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Poisonous Effects of Phosalone as an Insecticide on Rumen Degradability of Dry Matter according to *in situ* Technique

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Abstract **- In considering the interaction between microorganisms and insecticides, some certain effects may become significant. These effects involve the potential inhibition or possible germicidal action of insecticides on rumen microbes. Proper functioning of these microorganisms is essential, since they supply approximately 50 to 70% of the energy requirements of ruminants. Any deleterious influence, even of a temporary nature, could have a profound effect on the bacterial activity and subsequently upon the host animal. So a study to investigate the effects of different levels (0, 0.7, 2.8 and 5.6 mg) of phosalone as an insecticide with different levels of calcium bentonite (0 and 100 mg) on *in vitro* dry matter disappearance was carried out in the farm of animal science in Ferdowsi University of Mashhad, Iran in 2012. The study indicated that all the levels of phosalone had not any significant effect on *in situ* dry matter disappearance (ISDMD),only the effect of phosalone was significant after 24h incubation (p<0.05). There was a significant effect (P<0.01) for calcium bentonite for effective dry matter digestibility (EDMD), the rapidly soluble fraction (a) and the potentially degradable fraction (b). In this experiment calcium bentonite is tested as toxin binder for decreasing the probably deleterious effects of phosalone on rumen degradability of dry matter.**

Keywords **– Phosalone, Calcium Bentonite, *In Vitro*, Dry Matter Disappearance.**

I. Introduction

The use of insecticides is increasing in developing countries. The problem of insecticides contamination of soil, water and subsequent animal feed in the agricultural regions of the Iran has recently acquired greater importance due to the increasing number and amount of chemicals used. The toxins in the water and feed transfer to animal products and then these contaminated products are eaten by humans [3], so it can be deleterious effects in the future for them. Also these pollutants can enter the food chain. Phosalone is one of the most widely used organophosphate insecticides (OPIs) in agriculture and public health programs. Degradation rates after release to the environment vary extensively between substances, with half life from minutes to many years. OPIs ingested by ruminants by means of residues on forage crops or as dermal or inhalation exposure from spray drifts are exposed to potential metabolic attack by rumen microorganisms. Cook [5] found bovine rumen fluid active in metabolizing phosphate insecticides *in vitro*. Parathion was reduced to aminoparathion by bovine rumen fluid. Dauterman et al. [6] treated rats and cattle with radioactive dimethoate and analyzed blood, tissue, milk, and excreta. The insecticide was rapidly metabolized and excreted. Plapp and Casida [13] incubated an OPIs, Trolene, with bovine rumen fluid and showed hydrolysis at the phosphorus-oxygen methyl group to yield phenyl phosphoric acids. Ahmed et al. [1] demonstrated that bovine rumen fluid hydrolyzed many OPIs, particularly parathion. Oxidation reactions in the rumen fluid were of little importance, and reduction reactions were of great significance in metabolizing these compounds. Dauterman et al. [6] suggested that rumen microorganisms dissimilate malathion by phosphatase action to dimethyl phosphate and 0, 0-dimethyl phosphorothioate. In animal especially ruminant, there are three general types of antidotes for poisons. First, a mechanical antidote is one that binds a poison in the gut and prevents absorption of the poison. Second, a chemical antidote stimulates the body such that the poison is metabolized and detoxified at a faster rate. Third, a physiologic antidote counteracts the toxic effects of the poison [10]. Microbially mediated decomposition is the major, and sometimes the only, mechanism of partial removal or modification of organophosphates in rumen. Clays (such as bentonite) are assemblies of tetrahedral layers of silicate units and octahedral layers of aluminates' units which result in planar sheets. Thus, adsorbents are

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constrained to diffuse in two-dimensional space in contrast to three-dimensional reaction volume [9]. In the other hand, bentonites are clays with prevailing montmorillonite of the smectite group, and can be considered alternative raw materials. Their good capability to bind water and mineral nutrients and protect them from washing up is a useful prerequisite for enhancement of soil fertility (Trckova, [16]). Among various cleanup technologies, the adsorption by some toxin binder such as bentonite is one of the well-established and effective techniques. Growth and milk production from ruminants is dependent upon the digestion of feed by the microorganisms in the rumen. OPIs below the toxic levels in animals may cause suboptimal performance or carry over of residues in livestock products, *in vitro* fermentations techniques help to determine nutritive value of feeds and can be used to evaluate potential chemical toxicity to the ruminants. The objective of this experiment was to determinate the poisonous effects of phosalone as an insecticide with or without calcium bentonite as toxin binder on rumen degradability parameters.

II. Material and Methods

Alfalfa silage samples and chemical analysis

Analytical grade of phosalone (99.3 % pure), an insecticide and acaricide, was chosen. Since the percent dissolubility of phosalone is more in acetone than in any other solvent (Fluka Co., America), acetone was used as the solvent. Different levels of phosalone (0.7, 2.8 and 5.6 mg per 5g of 2mm screened alfalfa silage) was dissolved in acetone and then sprayed to each nylon bag. The solvent was allowed to evaporate off. Also to control group, acetone was added alone. Alfalfa silage sample were dried at 60°C in oven dryer for 48h and then milled with 2mm mesh screen. The ash content was determined after 8 h oxidation at 525°C. Crude protein (CP) (Kjeltec 2300 Auotoanalyzer, Foss Tecator AB, Hoganas, Sweden) were analyzed by a standard Kjeldahl method. Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) contents were determined according to Van Soest et al. [17]. Fat content was determined by ether extraction (AOAC, [2]).

Rumen in situ procedure

The *in situ* DM degradation was carried out according to the procedure described by Mehrez & Ørskov [11]. Four adult Holstein steers (420 ± 12kg, body weight) fitted with ruminal fistula from the flock of the research farm of the Agricultural College at Ferdowsi University were used in this study. Animals were fed 50% of alfalfa silage and 50% of concentrate per day at 8.00 a.m. and 6.00 p.m. Forage samples (5 g of DM with 2mm screen) were weighed into nylon bags (10×17 cm) with a 50 μm pore size. Samples were incubated in the rumen of each sheep after feeding at 08:00 h for 0 (bags were washed with cold tap water without incubation), 3, 6, 12, 24, 48, 72 and 96 h. After each incubation time, bags were removed from the rumen and rinsed with cold tap water, until the water remained clear. The bags were dried at 60ºC for 48 h in an oven then weighed to determine DM disappearance. Ruminal disappearance at each incubation time was calculated as the difference between the residues and original samples. In each incubation time, five replications were used for each sample of forage.

Calculation and statistical analysis

The DM degradation data were fitted to the exponential equation $p = a + b (1-e^{-ct})$ (Ørskov and McDonald, [12]) where p = DM disappearance in rumen at time t, a = the rapidly soluble fraction, b = the insoluble but fermentable fraction, c = the constant rate of degradation of b ($\%h^{-1}$). EDMD was calculated applying the equation of Ørskov & McDonald [12]: $EDMD = a + (bc / (c+k))$, where k = the rumen outflow rate (at level of 2, 3 and 4% per h). A 4×2 factorial design was applied for data analysis with a completely randomized design for experimental basal using the Statistical Analysis System (SAS) program General Linear Model procedure (SAS, 9.1). Significant means were compared; using the Duncan's multiple range tests. Mean differences were considered significant at $P < 0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance.

III. Result and Discussion

Chemical composition of applied alfalfa silage was shown in table 1. The DM, CP, EE, Ash, NDF and ADF were 26.5, 21.9, 2, 9.4, 45 and 33% respectively.

Table 1: Chemical composition of applied alfalfa silage in the experiment.

Chemical composition (% of DM)	
DM	26.5
CP	21.9
EE	2
NDF	45
ADF	33
Ash	9.4

Also Effect of phosalone with or without Calcium bentonite on some parameters of dry matter digestibility, effective degradability of dry matter with different passage rate and dry matter digestibility after two incubated different times was shown in table 2 and 3. The results indicated that phosalone had not any significant effect on b, c, a+b and EDMD as a result of *in situ* application, but there was a significant effect for dry matter digestibility after 24h incubation ($p<0.05$). Application of calcium bentonite resulted in increasing of dry matter digestibility after 24h incubation than to control group, Also effect of calcium bentonite on a, b and EDMD was significant($p<0.01$) but there was no significant effect for c, a+b, 24 and 48h incubation times. Only significant interaction effect was found for "a" parameters ($p<0.05$). It seems that

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Table 2: Effect of phosalone with or without Calcium bentonite on some parameters of dry matter digestibility.

Treatment	Levels of P[1] (mg)	Levels of CB[2] (mg)	a[3]	b[4]	c[5]	a+b[6]
1	0	0	38.28^{ab}	45.53^{a}	0.030^{b}	83.81^{a}
2	0	100	38.15^{ab}	43.91^{ab}	0.032^{b}	82.06^{ab}
3	0.7	0	37.00^{b}	43.26^{ab}	0.037^{ab}	80.26^{ab}
4	0.7	100	39.03^{a}	39.07^{b}	0.043^{ab}	78.10^{ab}
5	2.8	0	37.65^{b}	43.30^{ab}	0.034^{ab}	80.95^{ab}
6	2.8	100	37.54^{b}	38.60^{b}	0.050^{a}	76.14^{b}
7	5.6	0	37.28^{b}	42.19^{ab}	0.037^{ab}	79.47^{ab}
8	5.6	100	38.32^{ab}	40.29^{ab}	0.038^{ab}	78.61^{ab}
		SEM	0.42	1.93	0.005	1.94
Factorial Effect						
P			Ns[7]	Ns	Ns	Ns
CB			P<0.05	P<0.05	Ns	Ns
P×CB			P<0.05	Ns	Ns	Ns

[a and b] means in the same column with different superscript differ significantly (P < 0.05). [1]P: Phosalone; [2]CB: Calcium Bentonite; [3]a = The rapidly soluble fraction; [4]b = The insoluble but fermentable fraction; [5]c = The constant rate of degradation of b; [6]a+b = Potential degradability of dry matter; [7]Ns: Non significant.

Schwartz et al. [15] reported that a concentration of 1000 ppm malathion plus 1,000 ppm Sevin inhibited *in vitro* dry matter digestibility significantly (P<0.05) while 1000 ppm Sevin or 1000 ppm malathion did not significantly decrease dry matter digestion, also some insecticides (Aldrin, DDT, Dieldrin, EPN, Parathion and Toxaphene) significantly (P<0.05) reduced dry matter digestion below control levels at a insecticide concentration of 1000 ppm. In this experiment, most of dry matter digestibility parameters weren't influenced by the different levels of phosalone, but in some cases for example potentially degradability of dry matter was numerically lower with increasing of phosalone levels than to control group. A study indicated that all the levels of each insecticide (Endosulfan, Phosalone and Chlorpyrifos) significantly (P0.05) inhibited the *in vitro* dry matter disappearance (IVDMD), the rumen fluid volatile fatty acids and the rumen fluid total protozoa, and elevated the rumen fluid ammonia compared to the control. However, the rumen fluid pH was not affected. Also, the study revealed that endosulfan significantly (P<0.05) inhibited the rumen protozoa and the IVDMD percent than both phosalone and chlorpyrifos [7]. At another experiment, effect of six commonly used insecticides (endosulphan, monocrotophos, chlorpyriphos, methyl-parathion, dimethoate and cypermethrin) were tested for their effects on *in vitro* feed digestibility. These insecticides were tested at 100 ppm concentration during 48 h incubations period. All the insecticides reduced (P<0.01) dry matter digestibility, however, effects of endosulphan and chlorpyriphos were more prominent. Endosulphan followed by chloropyriphos adversely (P<0.01) affected total volatile fatty acid concentration in incubation medium compared to other insecticides. Thus, insecticides exhibited adverse effect on dry matter disappearance and rumen fermentation under *in vitro* system [14]. Calcium bentonite is an expanded lattice clay of the montmorillonite group of minerals [4] with high ion exchange capacity that binds a wide range of cations specially insecticides and aflatoxins [8]. Much of the variation in response to bentonite may be attributable to the very variable structural and chemical properties of the different types of bentonite clays mined in the all of the word.

Table 3: Effect of Phosalone with or without Calcium bentonite on effective degradability of dry matter with different passage rate and dry matter digestibility after two incubated different times.

Treatment	Levels of P[1] (mg)	Levels of CB[2] (mg)	EDMD[3] (K=0.02)	EDMD (K=0.03)	EDMD (K=0.04)	EDMD (K=0.05)	Disapp Earance (24h)	Disappearance (48h)
1	0	0	63.78^{bc}	59.30^{b}	56.19^{b}	53.89^{b}	56.43^{b}	73.24^{a}
2	0	100	64.26a^{bc}	59.98^{ab}	56.93^{ab}	54.64^{ab}	58.57^{ab}	74.50^{a}
3	0.7	0	63.25^{c}	59.20^{b}	56.29^{b}	54.07^{b}	59.86^{ab}	72.76^{a}
4	0.7	100	65.64^{a}	61.98^{a}	59.21^{a}	57.04^{a}	62.80^{a}	75.32^{a}
5	2.8	0	65.03^{ab}	60.61^{ab}	57.51^{ab}	55.16^{ab}	62.66^{ab}	75.00^{a}
6	2.8	100	65.23^{ab}	61.81^{a}	59.14^{a}	57.01^{a}	63.50^{a}	74.22^{a}
7	5.6	0	63.22^{c}	59.18^{b}	56.26^{b}	54.05^{b}	58.17^{ab}	73.42^{a}
8	5.6	100	64.46^{abc}	60.73^{ab}	57.71^{ab}	55.49^{ab}	59.12^{ab}	75.12^{a}
		SEM	0.5	0.67	0.74	0.76	1.92	1.02
Factorial Effect								
P			Ns[4]	Ns	Ns	Ns	P<0.05	Ns
CB			P<0.01	P<0.01	P<0.01	P<0.01	Ns	Ns
P×CB			Ns	Ns	Ns	Ns	Ns	Ns

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[a and b] means in the same column with different superscript differ significantly ($P < 0.05$)). [1]P: Phosalone; [2]CB: Calcium Bentonite; [3]EDMD=effective degradability of dry matter with different outflow rate (k= 0.02, 0.03, 0.04 and 0.05); [4]Ns: Non significant.

IV. Conclusion

Animal feeds and milk may serve as a vector for the transmission of substances of extrinsic origin which can be potentially toxic to the consumer. These toxins may originate in cow's milk from the ingestion of plants known to contain toxic substances or feeds contaminated with OPIs. It is well established that *In situ* dry matter digestibility technique can be used to determine nutritive value of feeds; it now appears that this procedure can be used to evaluate potential chemical toxicity of insecticides to ruminants, although most studies have suggested that general toxicity to cattle was not produced without exposure to large doses of insecticides. In this experiment, at the tested concentrations, although phosalone had not significant effects on *in situ* dry matter digestibility parameters (except dry mater digestibility after 24h incubation), but it numerically exerted an adverse effect on most of digestibility parameters. Much attention recently has been focused on use of supplemented bentonite to detoxification of some insecticides in the ruminants ration. Also it is confirmed that application of bentonite in animal diet can be effective for improving digestibility of nutrients, daily gain and feed intake and it can absorb toxic product of digestion and decrease the accumulation of toxic substances in tissues, thus decreasing the incidence of internal disorders. Also consumption of only few milligrams of insecticides is sufficient to exceed established regulatory limits in food products. Adoption of scientifically recommended practices in the use of insecticides by the farmers will ensure that they will not contaminate the feedstuffs and thereby not affect the health of livestock.

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