

## STUDY ON THE EFFECT OF SOME INSECTICIDES ON FORAGE CULTURES AND THEIR INFLUENCES IN THE HOMEOSTASIS IN RUMEN ANIMALS

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**INTRODUCTION:** Insecticides are compounds of different physical and chemical properties. The toxicity and specific biological effect, as well as the metabolic study of any insecticide preparation intends to investigate metabolism, distribution, retention, accumulation, Soil-Leaching, translocation from soil plant-product to consumer (animal or human being). The product which has been got out of treated plant must to be hygienic clean from any residues of Pesticide. The presence of residues of the insecticide or its metabolites in the forage cultures may affect on the homeostasis of the rumen animals, although they don't show any clinical symptoms (3.5).

The aim of the present work is to investigate the character of the degradation products of insecticides Dursban 4EK, Tiodan 35EK and Sumi-alpha 5EK in some forage cultures. The intensive insecticides effect under the conditions of the regions and their upon treated forages on the homeostasis of animals fed with such fodders.

In additional, we have tried to test the effect of the oral administration of same insecticider to animals demonstrating the indices characterized homeostasis.

### Materials and methods

The field experiment has been carried out on Lucerne, barely and wheat to study the degradation of Dursban 4EK (chloririfusethyl), Tiodan 35EK (endosulfan) and Sumi-alpha 5EK (Esfenvalerate)

Lucerne was treated before 20 days from the first swathing with 5 replications of 100 m<sup>2</sup>. Wheat, and barely had been treated at the phetophase milky-was ripeness. Dursban was sprayed at the dose 150 cm<sup>3</sup>/dec., Tiodan – 300 cm<sup>3</sup>/dec.

Tests had been taken at 1, 5, 10, 20 and 30 day after treating. Dynamics of degradation was determined by gas-shromatography (MNZ-1985).

The effect of residues and metabolites of Dursban 4EK and Tiodan 35EK in forages treated with them and the dosing (per os) have been studied in sheep of starazagora breed of falf-fine wool. All animals were shosen of about same weight and age and kept in same condition, fed with ration consisted.

Lucerne 1,5 kg, barely 150 g., wheat 150 g. and salt 7.0g. By mean of dry matter 1,25 the ration contained 0,95 nutritional units, 0,64 digestible protein, calcium – 18,0, phosphor – 4,0g, and carotin – 31 mg.

The animals were divided into 2 groups. The first was fed with Lucerne, barely and wheat which had been treated with the up mentioned insecticides, meanwhile the second group was divided into 6 sup-groups of 4 animals in each. The first and second were administered per os Dursban 4EK at dose 10 and 20 mg./kg.b.w. respectively, the third and fourth with Tiodan at the dose 3,5 and 7 mg/kg. b.w. The fifth and sixth with sumi-alpha 5 and 10 mg/kg. b.w. All animals in the 6 sup-groups were fed with untreated fodder. Blood was collected from the jugular vein from all animals

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Tab. 1. The residues of Tiodan 35EK (isomers and metabolites) in the green mass of lucerne, mg/kg.

days after treatment	Tiodan 35EK (isomers and metabolites)				
	total	isomer $\alpha$	isomer $\beta$	metabolit endosulfan sulfat	metabolite endosulfan lacton
10	1.038	0.391	0.448	0.199	*
20	0.289	0.047	0.038	0.203	*
30	0.034	0.034	*	*	*

Tab. 2. The residues of Dursban 4EK in wheat-ear and stems, mg/kg.

The tests	Days after treatment				
	1	5	12	20	30
Ears (fon)	0.038	–	–	–	–
Ears	0.448	0.623	0.451	0.569	*
Grains	–	–	–	–	0.023
Grains covers	–	–	–	–	0.238
Steam (Fon)	0.017	–	–	–	–
Steam	0.493	0.648	0.201	0.714	0.076

\* – Unsenscable by the determining method

Tab. 3. Residues of Tiodan 5EK in barely-ears and stems mg/kg.

days after treatment	ears				stems			
	isomer		endosulfan		isomer		endosulfan	
	$\alpha$	$\beta$	sulfat	lacton	$\alpha$	$\beta$	sulfat	lacton
	0.025	*	*	*	0.016	*	*	*
1	0.068	*	*	*	0.018	*	*	*
5	0.301	*	*	*	0.659	0.010	*	*
12	0.288	*	*	*	0.357	0.003	*	*
20	0.020	*	0.021	*		*	0.059	*
					0.0214			
30	0.281	0.621	0.225	*	0.084	0.495	0.369	*

\* – Unsenscable by the determining method

of the second group as control tests before administration with the insecticides which was held at 10 a.m. after morning feeding. Then after 2 hours blood was withdrawn.

Blood glucose, total protein and lipids were determined as the methods and chemicals pro-

vided by Huma-Lab, Multitrakt, Bulgaria. The activities of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), lactate dehydrogenase (LDH) and cholinestrase were determined as methods described by Baoringer, monoamino oxidase (MAO) by the method described by

Tab. 4. Residues of Tiodan 35EK (isomers and metabolites) in wheat mg/kg.

days after treatment	ears				stems			
	isomer		endosulfan		isomer		endosulfan	
	$\alpha$	$\beta$	sulfat	lacton	$\alpha$	$\beta$	sulfat	lacton
	0.007	*	*	*	0.032	*	*	*
1	1.492	*	*	*	0.321	*	*	*
5	0.694	*	*	*	0.310	0.003	*	*
12	0.388	*	*	*	0.380	*	*	*
20	0.004	0.002	*	*	0.022	*	0.120	*
30					0.144	0.150	0.140	*
Grains	0.101	0.151	*	*				
grain								
covers	0.190	0.288	0.034	*				

\* – Unsenseable by the determining method

Tab. 5. The effect of Tiodan 35EK following oral dosing

Indexes	Tiodan 35EK		
	Control	3.5 mg./kg.	7 mg./kg.
Total protein g/L	88.0 ± 4.88	88.9 ± 3.25	72.5 ± 3.5 <sup>+</sup>
Total lipids G/L	3.28 ± 0.18	1.97 ± 0.06 <sup>++</sup>	1.05 ± 0.04 <sup>++</sup>
Blood glucose mg%	19.3 ± 0.18	66.6 ± 1.45 <sup>++</sup>	72.1 ± <sup>++</sup>
MAO mmol/s/L	196.1 ± 28.2	298.8 ± 11.44	324.9 ± 13.05 <sup>++</sup>
LDH mmol/s/L	4.2 ± 0.08	6.2 ± 0.03 <sup>++</sup>	6.8 ± 0.02 <sup>++</sup>
ASAT nmol/s/L	22.4 ± 1.42	42.9 ± 1.55	38.9 ± 1.13 <sup>++</sup>
ALAT nmol/s/L	324.8 ± 15.3	456.6 ± 17.44	398.7 ± 13.48 <sup>++</sup>

note: The control n = 12.2 experimental groups of n = 4

+ P 0.05

++ P 0.01

+p < 0.05

Krastev (1988). Obtained results stastically were analyzed as the method of Student.

## Results and discussion.

### The residues in forage

In table 1 are established the results of Tiodan 35EK and its degradation residues in the green mass of Lucerne. It is shown that after 10 days from sprinkle the residues began to redu-

ce, total as well as the isomers (alpha-beta), degradation metabolite endosulfan sulfat increased as shown at the 20 day and the endosulfan lacton haven't been discovered by the detirming method.

The residues of Dursban 4EK in the ears and stems of wheat as shown in table 2 at 12-20 days after sprinkle, are comparatively high. The higher levels of residuers were found in grain covers.

12 days after spraying Tiodan 35EK to barely (tab. 3), the alpha isomer residues in the ears of

Tab. 6. The effect of Dursban 4EK following oral dosing

Indexes	Dursban		
	Control	10 mg/kg	20 mg/kg
Total protein g/L	88.0 ± 4.88	85.1 ± 4.76	72.1 ± 3.22 <sup>++</sup>
Total lipids g/L	3.28 ± 0.18	1.26 ± 0.03 <sup>++</sup>	1.27 ± 0.02 <sup>++</sup>
Blood glucose mg%	19.3 ± 0.98	88.26 ± 2.35 <sup>++</sup>	93.2 ± 3.98 <sup>++</sup>
MAO nmol/100/ml	196.0 ± 28.2	246.2 ± 12.53	301.9 ± 9.48 <sup>++</sup>
ASAT nmol/S/L	22.4 ± 1.32	44.6 ± 1.15 <sup>++</sup>	42.1 ± 1.69 <sup>++</sup>
ALAT nmol/S/L	324.8 ± 15.30	486.1 ± 12.44	444.8 ± 11.23 <sup>++</sup>
Cholinestrase	17.3 ± 0.09	6.24 ± 0.03 <sup>++</sup>	4.38 ± 0.02 <sup>++</sup>
mic. mol/S/L			

note: Control n = 12,2 experimental groups of n = 4

+p < 0.05

++p < 0.01

Tab. 7. The effect of Sumi - alpha following oral dosing

Indexes	Sumi - alpha		
	Control	5 mg/kg	10 mg/kg
Total protein g/L	88.0 ± 1.88	96.5 ± 2.34	81.5 ± 2.62
Total lipids g/L	3.28 ± 0.18	1.82 ± 0.25	1.42 ± 0.35
Blood glucose mg%	19.3 ± 0.18	82.8 ± 0.85	72.8 ± 0.71
MAO nmol/100/ml	196.1 ± 28.2	348 ± 19.15	225 ± 23.51
ASAT nmol/S/L	22.4 ± 1.48	62.8 ± 3.32	68.1 ± 2.98
ALAT nmol/S/L	324.8 ± 55.30	398.2 ± 30.50	412.3 ± 34.51

Tab. 8. The effect of treated forages with Tiodan 35EK and Dursban 4EK following feeding to sheep

Indexes	Control	Tiodan 35 EK	Dursban 4 EK
Total orotein g/L	6.82 ± 0.32	7.09 ± 0.17	6.97 ± 0.33
Blood glucose mg%	42.98 ± 2.24	48.5 ± 2.63 <sup>+</sup>	62.3 ± 0.33 <sup>++</sup>
Total lipids g/L	1.65 ± 0.07	1.22 ± 0.04 <sup>+</sup>	1.32 ± 0.02 <sup>++</sup>
MAO nmol/100/ml	24.8 ± 1.06	29.8 ± 1.09	34.2 ± 1.44 <sup>++</sup>
ASAT nmol/S/L	314.3 ± 59.8	298.7 ± 23.9	332.1 ±
ALAT nmol/S/L	4.32 ± 0.09	5.98 ± 0.14	6.22 ± 0.19 <sup>+</sup>
Cholinestrase mic. mol/S/L	18.4 ± 0.08	-	12.3 ± 0.06 <sup>+</sup>

note: Control n = 18,2 experimental groups of n = 8

+p < 0.05

++p < 0.01

the plant significantly decreased. The beta isomer was found in the last test. The metabolite endosulfan sulfat was found after 20 day meanwhile the another metabolite endosulfan lacton have not been discovered in the plant tissues during the whole period of the experiment. This metabolite hadn't established by other works too.

The geneal tendency of the residues of Tiodan 35EK alpha isomer in wheat plant ears goes into reduction (tab. 4).

#### **The effect of the oral administrations and feeding forages sprayed with insecticide on sheep**

Levels of blood glucose, total protein, lipids and shoeostrol as well as the activities of ASAT, ALAT, LDH and MAO in sheep blood administered orally Tiodan 35EK at the dose 3,5 and 7 mg./kg. b.w. are shown in table 5. The chlororganic at the two doses caused reduction in total lipids ( $P < 0,01$ ), at the lower dose didn't show changes in total protein, but at the higher dose hypoproteinemia was observet ( $P < 0,05$ ). Tiodan 35EK caused increasing in blood glucose and the activities of MAO, LDH, ALAT and ASAT ( $P < 0,01$ ). Such alterations may be explained that the organism reacts differently at the higher dose (7mg/kg) by which the blood metabolites indicies and the activities of oxidases, dehydrogenases and transaminases.

The profile of the same indicies when sheep were administered the phosphoorganic insecticide Dursban 4EK at the dose 10 and 20 mg/kg. b.w. (tab. 6) seems unidirectionally and similarly to the effect of chlororganic insecticide Tiodan 35EK. In addition here could be declared that the activity of cholinestrace was inhabited by the cause of the phosphoorganic insecticide.

After 2 hours of oral administration to the sheep with Sumi-alpha 5EK at the dose 5 and 10mg/kg. b.w. (tab.7), blood glucose significantly increased at the higher dose. The both doses caused increasing in total lipids. Total protein didn't shov apreciable changes by dosing the insecticide; At the higher dose ASAT and MAO increased significantly ( $P < 0,01$ ) and ALAT reduced.

Forage which had sprayed with Tiodan 35EK fed to shep, as shown in table 8, blood glucose didn't rise noticeably agter 30 days of feeding. Total lipids decreased ( $P < 0,05$ ). The activity if ASAT and LDH increased ( $P < 0,05$ ). After the same duration of feeding with forage treated with Dursban

4EK, blood glucose increased ( $P < 0,05$ ), and at the same moment hypolipemia was reported.

The obtained results demonstrate that Tiodan 35EK, Dursban 4EK and Sumi-alpha 5EK after dosing cause alteration in the studied indicies chracterezied the homestasis in the organism, similar frindings were observed in monogastric animals (2,3 and 4), as well as the rumen animals in spite of the lower levels of glucose, in which was observed hyper glucamia, and the low levels total lipids and the high activity of transaminases after 2 hours of the oral administration. Dosing the insecticides to the animals caused stress reaction which can be seen by the changes in the activity of MAO and the high level of glucose in the bllood, decreasing in the lipids. Such reaction had been reported in small rumen animals (1). Other autthers found liver dystrophia after treatment with such insecticides (3,4). After 2 hours from dosing we can't conclude weather the treated doses cause liver dystrophia.

The effect of the residues and the metabolites of Tiodan 35EK and Dursban 4EK by feedint the animals forages treated with such insecticides after 30 days from mowing and the technological terms for cereal cultures, as were demonostrated in the results shows that there are changes in the homestastical parameters.

#### **Animal behaviour after exposing to the insecticides**

Animals behaviour after oral administratins at the lower doses of Tiodan 35EK, Dursban 4EK and Sumi-alpha 5EK didn't show any changes, meanwhile at the higher doses, observed slight inconvenience. Exposure to Dursban caused profuse salivation and frequentive rumen contractions and hyperactivity, which faded at 2 hours. Feeding animals with treated forages with the insecticides didn't cause any changes in the behaviour.

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## PROUČAVANJE UTICAJA NEKIH INSEKTICIDA NA KRMNE KULTURE I NJIHOVI UTICAJI NA HOMEOSTAZU U PREŽIVARA

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### SUMMARY

Insekticidi su jedinjenja različitih fizičkih i hemijskih osobina. Toksičnost i specifičan biloški efekt, kao i metaboličko proučavanje bilo kojeg preparata insekticida namerava da ispita metabolizam, distribuciju, retenciju, nagomilavanje, odliv iz zemljišta translokaciju iz zemljišta, biljnog proizvoda do potrošača (životinje ili ljudskog bića). Proizvod koji je dobijen iz tretirane biljke mora biti higijenski čist od bilo kakvih rezidua pesticida. Prisustvo rezidua insekticida ili njegovih metabolita u krmnim kulturama mogu da utiču na homeostazu preživara, iako oni ne pokazuju nikakve kliničke simptome (3,5).

Cilj ovog rada je da se ispita karakter degradacionih proizvoda insekticida Dursban 4EK, Tiodan 35EK i Sumialfa 5EK u nekih krmnih kultura. Intenzivan efekt insekticida u uslovima regiona i njihov uticaj na tretiranu kabastru hranu na homeostazu životinja hranjenih sa takvim hranivima. Dodavši tome, pokušali smo da ispitamo efekt oralnog davanja nekih insekticida životinjama koje su ispoljile znake karakterisane homeostazom.