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Effects of Sodium Bentonite on Blood Parameters, Feed Digestibility and Rumen Fermentation Parameters of Male Balouchi Sheep Fed Diet Contaminated by Diazinon, an Organophosphate Pesticide

Research Article

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ABSTRACT

The remnants of pesticides in livestock feeds have been increased by excessive using of these pesticides so as to meet extreme demands for more feeds. Finding a new strategy for reducing pesticides negative effects is absolutely necessary. Therefore, evaluation of the effects of sodium bentonite on blood parameters, feed digestibility and rumen fermentation parameters in sheep fed diets contaminated by diazinon, an organophosphate pesticide, was the aim of this study. Eight canulated male Balouchi sheep (40±2 kg) assigned to a 2 × 2 factorial arrangement in four 21-day-period. Treatments were: 1) control group, 2) control + 4% sodium bentonite, 3) control + 21 ppm diazinon and 4) control + 4% sodium bentonite + 21 ppm diazinon. Dry matter, organic matter, crude protein, neutral detergent fiber (NDF) and acid detergent fiber (ADF) digestibility were not affected by treatments. Sodium bentonite decreased pH variation after feeding. Ammonia nitrogen of rumen liquor was the same among treatments before feeding however 3 and 6 hours after feeding, amount of ammonia nitrogen was significantly higher in groups fed pesticide contaminated diets ($P<0.05$). Hemoglobin, white blood cells and hematocrit were not affected by treatments but red blood cells and acetylcholinesterase activity were significantly reduced in groups fed pesticide contaminated diets ($P<0.05$). Results of present study showed that using sodium bentonite as a binder was effective to reduce negative effects of diazinon on pH variation and acetylcholinesterase activity and it has no effect on hemoglobin, white blood cells, hematocrit and nutrient digestibility. Therefore, sodium bentonite can be used as an effective diazinon binder in sheep diet.

KEY WORDS blood parameter, diazinon, male Balouchi sheep, nutrient digestibility, rumen parameter, sodium bentonite.

INTRODUCTION

Most of animal feeds are grown using pesticides. Chemical control of weeds, insects and other pests has increased agricultural productivity. However, these economic benefits are not without risks to animal health and environmental damage. Feed and fodder offered to animals are often contaminated with pesticide residues (Raikwar and Nag, 2003) and after feeding, these residues assimilated into the body systems of the animals (Prasad and Chhabra, 2001). The occurrence of pesticides residues in milk of ruminants is a matter of public health concern, since milk and dairy products are widely consumed by infants, children and adults throughout the world. Similarly, the toxins in the water and feed transfer to animal products and then these contaminated products are eaten by humans (Ashraf *et al.* 2010), therefore it may have deleterious effects in the future for them. Moreover, these pollutants can enter the food chain and environment. Degradation rates of pollutants after release to the environments vary extensively between substances, with half-life from minutes to many years. One of the pesticides which is widely used is diazinon. Diazinon belongs to organophosphate pesticides. Diazinon's biological half-life in mammals equals about 12 hours and after 2 weeks only traces of this pesticide may be found in the bodies (Debski *et al.* 2007). Diazinon is still extensively used in sheep dip to control ectoparasites (Boucard *et al.* 2004; Gaworecki and Klaine, 2008; Jadhav and Rajini, 2009; Jemec *et al.* 2007). Organophosphate pesticides are among the leading chemicals used extensively for agricultural pests control throughout the world (Gaikwad *et al.* 2015). The presence of organophosphates in water and crops represents a potential hazard because of their high toxicity to mammals. Organophosphorous and carbamate insecticides affect acetylcholinesterase activity in the nervous system that lead to subsequent accumulation of toxic levels of endogenous acetylcholine in nerve tissues that affect organs in both insects and mammals (Kovac *et al.* 1998; Moretto and Johnson, 1987; Nistiar *et al.* 1984), including humans (Chambers and Levi, 1992; Lotti, 1995). Continuing emphasis on treating and preventing disease conditions caused by toxic substances is necessary because of the increasing incidence of poisoning of domestic animals resulting from the wide-spread use of pesticides in agriculture. There are three general types of antidotes for poisons and toxins. 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 so that the poison is metabolized and detoxified at a faster rate. Third, a physiologic antidote counteracts the toxic effects of the poison. Several studies have used independent or in conjunction methods so as to removal organophosphate pesticide including chemical oxidation with ozone, photo degradation (Zertal *et al.* 2005), combined ozone and UV irradiation (Malato *et al.* 1999), biological degradation (Chen *et al.* 2009), ozonation (Hua *et al.* 2006), membrane filtration (Hofman *et al.* 1997) and adsorption (Daneshvar *et al.* 2007). An example of a mechanical antidote is sodium bentonite. Bentonite is the terminology used to describe the clay rock material composed mainly of montmorillonite clay (Eisenhour and Brown, 2009) with the presence of common impurities such as quartz, feldspars and other minerals. Montmorillonite is a phyllosilicate mineral belonging to the smectite group (Dixon and Schulze, 2002). Smectites have a structural layer formed by two tetrahedral sheets and one octahedral sheet that was sandwiched by the tetrahedral sheets. Montmorillonite contains predominantly Al^{3+} in the octahedral positions with some substitution by Fe^{3+} and Mg^{2+}.

The tetrahedral sheets are dominated by Si^{4+}. The unbalance charges that arise from the isomorphic substitutions give an overall negative charge to montmorillonites. The charge is compensated by exchangeable cations between the layers.

This interlayer space is highly expandable depending on hydration and cation valence. The high surface area (~800 m^2/g) and the expandable structure are important properties in smectites that allow for a wide range of applications. Kazemi *et al.* (2012) used increasing levels of diazinon (0, 0.7, 2.8 and 5.6 mg) as an organophosphorous pesticide and (0 and 100 mg) calcium bentonite, as a toxin binder to evaluate *in vitro* and *in situ* dry matter disappearance. They declared that effect of diazinon in different levels was significant ($P<0.05$) for the entire estimated parameters exception "a" fraction and dry matter degradability after 24 h incubation.

Moreover, effect of calcium bentonite on entire estimated parameters for dry matter degradability was insignificant exception dry matter degradability after 48 hours incubation. Donia *et al.* (2010) assessed accumulation of toxins in raw milk and found that no kind of organophosphorous pesticide were detected in cow and buffalo raw milk but organochlorine (OC) pesticides, hexachlorobenzene, lindane, aldrin, heptachlor epoxide, chlordane, endrin and dichloro diphenyl trichloroethane (DDT), were detected at a value exceeded the tolerance levels of FAO/WHO. Elucidation of the effects of sodium bentonite on blood parameters, feed digestibility and rumen fermentation parameters in sheep fed on diazinon, an organophosphate pesticide, was the aim of this study.

MATERIALS AND METHODS

The study was conducted under the supervision and approval of the animal care and use committee of Ferdowsi University of Mashhad, Iran, concerning the use of animals in research. Eight Balouchi lambs (40±2 kg, body weight (BW)) fitted with ruminal cannulas (2.5 cm i.d.) in a 2 × 2 factorial arrangement were housed in individual metabolic cages (0.5×1.2×1 m) in a temperature controlled house (approximately 22 ˚C). The experiment consisted of 4 periods. Each period lasted 21 days, comprising 14 days of adaptation to the experimental diet and followed by 7 days of data collection. Treatments were 1) control group, 2) control + 4% sodium bentonite, 3) control + 21 ppm diazinon and 4) control + 4% sodium bentonite + 21 ppm diazinon. The ingredients and chemical composition of the experimental diet is shown in Table 1. All diets were supplied as total mixed ration (TMR), and offered at maintenance level twice daily in equal portions at 08:00 and 20:00. Clean water was freely available *ad libitum*.

Table 1 Ingredients and chemical composition of experimental diets (DM basis)[a]

Items	(%)
Ingredient (%)	
Alfalfa hay	30
wheat straw	20
Barley	30
Wheat bran	12.5
Canola meal	6
Vitamin and mineral premix[b]	1
NaCl	0.2
Limestone	0.3
Total	100
Chemical composition	
Dry matter	90.4
Crude protein (CP)	11.9
Ether extracts (EE)	2.4
Neutral detergent fiber (NDF)	40.8
Acid detergent fiber (ADF)	25.2
Non fiber carbohydrates (NFC)	40.4
Ca	0.8
P	0.5

[a] Ration were formulated to supply nutrient requirement at maintenance level (NRC, 2001).
[b] Chemical composition was calculated based on tabulated composition of individual feedstuffs (Ministry of Agriculture, MOA, PRC, 2004).
[c] The mix contained (/kg of premix; DM basis): vitamin A: 330000 IU; vitamin D: 60000 IU; vitamin E: 1000 IU; Ca: 160 g; P: 85 g; Na: 63 g; Mg: 45 g; Zn: 2100 mg; Mn: 1500 mg; Cu: 535 mg; Se: 12 mg and I: 45 mg.

Ruminal fluid was collected by suction through the rumen cannula from before the morning feeding (0.0 h) to 6 hours after feeding at 15-min intervals for the determination of rumen pH on day 18 of each period. The pH of each rumen fluid sample was measured immediately with a portable pH meter (Metrohm 744, Herisau, Switzerland). Samples of ruminal fluid were taken at 0, 3 and 6 hours after feeding for the determination of ammonia nitrogen concentrations. The rumen fluid was then strained through four layers of cheesecloth and prepared for subsequent ammonia-N analyses. On the last day of each experimental period, blood samples were collected from the jugular vein (10 mL into sterile tubes containing ethylenediamine-tetraacetic acid (EDTA) solution).

The samples were immediately placed on ice for processing in the laboratory. Blood samples were centrifuged (3000 g for 15 min at 5 ˚C). Plasma was harvested and frozen at -20 ˚C for later analysis. The count of white blood cells, red blood cells, hemoglobin and hematocrit were calculated using cell counter. Isolated serum was used for determining the activity of the acetylcholine esterase enzyme by titrimetric method. Feed samples were collected at each feeding and composited for later analysis. During the last 7 days of collection period, feces and refused feed were also collected and frozen. Feed, refusals and fecal samples thawed, mixed and were analyzed for DM (48 h at 60 ˚C) and ash (4 h at 550 ˚C).

Statistical analysis

All data obtained from the experiment were subjected to ANOVA for a completely randomized design with 2 × 2 factorial arrangement of treatments using the general linear models (GLM) procedures of the statistical analysis system Institute (SAS) (SAS, 1999). Treatment means were compared by Duncan's new multiple range test (Steel and Torrie, 1960).

The statistical model was:

$$X_{idbjn} = \mu + \alpha_d + \beta_b + (\alpha\beta)_{db} + P_j + T_n + \varepsilon_{idbjn}$$

Where:
X_{idbjn}: observation idbjn.
μ: overall mean.
α_d: effect of diazinon.
β_b: effect of sodium bentonite.
$(\alpha\beta)_{db}$: interaction of two factors.
P_j: effect of period.
T_n: effect of animal.
ε_{idbjn}: experimental error.

Laboratory analyses

The dry matter content of feed ingredients was determined by oven-drying at 60 ˚C for 24 hours then analyzed for concentrations of DM, CP, ether extract (AOAC, 2002), NDF and ADF (Van Soest *et al*. 1991). For ammonia nitrogen determination, 5 mL of rumen fluid from each collection point was acidified with 5 mL of 0.2 NHCl and then analyzed for ammonia-N concentration using the distillation method (Kjeltec Auto Analyzer, Model 1030, Tecator Co. Sweden). Samples of feeds offered and individual refusals were also retained for dry matter determination after drying in a forced drought oven at 80 ˚C for 48 h. The stored subsamples of feeds offered and refusals dried at 50 ˚C then ground to pass through a 1mm screen and stored until analyzed.

RESULTS AND DISCUSSION

Rumen pH

Rumen pH changes during 6 hours after feeding is presented in Figure 1 and effects of experimental treatment in 0, 3 and 6 hours after feeding are presented in Table 2. For all treatments rumen pH decreased 3 hours after feeding and again increased gradually till 6 hours after feeding. Diazinon pesticide had no significant effect on rumen fluid pH (P>0.05) while sodium bentonite significantly affected rumen pH of different experimental groups.

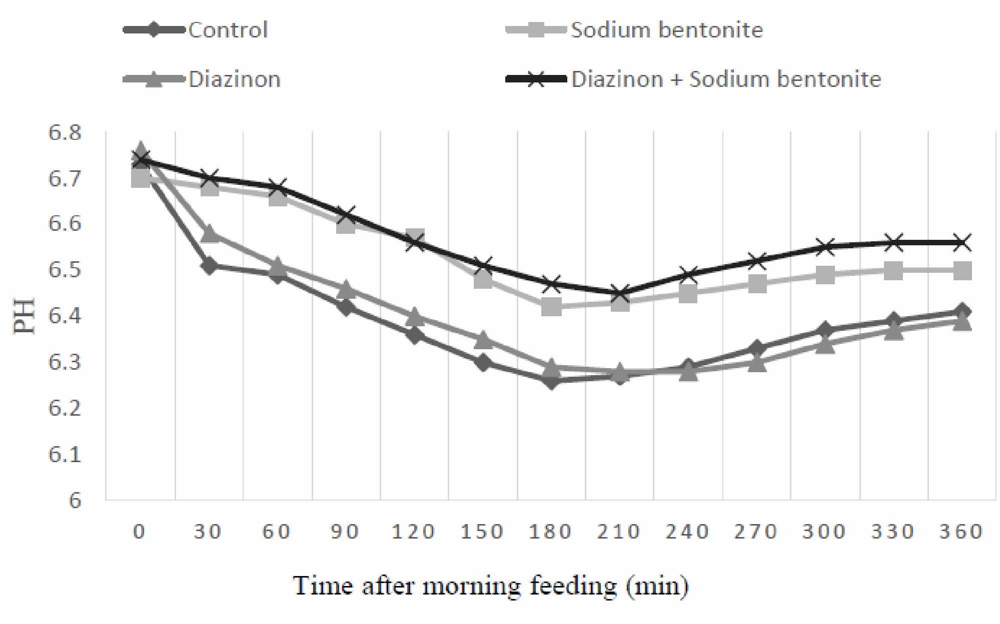

Figure 1 Effect of adding diazinon pesticide and sodium bentonite on ruminal pH

Table 2 Effect of adding diazinon pesticide and sodium bentonite on ruminal pH

Items	- Diazinon		+ Diazinon		P-value			SEM
	Sodium bentonite		Sodium bentonite					
	-	+	-	+	D	S	D × S	
Before feeding	6.73	6.70	6.76	6.74	0.24	0.39	0.95	0.12
3 h after feeding	6.26[a]	6.42[a]	6.29[a]	6.47[b]	0.56	0.04	0.88	0.05
6 h after feeding	6.41[a]	6.51[b]	6.39[a]	6.56[b]	0.79	0.04	0.50	0.05

(-) and (+) signs show respectively absence and presence of experimental treatment.
D: effect of diazinon; S: effect of sodium bentonite and D × S: interaction effect.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Ammonia nitrogen of rumen fluid

Before feeding, ruminal ammonia nitrogen level was similar among different experimental treatments (P>0.05). Three hours after feeding ammonia nitrogen rose in all treatments and this increase was significantly higher in groups containing diazinon pesticide rather than groups containing sodium bentonite (P<0.05). Six hours after feeding ammonia nitrogen showed trend toward reduction in all groups (Table 3). Adding sodium bentonite caused a mild decrease in ammonia nitrogen content of rumen but this effect was not statistically significant which was in agreement with Helal and Abdel-Rahman (2010). Decreasing pattern of after feeding rumen ammonia nitrogen level was similar among treatments without diazinon. Groups containing diazinon and without diazinon groups showed significant differences (P<0.05). Differences among the groups containing sodium bentonite plus diazinon and the groups containing just diazinon was not statistically significant (P>0.05).

Presence or absence of diazinon resulted in a significant difference of rumen ammonia nitrogen level of treatments.

Blood parameters

Blood parameters (red blood cell count, white blood cell count, hemoglobin concentration and hematocrit) are presented in Table 4. Hemoglobin, white blood cell count and hematocrit remained unaffected by adding diazinon and sodium bentonite (P>0.05). There were significant differences in red blood cell count among groups which receive diazinon pesticide and control group (P<0.05). Adding sodium bentonite as a binder was effective to protect RBC in a same count of control group. Presence of diazinon in Balouchi sheep diet significantly reduced acetylcholine esterase activity (P<0.05).

Apparent nutrients digestibility

Means of apparent nutrients digestibility (%) including dry matter (DM), organic matter (OM), crud protein (CP) neutral detergent fiber (NDF) and acid detergent fiber (ADF) are presented in Table 5. In the present study, apparent digestibility of DM, OM, CP, NDF and ADF did not significantly differ among treatments (Table 5).

Rumen pH

Sodium bentonite because of its high capacity of cation exchange, prevented rumen pH steep reduction during first 3 hours after feeding and sharp increase till 6 hours after feeding (next 3 hours). Diets with bentonite because of exchangeable cations, Na and K, maintain rumen pH in a stable level (Stephenson *et al.* 1992). Rumsey *et al.* (1975) stated that adding organophosphate pesticide and binder have no effect on rumen parameters. Hershberger *et al.* (1971) reported that supplying diet of lambs which was exposed to pesticide with sodium bentonite had no significant effect on rumen pH. Schwartz *et al.* (1973) in a 12-day *in vivo* study and an *in vitro* study found feed contaminated with pesticide did not change rumen fluid pH pattern. Aazami *et al.* (2013) stated that saponin as a natural antinutritional component has no effect on rumen pH and rumen ammonia nitrogen level.

Ammonia nitrogen of rumen fluid

Fluctuation of ammonia nitrogen in group containing sodium bentonite during six hours after feeding was lower. Aghashahi *et al.* (2005) reported adding 2% sodium bentonite had no effect on rumen ammonia nitrogen level but it reduced rumen ammonia nitrogen level variations. Meanwhile, adding fourpercent of sodium bentonite significantly reduced rumen ammonia nitrogen level especially after 4 hours. Stephenson *et al.* (1992) reported that adding sodium bentonite prevented rumen ammonia nitrogen raising after feeding probably due to influence of sodium bentonite on rumen microorganism metabolism. Limiting archaea movements, sodium bentonite ceases bacteria hunting (Saleh, 1994). The more bacterial population, the more ammonia nitrogen utilization in rumen. Moreover, Williams and Withers (1993) declared that protozoa population decrease will be resulted in ammonia nitrogen level alleviation. Additionally, Wallace and Newbold (1991) stated that sodium bentonite caused interference with the motion of cilia and thereby prevented motility of protozoa, particularly the holotrichs. McCullough (1974) interpreted cows which received sodium bentonite had a lower rumen ammonia level and a bigger population of micro-organisms except protozoa. As mentioned above, in this study presence or absence of diazinon resulted in a significant difference of rumen ammonia nitrogen level of treatments. One possible reason of this significant differences can be the reduction of microbial population due to presence of diazinon.

Nistiar *et al.* (2000) have verified negative effects of organophosphate pesticides on rumen microbial population. By reduction in microbial population, fewer nitrogen will be used for microbial production so nitrogen is cumulated in rumen (McCullough, 1974). Rumsey *et al.* (1975) observed no effect on ammonia nitrogen in cows fed runnel which this is inconsistent with our findings.

Blood parameters

Similar to our findings, Garillo *et al.* (1995) reported that binder was not effective to prevent dropping red blood cell counts in group fed with diazinon contaminated fodders. Effect of diazinon on red blood cell producing organs (liver and bone marrow), inefficiency of bone narrow stem cells, shortening life span of red blood cells and preventing heme biosynthesis could be mentioned as reasons of reducing red blood cell count in groups containing diazinon (Betrosian, *et al.* 1995).

Rumsey *et al.* (1975) reported insignificant effect of organophosphate pesticide on blood parameter namely, red blood cell count and white blood cell count. In this study the lowest red blood cell count (8.83) appertained to the group containing diazinon and without sodium bentonite. Therefore, in this case sodium bentonite was effective in order to relieve negative actions of dizinon pesticide.

Reducing acetylcholine esterase activity in group fed diazinon are consistent with Khan (2001) that interpreted dry cows and replacement heifers fed with diazinon contaminated diet had lower acetylcholine esterase activity.

Moreover, he has verified binders can reduce organophosphate pesticide absorption in gastrointestinal tract and keep acetylcholine esterase activity in a normal range. Judge *et al.* (2016) reported that acute *in vivo* diazinon exposure caused a small but statistically significant inhibition of blood and brain acetylcholine esterase activity and this was dependent on dose and tissue (blood and brain regions) but not time since exposure. Enzyme acetylcholine esterase is present in synaptic regions of neurons and mediates transmission of impulse by breaking acetylcholine into acetic acid and choline.

Diazinon induced suppressed activity of Acetylcholine esterase results in accumulation of acetylcholine at neural and neuromotor regions that causes hyper excitability (Van Cong *et al.* 2009).

Apparent nutrients digestibility

Ivan *et al.* (2001) stated adding 2% sodium bentonite had no significant effects on apparent nutrients digestibility of sheep fed with palm kernel cake by-product. Galyean and Chabot (1981) reported that supllying sudium bentonite in ruminally cannulated Hereford steers had no effect on nutrient digestibility.

Table 3 Effect of adding diazinon pesticide and sodium bentonite on ruminal ammonia nitrogen

Items	- Diazinon		+ Diazinon		P-value			SEM
	Sodium bentonite		Sodium bentonite					
	-	+	-	+	D	S	D × S	
Before feeding	17.04	17.63	18.11	19.94	0.26	0.42	0.68	1.46
3 h after feeding	21.37[a]	21.25[a]	27.87[b]	27.98[b]	0.02	0.99	0.96	1.67
6 h after feeding	20.58[a]	19.05[a]	26.53[b]	25.65[b]	0.04	0.34	0.92	1.77

(-) and (+) signs show respectively absence and presence of experimental treatment.
D: effect of diazinon; S: effect of sodium bentonite and D × S: interaction effect.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effect of adding diazinon pesticide and sodium bentonite on blood parameteres

Items	- Diazinon		+ Diazinon		P-value			SEM
	Sodium bentonite		Sodium bentonite					
	-	+	-	+	D	S	D × S	
Hemoglobin (g/dL)	10.40	9.63	8.80	9.43	0.09	0.89	0.17	0.57
Hematocrit (%)	30.47	29.67	25.40	26.70	0.06	0.89	0.58	1.84
RBC count	11.02[a]	10.13[ab]	8.83[b]	9.27[ab]	0.04	0.73	0.33	0.64
WBC count	7.50	7.56	7.43	7.13	0.52	0.76	0.63	0.37
AChE activity	18.67[a]	18.33[a]	12.03[b]	20.00[a]	0.03	0.08	0.06	1.91

(-) and (+) signs show respectively absence and presence of experimental treatment.
D: effect of diazinon; S: effect of sodium bentonite and D × S: interaction effect.
RBC: red blood cell count; WBC: white blood cell count and AChE: acetylcholine esterase activity.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of adding diazinon pesticide and sodium bentonite on apparent nutrients digestibility

Items	- Diazinon		+ Diazinon		P-value			SEM
	Sodium bentonite		Sodium bentonite					
	-	+	-	+	D	S	D × S	
Dry matter	54.33	55.10	52.66	55.47	0.57	0.14	0.22	1.41
Organic matter	58.60	58.00	56.00	60.66	0.69	0.06	0.1	1.54
Crud protein	58.17	57.92	57.87	58.22	0.09	0.82	0.44	0.91
Neutral detergent fiber	51.97	53.01	52.05	53.13	0.49	0.72	0.69	1.02
Acid detergent fiber	44.06	45.01	43.08	44.26	0.05	0.07	0.09	1.11

(-) and (+) signs show respectively absence and presence of experimental treatment.
D: effect of diazinon; S: effect of sodium bentonite and D × S: interaction effect.
SEM: standard error of the means.

Although the same result have been published by some other authors (Aguilera-Soto *et al.* 2009; Ha *et al.* 1983), but Fisher and MacKay (1983) reported that adding 0.6 to 1.6% of dry matter, sodium bentonite to diet decreased nutrients digestibility. Schwartz *et al.* (1973) examined pesticide effect on rumen function and *in vitro* nutrients digestion. They found that bordeaux mixture, toxaphene, Mema RM, dichloro diphenyl trichloroethane (DDT), O-Ethyl O-(4-nitrophenyl) phenylphosphonothioate (EPN), Zectran, dieldrin, parathion, mobam and aldrin significantly (P>0.05) reduced *in vitro* dry matter and cell wall constituent digestion at pesticide concentrations of 1000 ppm. No effect was noted with Baygon, Black-Leaf 40, malathion and 2, 4-D acid on either cell-wall constituent digestion or dry matter digestion *in vitro*. Kazemi *et al.* (2012) used different levels (0, 0.7, 2.8 and 5.6 mg) of diazinon with different levels of calcium bentonite (0 and 100 mg) as a toxin binder in order to test their toxicological effects on *in vitro* dry matter disappearance.

Authors reported that effect of diazinon with adding the different levels was significant for the entire estimated parameters exception "a" fraction and dry matter degradability after 24 h incubation. Moreover, effect of calcium bentonite on entire estimated parameters for dry matter degradability was insignificant except dry matter degradability after 48 h incubation. Chegeni *et al.* (2013) stated adding 2.5% of diet sodium bentonite had no significant effect on total tract digestibility of organic matter, NDF and ADF in Han × Dorper crossbreed sheep.

CONCLUSION

Different researches in sphere of diazinon and somewhat other organophosphate pesticide, emphatically evaluated the effects of diazinon on acetylcholineesterase activity especially in dipped sheep. Results of present study showed that using sodium bentonite as a binder was effective to reduce negative effects of diazinon on pH variation and acetylcholineesterase activity and it has no effect on hemoglobin, white blood cells, hematocrit and nutrient digestibility. So sodium bentonite can be used as an effective diazinon binder.

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