

## EDUCATION AND PRODUCTION

### Retention of Larvicidal Activity After Feeding Cyromazine (Larvadex®) for the Initial 20 Weeks of Life of Single Comb White Leghorn Layers<sup>1</sup>

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(Received for publication November 26, 1990)

**ABSTRACT** Single Comb White Leghorn pullets were fed cyromazine (Larvadex®) continuously at levels of 0, 25, 250, and 1,000 mg/kg diet (ppm) from hatch to 20 wk of age. Fresh manure was bioassayed for toxicity to housefly, *Musca domestica*, larvae beginning at the 6th wk after removal of cyromazine from the feed, and at weekly intervals thereafter.

At 6 wk after removal of the feed additive there was 51.6% fly mortality at 25 ppm, 75.7% at 250 ppm, and 86.5% at 1,000 ppm relative to the 0-ppm control. Fly mortality decreased to less than 10.7% mortality at 13 and 15 wk postremoval for hens grown on 25 ppm and 250 ppm cyromazine, respectively. Hens grown on 1,000 ppm cyromazine produced manure that was still exhibiting more than 50% fly mortality 20 wk after removal of the feed additive. These data demonstrate retention of cyromazine in laying hens for up to 20 wk after feeding the chemical to the birds at 5 to 200 times greater than the maximum recommended rate for the initial 20 wk of life.

(Key words: cyromazine, larvicide, commercial layers, fly control, body retention)

1991 Poultry Science 70:1873-1875

#### INTRODUCTION

Fly control is a major concern in poultry production (Axtell, 1986; Axtell and Arends, 1990). Cyromazine or CGA-72662 (N-cyclopropyl-1, 3, 5-triazine-2, 4, 6-triamine), is marketed for fly control as a poultry feed additive under the trade name of Larvadex®.<sup>4</sup> This compound is effective as a feed additive at levels of 5 mg/kg (ppm) or lower (Miller and Corley, 1980; Miller *et al.*, 1981; Axtell and Edwards, 1983).

Cyromazine has no observable effects on the reproductive performance of broiler breeders (Brake *et al.*, 1984), commercial laying hens (Brake *et al.*, 1985), or turkey breeders (Brake *et al.*, 1989) when fed at levels of 250

ppm or lower. When fed at levels of 1, 5, 10, and 50 ppm for 3 wk to White Leghorn laying hens, no detectable residual levels were found in the liver, fat, or muscle after returning to untreated feed for 1 wk (Cecil *et al.*, 1981). Miller and Corley (1980) reported detectable levels (100 ppb) of cyromazine in eggs but not liver and muscle when White Leghorn hens were fed 2.5 ppm for 5 wk. These investigators also found that feeding levels of cyromazine of 12.5, 25, and 125 ppm produced a dose-related increase in residues in eggs and detectable levels in liver and muscle, as well as feces. The objective of the present study was to determine how long after being fed cyromazine for the first 20 wk of life would laying hens produce feces that possessed significant larvicidal activity.

#### MATERIALS AND METHODS

Chicks of a commercial laying hen strain (Hy-Line® W-77) were obtained at 1 day of age and reared in floor pens as described by Brake *et al.* (1985). Cyromazine was added at 0, 25, 250, and 1,000 ppm to basal starter and grower diets and fed through 20 wk of age. Cyromazine was not included in the layer diet

<sup>1</sup>The research in the publication was funded in part by the North Carolina Agricultural Research Service. Trade names used in this publication do not imply endorsement of the products mentioned nor criticism of similar products not mentioned.

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TABLE 1. Survival of housefly larvae in manure collected from chickens for 6 to 20 wk after feeding cyromazine for the initial 20 wk of life

Week after treatment ended	Cyromazine (mg/kg) <sup>1</sup>			
	0	25	250	1,000
	— Mean number adult flies —			
6	33.3 <sup>a</sup>	16.1 <sup>b</sup>	8.1 <sup>b</sup>	4.5 <sup>b</sup>
7	34.7 <sup>a</sup>	20.4 <sup>b</sup>	2.7 <sup>b</sup>	3.1 <sup>b</sup>
8	31.0 <sup>a</sup>	24.4 <sup>a</sup>	20.0 <sup>a</sup>	1.9 <sup>b</sup>
9	32.7 <sup>a</sup>	27.4 <sup>a</sup>	24.7 <sup>a</sup>	12.0 <sup>b</sup>
10	35.6 <sup>a</sup>	26.9 <sup>b</sup>	25.1 <sup>b</sup>	13.7 <sup>c</sup>
11	36.0 <sup>a</sup>	27.9 <sup>a</sup>	27.7 <sup>a</sup>	14.2 <sup>b</sup>
12	35.5 <sup>a</sup>	31.2 <sup>a</sup>	25.7 <sup>a</sup>	12.2 <sup>b</sup>
13	37.4 <sup>a</sup>	33.4 <sup>ab</sup>	25.4 <sup>b</sup>	1.9 <sup>c</sup>
14	31.0 <sup>a</sup>	32.6 <sup>a</sup>	27.1 <sup>a</sup>	8.9 <sup>b</sup>
15	34.7 <sup>a</sup>	32.5 <sup>a</sup>	31.0 <sup>a</sup>	5.2 <sup>b</sup>
17	37.6 <sup>a</sup>	37.5 <sup>a</sup>	34.6 <sup>a</sup>	13.1 <sup>b</sup>
18	34.7 <sup>a</sup>	35.1 <sup>a</sup>	33.0 <sup>a</sup>	1.9 <sup>b</sup>
19	37.5 <sup>a</sup>	36.5 <sup>a</sup>	36.9 <sup>a</sup>	15.3 <sup>b</sup>
20	36.9 <sup>a</sup>	34.6 <sup>a</sup>	37.2 <sup>a</sup>	9.9 <sup>b</sup>

<sup>a-c</sup>Means within a row with no common superscripts were significantly different ( $P \leq .05$ , Tukey's test [SAS Institute, 1982]).

<sup>1</sup> $n = 8$ .

that was fed after 20 wk of age. All diets met or exceeded the National Research Council (1984) requirements as reported earlier (Brake *et al.*, 1985). A portion of the pullets were moved to laying cages and evaluated for possible reproductive effects of the long-term feeding. The remaining pullets were moved to another facility for use in another experiment.

When it was observed that the fresh manure of the second group of hens would not support the growth of fly larvae, the hens were sorted by wing-band number into the four original treatment groups: 0, 25, 250, and 1,000 ppm. There were 10 birds, held 2 per cage, in each of eight groups per treatment beginning at 25 wk of age. The birds had not received cyromazine for 5 wk at this time. The birds were given *ad libitum* access to water and feed in the presence of a 15 h light:9 h dark lighting cycle.

Manure samples were taken from the birds for the first time when the birds were 26 wk old and the feed additive had been withdrawn for 6 wk. Sampling was repeated at weekly intervals with the last sample taken when the feed additive had been removed from the birds for 20 wk. Manure was collected weekly for a 2-day period from each of the eight groups of birds for each treatment by catching the fresh

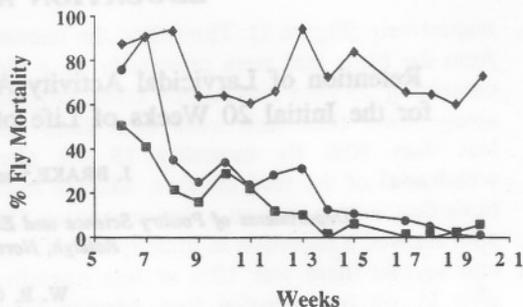


FIGURE 1. Percentage mortality (corrected for control mortality by Abbott's formula) of house fly larvae in manure from chickens fed cyromazine at 25 (■), 250 (●), and 1,000 (◆) mg/kg (ppm) in the diet for the first 20 wk of life. Weeks shown represent the number of weeks after cyromazine was removed from the feed.

droppings on plastic sheets spread beneath the cage with new plastic used at each sampling time. The accumulated manure under each cage was mixed and frozen for 24 h after which an aliquot (150 g) was placed in a cup (355 mL). To each cup of manure 50 eggs of the house fly (*Musca domestica*) were added and a screen lid attached. The house flies were collected in North Carolina at a poultry farm and had been in culture for 2 mo at the beginning of this experiment.

The cups of manure were held until house fly adults emerged and died, at which time the number of adult flies per cup were counted. Holding conditions were 22 to 26 C and 10 to 14 days were allowed for total fly emergence before counting.

Data regarding fly emergence were subjected to a one-way ANOVA and Tukey's test to separate means (SAS Institute, 1982) on a weekly basis. Statements of statistical difference are based on  $P < .05$ .

## RESULTS AND DISCUSSION

The numbers of house fly adults produced from the manure from the treated and control birds are shown in Table 1. The percentage mortalities in the treatments were corrected for nonspecific mortality by comparison to control mortalities using Abbott's formula (Abbott, 1925), and presented graphically in Figure 1.

At 6 wk following withdrawal of cyromazine from the feed, there was 51.6% fly mortality at the 25 ppm, 75.7% at 250 ppm, and 86.5% at 1,000 ppm pullet treatments,

respectively (Figure 1). Thereafter, the manure from the birds that were previously on a diet containing 25 ppm cyromazine killed progressively fewer flies each week until there was less than 10% fly mortality 13 wk after withdrawal of the feed additive. Manure from birds that were reared on a diet containing 250 ppm showed a reduction in toxicity at a slower rate so that there was 10% or less mortality after 15 wk on untreated feed. Manure from birds reared on the diet containing 1,000 ppm cyromazine remained highly toxic (more than 50% fly mortality) to house fly development for the entire 20 wk production period.

Birds fed relatively high concentrations of cyromazine for the first 20 wk of age will continue to pass feces that are toxic to fly development for many weeks after being placed on untreated feed. The duration of this residual toxicity was related to the concentration of cyromazine in the diet during the treatment period. At 25 ppm some fly toxicity occurred 13 wk after removal of the additive from the diet suggesting that there was some retention of the chemical in the tissues and organs of the birds, and that gradual release of the chemical (or its metabolites) occurred subsequently. There was longer retention of fly toxicity in the manure at higher concentrations of cyromazine in the growing diets. Although it is tempting to attribute all of this retention to dietary intake, the fact that the pullets were reared in litter-covered floor pens may have contributed to recycling of cyromazine from the litter back into the birds. The results may have been different had the pullets been reared in cages.

The current recommended use of cyromazine for fly control is 1 to 5 ppm in the feed. This creates a wide safety margin for poultry reproduction because levels of 250 ppm or lower have been reported to have no observable effect on broiler breeders or progeny (Brake *et al.*, 1984), commercial laying hens (Brake *et al.*, 1985), or turkey breeders (Brake *et al.*, 1989).

The present data suggest that there is retention of some larvicidal activity in commercial laying pullets for many weeks following removal of cyromazine when fed levels 5 to 200 times greater than the minimum recommended level during the growing period.

The manner in which this activity is retained is unclear, as there is no apparent effect on the bird. However, this may partially explain why satisfactory fly control may be observed in poultry operations where cyromazine is included in the diet on alternate weeks.

#### ACKNOWLEDGMENT

This project was funded in part by Ciba-Geigy Corporation, Greensboro, NC 27419.

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