

EVALUATION OF BIPYRIDYLIUM HERBICIDES RESIDUES IN MEAT.

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New proposed use of paraquat as bloat preventive, on pastures where species of Fabaceae are dominant, has made necessary the study of its residues in animal products. Three trials were carried out. Experiment 1: During 101 days pastures were sprayed with paraquat at 56 g/Ha and steers were admitted to the area 48 hours after spraying. At the end of the period 16 animals that had grazed cured pasture were sacrificed. Samples of kidney, liver, meat and fat were taken and analyzed for paraquat. No residues were detected by a method capable of detecting 0.05 ppm. Experiment 2 was performed with 150 days of grazing, the dose used to treat alfalfa 48 hours before grazing was 50 g/Ha. At sacrifice, samples from 8 animals fed with grasses containing paraquat residues were taken. No residues above the method sensibility were found. In experiment 3, dairy cows were allowed to graze alfalfa treated with paraquat at 50 g/Ha and diquat at 100 g/Ha 48 hours after spraying. Each treatment had 4 animals. Milk samples were taken during 15 days every other day. Paraquat residues over the method sensibility (0.0001 ppm) was detected just in milk from one animal (0.00042 ppm) the first day of grazing. Diquat residues above method capability (0.001) were not found. The results obtained showed that Bipyridylum herbicides don't originate residues in animal food above Codex LMRs, when used at low dose on pastures in order to prevent bloat in grazing animals.

Introduction

Bloat is cause of important economic losses in cattle production systems based on pastures where species of Fabaceae (Leguminoseae) are dominant, mainly alfalfa cultivars without winter dormancy.

Paraquat and diquat are the common names for 1 - 1' - dimethyl - 4 - 4' bipyridylum and 1 - 1' - ethylene - 2 - 2' bipyridylum ion, manufactured as dichloride, sulphate or dibromide are the active ingredients of Gramoxone and Reglone herbicides, world wide used as harvest aids or desiccants for grains and fodder crops. In Argentina use of paraquat and diquat has been authorized for some cultures and seed production alfalfa with the restriction of no grazing the stubbles before 30 days after the herbicide application.

Paraquat and diquat were evaluated by the FAO/WHO Joint Expert Committee on Pesticide Residues (CCPR) in several opportunities (1970, 1972, 1976, 1977, 1978, 1981, 1982, 1985, 1986) and the following Maximum Residue Limits (MRL) were set for cattle products; meat and by-products (except kidney): 0.05 p.p.m. kidney: 0.5 p.p.m. milk: 0.01 p.p.m.

Studies have been done on the metabolism of bipyridylum herbicides, mainly on paraquat, and on residue occurrence in meat, meat by-products and milk. Calderbank et al (1965), Daniel et al (1971) and Edwards et al (1974) have proved that oral doses of paraquat are excreted principally in the feces as unchanged paraquat. Hemingway et al (1974) informed that when cows were given a single oral dose of 14- C-methyl paraquat at 8 mg/Kg during three days, only 0.003 - 0.004 % of the radioactivity was recovered in milk and the maximum radioactive residue (0.005 p.p.m. ion equivalents) occurred on the day after the first dose. Calderbank et al (1965) reported that after consuming for fourteen days herbage containing an average of 300 p.p.m. of paraquat on a dry weight basis, cows were returned to unsprayed pasture and slaughtered

after three days detecting paraquat residues just in kidney (0.03 p.p.m.).

Recently, in Argentina, experimental use of paraquat on pastures at low dose, 48 hours before grazing, showed good results regarding animal health and weight gain, however there was no information available on food residue occurrence when used the conditions proposed for herbicides bipyridylum as bloat preventives, to uncertain the safety of this practice this study was made.

Material and Methods.

Experimental

First trial - Beef cattle: The trial was performed in a cattle farm located in the Humid Pampa (Buenos Aires province) during the 1990 spring - summer period, when the incidence of bloat is greater. One dominating Fabaceae pasture in its second year was divided in two in order to have a control plot. In each plot subdivisions were made with the purpose of using rotational grazing. Aberdeen Angus steers with an average initial weight of 302 Kg were divided in two homogeneous groups according to age and weight. During 101 days in one plot pastures were sprayed with paraquat at 56 g/Ha, 48 hours before steers were admitted for grazing. At the end of the period sixteen animals that had grazed cured pasture and four grazing the untreated one, were sacrificed at an average weight of 358 Kg. Samples of kidney, liver, meat and fat were taken for paraquat residues analysis.

Second trial - Beef cattle: It was carried out in INTA Marcos Juarez Experimental Station, located in the Humid Pampa (Cordoba province) during the spring-summer of 1990 using twenty Aberdeen Angus steers. The pasture, alfalfa in its second year of implantation, was divided in two plots (12 Ha each) one served as control, and the other was treated with paraquat at 50 g/Ha, 48 hours before grazing all along five months. Rotational grazing was used. One group of eight Aberdeen Angus steers receiving cured pastures and another group of four, acting as control were sacrificed after 150 days of grazing. Samples of kidney, liver, meat and fat were taken for residue analysis. From that time the remaining animals stayed in their respective plots but no more treatments with paraquat were done. After 30 days four steers from the treated group were sacrificed and the same samples of tissues obtained.

Third trial - Dairy cattle: The experiment was performed at INTA Rafaela Experimental Station, located in Santa Fé province in a prevailing dairy farms zone during November and December 1991. One alfalfa pasture was so divided to evaluate two treatments disposing of a control area and using rotational grazing. The treatments were Paraquat 50 g/Ha and Diquat 100g/Ha 48 hours before grazing. Eight primiparous Holstein dairy cows in late lactation averaging 18 Kg/milk/day were selected taking account of milk production and time post-partum. Herbage allowance was more than 25 Kg/dm/day cow, (value considered no consumption limiting) without any supplementation provided Samples of 4 lt. from a pool of morning and afternoon milkings of each cow were taken, as auto control samples before the herbicide residues intake began, time 0, and 1, 3, 5, 7, 9, 11, 13, and 15 days after the grazing of cured alfalfa commenced. Alfalfa control and alfalfa cured samples at 48 hours were taken in order to know the animal intake of herbicide residues. Additional samples of paraquat and diquat treated alfalfa were taken 7, 11 and 15 days after the treatment in order to know the herbicides depletion in pastures.

Methods of analysis.

Paraquat and diquat residues were determined using the method of Calderbank (1965), that after different prior treatments according to the sample retains the paraquat or diquat and some of the naturally occurring constituents with cation exchange resin. Washing of the so formed column is followed by elution of paraquat

or diquat residues. A portion of the effluent is treated with sodium dithionite in alkali, this reduces paraquat and diquat to a free radical whose light absorption are measured with a spectrophotometer at 396 and 380 nm respectively. Some modifications were introduced to the original method by ICI Plant Protection Limited Research Station and Meat Technology Institut as follows:

Pastures: After the acid digestion, 5 g of decolorizing carbon were added.

Milk: An aliquot of 2000 ml of milk was mixed with 5 g of a cation exchange resin using a roller, 2 hours, at 40-50 rpm 2 hours. The resin was allowed to settle during 2 hours and 1500 ml of milk were decanted off.

After centrifugation, 20 minutes, at 1400 rpm, 350 ml were decanted-off and the milk remaining washed twice with 50 ml of de-ionised water. The resin was suspended in 50 ml of de-ionised water and poured-out in a burette forming a column. After washing with 200 ml of ammonium chloride 2,5% (w/v), paraquat or diquat were eluted with 50 ml of saturated ammonium chloride solution. The absorbance was measured using 100 mm cuvettes.

Tissues. These were analysed according to the ICI method of Kennedy (1986).

All samples were analyzed in duplicate and average results are reported. Taking in account recoveries.

Results and Discussion.

Method performance was evaluated for detection limits (D.L) of paraquat and diquat in each matrix taking as acceptable certainty as instrumental signal to noise ratio greater than 5:1. Recovery experiments were carried out by adding known amounts of paraquat or diquat to blank samples, coefficients of variation (C.V.) were calculated. Results are given in table 1.

TABLE 1. LIMITS OF DETECTION AND RECOVERIES FOR PARAQUAT AND DIQUAT RESIDUES ANALYSIS

MATRIX	G OF SAMPLE	HERBICIDE	CONCENTRATION OF ADDED HERBICIDE IN THE SAMPLE	% RECOVERY		DL p.p.m.
				n = 5	C.V.	
KIDNEY	25	Paraquat	0,5	85,3	5	0.05
LIVER	25	Paraquat	0,1	74,5	4	0.05
FAT	25	Paraquat	0,1	90,5	2	0.05
MEAT	25	Paraquat	0,1	94,2	2	0.05
ALFALFA	20	Paraquat	3	80,6	3	0.08
MILK	2000	Paraquat	0,01	86,5	4	0.0001
ALFALFA	20	Diquat	3	79,9	4	0.1
MILK	2000	Diquat	0,01	83,4	5	0.001

Paraquat residues in tissues above method sensitivity could not be detected in any of the trials in agreement with previous work of Calderbank (1968) who using doses of 453 g/ACRE found negligible residues in tissues.

Analysis of milk could detect paraquat residues above 0.0001 p.p.m. just in one sample obtained after one day of grazing at 0.00042 p.p.m. very small value compared to milk MRL (0.01 p.p.m.), however it is necessary to call the attention over the fact that milk MRL was set considering 0.01 p.p.m. as D.L. for paraquat and in this work, greater sensitivity was achieved increasing sample size and using 100 mm cuvettes.

Alfalfa samples taken in third trial were analyzed for paraquat and diquat residues, results are shown in table 2 and figure 1. It can be seen more rapid depletion for diquat residues, this confirmed previous

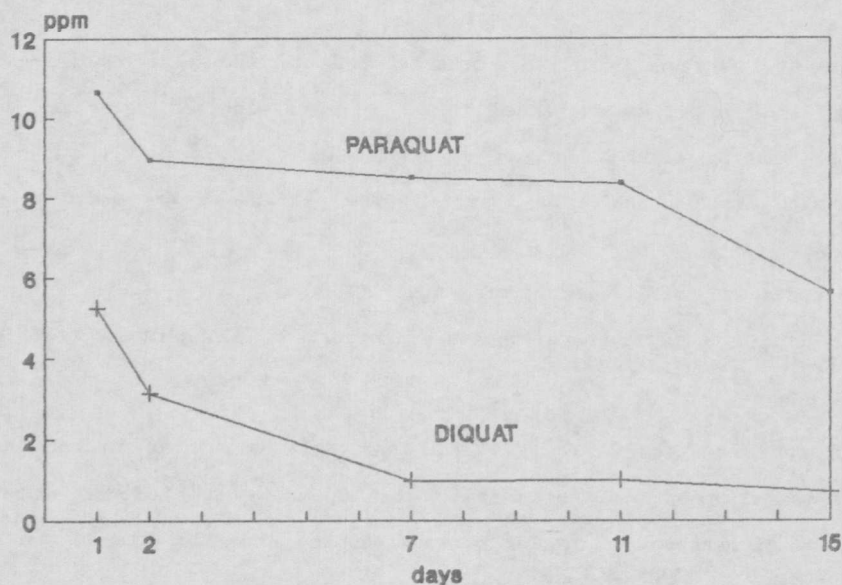
TABLE 2 - TRIAL 3. PARAQUAT AND DIQUAT RESIDUES IN ALFALFA DRY MATTER BASIS

DAYS AFTER TREATMENT	1	2	7	11	15
PARAQUAT p.p.m.	10.66	8.96	8.50	8.36	5.61
DIQUAT p.p.m.	5.25	3.14	0.98	0.99	0.71

information provided by I.C.I. reason why double diquat dose was used. Concentration of both herbicides attained a plateau after a great initial decrease. It was rather surprising not to detect paraquat residues

in milk samples with increasing time of grazing but this agree with the work of Hemingway (1974) who using ^{14}C paraquat orally (at higher dose than in our work) found that levels of radioactivity did not increase with multiple dosing.

FIGURE 1
PARAQUAT AND DIQUAT ALFALFA RESIDUES



Conclusion

Results obtained using bipyridylum herbicides on pastures as bloat preventive, suggest that should be no hazard to humans of consuming beef products and milk with bipyridylum residues above MRL,s.

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