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Effects of Feeding Diflubenzuron to Young Male Holstein Cattle[1]

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Diflubenzuron, an insect growth regulator that interferes with the formation of insect cuticle (MULDER & GIJSWIJT 1973), is active against a variety of insect pests (ELINGS & DIEPERINK 1974). Specifically MILLER (1974) and others (WRIGHT 1975, BARKER & NEWTON 1976, BARKER & JONES 1976) have shown that diflubenzuron is active against manure breeding flies when fed to cattle at a rate of 0.5 mg or less/kg body wt per day. However, the only information concerning residues of diflubenzuron in cattle was obtained by MILLER et al. (1976) who reported that diflubenzuron was not detectable (<0.02 ppm) in milk of cows fed up to 8 mg diflubenzuron/kg body wt per day. When fed at 16 mg/kg body wt per day 0.02 ppm was detected in the milk, and a maximum of 0.25 ppm was found in the tissues.

In the present experiment diflubenzuron was fed to growing dairy bulls at a rate of 2.8 or 1 mg/kg body wt per day to determine tissue residues and to study the effect of diflubenzuron on growth rate, serum testosterone, reproductive characteristics, and organ histopathology. Thus, the amount fed was ca. 2-6 times more than needed for control of manure breeding flies and greatly in excess of the amount cattle could be expected to obtain from feed byproducts of crops treated with diflubenzuron to control insects such as the boll weevil.

MATERIALS AND METHODS

Four pairs of Holstein bull calves (including two sets of twins) were used. Animals that were not twins had similar birthdates. Table 1 identifies the bulls and the time frame of the study.

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TABLE 1. Date on Experimental Bulls

Animal no.	Diet	Birthdate	Slaughter date
1	Control	2/15/76	6/29/76
2	Diflubenzuron	2/04/76	6/29/76
3	Control	3/12/76	3/01/77
4	Diflubenzuron	3/17/76	3/01/77
5[a]	Control	2/28/76	9/21/77
6[a]	Diflubenzuron	2/28/76	9/21/77
7[a]	Control	3/01/76	9/21/77
8[a]	Diflubenzuron	3/01/76	9/21/77

[a]Twins.

For the first 4 wk of life the bulls were fed whole milk from open pails at 8% of body weight per day. At 4 wk, the milk was reduced by 0.25 kg per day until the calves were weaned completely. Starting at 3 days of age a calf starter (Concentrate 1, Table 2) and alfalfa hay were offered. The bulls continued on this diet (Concentrate 1 fed at a rate of 2.8% of body weight per day and 1 kg of alfalfa hay) until October, 1976. After this time the bulls were fed Concentrate 2 (Table 2) at a rate of 1% of body weight per day and alfalfa hay ad libitum. Ingredients of the two concentrate rations are given in Table 2.

The concentrates (both 1 and 2) for the treated animals contained 100 ppm of diflubenzuron.

The bulls were weighed weekly until October 1976 and twice monthly thereafter. Changes in body weight were calculated by regression techniques.

On ca. 1300 hr on September 16, November 3, December 27, 1976, and February 25, 1977 blood samples were drawn via jugular puncture from the 6 animals still on the experiment. The blood was held overnight in a refrigerator and centrifuged. The serum was pipetted off and stored frozen at -20° C until analyzed for testosterone.

Serum testosterone was measured by radioimmunoassay by using a commercially-available testosterone RIA kit (Testosterone[^{3}H] Radioimmunoassay Pak, New England Nuclear). Duplicate samples of 0.5 mL serum were extracted with 7 mL petroleum ether-ethyl acetate (1:1) for 1 hr with shaking at room temperature. An aliquot of the ethereal layer was dried under a gentle air stream. The residue was dissolved in buffer and assayed without chromatographic purification. The antiserum (New England Nuclear lot # PG387) does not cross-react significantly with other steroids except dihydrotestosterone (DHT, cross reactivity, 56%); thus, some of the testosterone measured may have been DHT. Free and bound

TABLE 2. Composition of Concentrates (%)

Ingredients	Concentrate 1	Concentrate 2
Cracked corn	34.3	40.2
Corn gluten feed		17.9
Middlings		17.9
Whole oats	14.7	13.4
Soybean meal, 44%	14.7	
Wheat bran	14.7	
Linseed meal	9.8	
Alfalfa meal	4.9	
Blackstrap molasses	4.9	8.9
Iodized salt	1.0	0.9
Meat scraps	1.0	
Dicalcium phosphate		0.9
Vitamin A	.02	

testosterone were separated by using dextran-coated charcoal, and the activity of the bound fraction was counted. A linear standard curve was calculated by logit transformation. Coefficient of variation between assays was 9.8%.

On September 13, 1977, semen was collected from the 2 sets of twins by using a standard artificial vagina when the bulls mounted a teaser cow. The fresh semen from each bull was examined by phase contrast microscopy. Also the undiluted semen was stored frozen at -5° C until December 1978, when the sperm concentration was assessed by the hemocytometer method described by HERMAN & MADDEN (1974).

The animals were slaughtered and autopsied on the dates indicated in Table 1. Samples of kidney, liver, lung, and spleen were stored in formalin for histopathological examination; samples of liver, kidney, muscle, omental fat, and subcutaneous fat were stored frozen for residue analyses. These analyses were conducted as previously described (MILLER et al. 1976).

RESULTS AND DISCUSSION

Since the concentration of diflubenzuron in the concentrate portion of the diet remained constant throughout the experiment, the amount fed was 2.8 mg/kg body weight per day until October 1, 1976 (average age 208 days), and 1.0 mg/kg body weight per day thereafter.

Regression analyses of the body weight data showed that the control animals gained an average of 995 g per day and that the treated animals gained an average of 983 g per day. This difference was not significant at the 5% level of probability.

There were no significant ($P > 0.05$, two-way analysis of variance) differences in serum testosterone concentrations between control and treated animals (Table 3). At a particular sampling time the serum testosterone concentration of one twin was very similar to his twin

mate. However, the testosterone concentrations between animals in the non-twin pair and between sets of twins varied considerably.

TABLE 3. Serum Testosterone of Control and Diflubenzuron-fed Bulls

Animal no.	Diet	Date sampled 9/16/76	11/3/76	12/27/76	2/25/77
		ng/mL serum			
3	Control	1.05	0.82	9.56	1.04
4	Diflubenzuron	5.85	1.35	0.99	13.90
5[a]	Control	9.40	3.80	5.43	4.30
6[a]	Diflubenzuron	11.01	5.30	3.91	1.41
7[a]	Control	6.34	1.16	6.58	1.44
8[a]	Diflubenzuron	5.76	0.58	6.49	1.44

[a]Twins.

Other workers have shown that the plasma levels of testosterone in prepubertal and pubertal bulls to fluctuate in a pronounced rhythm throughout the day (KARG et al. 1976, SCHAMS et al. 1978). With a single sampling on each sampling day, we cannot distinguish the pattern for individual bulls, but during the 4 sampling dates, each bull had a serum testosterone concentration equivalent to or exceeding the maximum concentration reported for 11-mo-old bulls (SCHAMS et al. 1978, RAWLINGS et al. 1972).

Diflubenzuron had no apparent effect on libido, as both treated and control bulls mounted the teaser cow without hesitation. All semen samples collected appeared to have normal color and density. Although sperm concentration was not determined at the time the semen was collected, microscopic examinations revealed good progressive motility in all samples and no evidence of an excessive number of abnormal sperm. Sperm concentrations determined after 15 mo of storage of the undiluted semen were as follows: 0.52, 0.92, 1.17, and 0.40 billion/mL for bulls 5, 6, 7, and 8, respectively. These values are lower than the 1.7 billion/mL reported by HAHN et al. (1969) for bulls of comparable age. However, semen was collected only once in our study, and the sperm counts, made on samples stored in a freezer without cryoprotectant or any other treatment for 15 mo, cannot be considered reliable indicators of the ability of the bulls to produce sperm. However, there was no apparent difference between control and diflubenzuron-fed bulls.

No abnormalities were found at autopsy in either the control or treated animals. Histopathological examination of liver, lung, kidney, and spleen tissues revealed no significant difference between the treated and control bulls.

Diflubenzuron was not detected (<0.02 ppm) in any of the tissue samples analyzed from any of the 4 treated bulls with the exception of bull 2, which was slaughtered at 5 mo of age. This bull had 0.02 ppm diflubenzuron in the liver and kidney, 0.04 ppm in the subcutaneous fat, and 0.08 ppm in the renal and omental fat. No (<0.02 ppm) diflubenzuron was found in the muscle of bull 2. Although the other 3 treated bulls were fed diflubenzuron for 8-14 mo longer than bull 2, they were fed only 1 mg diflubenzuron/kg body weight per day for the last 6-12 mo of their lives. In contrast, bull 2 was fed 2.8 mg diflubenzuron/kg body weight per day up until the time of slaughter when the animal was ca. 5 mo old. The distribution of residues was generally what we (MILLER et al. 1976) reported previously, except that the kidneys of bull 2 contained diflubenzuron, which we did not find in animals in our earlier study.

Our results indicate that dairy bull calves can consume on the order of 1 mg diflubenzuron/kg body weight daily without acquiring any resultant tissue residues. Growth and organ histopathology were also normal. Although no cows were inseminated with semen from the bulls in this study, there would appear to be no reason to suspect effects on reproduction. Semen volume, sperm concentration, serum testosterone and libido did not appear to be abnormally reduced after diflubenzuron was fed.

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