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RESEARCH ARTICLE

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Long-term Feeding Effects of Calcium-bentonite Clay in Aflatoxin B_1 Contaminated Diet on Egg Production, Egg Quality and Immune Status of Laying Hens

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ABSTRACT

Background: This experiment was conducted to determine the effect of synthetic aflatoxin B_1 and calcium bentonite clay as a mycotoxin binder in the laying hen diet on performance, egg quality and immune response to vaccination.

Methods: A total of 144 Lohmann brown classic hens (22 weeks old) were divided into 4 groups of 6 replications with 6 hens each. This experimental design was completely randomized design and there were 4 experimental diets: (1) Basal diet (Positive control), (2) Basal diet +0.25% calcium bentonite clay, (3) Basal diet+200 ppb aflatoxin B_1 (negative control) and (4) Basal diet+200 ppb aflatoxin B_1 and +0.25% calcium bentonite clay. All hens were vaccinated with Newcastle (ND) and Infectious bronchitis (IB) vaccines during 24 weeks feeding trial.

Result: At the end of the 24 weeks of feeding trial, dietary treatments did not influence the egg production, egg mass, feed conversion ratio (FCR) and IB titer of hens; however, aflatoxin B_1 significantly ($P<0.05$) deceased final body weight and deceased the eggshell color score ($P<0.05$). Moreover, the ND titer was reduced by aflatoxin B_1 at 8th and 12th weeks of trial ($P=0.05$ and <0.05; respectively). Thus, supplementation of 0.25% calcium bentonite clay in feed improved body weight, eggshell color and ND titer of laying hens similar to those fed the positive control diet.

Key words: Aflatoxins, Calcium bentonite clay, Egg production, Egg quality, Immune system, Laying hens.

INTRODUCTION

Hot and humid conditions in the tropical counties are provisionally to the growth of molds and occasionally generate the Aflatoxins (Kumar *et al.* 2017 and Chukwudi *et al*., 2021). It has been reported the aflatoxin can be developed within 24 hours in corn stored conditions, even though corn was previously without any aflatoxin contamination (Villers, 2014 and Lavkor *et al*., 2019). Aflatoxins are mainly including aflatoxins B_1, B_2, G_1 and G_2 (Peles *et al*., 2019). Nevertheless, aflatoxin B_1, the most commonly occurring aflatoxin and potentially decrease the productive performance of animals (Lakkawar *et al*., 2016 and Rather *et al*., 2017) and can cause significant depression on immune response and antibody titers (Yunus *et al*., 2011).

Aflatoxins may be contaminated in the feedstuffs in the field before harvest or during post-harvest handling and storage and during feed processing in the feed mill. Thus, laboratory screening is need in order to reduce the contamination in the feed. Calcium bentonite clay is natural clay that binding with the toxic substances and acts like a "chemical sponge". The calcium bentonite clay adsorbed toxins are both eliminated together; this keeps the toxins from being reabsorbed into gastro-intestinal tract and bloodstream (Udomkun *et al*., 2017). Therefore, the aim of this study to evaluate the effects of calcium bentonite clay in synthetic aflatoxin contaminated feed on egg production, egg quality and antibody titer of ND and IB of hens.

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MATERIALS AND METHODS

The study was conducted at Animal Research Farm, Department of Animal Science, Faculty of Agriculture, Kasetsart University, Thailand in February, 2019 to August, 2019. The number of Approval for Animal Care and Use for Scientific Research, Kasetsart University is ACKU62-AGR-001.

Experimental birds

One hundred twenty Lohmann Brown-Classic hens were used. At 20 weeks of age, the hens were divided into 24 units and each unit consists of 6 hens. At 22 weeks of age, all 24 units were randomly divided into 4 groups each group consisted with 6 replications with 6 hens in each replication. During 24 weeks of experiment (22-46 weeks of age), the hens were house in wire cages individually. Therefore, 6 adjacent cages were used as the replicate in 20×40×36 cm^3 cage and raised in evaporative cooling houses and temperature was maintained 26 ± 3°C. The lighting program was set 16 hours from 5 am to 9 pm daily. All hens received the vaccination schedule; Newcastle-Bronchitis Vaccine and Newcastle disease (ND) and Infectious bronchitis (IB) at 0 (initial), 4, 8, 12, 16 and 20 weeks of the experiment (4 weeks interval), respectively by 1 drop intranasally into the nostril of hens.

Experimental design and diets

The Completely Randomized Design (CRD) was used as the experimental design. Feed (mash form) and water were provided *ad libitum* throughout the trial. The artificial aflatoxin B_1 was procured from Fermentek Ltd. Israel. Calcium bentonite clay (Novasil™ Plus) was provided by BASF SE, Germany. Basal diet was prepared without addition of aflatoxin and adsorbent as T1) Basal diet (Positive control); T2) Basal diet+0.25% calcium bentonite clay; T3) basal diet+200ppb artificial aflatoxin B_1 (Negative control) and T4) basal diet+200ppb artificial aflatoxin B_1 and + 0.25% calcium bentonite clay.

Parameters

Egg productions

At the end of the 4-weeks interval, the egg performances were accomplished as follow; egg production, egg weight, feed consumption, egg mass, feed conversion ratio, feed cost per egg, feed cost per kg egg, mortality. Body weight were measured individually at initial and the end of trial in order to calculation of body weight uniformity from coefficient of variation of body weight.

Egg quality

Hen-day egg production was recorded daily whereas egg weights were measured at 8 weeks interval. 4 eggs from each replication that have weight close to the replication's mean were chosen to analyze egg qualities The eggshell color was measured by Zinpro eggshell color fan. The shell breaking strength was measured using a texturometer (LLOYD Instruments, England). The egg weight was measured and the eggs were opened to measure the albumen height and yolk color by BASF yolk color fan. The egg was separated the shell, yolk and albumen. The yolk and shell weight were measured and the albumen weight was calculated as the difference between egg weight and the weight of the yolk and shell. The shell thickness was measured by micro vernier caliper.

Antibody titers

At 4-weeks interval, blood was collected from 2 bird/ replication unit to determine ND Haemagglutination Inhibition test (HI-test) Two-fold serial form Choi *et al.,* (2013) and IB titers using technique Indirect ELISA (Enzyme linked immune sorbent assay) test as described by Chen *et al.*, (2011) with commercial ELISA kit (BioChek, Holland).

Reproductive organ

At the end of the trial, 2 birds from each experimental unit which were closed to average weight of each experimental were sacrificed by carbon dioxide (CO_2). The visceral were then opened and hens were dissected from neck to anus to observe the various internal organs. Ovary and oviduct were removed and weighed on digital top pan electronic balance and measure the length.

Statistical analysis

All data were statistically analyzed using analysis of variance (ANOVA) of SAS (SAS, 1988). The differences between the means of groups were separated by Duncan's new multiple range test (Duncan, 1955). Statements of statistical significance were based on $P<0.05$. All statistical analyses were done in accordance with the method of Steel and Torrie (1980).

RESULTS AND DISCUSSION

Egg production

The results indicated that adding aflatoxin B_1 without calcium bentonite clay seems to decrease feed intake of hens but statically were not found. However, aflatoxin B_1 significantly ($P<0.05$) decreased the body weight gain of hens at the end trial. On the other hands, the aflatoxin B_1 supplementation without calcium bentonite clay decreased the egg production and results in decreased the egg mass and poor FCR egg, however, the statically difference were not found. Interestingly, the calcium bentonite clay 0.25% supplementation in aflatoxin B_1 diet improved the egg production, egg mass and FCR egg of hens similar to those fed the control diet or control diet with calcium bentonite clay 0.5% (Table 1).

The dietary treatments not influenced the mortality of hens. Aflatoxin significantly decreased body weight (Table 1, $P>0.05$) and 200 ppb of aflatoxin not significantly decreased feed intake, however, the adding 0.25% calcium bentonite clay in feed not influenced the feed intake when supplement in to the basal diet. Interestingly, during 24 weeks feeding trail, adding 200 ppb of aflatoxin in diet not significantly influenced the egg production, egg weight, egg mass, FCR egg, feed cost per egg and mortality of hens.

Egg quality

Eggshell color was significantly decreased in 200 ppb of aflatoxin group or adding 0.25% calcium bentonite clay in control diet. Interestingly, adding 0.25% calcium bentonite in aflatoxin contaminated feed increased the eggshell color. The dietary treatments did not influence the others egg quality parameters. However, in the study, the aflatoxin or calcium bentonite clay not influenced the eggshell breaking strength but aflatoxin reduced the eggshell color and adding 0.25% calcium bentonite clay in 200 ppb aflatoxin contaminated feed improved the eggshell color (Table 2).

Antibody titers (ND, IB)

Adding aflatoxin 200 ppb significantly decreased the ND titer (log2) at 8^{th} and 12^{th} weeks, however, adding 200 ppb

Table 1: Effect of calcium bentonite clay in diet on body weight and egg production at 1st-24th trial weeks of hens.

Item	Control	+0.25% calcium bentonite clay	+200 ppb aflatoxin	+200 ppb aflatoxin +0.25% calcium bentonite clay	Pooled SEM	P-Value
Initial body weight (g)	1,709.78	1,708.89	1,709.31	1,709.64	15.61	1.00
Initial uniformity (%)	94.18	94.18	94.20	94.28	0.07	0.75
Final body weight (g)	1,890.90^{a}	1,874.43^{ab}	1,827.59^{b}	1,870.87^{ab}	17.22	0.01
Final uniformity (%)	93.16	93.09	92.79	93.77	0.71	0.80
Body weight changed (g)	203.76^{a}	179.99^{ab}	139.15^{b}	176.79^{ab}	17.80	0.02
Feed intake (g/hen/day)	115.96	115.93	114.08	114.18	1.07	0.44
Hen-day egg production (%)	98.11	97.12	95.44	97.91	1.62	0.65
Egg weight (g)	61.11	60.84	61.41	61.21	0.58	0.92
Egg mass (g/hen/day)	58.93	59.14	57.74	59.42	1.00	0.66
FCR Egg	1.97	1.96	1.99	1.92	0.03	0.50
Feed cost per egg (฿)	1.65	1.65	1.67	1.61	0.02	0.50
Feed cost per kg egg (฿)	26.92	27.11	27.12	26.44	0.39	0.57
Mortality (%)	0.00	0.46	0.00	0.00	0.24	0.43

^{a,b}Means in the same row with different superscript differ significantly (P<0.05).

Table 2: Effect of calcium bentonite clay in diet on egg quality of hens.

Item	Control	+0.25% calcium bentonite clay	+200 ppb aflatoxin	+200 ppb aflatoxin +0.25% calcium bentonite clay	Pooled SEM	P-Value
Shell color	6.67^{ab}	6.32^{b}	6.49^{b}	6.88^{a}	0.12	0.03
Shell breaking strength (N)	39.91	39.96	39.53	38.26	0.77	0.39
Yolk color	8.81	8.61	8.78	8.76	0.09	0.49
Albumen height (mm)	8.00	8.03	8.10	8.06	0.16	0.97
Haugh units	88.81	89.07	89.27	89.43	0.81	0.95
Yolk weight ratio (%)	25.19	25.05	24.73	24.82	0.28	0.63
Albumen weight ratio (%)	64.76	64.84	65.41	65.05	0.33	0.51
Shell weight ratio (%)	10.05	10.09	9.85	10.12	0.15	0.59
Yolk: Albumen ratio	38.91	38.52	37.77	38.16	0.58	0.56
Shell thickness (mm)	0.402	0.401	0.396	0.405	0.005	0.63

^{a,b}Means in the same row with different superscript differ significantly (P<0.05).

Table 3: Effect of calcium bentonite clay in diet on antibody titre against ND and IB in hens.

Item	Control	+0.25% calcium bentonite clay	+200 ppb aflatoxin	+200 ppb aflatoxin +0.25% calcium bentonite clay	P-Value
ND titer (Log2)					
4thwks	4.58±0.38	4.67±0.38	4.25±0.38	4.59±0.38	0.86
8thwks	5.47±0.41^{ab}	5.28±0.41^{ab}	4.50±0.39^{b}	5.68±0.43^{a}	0.05
12thwks	5.95±0.42^{a}	5.58±0.40^{a}	4.15±0.44^{b}	5.42±0.40^{a}	0.04
16thwks	6.06±0.48	6.19±0.46	5.56±0.44	6.33±0.46	0.64
20thwks	6.19±0.53	5.90±0.55	5.75±0.51	6.79±0.56	0.54
24thwks	5.60±0.31	5.50±0.29	5.08±0.29	5.42±0.29	0.62
Av. ND titer,4th -24thwks)	5.68±0.36	5.42±0.36	4.95±0.36	5.56±0.36	0.51
IB titer (Log2)					
4thwks	4.00±0.05	3.90±0.05	3.94±0.05	3.94±0.05	0.56
8thwks	4.00±0.05	3.90±0.05	3.93±0.05	3.89±0.05	0.09
12thwks	4.07±0.05	3.97±0.05	3.98±0.05	3.94±0.05	0.07
16thwks	3.93±0.05	3.89±0.05	3.92±0.05	3.87±0.05	0.80
20thwks	4.05±0.05	3.99±0.05	4.01±0.05	3.99±0.05	0.79
24thwks	4.05±0.05	3.99±0.05	3.98±0.05	4.01±0.05	0.79
IB titer, 4th -24thwks)	4.02±0.04	3.94±0.04	3.96±0.04	3.94±0.04	0.57

^{a,b}Means in the same row with different superscript differ significantly (P<0.05).

aflatoxin with 0.25% calcium bentonite clay in diet significantly increased the IB titer of hens (P<0.05). However, an average antibody against Newcastle disease had no significant throughout the trail. Adding 0.25% calcium bentonite clay within 200 ppb aflatoxin B_1 improved the ND titer of laying hens when compared to 200 ppb aflatoxin diet without calcium bentonite clay and gave the ND titer similar to those fed the control diet (Table 3).

Reproductive organ, liver and spleen weight

The aflatoxin B_1 and calcium bentonite clay did not have any negative effect on weight of the ovary, oviduct, infundibulum, magnum, isthmus, uterus and liver of laying hens. However, adding aflatoxin B_1 200 ppb tended to increase the weight of spleen (Table 4). Aflatoxin B_1 200 ppb significantly reduced the length of oviduct of hens. However, the supplementation of calcium bentonite clay 0.25% in aflatoxin diet significantly improved the length of oviduct of hens (Table 5).

Feed contaminated with aflatoxin cause adverse effect on egg weight, egg mass and body weight. In addition, aflatoxin B_1 significantly decreased the body weight gain of laying hens. Similarly, Pandey and Chauhan (2007) found that body weight was significant low in all aflatoxin fed groups, especially in group received highest aflatoxin. Moreover, Tessari *et al.,* (2010) reported that toxin binder could increase body weight gain of birds. In this study, supplementation of calcium bentonite clay in diet improved the final bodyweight of hens. In this study, 200 ppb of aflatoxin significantly decreased body weight and not significantly influenced the feed intake. As the body needs energy to eliminate toxins, thus hens received the aflatoxin the body weight decreased may due to the negative energy balance.

Tessari *et al.,* (2010) observed that supplemented calcium bentonite in diet decrease the moisture proportion of digesta can affect the feed intake negatively. Moreover, Damiri *et al.*, (2010) reported that adding 3% sodium bentonite increase feed intake, therefore increasing retention time had no negative effect on feed intake and even increase performance. It may be due to viscose nature of bentonite which absorbs much water and decrease passage rate of digesta. In contrast, the results for this study reported that adding calcium bentonite clay as a toxin binder up to 0.25% in feed not influenced the feed intake of birds. The supplementation 0.25% calcium bentonite clay may neutralize the effect of mycotoxin toxin binder in layer hen diet which not give the negative effect on feed intake.

The diet contaminated naturally mycotoxin may reduce the egg production due to the hepatotoxic effect of aflatoxin to the protein and lipid metabolism, impairing egg synthesis. However, in this study, addition 0.25% bentonite not influenced egg production compared to contaminated diet. Fowler *et al.*, (2015) reported that group fed aflatoxin 170 ppb gave lowest egg production when compared to the group fed aflatoxin 170 ppb combined with sodium bentonite 2 and 2.5%. It is believed that aflatoxins adversely affected egg production in layers and the addition of mycotoxin toxin binder effectively increase production of eggs because this binder neutralized the major effects of aflatoxins as compared to group fed aflatoxin. In addition, Lee *et al.*, (2012). observed that eggs production during the recovery period in the high toxin administration treatments increased following the removal of mycotoxin to level comparable with all other treatments. However, aflatoxin in this study is a synthetic aflatoxin and mainly contained the aflatoxin B_1. Thus, the absorption and mechanism in animal may differ

Table 4: Effect of calcium bentonite clay in diet on reproductive organ, liver and spleen weight (% of live weight) at 24th trial weeks of hens.

Item	Control	+0.25% calcium bentonite clay	+200 ppb aflatoxin	+200 ppb aflatoxin +0.25% calcium bentonite clay	P-Value
Ovary (%)	2.29±0.39	2.37±0.53	2.32±0.36	2.26±0.32	0.92
Oviduct (%)	3.42±0.53	3.44±0.42	3.57±0.50	3.64±0.54	0.68
Infundibulum (%)	0.11±0.02	0.11±0.03	0.12±0.05	0.13±0.05	0.50
Magnum (%)	1.59±0.22	1.55±0.26	1.63±0.27	1.74±0.40	0.43
Isthmus (%)	0.32±0.06	0.28±0.03	0.28±0.04	0.29±0.06	0.16
Uterus (%)	1.47±0.19	1.46±0.18	1.51±0.20	1.47±0.15	0.93
Liver (%)	2.56±0.23	2.68±0.47	2.57±0.65	2.47±0.44	0.77
Spleen (%)	0.09±0.02	0.12±0.03	0.12±0.02	0.12±0.03	0.06

Table 5: Effect of calcium bentonite clay in diet on reproductive organ length at 24th trial weeks of hens.

Item	Control	+0.25% calcium bentonite clay	+200 ppb aflatoxin	+200 ppb aflatoxin +0.25% calcium bentonite clay	P-Value
Oviduct (cm)	68.12±1.44^{a}	68.94±1.47^{a}	63.29±1.54^{b}	68.31±1.43^{a}	0.03
Infundibulum (cm)	11.73±0.84^{a}	8.97±0.86^{b}	9.92±0.89^{ab}	10.03±0.83^{ab}	0.02
Magnum (cm)	34.85±1.15	36.96±1.18	33.15±1.23	36.40±1.15	0.06
Isthmus (cm)	11.56±0.42	10.86±0.43	10.92±0.45	11.22±0.42	0.62
Uterus (cm)	9.98±0.68^{b}	12.15±0.69^{a}	9.30±0.72^{b}	10.66±0.67^{ab}	0.03

^{a,b}Means in the same row with different superscript differ significantly (P<0.05).

from the naturally mycotoxin which contain variety of mycotoxins.

It has been reported decrease of shell breaking strength due to feeding of aflatoxin might be due to poor calcium and phosphorous adsorption and interference in vitamin D3 metabolism resulting in poor shell quality at the high level of aflatoxin (Fowler *et al*., 2015). In this study, adding aflatoxin 200 ppb within or without calcium bentonite clay 0.25% tended to increase the spleen and may be caused by the presence of congested red pulp in the organ (Peng *et al*., 2014). Similarly, Phillips *et al.* (2019). found that broilers group fed aflatoxin increase the relative weight of spleen and liver weight, in addition, the discolored liver was observed in birds fed on aflatoxin-containing diets and the ovaries were atrophied. In addition, Lee *et al*. (2012) reported that relative hen liver weights were increased due to toxins administration compared with the control group.

It has been reported, adding zeolite as a mycotoxin binder decreased the pigmentation accumulation in egg yolks (Fendri *et al*., 2012). It might be believed that pigment might absorption by clay as exhibit variation factors as concentrations. Thus, the color of egg yolk might be connected with aflatoxin interference with lipid metabolism and pigment deposition in yolk. However, in this study the aflatoxin or calcium bentonite clay not influenced the yolk color and eggshell composition of laying hens.

The major pigment in eggshells of brown-egg laying hens is protoporphyrin IX, in addition, traces of biliverdin and its zinc chelates are also present. In this study, Eggshell color was significantly decreased in 200 ppb of aflatoxin group or adding 0.25% calcium bentonite clay in control diet. The calcium bentonite clay or aflatoxin may reduce the absorption of the microminerals such as Fe and Zn which results in reduces the color of eggshells. Interestingly, adding 0.25% calcium bentonite in aflatoxin contaminated feed increased the eggshell color.

ND titer was reduced by aflatoxin B_1 at 8^{th} and 12^{th} weeks of feeding trial. These results are in agree with Yunus *et al*., (2011) indicated that aflatoxin significantly reduced antibody titer in birds, aflatoxin could inhibition of antibody production through the toxin’s effects on lymphocytes leading to enhanced turnover of serum antibodies and consequently to decrease antibody titer half-life. Interestingly, supplementation of 0.25% calcium bentonite clay in feed improved ND titer of laying hens similar to those fed the control diet.

Aflatoxin B_1 200 ppb significantly reduced the length of oviduct of laying hens. Although the mechanisms of Aflatoxin B_1 for their effects on the oviduct are not clear yet and need to be investigated. Aflatoxin may delay sexual maturity of birds, therefore adversely affecting the production and quality of eggs (Pandey and Chauhan, 2007). In this study, aflatoxin decreased the oviduct length and the eggshell color were decreased when adding aflatoxin in diet. Thus, aflatoxin decrease the reproductive and egg quality of laying hens. However, the supplementation of calcium bentonite clay 0.25% in aflatoxin diet significantly improved the length of oviduct, magnum of laying hens. Interestingly, the reproductive and egg quality might be improved when adding calcium bentonite clay into the aflatoxin contaminated diet.

CONCLUSION

Aflatoxin B_1 at 200 ppb decreased the body weight gain accumulation, eggshell color and ND titer of laying hens. The supplementation of calcium bentonite clay as a mycotoxin binder improves the body weight gain accumulation, eggshell color and ND titer response of laying hens.

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REFERENCES

Chen, H.W., Wang, C.H. and Cheng, I.C. (2011). A type-specific blocking ELISA for the detection of infectious bronchitis virus antibody. Journal of Virological Methods. 173(1): 712.

Choi, K.S., Kye, S.J., Jeon, W.J., Park, M.J., Kim, S., Seul, H.J. and Kwon, J.H. (2013). Preparation and diagnostic utility of a hemagglutination inhibition test antigen derived from the baculovirus-expressed hemagglutinin-neuraminidase protein gene of Newcastle disease virus. Journal of veterinary Science. 14(3): 291-297.

Chukwudi, U.P., Kutu, F.R. and Mavengahama, S. (2021). Mycotoxins in maize and implications on food Security: A review. Agricultural Reviews. (42): 42-49.

Damiri, H., Chaji, M., Bojarpour, M., Eslami, M. and Mamoei, M. (2010). The effect of sodium betonites on economic value of broiler chickens diet. J Anim Vet Adv 9:2668-26670.

Duncan, D.B. (1955). Multiple Range Test. Biometrics. Washington, DC.

Fendri, I., Khannous, L., Mallek, Z., Traore, A.I., Gharsallah, N. and Gdoura, R. (2012). Influence of zeolite on fatty acid composition and egg quality in Tunisian laying hens. Lipids in Health and Disease. 11: 71.

Fowler, J., Li, W. and Bailey, C. (2015). Effects of a calcium bentonite clay in diets containing aflatoxin when measuring liver residues of aflatoxin B in starter broiler chicks. Toxins. 7(9): 3455-3464.

Kumar, P., Mahato, D.K., Kamle, M., Mohanta, T.K. and Kang, S.G. (2017). Aflatoxins: A global concern for food safety, Human health and their management. Frontiers in Microbiology. 7: 2170.

Lakkawar, A.W., Sathyanarayana, M.L., Narayanaswamy, H.D., Sugunarao, S. Yathiraj, Isloor S.K., Shridhar, N.B. and Krishnaveni, N. (2016). Efficacy of diatomacious earth in amelioration of aflatoxininduced toxicity in broiler chicken. Indian Journal of Animal Research. (50): 529-536.

Lavkor, I., Var, I., Saglam S., Uckun, O., Tekin, A. and Savas, O. (2019). Presence of some mycotoxins in peanuts from harvest to storage. Legume Research. (42): 862-866.

Lee, J.T., Jessen, K.A., Beltran, R., Starkl, V., Schatzmayr, G., Borutova, R. and Caldwell, D.J. (2012). Mycotoxin-contaminated diets and deactivating compound in laying hens: 1. Effects on performance characteristics and relative organ weight. Poult Sci. 91(9): 2089-2095.

Pandey, I. and Chauhan, S.S. (2007). Studies on production performance and toxin residues in tissues and eggs of layer chickens fed on diets with various concentrations of aflatoxin AF B_1. Br Poult Sci. 48(6): 713-723.

Peles, F., Sipos, P., Győri, Z., Pfliegler, W.P., Giacometti, F., Serraino, A., Pagliuca, G., Gazzotti, T. and Pócsi, I. (2019). Adverse effects, transformation and channeling of aflatoxins into food raw materials in livestock. Frontiers in microbiology. 10: 2861.

Peng, X., Zhang, K., Bai, S., Ding, X., Zeng, Q., Yang, J., Fang, J. and Chen, K. (2014). Histological lesions, cell cycle arrest, apoptosis and T cell subsets changes of spleen in chicken fed aflatoxin-contaminated corn. Int. J. Environ. Res. Public Health. 11: 8567-8580.

Phillips, T.D., Wang, M., Elmore, S.E., Hearon, S. and Wang, J.S. (2019). NovaSil clay for the protection of humans and animals from aflatoxins and other contaminants. Clays and clay minerals. 67(1): 99-110.

Rather, I.A., Koh, W.Y., Paek, W.K. and Lim, J. (2017). The sources of chemical contaminants in food and their health implications. Frontiers in pharmacology. 8: 830.

SAS. (1988). SAS User's Guide, Statistics. SAS Institute, Cary, North Carolina.

Steel, R.G.D. and Torrie, J.H. (1980). Principles and procedures of statistics. McGraw-Hill, New York.

Tessari, E.N., Kobashigawa, E., Cardoso, A.L., Ledoux, D.R., Rottinghaus, G.E. and Oliveira, C.A. (2010). Effects of aflatoxin B(1) and fumonisin B(1) on blood biochemical parameters in broilers. Toxins. 2(4): 453-460.

Udomkun, P., Wiredu, A.N., Nagle, M., Müller, J., Vanlauwe, B. and Bandyopadhyay, R. (2017). Innovative technologies to manage aflatoxins in foods and feeds and the profitability of application-A review. Food control. 76: 127-138.

Villers, P. (2014). Aflatoxins and safe storage. Frontiers in Microbiology. 5: 158.

Yunus, A.W., Razzazi-Fazeli, E. and Bohm, J. (2011). Aflatoxin B (1) in affecting broiler's performance, immunity and gastrointestinal tract: a review of history and contemporary issues. Toxins. 3(6): 566-590.