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Effect of Urea on the Development and Survival of *Haemonchus contortus* Eggs and Larvae

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ABSTRACT

Effect of urea on the development and survival of *Haemonchus contortus* eggs and larvae was investigated with an objective to workout its feasibility for control on pastures/farm premises. Urea solutions delayed hatching and development of eggs of *Haemonchus contortus* at lower concentrations (2.5 to 5.0%) and proved lethal at higher (>5-10%) concentrations. Therefore, urea may be used on farm premises or on pastures in 5-10 % concentrations for the control of Haemonchosis. However, further studies are suggested for standardisation of rate and mode of application of urea.

Key Words: *Haemonchus contortus*; Urea; Survival; Development

INTRODUCTION

The prevalence of *Haemonchus (H.) contortus* in sheep has been reported very high (25.1 to 92%) by many workers in Pakistan (Durrani *et al*., 1981; Mohiuddin *et al*., 1984; Khan *et al.,* 1989; Iqbal *et al*., 1993; Qayyum, 1996). The estimated production losses amount to Rs. 31.43 million per annum in sheep and goats slaughtered at Faisalabad abattoir (Pakistan) (Iqbal *et al.,* 1993). Major effects of parasite on indigenous animals are severe anaemia along with other haematological and biochemical disturbances (Rasool *et al.,*1995; Iqbal *et al.,*1998). Although effective control can be achieved by treating the Haemonchus infected animals using anthelmintics, yet development of resistance in parasites against anthelmintics and chemical residual/toxicity problems limit their scope (Kaemmerer & Butenkotter, 1973; Waller, 1987; Van Wky & Malan, 1988). Likewise, immunologically maneuvered vaccination strategies have also been partially successful due to antigen complexity and non-availability of required antigens in sufficient quantity. Such problems complemented with low education and limited purchasing power of people in developing countries necessitate exploring alternate approaches for Haemonchus control. One of the alternate strategies may be inhibiting/killing the Haemonchus on the pasture to break its life cycle. This paper describes the effect of urea on the development and survival of *H. contortus* eggs and larvae.

MATERIALS AND METHODS

Collection of adult *H. contortus.* Abomasa of sheep, slaughtered at Faisalabad abattoir were collected, incised longitudinally and examined for the presence of adult *H. contortus* (Maqsood *et al.,* 1996). The worms present in ingesta or attached to the abomasal epithelium were picked manually using artery forceps and placed in a bottle containing PBS (pH 7.2). Female worms were separated from males by grossly witnessing the blood filled intestine spirally coiled around white ovary giving an appearance of barber's pole worm. The female worms were washed thrice in PBS (pH 7.2).

Extraction of *H. contortus* eggs. The worms were transferred to 0.9% normal saline solution, incubated at 37°C for 24 hours and ova laid by them were collected by sedimenting them using slow centrifugation. Inoculi containing 25,000 eggs in 10 ml of distilled deionised water were prepared for further use.

Preparation of medium for culture. The culture medium was prepared by mixing sheep faeces, soil and supernatant derived from ovine gastric contents in 25 g: 25 g: 8 mL ratio as follows: The faeces of sheep were broken up finely, using a large pestle and mortar, mixed with equal quantity of soil (low porosity sandy loam) and sterilised at 15 lb pressure 121°C using an autoclave. The ovine gastric contents were obtained from slaughtered sheep. The pH of the gastric contents was measured electrometrically (2.7) immediately with standard buffers. The contents were filtered through gauze, centrifuged and the clear supernatant sterilized by filtration (0.2 um, Millipore). The supernatant fluid from sheep was stored at –20°C before use.

Experiment. The culture medium was added to 16 culture dishes, each inoculated with 25,000 eggs of *H. contortus* in 10 mL water and assigned to control (distilled water), and four concentrations (each having three replicates) of urea as follows:

A1= Distilled water (control); A2= 2.5% urea solution spray; A3= 2.5% urea solution spray; A4= 2.5% urea solution spray; B1= Distilled water (control); B2= 5% urea solution spray; B3= 5% urea solution spray; B4=

5% urea solution spray; C1= Distilled water (control); C2= 7.5% urea solution spray; C3= 7.5% urea solution spray; C4= 7.5% urea solution spray; D1= Distilled water (control); D2= 10% urea solution spray; D3= 10% urea solution spray; D4= 10% urea solution spray

The cultures were stirred gently daily, covered with lids and incubated at 27°C (relative humidity 75%) for eight days. Three aliquots each of one gram medium from different places of each culture dish were taken randomly at alternate days up to eight days of culture. The aliquots were dissolved in distilled deionised water and eggs/larvae were counted using McMaster counting chamber (Soulsby, 1982). The average of all counts from each culture dish for different days and concentrations were tabulated.

RESULTS AND DISCUSSION

In 2.5% urea concentration, the egg hatching was observed on day 2 post-treatment (PT) when L1 were identified from the culture aliquots. The percentage of hatched L1 was, however, much lower in urea treated groups compared with control (70%) on day 2 PT (Table I). Rare eggs hatched in 5% (Table II); whereas, no hatching of Haemonchus eggs took place on higher concentrations i.e. 7.5 and 10.0% (Table III and IV). L2 and L3 started appearing in cultures (2.5% urea treated) from day 4 and 6 PT, respectively; whereas, in control L2 and L3 started appearing in cultures from day 2 and 4 PT, respectively (Table I). It was found that urea in addition to causing reduction in the number of hatched eggs, also delayed hatching of eggs and moulting from L2 to L3. This conclusion was further strengthened when hatching of the eggs was totally stopped in higher concentrations of urea. The eggs of *H. contortus* hatched to L1, L2 and L3 in 2.5% urea solution. However, the per cent hatching and subsequent moulting to L2 and L3 was lower as compared to control. It was found that only 6.4% eggs hatched and consequently developed to L3 by day 8 PT compared with 49.2% in control group (Table I).

Table I. Effect of 2.5% urea solution on hatching, moulting and survival of eggs and larvae of *Haemonchus contortus*

Days PT	2.5 % Urea (Control)			
	Egg/gm	L_1	L_2	L_3
0	500 (500)	–	–	–
2	340 (65)	11.2 (70.0)	– (5.6)	–
4	128 (R)	16.0 (39.6)	6.8 (45.2)	– (19.2)
6	R (–)	8.4 (10.4)	10.8 (6.4)	5.6 (36.8)
8	– (–)	R (–)	6.4 (R)	6.4 (49.2)

PT= Post-treatment; R= < 20 eggs or larvae; Figures in parenthesis indicate per cent values of control (distilled water treated) group

Table II. Effect of 5% urea solution on hatching, moulting and survival of eggs and larvae of *Haemonchus contortus*

Days PT	5.0 % Urea (Control)			
	Eggs/gm	L_1	L_2	L_3
0	500 (500)	– (–)	– (–)	– (–)
2	R (78)	– (68.4)	– (5.2)	– (–)
4	R (R)	– (48.4)	– (43.6)	– (11.2)
6	– (–)	– (R)	– (7.2)	– (42.2)
8	– (–)	– (–)	– (–)	– (45.2)

PT= Post-treatment; R= < 20 eggs or larvae; Figures in parenthesis indicate per cent values of control (distilled water treated) group

Table III. Effect of 7.5% urea solution on hatching, moulting and survival of eggs and larvae of *Haemonchus contortus*

Days PT	7.5 % Urea (Control)			
	Eggs/gm	L_1	L_2	L_3
0	500 (500)	– (–)	– (–)	– (–)
2	– (68)	– (64.4)	– (9.6)	– (–)
4	– (R)	– (38.4)	– (59.6)	– (15.6)
6	– (–)	– (R)	– (6.4)	– (47.6)
8	– (–)	– (–)	– (–)	– (53.2)

PT= Post-treatment; R= < 20 eggs or larvae; Figures in parenthesis indicate per cent values of control (distilled water treated) group

Table IV. Effect of 10% urea solution on hatching, moulting and survival of eggs and larvae of *Haemonchus contortus*

Days PT	10 % Urea (Control)			
	Eggs/gm	L_1	L_2	L_3
0	500 (500)	– (–)	– (–)	– (–)
2	– (46)	– (69.6)	– (4.4)	– (–)
4	– (R)	– (40.8)	– (45.6)	– (19.2)
6	– (–)	– (R)	– (14.8)	– (41.6)
8	– (–)	– (–)	– (–)	– (46.0)

PT= Post-treatment; R= < 20 eggs or larvae; Figures in parenthesis indicate per cent values of control (distilled water treated) group

The effect of urea on development of larvae of *H. contortus*, Ostertagia spp., Trichostrongylus spp. and *Chabertia ovina* was also reported by Helle *et al.* (1989). They reported that larval development was completely blocked in cultures sprinkled either with sheep urine, with solutions of 2 or 4% urea or with urine from which urea or the phenol components had been extracted. Only a few third-stage larvae developed in cultures sprinkled with 1% urea. Normal larval development occurred in cultures sprinkled with water, including one culture where there was urine in the space between the outer and inner beaker used for cultivation. It is suggested that the inhibitory effect of urine on larval development is mainly caused by ammonia produced when urinary urea is brought into contact with urease of faecal origin. It is, however, unclear why urine, from which urea had been removed, also inhibited larval development.

The delayed or blocked hatching of *H. contortus* eggs may be attributed to the affect of urea on pH of the

soil media not favorable for hatching or development of larvae. A consistent increasing trend in soil pH was observed as the hydrolysis proceeded; after that the samples with complete hydrolysis of urea showed a decrease in pH. Detrimental effects of urea on parasitic forms as found in this study, have also been reported for plants at higher doses of urea. The adverse effects of urea fertilizer have also been reported on seed germination, seedling growth and early plant growth in soil (Cummins & Parks, 1961; Gasser, 1964; Goyal & Huffaker, 1984). Studies to account for these effect have given divergent results and several explanations have been advanced for each effect (Wilkinson & Ohlorgge, 1960; Goyal & Huffaker, 1984). For example studies to account for the adverse effect of urea on seed germination have suggested that it is due to the high pH or the high concentration of ammonium ions resulting from hydrolysis of urea fertilizer by soil urease (Widdowson *et al.,* 1960; Court *et al.,* 1964a, 1964b). Therefore, the adverse effects of urea on development and survival of *H. contortus* eggs and larvae may be attributed to similar reasons.

CONCLUSIONS

Urea solutions delayed hatching and development of eggs of *H. contortus* at lower concentrations and proved lethal at higher (>5-10%) concentrations. Therefore, urea may be used on farm premises or on pastures in 5-10% concentrations for the control of Haemonchosis. However, further studies are suggested for standardisation of rate and mode of application of urea.

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