IJAS Iranian Journal of Applied Animal Science

Effects of Different Toxin Adsorbents on the Amount of Diazinon Residue in White Grape Pomace and Milk Production and Composition and Toxin Residues in Mohabadi Lactating Goats

Research Article

B. Pourmahmoud[1], R. Pirmohammadi[1] and H. Khalilvandi Behroozyar[1*]

[1] Department of Animal Science, Faculty of Agriculture, Urmia University, Urmia, Iran

Received on: 17 Nov 2018
Revised on: 10 Mar 2019
Accepted on: 16 Mar 2019
Online Published on: Dec 2019

*Correspondence E-mail: h.khalilvandi@urmia.ac.ir

Online version is available on: www.ijas.ir

ABSTRACT

Today, researches have shown that the best and most effective way to reduce the incidence of disorders related to pesticides and prevent their transfer to the milk and other animal products is the application of adsorbents or bonding compounds. This study aimed to investigate the effects of different toxin adsorbents (Mycofix-Plus, Bio-Tox, Bio-Acid) on the amount of diazinon residues in grape pomace, and their effects on milk production and composition and toxin residues in it. In this study, 20 Mahabadi breed dairy goats were used with 4 experimental treatments and 5 replications in a completely randomized design. The milk production was significantly affected by Bio-Tox and Mycofix-Plus adsorbents (P<0.05). Adsorbents had a positive and significant role in reducing the amount of diazinon toxins in the feed (P<0.05). At the same time, Bio-Tox adsorbent had the greatest impact on the reduced amount of diazinon toxins in diets with high levels of grape pomace. The amount of toxins transferred to the milk was significantly reduced under the effect of Bio-Tox and Bio-Acid (P<0.05) which was lower than maximum residues limit for diazinon in milk. Generally, the results revealed that the toxin adsorbent compounds decreased the amount of diazinon toxins in diets with high levels of grape pomace and resulted in healthier products in addition to assuring the safety of milk and increasing the efficiency of milk yield. In this study, Bio-Tox and Bio-Acid were more effective in reducing diazinon residue in milk.

KEY WORDS diazinon, grape pomace, Mahabadi goat, milk production, toxins adsorbent.

INTRODUCTION

Today, feed security is among the most important issues in human life. Along with this, feed health has become important to the consumers. Providing feed for human beings and protecting agricultural crops from damages due to drought, pests and diseases have forced farmers to use different pesticides to fight with pest in agriculture (Cengiz *et al.* 2006). Pesticides are materials with the chemical or biological origin that used extensively by human society and can be extremely toxic and harmful to both people and animals (Kazemi *et al.* 2017). Animal feeds may be contaminated with pesticide residues, and these toxin residues may be passed into the animal's body and its products (Nag and Raikwar, 2011). Today, 320 active pesticide compositions as thousands of commercial formulations are used all over the world (Deshpande, 2002; Shibamoto and Bjeldanes, 2009). In order to control pests in Iran, about 5261.80 tons pesticides are used in 2013 (FAO, 2013). Organophosphate pesticides are among the leading chemicals used extensively for agricultural pests control throughout the world (Gaikwad *et al.* 2015). Diazinon is an organophosphate pesticide that has been widely used by Iranian farmers. 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 residues in food and feed with different concentration have been reported by researchers in Iran and other countries (Golghasem Gharehbagh, 2017; Kazemi, 2012). In places with high diazinon residues, more species are endangered by extinction (United State Environmental Protection Agency, 2004).

The presence of phosphoric and chloric pesticides has been proven in milk and related products, meat and feed prepared from contaminated ingredients as well as feed products prepared from raw materials sprayed before the expiration of the occurrence period of the insect all of which create chronic toxic effects of accumulation of pesticides in human and livestock (Bakore *et al.* 2002). Residues pesticides in feed after consumption (Raikwar and Nag, 2003) in addition to absorption from the digestive system appear in the blood and most of the animal organs such as fat tissue milk (Darko and Acquaah, 2008). Clinical and sub-clinical effects in the animals consuming contaminated feed (Kutches *et al.* 1970), the possibility of accumulation of pesticide toxins in animal tissues and milk can create serious problems such as liver tissue damage and neural, reproductive, skin and digestive diseases and even some cancers in human (Ceron *et al.* 1995; Abd-El Ghaney, 2002).

Due to the importance of public health and sanitation, and possible economic losses in animal husbandry, detection, and control of toxin transfer through the feed to animals is especially important. 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 (Aazami *et al.* 2017). An example of a mechanical antidote is bentonite and saccharomyces. Recent research has shown that the best and most cost-effective way to decrease the side effects of pesticides and prevent their transfer into milk and other animal products is the application of adsorbents or bonding materials (Kiyothong *et al.* 2012; Aazami *et al.* 2017; Kazemi *et al.* 2017). Generally, these compounds bond with the effective material in the toxin present in animal feed and ultimately are removed through urination or defecation of the animal preventing or minimizing the digestion of toxins (Kazemi *et al.* 2017). Therefore, due to extensive application of different pesticides and considerations of the effects of residual toxins on the health of consumers; one can conclude that controlling the amount of pesticides applied to agricultural crops is very essential (Park *et al.* 2015).

Scientific information about the effect of different toxin adsorbents on residue pesticides in grape pomace and their effects on ruminal environment, production and composition of milk and also transfer of toxins into milk is incomplete; it is furthermore, unknown whether Mycofix-Plus, Bio-Tox, and Bio-Acid would have required efficiency for neutralizing their adverse effects when exposed to these pesticides.

Therefore, the aim of this study was to investigate the effects of varied adsorbents as commercial toxin binders on the amount of diazinon toxins in grape pomace, and Mahabadi dairy goats under *in vivo* conditions.

MATERIALS AND METHODS

Animals, samples and chemical analysis

All the experimental protocols were approved by the Animal Use Committee in Urmia University, Urmia, Iran (proposal No. 1334569; 25.02.2017). The animals were cared according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching (NRC, 2001). In this study, 20 mature Mahabadi dairy goats with the average live weight of 55 ± 5 kg have been studied in a completely randomized design with 4 treatments and 5 replicates for 30 days.

The animals were fed with total mixed ration (TMR) feeding system (the concentrate-to-forage ratio in the TMR was 60:40 on DM basis, Table 1) containing 27.71% grape pomace, 18.95% chopped alfalfa hay, 14.98% corn silage, 25.87% barley grain, 6.58% soybean meal and 4.79% wheat bran plus mineral/vitamin supplement according to their requirements for two times daily at 8:00 and 18:00 hr.

Grape pomace used in this research after obtaining from Urmia Pakdi's factory was stored in silos under anaerobic conditions before addition to the diets, and it was used without processing or additives for the control group. In treatments 2, 3 and 4 were used 5 g per animal in the day from adsorbents Mycofix-Plus, Bio-Tox and Bio-Acid. The amount of dry matter and chemical composition for all feed samples were measured with three replications. Feed samples were analyzed for dry matter (DM), at 55 °C for 48 hr (AOAC, 2002), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin (Van Soest *et al.* 1991). The neutral detergent solution contained sodium sulfate and heat stable alpha-amylase.

Ash content was determined by ignition of the dried samples at 500 ˚C for 4 hr (Mertens *et al.* 2002). Ash was determined in the bag residues and NDF was expressed free of residual ash. The lignin content was determined by solubilizing of the ADF fraction in 12 M sulfuric acid (Gomes *et al.* 2011).

Table 1 Ingredients and chemical composition of the basal diet (% of DM)

Ingredients	Amount (%)
White grape pomace	27.71
Alfalfa hay	18.95
Corn silage	14.98
Barley grain	25.87
Soybean meal	6.58
Wheat bran	4.79
Mineral and vitamin premix	0.51
Calcium carbonate	0.61
Mycofix-Plus[1]	-
Bio-Tox[2]	-
Bio-Acid[3]	-
Chemical composition	
Crude protein (%)	14.7
Metabolizable energy (Mcal/kg DM)	2.49
Neutral detergent fiber (%)	39.2
Acid detergent fiber (%)	21.22
Ether extract (% of DM)	3.21
Nonfiber carbohydrate	37.1
Ash	8.08

[1] Mycofix-Plus including: 1-synergistic blend of minerals; 2-biological constituent and 3-BBSH 797 4-phytogenic substances 5-phycophytic.
[2] Bio-Tox including: silicates and yeast cell wall extract (*Saccharomyces cerevisiae*).
[3] Bio-Acid containing: formic acid, propionic acid, lactic acid and ammonium formate *vs*. ammonium propionate.

Milk production and collection

To compensate for the effect of stresses due to milking, the amount of milk production of goats was measured by weighing the kids. To do so, one week after being born, the kids were separated from their mothers and were transferred to another place, and they were breastfed twice daily in the morning and afternoon each time for 20 minutes. To measure and record the amount of milk, each kid was weighed before and after breastfeeding using a digital scale and their difference was recorded as the received milk. Hand milking was also performed in order to ensure total discharge of milk from the breast of the mother, and the amount of daily milk was calculated by summing the two portions. About 50 mL samples of a thoroughly mixed composite of milk (morning and afternoon; 60:40 v/v) of individual goats were taken weekly for determination of milk composition.

The milk samples were analyzed in Urmia Pegah Factory for fat, protein, lactose, solids-not-fat, total solids, using the Milko-Scan (Milkoscan^{TM} Minor FOSS, Denmark); somatic cell counts (SCC) using the (Fossomatic^{TM} Minor Integrator, Denmark).

Sample preparation for measuring diazinon toxin

Diazinon toxin residues were identified and measured in the comprehensive laboratory of West Azarbaijan Jahade Daneshgahi using HPLC device with 3 replications. For sample preparation, the first 5 g sample was weighted, and 10 mL acetonitrile was added as extracting solvent. Then 200 µL of acetic acid was added to acidify the medium.

The medium was thoroughly mixed with ultrasonication for 3 minutes; then the mixture was centrifuged at 5000 rpm for 10 minutes and filtered and injected into HPLC device.

Conditions of HPLC device

Solid phase: C_{18} (25 cm), Mobile phase: acetonitrile/phosphate buffer solution pH=3 (20:L80), Absorption wavelength: 230 nm, Detector: DAD (direct array detector), Mobile phase flow: 1 mL/min

Statistical analysis

The complete data set was analyzed as a completely randomized design using generalized linear model (GLM) of SAS (2004). Least square means were adjusted by Tukey and separated using PDIFF option. Additionally, PROC REG was used to investigate the relationship between different measurements. Data were presented as the least square means and corresponding standard errors.

RESULTS AND DISCUSSION

Milk production and composition

The experimental data by using toxin adsorbent materials on milk production and composition are shown in Table 2. The results showed that milk production was affected by experimental treatments such that the highest daily milk production was obtained for treatment Bio-Tox and was 18.30% higher than control, which was a significant difference (P<0.05).

Table 2 Lactation yield and milk composition of goats fed grape pomace-base diets supplemented with different toxin adsorbents

Item	Treatment				SEM	P-value
	T1	T2	T3	T4		
Yield						
Milk (kg/day)	1.42[c]	1.62[ab]	1.68[a]	1.53[bc]	0.04	0.015
Milk (4% FCM)	1.19[b]	1.33[a]	1.37[a]	1.27[ab]	0.02	0.026
Milk components (%)						
Fat	2.93	2.79	2.76	2.87	0.07	0.247
Protein	4.99	4.69	4.61	4.69	0.12	0.22
Lactose	4.89	5	4.99	4.87	0.12	0.83
Total solids	13.29	12.85	12.74	12.80	0.19	0.247
Solids not fat	10.96	10.65	10.55	10.52	0.11	0.089
Somatic cells	4.70	4.96	4.77	5.05	0.23	0.68
Milk components (g/day)						
Fat	41.66	44.93	46.34	43.92	1.33	0.665
Protein	70.89	76.33	77.41	71.76	1.08	0.076
Lactose	69.47	80.30	83.60	74.50	2.24	0.065
Total solids	188.22	207.76	213.25	195.53	3.87	0.107
Solids not fat	155.58	172.25	176.95	160.56	3.51	0.061

T1: control: diet containing unprocessed grape pomace; T2: diet containing grape pomace processed with toxin binder Mycofix-Plus; T3: diet containing grape pomace processed with toxin binder Bio-Tox and T4: diet containing grape pomace processed with toxin binder Bio-Acid.
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.

In treatment with Mycofix-Plus, there was no significant difference compared with other toxin's adsorbent compounds, but there was a significant difference compared to the control group, and a 14.08% increase was observed in milk production. Bio-Acid gave lower milk production compared to Bio-Tox treatment and reached 1.53% compared to 1.68% for Bio-Tox, which was a significant difference (P<0.05) but no significant difference was observed in treatment with Bio-Acid compared to Mycofix-Plus and control treatments. Although 7.75% increase in milk production was obtained by treatment with Bio-Acid compared to the control group, this was not a significant difference. The results obtained from the milk amount corrected according to 4% fat showed that the highest amount of this parameter was in treatments 2 and 3 which were 1.33 and 1.37 kg compared to 1.19 kg for a control group, respectively, which showed a significant difference (P<0.05). Treatment 4 also showed no significant difference compared to treatments with different toxin adsorbent and control. The fat percentage of milk in different experimental treatments did not show significant differences, but milk fat was numerically higher for control and Bio-Acid, which produced lower amounts of milk compared with other treatments. Parameters such as protein, lactose, total solid, solids not fat and somatic cells percentages were not significantly different. The values for milk composition which were calculated in terms of g per day are presented in Table 2. There were no significant differences between experimental treatments in terms of production of fat, protein, lactose, solids not fat, total solid, and somatic cells.

However, in all investigated parameters regarding milk composition, the amount of components was higher in treatments with different toxins adsorbent compared to the control group.

Diazinon residues

Diazinon residues in different experimental treatments are presented in Table 3. As can be seen, the lowest amount of diazinon toxins was obtained in treatment 3 (0.67 mg/kg), and a significant difference was observed between this amount and those obtained for control (4.08 mg/kg) and other treatments (P<0.05). Grape pomace processed with Bio-Acid and Mycofix-Plus decreased the amount of diazinon toxins in treatments 4 and 2 to 1.01 and 1.36 mg/kg, respectively, which were significant and meaningful compared to the control group (P<0.05). Compared to treatment 3, treatments 2 and 4 showed a significant difference (P<0.05), but the amount of diazinon toxin in treatment 3 was the lowest. The results obtained from the percentage of decrease in toxin residues in different treatments due to processed grape pomace compared to the control group are summarized in Table 3. Based on the obtained results, treatment 3 showed the highest percentage of diazinon toxin reduction (83.58%) compared to the control group and had a significant difference compared with other treatments (P<0.05). Treatment 4 also decreased the amount of diazinon toxins (75.25%) compared to the control group and showed significant differences with treatments 3 and 2 with the highest and lowest toxin decreases compared with the control group, respectively (P<0.05).

Table 3 Diazinon residues in different experimental treatments and the percentage reduction of toxin residues in treatments compared to control treatment

Item	Treatment				SEM	P-value
	T1	T2	T3	T4		
	Pesticide residues in Feed					
Diazinon (mg/kg)	4.08[a]	1.36[b]	0.67[d]	1.01[c]	0.51	< 0.0001
Decrease diazinon residues (%)	-	66.67[c]	83.58[a]	75.25[b]	3.09	< 0.0001

T1: control: diet containing unprocessed grape pomace; T2: diet containing grape pomace processed with toxin binder Mycofix-Plus; T3: diet containing grape pomace processed with toxin binder Bio-Tox and T4: diet containing grape pomace processed with toxin binder Bio-Acid.
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 The amount of diazinon residue and the percentage of transfer to milk in experimental treatments

Item	Treatment				SEM	P-value
	T1	T2	T3	T4		
	Toxin residue in the milk					
Diazinon (mg/kg)	0.136[a]	0.042[b]	0.014[c]	0.018[c]	0.01	< 0.0001
Transfer diazinon to milk (%)	3.33[a]	3.08[a]	2.09[b]	1.78[b]	0.25	0.001

T1: control: diet containing unprocessed grape pomace; T2: diet containing grape pomace processed with toxin binder Mycofix-Plus; T3: diet containing grape pomace processed with toxin binder Bio-Tox and T4: diet containing grape pomace processed with toxin binder Bio-Acid.
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.

Treatment 2 also showed the lowest diazinon toxin decrease compared to the control group among other treatments (66.67%) which had a significant difference with treatments 3 and 4 (P<0.05).

Transfer of diazinon toxins to milk

The amounts of diazinon toxins transferred to milk are summarized in Table 4. The obtained results showed that the amount of diazinon toxin transferred through the contaminated feed to milk was decreased by different toxin adsorbents.

The results obtained by investigations of standards of food commission (Codex), FAO and WHO, European Commission (EC), and European Medication Agency (EMA) showed that maximum residue limit (MRL) of diazinon toxins in milk was 0.02 mg/kg. Therefore, based on these global standards, treatments 3 and 4 showed the lowest amount of toxin transferred to milk (0.014 and 0.018 mg/kg, respectively) compared to the control group (0.136 mg/kg) and a significant difference was observed between them (P<0.05).

Treatment 2 with Mycofix-Plus also decreased the amount of diazinon toxins transferred to milk and showed a significant difference compared to the control group (P<0.05), but according to the standards, MRL did not reach an acceptable level and the amount of diazinon toxin in milk was higher than the allowed level. However, as can be seen in the obtained results, in all treatments with toxin adsorbents, the amounts of diazinon toxin transfer to milk were decreased compared to the control group with significant difference (P<0.05). The statistical information presented in Table 4 shows the percentages of diazinon toxins transferred to milk through processed grape pomace with different toxin adsorbents.

Treatments 3 and 4 which contained Bio-Tox and Bio-Acid adsorbents showed the lowest percentages of toxin transfer (2.09 and 1.78%, respectively) compared to treatment 2 with Mycofix-Plus (3.08%) and the control group (3.33%) and the significant statistical difference was observed (P<0.05).

Milk production and composition

In the present study, milk production was increased due to the application of different toxin adsorbents. Recent research has reported (Kazemi, 2012; Golghasem Gharehbagh, 2017) that by decreasing the amount of toxins in diets, milk production was increased, which complied with our findings. Kiyothong *et al.* (2012), in a research conducted using mycotoxin inhibitors, showed that mycotoxins entering the rumen decreased milk production. Different researchers also reported that mycotoxins decreased milk production (Diaz *et al.* 2005; Politis *et al.* 2005; Cheng *et al.* 2006). In line with our findings, Golghasem Gharehbagh (2017) reported that different toxin adsorbents could decrease negative effects of toxins and pesticides residue on different metabolic processes by inactivating them and therefore increased milk production such that the obtained data showed that milk production was significantly increased compared to control treatment, but no effect was observed on milk composition. Kazemi *et al.* (2017) reported that pesticides and toxin residues could have harmful long-term effects on ruminal environment and performance of animals. Kiyothong *et al.* (2012) have shown that toxin adsorbents in contaminated diets can increase DM intake in animals and have negative effects on the health and ruminal balance and therefore, increase milk production. Also, decreased DM intake and insufficient supply of microbial protein may be among the reasons for decreased milk production (Cardozo *et al.* 2005; Chichlowski *et al.* 2005). In this study, different toxin adsorbents showed positive effects on the ruminal environment and metabolic processes by decreasing the amounts of diazinon toxin and other mycotoxins, and therefore, increased milk production. However, decreasing diazinon toxin due to the application of different adsorbents in diets based on white grape pomace had significant effects on different compounds in the produced milk. Also, our results showed that adding different toxin adsorbents at dosages advised by Biomin Company had no negative effects on the production and composition of the produced milk.

Diazinon residues

Since diazinon is one of the important and most commonly used toxins in the fight against grape cluster warm, its overuse in Iranian vineyards, including vineyard gardens of West Azarbaijan province, has resulted in the detection of residues of this toxin in grapes and related products such as grape pomace produced in fruit juicer factories. Statistical results obtained from the toxicology of diazinon in grape pomace, which are presented in Table 3, proves this claim. Maximum residue limit for diazinon toxin in fruits such as grapes based on Food and Agriculture Organization of the United Nations (FAO, 2013) is 0.5 mg/kg. Since diazinon toxins have been overused and currency period has not been obeyed, the amount of this toxin in grape pomace was increased to above the allowed level in all treatments. However, as mentioned above, different toxin adsorbents significantly decreased the amount of diazinon toxins compared to control treatment but because of a high level of this toxin in grape pomace, only Bio-Tox treatment could decrease diazinon toxin to near its allowed threshold.

The decrease of diazinon toxins with treatment 3 could be due to the structure it, such that Bio-Tox supplement as an organic toxin adsorbent, combined with silicates and cell wall extracts of yeast (*Saccharomyces cerevisiae*), can absorb a wide range of toxins. Organic toxin binders based on β-glucan (inner wall of yeast) with 1, 3 and 1, 6 β-glucan structures are unnatural and charge-free molecules (Moschini *et al.* 2008). Due to helical, spring-like structure, and hydrogen and van der Waals bonding; these compounds have high absorption capacity for bonding to toxins (Nemati *et al.* 2015). On the other hand, positive effects of compounds containing yeast extract can be attributed to the capabilities of these compounds in increasing the growth of acetic acid consuming bacteria, pH fixation in the rumen, and increased food consumption (Nemati *et al.* 2015).

Savari *et al.* (2013) reported that organic toxin adsorbents have higher efficiencies compared to inorganic sorbents such that in this study Bio-Tox adsorbent was introduced as the best mycotoxin adsorbent. In treatment 2, a decrease of diazinon toxins may be due to the presence of sodium bentonite in the ingredients of Mycofix-Plus which has very high cation exchange capacity. Bentonite is a term used for describing clay rock materials which are mainly composed of montmorillonite clay with impurities such as quartz, feldspar, and other minerals (Eisenhour and Brown, 2009).

Montmorillonite is a phyllosilicate mineral belonging to the smectite group (Dixon and Schulze, 2002). Bentonite belongs to planar silicate family and has a three-layered structure with an aluminum layer with loose bonding between two silicate layers. Due to this three-layered structure, bentonite has colloidal properties. The important properties of minerals in the smectite family are an ionic substitution, formability, and ionic expansion and contraction and these properties can be considered to be responsible for decreasing toxins by the addition of bentonite.

The decrease of diazinon toxins in treatment 4 compared to the control group was due to the application of Bio-Acid. Using a combination of formic acid, propionic acid, and lactic acid along with ammonium formate and ammonium propionate salts can be effective in decreasing the amount of toxins. Golghasem Gharehbagh (2017) reported that apple pomace processed with Bio-Acid toxin significantly decreased diazinon toxin compared to the control group. Acidic adsorbents decrease ruminal pH and therefore, destroy ruminal toxins (Kim *et al.* 2005). Also, by being dispersed all over the body, acids can prevent the harmful effects of toxins and block them (Lukstadt, 2014). Similarly, Golghasem Gharehbagh (2017) reported a significant decrease in diazinon toxins by applying Mycofix-Plus and Bio-Tox adsorbents compared to the control group. Kazemi *et al.* (2013) also reported that sodium bentonite is an expanded lattice clay of the montmorillonite group (belong to minerals) with high ion exchange capacity that binds a wide range of cations specially organophosphorus and other toxins. Aazami *et al.* (2017) in a study on diazinon toxin reported that bentonite acted as a linker of diazinon and other organophosphorus toxins, and prevented their spread.

Transfer of diazinon toxins to milk

Research has shown that organophosphorus toxins enter the systematic cycle within the body after being consumed by the animal and are finally stored in tissues such as adipose, brain, liver, spleen, and milk (Sharma *et al.* 2007). In line with our findings, it has been reported that pesticide residues in contaminated feed supplies can be transferred to milk and other animal products (Nag *et al.* 2007). Organophosphorus toxins are covalently bonded to proteins and cause the survival of toxins in milk and also milk casein can bond to toxin residues through seryl and phosphoseryl moieties (Deiana and Fatichenti, 1992). Lipophilic nature of most organophosphorus toxins supports the accumulation of this toxin in a fat matrix of milk (Juhler, 1997). In dairy animals, which consume organophosphorus toxins, especially diazinon; the removal of toxins from the body takes place rapidly in a few days; however, a fraction of diazinon is observed in dairy products. Szerletics *et al.* (2000) reported that in the milk of cows and sheep treated with diazinon, this toxin was obviously detected on day 1 (0.1-0.2 mg/kg). Hamann *et al.* (1984) also found similar results showing that in the milk of animals, which had received organophosphorus toxins through their skin or air, toxin residue was observed. In line with our findings, Kazemi (2012) showed that diazinon and organophosphorus toxins could be significantly transferred to the milk after being consumed by the animal, and the application of toxin adsorbents (sodium bentonite) could significantly decrease this transfer process, and therefore, a large amount of toxins could be discharged through urination. Also, Golghasem Gharehbagh (2017) showed that in goats fed with different adsorbents, diazinon toxin could significantly be transferred into milk, and different toxin adsorbents could significantly decrease the amounts of transferred toxins, which complied with our findings.

CONCLUSION

In conclusion, our findings showed that addition of Mycofix-Plus, Bio-Tox, and Bio-Acid toxin adsorbent materials to grape pomace had no negative effect on the measured parameters and Bio-Tox and Mycofix-Plus toxin adsorbent increased milk production by 18.30 and 14.08%, respectively. Also, due to destroying toxin residues and enhancing metabolic performances and DM intake, adsorbents can somehow improve animal performance, especially in terms of milk production. Compared with diazinon residues in the control group (4.08 mg/kg), adsorbents played a positive and significant role in decreasing diazinon toxin in animal feed. However, Bio-Tox in diets containing high grape pomace showed the highest toxin decrease (0.67 mg/kg) in the diet and decrease in diazinon compared to the control group was 83.58%. Furthermore, adsorbents play a very important role in decreasing toxin transfer to milk and treatments with Bio-Tox and Bio-Acid adsorbents showed the least amount of toxin transfer to milk. Mycofix-Plus adsorbent also played a critical role in decreasing diazinon toxin, but it could not decrease the amount of this toxin in the milk to below the allowed standard level. According to the research conducted so far, this study was the first to investigate the effects of different toxin adsorbents on pesticide's residue amounts, including diazinon in white grape pomace. Generally, the amount of diazinon applied in vineyards are increasing due to lack of suitable alternative pesticides, neglecting durability and currency period of a toxin, and lack of sufficient control on toxin application in vineyards; therefore, due to the presence of a diazinon toxin in levels higher than the standard allowed level in stored grape pomace, it can be concluded that the application of toxin adsorbent supplement with fed diets high in grape pomace is essential for avoiding negative effects of the toxin on metabolism as well as on transfer of a toxin into milk.

ACKNOWLEDGEMENT

Authors would like to thank Urmia University for the approval and laboratory and technical supports of this research.

REFERENCES

Aazami M.H., Tahmasbi A.M., Forouhar V. and Naserian A.A. (2017). 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. *Iranian J. Appl. Anim. Sci*. **7(3),** 421-428.

Abd-El-Ghaney A. (2002). Study the effect of imidacloprid insecticide on some physiological parameters in Japanese quail. MS Thesis. Faculty of Science Al-Azhar University, Cairo, Egypt.

AOAC. (2002). Official Methods of Analysis. 18th Ed. Association of Official Analytical Chemists, Arlington, Washington, DC., USA.

Bakore N., John P.J. and Bhatanagar P. (2002). Evaluation or of ganochlorine insecticide residue levels in locally marketed vegetables of jaipur city, Rajasthan, India. *J. Environ. Biol*. **23,** 247-52.

Cardozo P.W., Calsamiglia A. and Kamel C. (2005). Screening for the effects of natural plant extracts at diffrerent pH on *in vitro* rumen microbial fermentation of a high-concentrate diet for beef cattle. *J. Anim. Sci*. **83,** 2572-2579.

Cengiz M.F., Certel M. and Gocmen H. (2006). Residue contents of DDVP (Dichlorvos) and diazinon applied on cucumbers grown in greenhouses and their reduction by duration of a pre-harvest interval and post-harvest culinary applications. *Food Chem*. **98,** 127-135.

Ceron J.J., Panizo C.G. and Montez A. (1995). Toxicological effects in rabbits induced by endosulfan, lindane and methyl parthion representing agricultural by products contamination. *Bull. Environ. Contam. Toxicol*. **48,** 105-110.

Cheng Y.H., Weng C.F., Chen B.J. and Chang M.H. (2006). Toxicity of different *Fusarium* mycotoxins on growth performance, immune responses and efficacy of a mycotoxin degrading enzyme in pigs. *J. Anim. Res*. **55,** 579-590.

Chichlowski M.W., Schroeder J.W., Park C.S., Keller W.L. and Schimek D.E. (2005). Altering the fatty acids in milk fat by including canola seed in dairy cattle diets. *J. Dairy Sci*. **88,** 3084-3094.

Darko G. and Acquaah S.O. (2008). Levels of organochloride pesticidesresidues in dairy products in Kumasi, Ghana. *Chemosphere*. **71,** 294-298.

Debski B., Kania B.F. and Kuryl T. (2007). Transformations of diazinon, an organophosphate compound in the environment and poisoning by this compound. *Ekologia*. **26(1),** 68-82.

Deiana P. and Fatichenti F. (1992). Pesticide residues in milk processing. *Italian J. Food Sci*. **4,** 229-245.

Deshpande S. (2002). Handbook of Food Toxicolicology. CRC Press Marcel Dekker, Inc., New York.

Diaz G.J., Cort'es A. and Rold'an L. (2005). Evaluation of the efficacy of four feed additives against the adverse effects of T-2 toxin in growing broiler chickens. *J. Appli. Poult. Res*. **14,** 226-231.

Dixon J.B. and Schulze D.G. (2002). Soil Mineralogy with Environmental Applications: Soil Science Society of America, Inc., Madison, Wisconsin, USA.

Eisenhour D.D. and Brown R.K. (2009). Bentonite and its impact on modern life. *Elements*. **5(2),** 83-88.

FAO. (2013). Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Gaikwad A.S., Karunamoorthy P., Kondhalkar S.J., Ambikapathy M. and Beerappa R. (2015). Assessment of hematological, biochemical effects and genotoxicity among pesticide sprayers in grape garden. *Int. J. Occup. Med. Toxicol*. **10,** 1-11.

Golghasem Gharehbagh A. (2017). Investigation of the effects of different processing methods and feed additives on patulin and pesticides residues concentration on apple pomace and its effects on ruminal fermentation and fermentative parameters *in vitro*, milk yield and composition and some of the blood metabolites in Mohabadi lactating does. Ph D. Thesis. Urmia univ., Urmia, Iran.

Gomes D.I., Detmann E., Filho S.C.V., Fukushima R.S., Souza M.A., Valente T.P. and Queiroz A.C. (2011). Evaluation of lignin contents in tropical forages using different analytical methods and their correlations with degradation of insoluble fiber. *J. Anim. Feed Sci. Technol*. **168,** 206-222.

Hamann J., Heeschen W.H. and Lubs M. (1984). Animal experimental tests on residues and physiologic-biochemical parameters in milk and blood after application of ectoparasiticide. *Milchwissenschaft*. **39(2),** 71-75.

Juhler R.K. (1997). Optimized method for the determination of organophosphorus pesticides in meat and fatty matrices. *J. Chromatogr*. **786,** 145-153.

Kazemi M., Eskandary A., Tahmasbi A.M., Valizadeh R. and Naserian A.A. (2017). Effects of phosalone consumption via feeding with or without sodium bentonite on performance, blood metabolites and its transition to milk of Iranian Baluchi sheep. *J. Anim. Sci. Technol*. **59,** 10-21.

Kazemi M., Tahmasbi A.M., Valizadeh R., Naserian A.A. and Sonei A. (2013). Toxicological effects of diazinon as an organophosphate pesticide on fermentation activity of microorganisms and evaluation of sodium bentonite as a toxin binder by using the *in vitro* batch culture. *J. Agric. Food Technol*. **1(4),** 52-58.

Kazemi M. (2012). Effects of organophosphate pesticides via feeding with or without sodium bentonite on performance, blood metabolites and its transition to milk. Ph D. Thesis. Ferdowsi University of Mashhad, Mashhad, Iran.

Kim Y.Y., Kil D.Y., Oh H.K. and Han K. (2005). Acidifier as an alternative material to antibiotics in animal feed. *Asian-Australasian J. Anim. Sci*. **18(7),** 1048-1060.

Kiyothong K., Rowlinson P., Wanapat M. and Khampa S. (2012). Effect of mycotoxin deactivator product supplementation on dairy cows. *J. Anim. Prod. Sci*. **52,** 832-841.

Kutches A.J., Church D.C. and Duryee F.L. (1970). Toxicological effects of pesticides on rumen function *in vitro*. *J. Agric. Food. Chem*. **18(3),** 430-433.

Lukstadt C. (2014). Acidifiers in Animal Nutrition: A Guide for Feed Preservation and Acidification to Promote Animal Performance. Technology, Business, and Nottingham University Press, Nottingham, United Kingdom.

Mertens D.R., Allen M., Carmany J. and Wolf M. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles. Collaborative study. *J. AOAC Int*. **85,** 1217-1240.

Moschini M., Gallo A., Piva G. and Masoero F. (2008). The effects of rumen fluid on the *in vitro* aflatoxin binding capacity of different sequestering agents and *in vivo* release of the sequestered toxin. *J. Anim. Feed Sci. Technol*. **147,** 292-309.

Nag S.K. and Raikwar M.K. (2011). Persistent organochlorine pesticide residues in animal feed. *Environ. Monit. Assess*. **174,** 327-335.

Nag S.K., Mahanta S.K., Raikwar M.K. and Bhadoria B.K. (2007). Residues in milk and production performance of goats following the intake of a pesticide (endosulfan). *Small Rumin. Res.* **67,** 235-242.

Nemati Z., Janmohammadi H., Taghizadeh A., Maleki Nejad H. and Mogaddam G. (2015). Effect of Bentonite as a natural adsorbent to ameliorate the adverse effects of aflatoxin B1 on performance and immune systems in broiler chicks. *Anim. Prod. Res*. **4(3),** 67-77.

NRC. (2001). Nutrient Requirements of Dairy Cattle. 7th Ed. National Academy Press, Washington, DC, USA.

Park M., GBlitzer E.J., Gibbs J., Losey J.E. and Danforth B.N. (2015). Negative effects of pesticides on wild bee communities can be buffered by landscape context. *Proc. Biol. Sci*. **22,** 282-299.

Politis I., Fegeros K., Nitsch S., Schatzmayr G. and Kantas D. (2005). Use of *Trichosporon mycotoxinivorans* to supp-ress the effects of ochratoxicosis on the immune system of broiler chicks. *Br. Poult. Sci*. **46(1),** 58-65.

Raikwar M.K. and Nag S.K. (2003). Organochlorine pesticide residues in animal feeds. Pp. 54-57 in Proc. 40th Annu. Conv. Chem., Indian Chemical Society, India.

SAS Institute. (2004). SAS®/STAT Software, Release 9.4. SAS Institute, Inc., Cary, NC. USA.

Savari M., Dehghan Banadaki M., Rezayazdi K. and Nikkhah M.J. (2013). A comparison of mineral adsor-bents zeolite and bentonite *vs*. organic adsorbent (Mycosorb) and mineral – organic adsorbent (Biotox) as their regards their potential of adsorbing aflatoxin B1. *Iranian J. Appl. Anim. Sci.* **44(1),** 105-112.

Sharma H.R., Kaushik A. and Kaushik C.P. (2007). Pesticide residues in bovine milk from a predomina-ntly agricultural state of Haryana, India *Environ. Monit. Assess*. **129,** 349-357.

Shibamoto T. and Bjeldanes L.F. (2009). Introduction to Food Toxicolicology. Elsevier, Amsterdam, The Netherlands.

Szerletics T.M., Soos K. and Vegh E. (2000). Determination of residues of pyrethroid and organophosphorus ectoparasiticides in foods of animal origin. *Acta Vet*. **48,** 139-149.

United State Environmental Protection Agency. (2004). Interim Registration Eligibility Decision, Diazinon, Cornell University Press, United State.

Van Soest P.J., Robertson J.B. and Lewis B.A. (1991). Methods for dietary fiber, neutral detergent fibre and non-starch poly-saccharides in relation to animal nutrition. *J. Dairy Sci*. **74,** 3583-3597.