Effect of Urea Solution on *Haemonchus contortus* Inoculum from Intact Faecal Pellets or L3 Larvae

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Abstract

Internal parasites cause severe economic losses to the sheep industry globally. Of particular concern is Haemonchus contortus; a parasite widely resistant to synthetic anthelmintics and prevalent in the warm wet environments of the tropics. The ovicidal and larvicidal properties of urea solution were investigated in pot trials using Batiki grass (Ischaemum aristatum var. indicum) inoculated with either intact faecal pellets containing approximately 10,000 eggs or 10,000 infective stage larvae. Urea solution at 5% concentration was sprayed once onto grass on the day of deposition for pellets and after a 48 hour adaptation period for infective stage larvae. Foliage and soil (inclusive of faecal pellets) were sampled separately for the presence of infective stage larvae. Urea significantly reduced larval populations from foliage for both pellet (P = 0.0015) and infective stage larvae (P = 0.0034) inoculums at P = 0.05. Urea did not significantly reduce larval populations from soil of pellet inoculated pots (P = 0.4055) but did from pots inoculated with infective stage larvae (P = 0.0488). Urea did not significantly reduce cumulative larval populations in pots inoculated with intact faecal pellets (P = 0.1070) but significantly reduced cumulative larval populations from pots inoculated with infective stage larvae (P = 0.0079). Urea solution at 5% is effective in reducing infective stage larvae from foliage. Application rate is a factor in its efficacy against Haemonchus contortus.

Keywords: *Haemonchus contortus*, urea solution, Batiki

Introduction

Internal parasites are a major setback to productivity of grazing sheep and goats globally (Roeber et al., 2013) and in particular to small ruminant producers (Hepworth and Hutchens, 2006). Losses caused to the global livestock industry due to parasites are projected in the tens of billions annually (Roeber et al., 2013). A recent study in Australia revealed that *Haemonchus contortus* likely accounts for a significant percent of the 436 million Australian Dollars used by the sheep industry to annually combat nematodes (Lane et al., 2015).

Haemonchosis is widespread among sheep and goat flocks particularly in warm humid environments (Burke, 2005; Leite-Browning, 2006). Historically *H. contortus* was restricted to more tropical environments; however, its occurrence is becoming evident in temperate regions (Jones, 2014; Troell et al.,

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2005). As such, *H. contortus* is of economic importance to sheep farming worldwide (Silva et al., 2011; Troell et al., 2005).

A study in Samoa concluded that *Haemonchus* spp. is among the most common intestinal parasites in goats (Petaia, 1989). Furthermore, due to the high egg counts found, Petaia (1989) suggests *H. contortus* is the predominant species. No data is available on the economic cost of haemonchosis or other internal parasites in Samoa. Macfarlane (1998) suggested that successful sheep rearing in the Southwest Pacific is reliant on the effective use of anthelmintics for the control of internal parasites.

Though there are multiple approaches to internal parasite management, drenching animals with synthetic anthelmintics is most frequently used. Alarmingly, for many years it has been apparent that the use of synthetic anthelmintics is failing (Echevarria et al., 1996; Vercruysse and Claerebout, 2016; Waller, 1997). Consequently, there is growing concern about internal parasite resistance to widely used anthelmintics.

Multiple management techniques can reduce the incidence of parasite resistance and simultaneously improve small ruminant productivity. The implementation of an integrated parasite management programme includes good pasture management and rotational grazing systems, selection for genetic resistance among and between small ruminant breeds, and biological control of internal parasites (Kumar et al., 2013, Shalaby, 2013).

One intriguing avenue of investigation is the use of urea as a larvicide against the infective stage of *H. contortus*. Urea is a source of nitrogen for plants. It is the most popular form of nitrogen fertilizer worldwide (Bareja, 2013). Liquid urea at varying concentrations inhibited egg hatching and larval development of *H. contortus* in vitro (Iqbal et al., 2000). Similarly, Howell et al. (1999) found that urea, ammonium nitrate and liquid nitrogen fertilizer showed significant larvicidal properties against L_3 stage of *H. contortus* in vitro. Urea, in combination with phosphorus and potassium fertilizers has been shown to reduce *H. contortus* larval populations in plot trials (Roul et al., 2017). Urea is generally broadcast onto pastures but can be applied in foliar form. Urea has been successfully sprayed on plants up to a concentration of 10% without inducing phytotoxicity (Eichert & Fernández, 2012).

Field work on the toxic effect of urea on parasitic eggs on plants and sludge has been conducted. Prabhaker et al. (1999) evaluated the effect of urea on *Bemisia argentifolii* on cotton seeds and concluded that it was effective in reducing oviposition, but this reduction was not observed for soil application. In addition, urea was determined ineffective against immature *B. argentifolii* on seeds and soil application. Another study used urea to successfully inactivate eggs of *Ascaris suum*, a common parasitic nematode of pigs, in sludge (Katakam et al., 2014).

With ruminants being a key staple of agriculture worldwide, and the growing ineffectiveness of available anthelmintics, it is apparent that research of promising new anthelmintics is of utmost importance (FAO, 2011). The objectives of this study were to evaluate the efficacy of urea, applied in foliar form, as a larvicide against *Haemonchus contortus* eggs and larvae in artificially inoculated pasture under semi-controlled environment in Samoa.

Materials and Methods

Location and Pot Preparation

Urea, at 5% concentration, was chosen to test the hypothesis. Prior in vitro trials were conducted indicating this concentration completely inhibited egg hatch from intact faecal pellets. Batiki grass was selected due to its predominance in the region.

Pots (top diameter 26.0 cm, height 16.5 cm) were filled with 6.5 kg of soil. Soil was obtained from a field that had not been grazed by neither sheep nor goats (S 13° 51' 37.1", W 171° 47' 35.4") with an elevation of 86 meters above sea level. The soil was thoroughly mixed to ensure homogeneity of soil properties. Soil was sterilized using a steam soil sterilizer to ensure it was free of H. contortus. The temperature was set at 88 °C for two six-hour cycles. The soil was moistened prior to placing in the sterilizer and the sterilizer was covered for the duration of the cycle. It was allowed to cool for 48 hours prior to filling the pots.

Planting Material

Batiki (Ischaemum aristatum var. indicum) was established in pure stands in pots. Plants were obtained from a field not previously grazed by sheep or goats. The plants were collected once the dew had evaporated to further reduce the chance of nematodes. Planting material was washed in tap water then triple rinsed with distilled water. Planting material was soaked in 0.5% sodium hypochlorite for ten minutes to eliminate potential nematodes. Plants were then triple rinsed in distilled water. A composite sample was examined for nematodes using the Baermann technique (Taylor et al., 2016). Twenty pots were planted with seven stolons each having an average of seven to eight nodes. Each stolon was planted 1.25 cm deep. Each pot received 1 L of water on the day of planting and kept at 15 to 20% moisture thereafter using a portable soil moisture meter (Weather Monitor TZS Series).

Efficacy of Urea Against H. Contortus Eggs in Faecal Pellets or Larvae (L3) in Artificially Inoculated Pots

Ten pots, each containing approximately 10,000 eggs, were inoculated with intact faecal pellets. Fresh faecal pellets were obtained from one naturally infected hogget. A faecal egg count was performed using a modified McMaster technique. Prior to faecal pellet deposition the grass was clipped at a height of 10 cm to simulate grazing. Faecal pellets were deposited in the centre of the pots. On the day of faecal deposition five randomly selected pots were treated with 7 ml of urea solution (5%) equivalent to 30.3 kg N per hectare. The remaining pots received no urea treatment but were misted with 7 ml water. All pots were misted daily using a 1 L spray bottle between 6:30 and 7:00 A.M. to simulate night rainfall or heavy dew. Pots were misted with the same volume of water at a height of 5 cm above the swards.

Soil moisture content was monitored using the soil probe (Weather Monitor TZS Series) and maintained at 15 to 20%. Ambient temperature and relative humidity were recorded daily using a digital thermometer (Traceable ® Fisher Scientific). Ten days post treatment pots were destructively sampled by clipping foliage at ground level, weighing and placing in a Baermann tray for nematode extraction.

Faecal pellets and surrounding soil to a depth of 2 cm was collected. Sample was homogenized by gentle mixing and 200 grams, inclusive of faecal pellets, was weighed and placed in a Baermann tray for extraction.

A further ten pots were inoculated with 10,000 L3 larvae each. Larvae were pipetted into pots in concentric circles at the centre of the pot. Pots were misted daily as mentioned above. The larvae were given a 48 hour adaptation period after which five randomly selected pots were treated with foliar urea and the remaining pots were not. The treated pots were sprayed two hours post misting. Urea was applied as mentioned above. The soil moisture and temperature as well as the ambient temperature and relative humidity were recorded as above.

All pots were destructively sampled 48 hours post treatment. Foliage was clipped at ground level, weighed and placed in a Baermann tray for extraction. Nematodes were extracted and H. contortus were identified and enumerated.

Soil from a 10 cm radius from inoculation site and 2 cm deep was collected and placed in a pre-labelled Ziploc bag. The sample was homogenized by gentle manual mixing and 200 grams were used for nematode extraction in a Baermann tray. Nematodes were extracted and H. contortus were identified and enumerated.

Experimental Design and Statistical Analyses

The experimental design was a completely randomized design and data were analysed by ANOVA and student t-test using STAR statistical package.

Results

The total larvae recovered in 0% urea solution was significantly higher (P=0.0015) than that from 5% in pots inoculated with faecal pellets. The recovery rate expressed as a percentage of larval recovery of H. contortus L3 from Batiki foliage was significantly reduced by treatment with 5% urea solution in comparison to the control (Figure 1). Though the recovery rate for control was low (2.66%, N = 5) urea reduced the recovery rate further to 0.5% (N = 5) representing a 432.00% reduction in larval population from foliage.

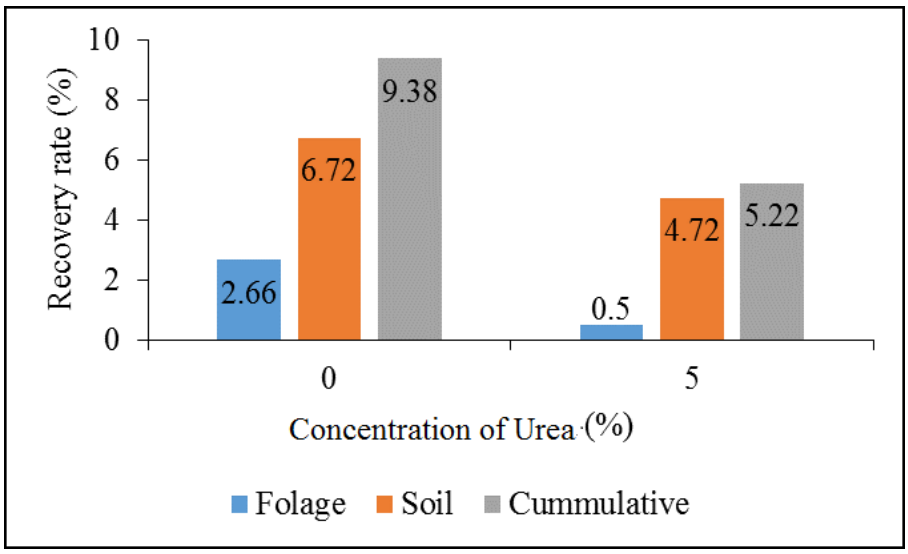

Figure 1. Recovery rate of Haemonchus contortus larvae from faecal sample inoculated pots.

|The recovery rate of L3 from soil in pots inoculated with pellets showed a 42% reduction in the mean number of L3 recovered in treatment versus control (Figure 1). However, the reduction in recovery rate between treatment and control pots was not significant (P = 0.4055).

The cumulative number of larvae recovered from pots inoculated with faecal pellets showed a 79% decrease in comparison to control (Figure 1). However, results indicate that the difference of total larvae recovered between control and treatment was not significant (P = 0.1070).|A recovery rate of 2.83% and 0.39% was obtained from foliage inoculated with L3 treatment and control pots respectively (Figure 2). This represents 625.42% fewer larvae in the treatment pots compared to the control pots and is significant (P = 0.0034).

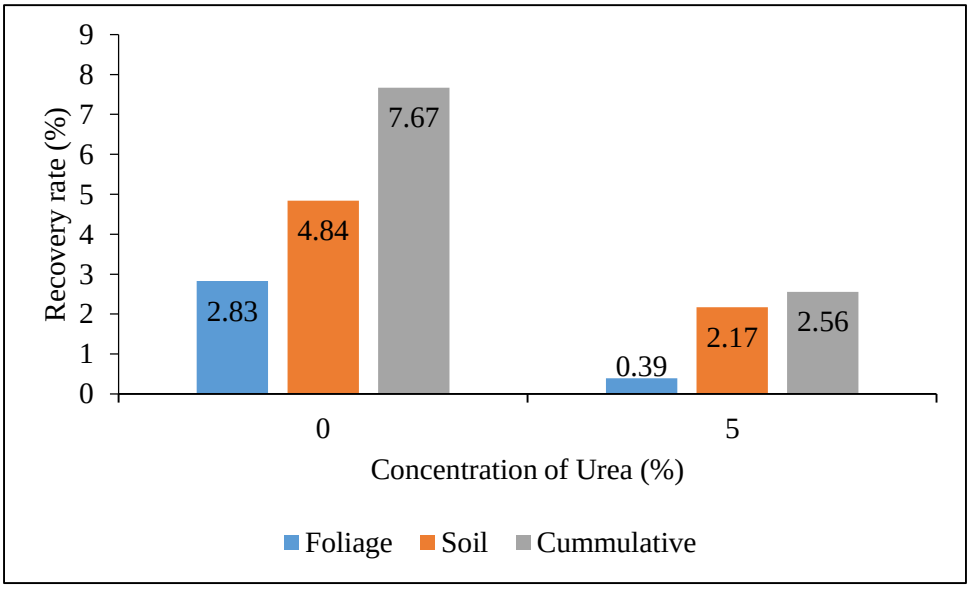

Figure 2. Recovery rate of Haemonchus contortus larvae from pots inoculated with L3 larvae.

Larval recovery from soil of pots inoculated with L3 was 2.17% and 4.84% for treatment and control respectively (Figure 2). This represents a 123.04% reduction of larvae found in soil of treatment pots in comparison to control pots. The difference between treatment and control was shown to be significant (P = 0.0488).|The cumulative recovery rate of 7.67% and 2.56% for control and treatment respectively is presented in Figure 2. This difference on recovery rate represents 199.61% fewer larvae overall in the treatment versus control pots; proving to be significant (P = 0.0079).

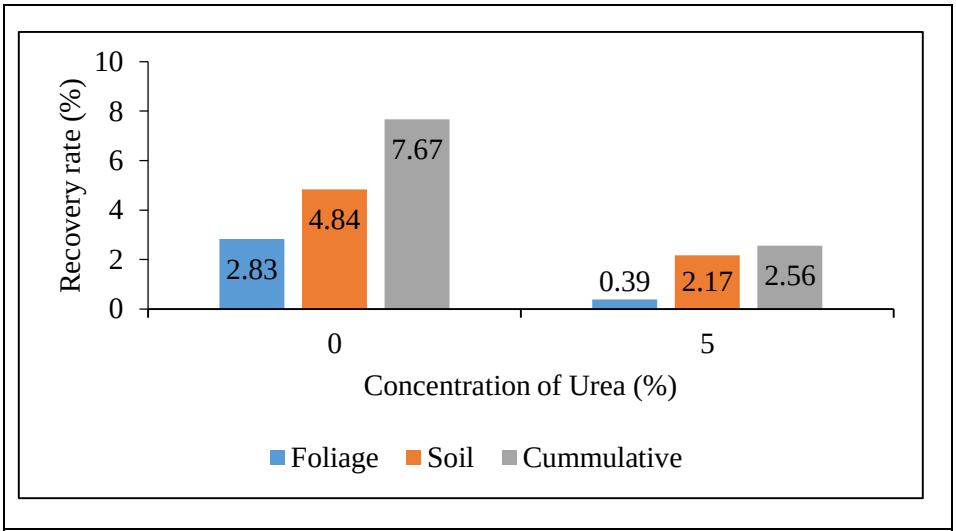

Figure 3. Soil moisture and temperature, ambient temperature and relative humidity during pot trial.

Discussion

Efficacy of Foliar Urea Against Haemonchus Contortus Egg Hatching from Intact Sheep Faecal Pellets

Urea had a deleterious effect on H. contortus, although urea was found to be less effective against the pellet than against the larval form. It is possible that larvae emerged from the faecal pellet but failed to migrate to foliage due to a change in pH immediately surrounding the faecal pellets. Iqbal et al. (2000) suggested that a change in the pH of soil may be deleterious to larval development as well. Another cause of fewer larvae recovered from the foliage may be a residual effect of urea on the swards. Since the grass was misted daily it is unlikely there was urea residue on foliage however urea residue was not measured. Consequently, it is not known if the potential toxic effect of urea on the swards caused fewer larvae to remain viable. A study in which plots were seeded with H. contortus eggs and subsequently treated with a complete fertilizer (urea, single super phosphate and muriate of potash) reported a significant reduction of L3 recovered from herbage (Roul et al., 2017). Given the more complex nature of a complete fertilizer versus urea it is difficult to draw comparisons. However, the authors suggest that the observed developmental inhibition was due to the efficaciousness of the increased application rates.

Fewer H. contortus larvae were recovered from soil treated with urea. It is plausible that upon hatching, larvae remained in the pellet or emerged from the faecal pellet but remained in the soil where a higher moisture content compared to the sward was favourable for their survival. The environment at the soil level in the pots provided ideal moisture and adequate shade for the free living larvae given that H. contortus is known to survive as long as three months under such conditions (Taylor et al., 2016). Rainfall and free water have been proposed as a requirement for larval emergence from faecal pellets (van Dijk and Morgan, 2011; Wang et al., 2014). In contrast, Khadijah et al. (2013) propose that rainfall is not a requirement but soil moisture (> 20%) promotes larval development. However, larval emergence is also influenced by the moisture content of the pellet given that partially desiccated and encrusted pellets require more than a single shower to induce emergence (Wang et al., 2014). Since experimental conditions of daily misting and adequate soil moisture were maintained throughout, it suggests that larvae had ideal conditions to emerge from pellets (Figure 3). These conditions are consistent with findings by Wang et al. (2014) who suggest that regular light rainfall on moist pellets promote larval emergence.

The cumulative data for foliage and soil recovery rates is provided in Figure 3. Although there was a difference between treatment and control, no significant difference was found. This is due to the high number of larvae from the soil of treatment pots. Though no significance of cumulative larval populations between treatment and control in pellet inoculated pots was found, the arithmetic reduction is noteworthy. Overall there were 78.54% fewer larvae in the treatment pots versus the control pots. Therefore, if a sustained reduction of larvae on the swards can be achieved by consecutive foliar applications it is within reason to speculate that parasitic burdens can be reduced over time. Furthermore, the larval population on foliage was 432% fewer in treatment pots in comparison to control pots. This elevated reduction of larvae present on the swards is an important consideration given that sheep and goats ingest L3 as they graze (Donald et al., 1978; Santos et al., 2012). It is therefore plausible to predict a lower infectivity rate of H. contortus from Batiki pasture treated with 5% foliar urea.

Efficacy of foliar urea against Haemonchus contortus larvae (L3) in artificially inoculated pots

The recovery rate from treated foliage in pots inoculated with pellets was 0.5% versus 0.39% from L3 inoculated pots. Given that both sets of pots received the same volume of water during misting the availability or lack of water for larval migration cannot account for the difference. Plants were misted to facilitate larval migration up the swards. Literature on water as a requirement for larval migration up swards is indecisive. Amaradasa et al. (2009) and Santos et al. (2012) found evidence indicating water, in the form of rainfall or humidity, increases larval migration up swards. On the other hand, van Dijk and Morgan (2011) suggest that free water is not necessary for trichostrongyloid of sheep larvae to migrate from soil onto pasture. Furthermore, van Dijk and Morgan (2011) suggested that rainfall induces larval emergence from pellets and as a result high larval populations are observed on pasture following rainfall. Khadijah et al. (2013) suggested that soil moisture greater than 20% suffices for egg hatch and subsequent larval migration up pasture. Considering that a water film on the swards, relative humidity and soil moisture were adequate (Figure 3) for larval migration a lack of moisture is unlikely to have negatively affected the larvae's ability to migrate up the swards. What might account for the difference is the absence of pellets in pots inoculated with larvae. As such, larvae lacked the protective feature of pellets. The inoculation with intact pellets more closely resembles field conditions where larvae can emerge when environmental conditions are ideal. Given that a portion of the larval population tends to remain in the pellets, multiple foliar applications can target larvae as they migrate up the swards. These applications must coincide with adequate temperature and moisture to enhance optimal migration up swards.

Environmental temperature also influences egg hatch, larval development and migration (Jasmer et al., 1987, O'Connor et al., 2006, Santos et al., 2012). Haemonchus contortus eggs are known to hatch after exposure to very low temperatures (Jasmer et al., 1987) but may also hatch at temperatures as high as 37 °C with the optimal being 26 °C (Ashad et al., 2011). The mean environmental temperature throughout this experiment was 28.8 °C. Considering minimal fluctuations were recorded it is likely that the environmental temperature was adequate for H. contortus survival.

Unlike the pots inoculated with pellets there was a significant ($P = 0.0488$) difference between the treatment and control of L3 inoculated pots. The fact that an adaptation period (48 hours) was allowed would suggest that even larvae on the bottom strata of grass and in the dense mat were adversely affected by the urea. It can be argued that once larvae are no longer harboured within the pellet they are more susceptible to fluctuations in temperature and moisture (O'Connor et al., 2006). Alternatively, the reduced larval recovery rate may be due to the potential change in pH provoked by the urea (Iqbal et al., 2000).

A number of studies on ovine nematodes have yielded consistently low recovery rates (Amaradasa et al., 2009; Carneiro and Amarante; 2008, Santos et al., 2012). Amaradasa et al. (2009) reported a recovery rate between 0.05 to 1% of the larvae seeded on pasture. The low recovery rate is attributed to potential factors including soil moisture at time of seeding, soil texture and composition. Similarly, Santos et al. (2012) had recovery rates generally below 1%. These authors suggest that the low recovery rates may be due to depleted lipid reserves in larvae exposed to "desiccation-revival" cycles induced by night fluctuations in pasture humidity. Furthermore, exposure to solar radiation may also account for larval mortality (van Dijk et al., 2009). Again this is likely not a factor in the present study since it was conducted in a screen house. Though the present study reports higher recovery rates it is likely that any combination of the above mentioned factors may have reduced the larval population. However, since all pots were exposed to similar conditions it is unlikely the reduced viability of larvae influenced the disparity between control and treatment.

As a result of lower recovery rates in foliage and soil from treatment pots the cumulative recovery rate was significant ($P = 0.0079$). The control pots had a recovery rate of 7.67 % compared to 2.56% in the treatment. Overall, there were 199.61% fewer larvae in treatment pots versus control pots.

Conclusions

Urea significantly reduced larvae from foliage in both trials using 5% concentration. It had no significant deleterious effect on larvae populations from soil inoculated with intact faecal pellets but significantly reduced larvae from soil inoculated with infective stage larvae. Urea can therefore be sprayed on pastures at 5% concentration to reduce the populations of infective larvae.

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