interaction between experimental phases and treatments (Repeated Measures ANOVA, $F_{(2,12)}$=0.097, $p$=0.908). Once again, of all the eggs treated with LiCl, which were predated during the treatment phase, the number of partly eaten eggs was much higher than that of fully eaten eggs (Table 7), unlike in the pre-treatment phase where eggs were fully consumed. During the post treatment phase, there was no significant difference between the number of control eggs and untreated eggs eaten by pigs in either the LiCl low or LiCl high dosage treatments.

### c. General Behavior

The responses of feral pigs to eggs treated with TBZ and LiCl were similar. Most pigs predated treated eggs during the treatment phase. During feeding in this phase, pigs would crack the eggs and test their contents, using careful tongue movements. This feeding behavior had not been observed in the pre-treatment phase. This change in feeding behavior suggests feral pigs were able to detect the TBZ and LiCl taste.

**Table 7:** The number of partly and fully predated eggs during the treatment phase

| **Treatment** | | **Days** | | | | | **Total** |
|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | |
| **LiCl Low dosage** | **Partly predated** | 6 | 6 | 5 | 5 | 6 | 28 |
| | **Fully predated** | 2 | 2 | 0 | 1 | 0 | 5 |
| **LiCl High dosage** | **Partly predated** | 12 | 16 | 12 | 11 | 14 | 65 |
| | **Fully predated** | 2 | 0 | 1 | 0 | 0 | 3 |

The pigs were slower to approach eggs during the treatment phase than during the pre- and post-treatment phases. The pigs usually hesitated before breaking the treated eggs. However, they would still crack the eggs and taste them. After tasting, they tended to avoid the treated eggs. The feral pigs were active from late afternoon to early morning, and were inactive during the day. During Experiment 1, pigs consumed all presented commercial grain. All pigs approached the grain as soon as it was presented, and their appetite for grain food was not influenced by their aversion to the tested eggs. Their good appetite indicates the pigs were healthy. Moreover, there was no general change in the appearance of subjects' excrement during the research phase. Only one pig (pig no. 2 that received TBZ high dosage treatment) vomited after fully eating treated eggs. But, even this pig continued to taste eggs during the treatment phase and to fully consume eggs in the post-treatment phase.

### Experiment 2

### a. Creosote Treatment

There were no significant differences in predation rates between the three experimental phases for both creosote – TBZ and creosote – LiCl treatments (Repeated Measures ANOVA, $F_{(2,12)}=0.00$, $p=1.00$) (Figure 4).

During the pre-treatment phase, of the total 112 untreated eggs presented to the pigs, 108 (96.4%) were eaten. During the treatment phase, 77 of 80 (96.25%) treated eggs presented in the creosote-TBZ treatment were predated, compared with 79 of 80 (98.75%) treated eggs presented in the creosote-LiCl treatment (Table 8). In addition, all 160 untreated eggs presented to the pigs were eaten.

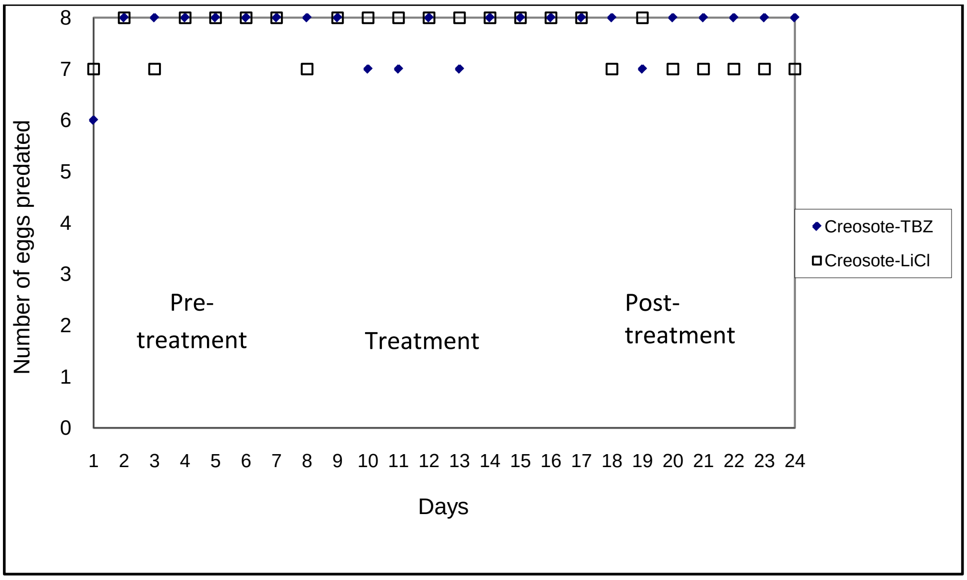

**Figure 4:** The number of eggs predated daily for creosote-TBZ and creosote LiCl treatment

**Table 8:** The number of eggs scented with creosote treated with TBZ and LiCl predated during the treatment phase

| **Treatment: Creosote** | **Days** | | | | | | | | | | **Total predated** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| **TBZ treated eggs** | 8 | 8 | 7 | 7 | 8 | 7 | 8 | 8 | 8 | 8 | 77 |
| **Untreated eggs** | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 80 |
| **LiCl treated eggs** | 7 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 79 |
| **Untreated eggs** | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 80 |

As before, most predated eggs during the treatment phase were only partly eaten, in contrast to the pre-treatment phase when eggs were all fully eaten (Table 9). The proportion of eggs predated between the creosote - TBZ and creosote-LiCl treatments did not differ significantly (Repeated Measures ANOVA, $F_{(1,6)}$=0.000, $p$=1.00). Moreover, there was no interaction between experimental phases and treatments (Repeated Measures ANOVA, $F_{(2,12)}$= 0.720, $p$=0.507).

During the post-treatment phase, the number of eggs predated did not significantly differ between untreated and control-scented eggs. Pigs that had been exposed to creosote - TBZ treatment eaten 27 (96.4%) control-scented eggs and all 28 untreated eggs in the post treatment phase. Pigs that had been exposed to creosote-LiCl treatment eaten 25 (89.2%) control-scented eggs and all 28 untreated eggs. Overall, the average proportion of eggs predated in the pre-treatment phase were similar to the average proportion of eggs eaten in the post-treatment phase, regardless of whether the eggs were scented with creosote and were treated with TBZ and LiCl.

**Table 9:** The number of fully and partly predated eggs during the treatment phase

| **Treatment:** | | **Days** | | | | | | | | | | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Creosote +** | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| **TBZ** | **partly** | 6 | 6 | 7 | 7 | 6 | 8 | 8 | 7 | 7 | 6 | 68 |
| | **fully** | 2 | 2 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 9 |
| **LiCl** | **partly** | 6 | 6 | 7 | 7 | 7 | 7 | 8 | 7 | 8 | 7 | 73 |
| | **fully** | 2 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 6 |

**b. Aftershave Treatment**

There were no significant differences in predation rates between the three experimental phases for both aftershave – TBZ and aftershave – LiCl treatments (Repeated Measures ANOVA, $F_{(2,12)}=2.00$, $p=0.18$) (Figure 5). Overall, the average proportion of eggs predated in the pre-treatment phase were similar to the average proportion of eggs eaten in the post-treatment phase, regardless of whether the eggs were scented with aftershave and were treated with TBZ and LiCl.

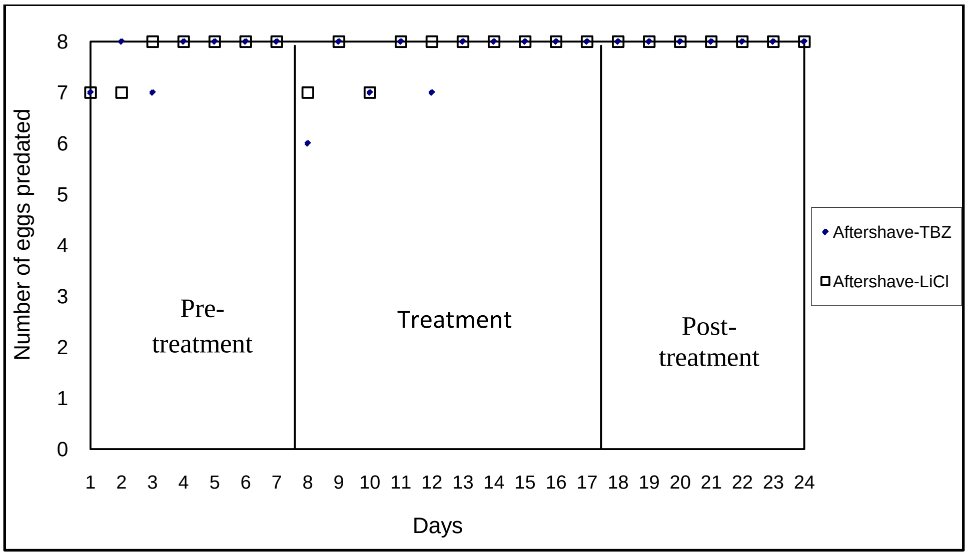

**Figure 5:** The number of eggs predated daily for aftershave-TBZ and aftershave-LiCl treatment

During the pre-treatment phase, of the total 112 untreated eggs presented to the pigs, 108 (96.4%) were eaten. During the treatment phase, 78 of 80 (97.5%) of aftershave-TBZ eggs were predated and 78 of 80 (97.5%) of aftershave-LiCl treated eggs were predated (Table 10). In addition, of the total 160 untreated eggs presented to the pigs, 158 (98.75%) were eaten.

**Table 10:** The number of eggs scented with aftershave and treated with TBZ and LiCl predated during the treatment phase

| **Treatment:** | **Days** | | | | | | | | | | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Aftershave** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | **predated** |
| **TBZ treated eggs** | 7 | 8 | 8 | 8 | 7 | 8 | 8 | 8 | 8 | 8 | 78 |
| **Untreated eggs** | 7 | 8 | 7 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 78 |
| **LiCl content eggs** | 7 | 8 | 7 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 78 |
| **Untreated eggs** | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 80 |

Once again, in contrast to the untreated eggs which pigs fully eaten, of all the scented-treated eggs that were predated, the number of partly eaten eggs was much higher than that of fully eaten eggs (Table 11). The proportion of eggs consumed in the aftershave - TBZ and aftershave - LiCl treatments did not differ significantly (Repeated Measures ANOVA, $F_{(1,6)}=0.00$, $p=1.00$). Moreover, there was no interaction between experimental phases and treatments (Repeated Measures ANOVA, $F_{(2,12)}=0.00$, $p=1.00$).

**Table 11:** The number of fully and partly predated eggs during the treatment phase

| **Treatment:** | | **Days** | | | | | | | | | | **Total** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **aftershave** | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| **TBZ treated** | **partly** | 6 | 7 | 8 | 7 | 7 | 8 | 8 | 7 | 7 | 8 | 73 |
| | **fully** | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 5 |
| **LiCl treated** | **partly** | 6 | 7 | 7 | 8 | 8 | 7 | 8 | 7 | 8 | 7 | 73 |
| | **fully** | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 5 |

During the post-treatment phase, the number of eggs predated did not significantly differ between untreated and control-scented eggs. All 224 eggs presented were eaten, regardless of whether they were control-scented or untreated.

### c. General Behavior

During Experiment 2, the general behavior of pigs was reasonably similar to that observed in Experiment 1. In the treatment phase, most of the pigs refused to fully eat treated-scented eggs. They would taste the treated-scented eggs, reject them, and move on to eat the untreated eggs. Conversely, if they approached the untreated eggs first, they would fully eat them and then move on to taste the treated-scented eggs. During feeding in this phase, the pigs followed the same eating pattern as was observed in Experiment 1. Thus, their feeding behavior suggests that as in Experiment 1, the feral pigs were detecting and showing an aversion to the TBZ and LiCl taste in the treated eggs as other pigs did in Experiment 1.

As in Experiment 1, the feral pigs were slower to approach the treated-scented eggs than the untreated eggs, and usually hesitated before breaking the treated eggs. However, they would still crack and taste the eggs. After tasting, they tended to avoid the treated eggs. Again, as in Experiment 1, the pigs eagerly ate all grain as soon as it was presented. Once again therefore, their appetite for grain food was not influenced by their aversion to the tested eggs. Similarly, there was no general change in the appearance of the subjects' excrement, and no symptoms of poisoning appeared during the treatment phase.

## 4. Discussion

### Experiment 1.

The first experiment demonstrated that feral pigs consumed similar proportions of untreated eggs during the pre-treatment and untreated plus control eggs in the post-treatment phases, and that they predated a similar proportion of eggs treated with a low and high dosage of TBZ during the treatment phase. Untreated and control eggs placed in each pen during the post-treatment period were predated immediately by the feral pigs. This behavior indicates that aversion to depredation had not been established in the feral pigs, and that aversion to the eggs' flavor had similarly not been established. Thus, in the absence of CTA in the post-treatment phase, pigs were able to 'discover' that these eggs lacked the treatment chemicals, and proceed to fully consume them.

Despite attempts to produce aversion to the eggs, this study found that CTA was not established. Ideally, predators should develop a CTA to the treated eggs, generalize that aversion to all eggs and, as a result, stop predating eggs [30]. However, as the number of eggs depredated in the post-treatment phase remained high, it can be seen that this CTA pattern did not occur in this study. Feral pigs in the high-dosage TBZ treatment reduced their consumption of treated eggs, but not significantly so. Feral pigs did not cease predation of treated eggs over days. Indeed, on the last day of the treatment phase, the proportion of predated eggs slightly increased again for both low and high TBZ. Importantly, after the treatment phase ended, egg predation returned to pre-treatment rates, indicating no long-term conditioned taste aversion occurred.

In this study, it seems likely that (1) aversion was associated with the inoculums only, and (2) pigs avoided the treated eggs after tasting them. The behavior of pigs in handling eggs treated with both chemicals indicated that they could detect TBZ and LiCl. The large number of partly predated eggs showed that pigs seemed to be able to eat around the chemicals, and perhaps consume only the untainted portions of the eggs. Due to their only partial consumption of the treated eggs, it is likely that pigs ate insufficient amounts of TBZ and LiCl to induce detectable signs of illness. Provenza and his colleagues [31] stated that a critical feature in the development of CTA is the occurrence of a nauseous feedback in the gut. Results of other studies suggest that deterrence and toxicities are intimately related via feedback mechanisms. In these experiments, treated eggs were only partly eaten and insufficient dosages of the chemicals were therefore consumed, CTA might have failed to develop because the experiment did not produce illness in the feral pigs. Even fully consumed eggs only produced one incidence of sickness. After fully consuming the treated eggs, this pig vomited but then recovered, and continued to predate treated eggs on subsequent days. Ideally, the novel taste should be quickly followed by illness, if a strong aversion is to be created to that food [32]. If illness does not ensue, conflicting messages about the value of the egg will be sent to the subject. Memory retrieval will be confused by the eggs' acceptance during pre-treatment phase and bad taste of egg during the treatment phase, thus any aversion would be weak or non-existent. This phenomenon has been referred to as learned safety or learned non-correlation [33]. Studies of other species concluded that aversions tended to be formed readily with novel foods after their total consumption was followed by illness. This observation is in accordance with the lack of symptoms observed in the feral pigs, and the fact that they produced normal feces. These factors indicate that illness did not occur.

Pigs were presumably able to detect the presence of chemicals, due to their excellent sense of taste and smell. This is not surprising for LiCl. Ziegler and his colleagues [34] questioned the use of LiCl as an oral illness producing agent in taste aversion studies due to its detectability. Moreover, Gustavson and his colleagues [29] suggested that when the concentration of LiCl in bait is high, subjects will easily recognize this, and form aversions to the flavor of the LiCl, not to the bait. But, my observations are more surprising for TBZ. In low concentrations, TBZ, which has little taste [28], cannot be easily detected by most animals, allowing the original taste of the eggs to persist. However, due to their excellent sense of smell and taste (Mitchell, *pers. comm*), the pigs appeared able to detect the TBZ in the treated eggs. Thus, taste aversion may not have developed because acquired aversions are dependent: (1) on temporal contiguity between food ingestion and toxicities [35], (2) on food novelty [36], and (3) on the amount of chemicals ingested. It is also possible that the limited period of the treatment phase might not have been sufficient for pigs to develop a taste aversion of the treated eggs. As stated by Ralphs and his colleagues , [32] taste aversion might be difficult to be generated over a short period. However, the fact that even the proportion of consumed TBZ eggs began to rise again towards the end of the treatment period in experiment one indicated that even a longer conditioning period may not have worked. In addition, as already stated, the treated eggs were not fully consumed during the treatment phase.

In summary, there are four explanations, which are not mutually exclusive, for why taste aversion did not occur in this study, and hence why pigs continued to predate the eggs [31]. First, novelty might be important. Pigs initially predated untreated eggs, which may have taught them early in the experiment that eggs are a desirable food source. Secondly, taste aversions may not have occurred in this study because the small amount of chemicals that were perhaps ingested failed to produce illness. Finally, social facilitation might be a factor. As single pens consisted only of galvanized wire cage, individual pigs may have been easily able to learn from the behavior of adjacent pigs, allowing for the easy spread of chemical avoidance behavior through the experimental population.

### Experiment 2

The second experiment yielded similar results to the first experiment, with feral pigs depredating eggs during all phases of the experiment. During the treatment phase, feral pigs did not reduce their predation of scented-treated eggs, regardless of whether the treatment chemical was TBZ or LiCl. These trends are not consistent with the outcome of Experiment 1, in which pigs predated a lower proportion of eggs during the treatment phase, especially for those treated with TBZ. This suggests that the scents were actually attractive to the pigs, overcoming the deterrent effects of the chemicals. Overall, pigs predated an average of 90% of the scented-treated eggs, although, once again, they usually only partially consumed the treated eggs. Once again, this mitigated against developing CTA to treated eggs because illness did not follow egg consumption. They also failed to learn to associate the scent with ‘bad’ eggs. Therefore, feral pigs did not develop an aversion to either scented-treated or untreated eggs.

Pigs did not readily associate external scent cues with the treated eggs. Theoretically, the predation response is a complex integration of the physiological state of the animal (degree of hunger), the flavor of the food and its nutrient value ([19]. Their prior acceptance of egg as a legitimate food apparently overwhelmed the subsequent association of the scent and bad taste. Therefore, pigs did not acquire a taste aversion or reduce their consumption of the treated eggs. It is possible that treated-scented eggs lost odor over time. Yet, this possibility appears unlikely, as the single pens were kept clean and the scent used had a strong odor which persisted throughout the experiment. Nevertheless, some pigs did seem to recognize eggs by odor. In the morning, they selectively predated untreated eggs, avoiding the treated eggs at that time. However, in the afternoon, pigs would still crack and taste the treated eggs, and pigs that ate untreated eggs did not subsequently avoid scented-treated eggs. The next day in the treatment phase, pigs started predating scented-treated eggs again. Therefore, pigs viewed scented-treated eggs as possible food, and tasted them to see if they were edible or not. The pigs’ behavior appears similar to that of the carnivores studied by Nicolaus [37], who found that some carnivorous animals may continue to break eggs or kill prey, but not eat them after conditioning. This outcome might be attributed to food preferences, the availability of food under control conditions and or/ the animals’ physiological state.

The feral pigs in the experiment 2 did not show a reduced propensity to break eggs after eating eggs treated with TBZ and LiCl. During the post-treatment phase, most pigs started eating eggs within a day. They learned that predating the untreated and control eggs was not associated with bad taste, and sampling the eggs without adverse consequences. As this experiment failed to produce CTA, pigs were able to quickly discern the absence of both chemicals in the untreated and the control eggs. Other experiments reported that while CTA may be an effective deterrent to consumption of certain foods, practical application often fails due to confounding factors. These confounding factors include, firstly, incomplete avoidance of the treated substance by conditioned subjects because subjects detected a marker in the treatment baits [38]. A second confounding factor during the experiment might be that the attempted treatment coincided with the availability of large amounts of untreated food of the same type [16]. During the treatment phase, untreated eggs were presented, and this may have conditioned the pigs to regard all eggs as legitimate food. This is a difficult problem to overcome experimentally, as some form of control is required. But perhaps it does in fact provide a good reflection of reality, as it would be difficult to ensure in the field that all pigs would never eat untreated eggs before encountering treated eggs. The two factors may have played an important role in hampering the development of CTA in these experiments.

Overall, these results do not indicate that feral pigs can be taught to avoid eggs through a taste aversion program. Several weaknesses have been identified in this study design. The pigs detected chemicals by taste. When the experiment was designed, this eventuality appeared very unlikely, and was therefore not taken into consideration. For future experiments, the problem of detectability might be overcome by inserting the chemicals into the eggs within a gel capsule, which would conceal the flavor of the chemical. This was tried prior to the experiment, but it proved difficult to insert gel capsules into the chicken eggs. A larger problem seems to be the lack of illness induced by the chemicals. But if pigs are able to detect and avoid the presence of chemicals in eggs, it may be impossible to overcome this problem with the present chemicals.

Another problem identified is that of pre-exposure. Studies with species other than feral pigs have shown that pre-exposure to a flavor interfered with the formation of flavor aversions. Conover and his colleagues [24] suggested that prior predation experience might have precluded formation of prey-killing aversions in their study. Feral pigs in this study also gained prior experience depredating eggs during the familiarization process which occurred before aversive conditioning began as is the case in a natural population of feral pigs. In addition, during the research, pigs were only presented with 70% of their usual daily intake of commercial food. Being opportunistic animals, they were willing to test anything that they had previously experienced as food. These factors may be viewed as reasons why egg avoidance was not established.

## 5. Conclusion

Experiment 1 was not successful in creating long-term aversion to egg predation. Although there was a drop in proportion of treated eggs predated during the treatment phase, this was not maintained, either in the post-treatment phase, or even towards the end of the treatment phase. Overall, at the concentrations used in these experiments, TBZ and LiCl were clearly detectable by feral pigs, and therefore aversions could not be established using these chemicals, presented in this form.

Experiment 2 also failed to support the effectiveness of creosote and aftershave as conditioning agents in eggs. Conditioned taste aversion did not develop in Experiment 2. The pigs seemed to test whether the eggs were edible or not and did not exclusively select either scented-treated or untreated eggs. There was no change in egg selection resulting from bad taste feedback. On a daily basis, there was a tendency for pigs to select untreated eggs in the morning, but they still sampled scented-treated eggs in the afternoon. These findings indicate that this model is incapable of producing aversions in feral pigs, and is unlikely to be effective in reducing their predation on wildlife eggs.

In both experiments, it is unclear how much of the chemicals were consumed during the treatment phase, but it is suspected that the amount was very low. Therefore, there was no post-ingestive feedback and the senses of taste and smell are not interrelated through affective and cognitive processes [39].

## 6. Management Implications

Overall, it seems that the prospects for using the chemicals tested in this study are poor, as there was evidence that the pigs were able to detect their taste, and selectively sample eggs for their presence. Constraints also posed by the size of the predator and size of egg. Nevertheless, CTA should not be abandoned as a technique for controlling egg predation by feral pigs. The results of this study should be considered in the development of future experiments on the possibility of using chemically-induced CTA in feral pigs. These results have improved our understanding of pig egg-selecting and feeding behavior, and the techniques used here can be used, with some modifications, to further explore the usefulness of other chemicals for the same purpose of controlling feral pig predation on wildlife eggs. Exploration should focus on alternative poisons with undetectable noxious chemicals that produce nauseous effects in the pigs.

Ideally, CTA experiment should be better replicated, both in terms of pig number and the number of eggs presented to the subjects, to fully realize the power of the repeated measures ANOVA. All this should also be combined with an extended treatment period. More generally, field data is also required, exploring the number of eggs pigs consume and their impacts on natural wildlife populations. This study demonstrates that feral pig readily consume chicken eggs yet, curiously, no quantitative figures exist on their impact on the eggs and hatchlings of wildlife, including sea turtles, though there is evidence to suggest that feral pigs have a negative impact on sea turtles' breeding success [10]). For the present, current allowable measures to control feral pig impact on sea turtle breeding success, including shooting, trapping and poisoning, remain critical whilst further work exploring the usefulness of CTA is carried out.

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