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Comparative Assessment of Thermal Treatment for Mycotoxins Adsorption Potential of Indigenous Clay from Balochistan, Pakistan

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Abstract

The current study aimed to investigate the physical characteristics and mycotoxins (Aflatoxins B_1 (AFB_1) and Ochratoxin A (OTA) binding efficacy of indigenous clays before and after thermal treatment (calcination). The clay sample was collected from various mountainous areas of Balochistan, Pakistan. The samples were ground into a 360 mesh size and thermally treated initially at 200 °C and thereafter at 800 °C for 30 minutes with the help of a muffle furnace. XRD analysis revealed montmorillonite as the most commonly occurring group of clay minerals. The thermally treated clays showed that the binding potential was significantly ($P < 0.05$) improved against mycotoxins. Enzyme-linked immunoassay (ELISA) determination showed significantly ($P < 0.05$) lower residual concentrations of mycotoxins in thermally treated clays. The mycotoxin adsorption capacity (%) was found to be higher in the clay samples procured from Pishin (79.17% for AFB1 and 67.34% for OTA) and Bolan (62.0% for AFB1 and 50.17% for OTA). Calcination also caused a significant decrease in Cation Exchange Capacity (CEC) in some clay samples. Overall, the findings of the study showed that thermal treatment enhanced the in vitro binding capacity of clays against the studied mycotoxins. An increase in the mycotoxin binding capacity of the clays not only reduces the bioavailability of mycotoxins but also decreases their toxic effects. It is recommended that Balochistan clay have the potential to be used as an adsorbent for the removal of mycotoxins from the environment and animal feeds.

Keywords: Aflatoxins B1, Ochratoxin A, Indigenous Clay, Thermal treatment, Adsorption

Introduction

Mycotoxins are an emerging issue in food safety and are associated with health risks for humans and livestock [1]. These are secondary metabolites produced by fungi at different steps of the food chain [2]. Crops are more vulnerable to mycotoxin contamination due to weak growth conditions and poor storage and transportation facilities [3]. Mycotoxins can interfere with growth and cause immunotoxicity [4]. Additionally, mycotoxins persist in agricultural commodities (egg, milk, and meat) and make their way into human bodies through different food sources, posing a threat to food safety [5]. Aflatoxins are regarded as the most significant mycotoxins due to their risk of carcinogenicity and

prevalence in different foods and animal feeds [6]. Aflatoxins are mostly produced by species of Aspergillus since they are found in both the field and storage [7]. Hence, the degree of contamination increases in storage due to favorable conditions. Aflatoxins affect the health and performance of poultry animals (cattle and chickens), resulting in economic losses. It reduces egg production and growth rate and induces changes in various organs, increasing the risk of diseases. The most vital aflatoxins are AFB_1, followed by AFB_2, AFG_1, and AFG_2. Particularly, AFB_1 is a powerful liver carcinogen known as hepatocellular carcinoma (HCC) [8]. The risk has been multiplicatively enhanced for people suffering from HIV and exposed to AFB_1 simultaneously [9]. It is regarded as a Group-1 carcinogen, and its occurrence in food cannot be completely avoided. Ochratoxins (OTA) are one of the identified mycotoxins having the greatest impact on agricultural production and public health [9]. It is produced by *Aspergillus Ochraceous* in tropical regions and *by Penicillium verrucosum* in temperate areas of the world [10]. It can induce immunotoxicity, nephrotoxicity, teratogenicity and hepatotoxicity in exposed animals [11]. It has been reported that it can also cause cancer in experimental animals and is designated as a group B2 carcinogen [12]. It is a naturally occurring pollutant usually found in animal feeds that could affect production, resulting in economic crises worldwide. The presence of OTA has been widely detected in meat and dairy products [13, 14]. A broad range of preventive strategies have been reported in the literature to overcome the negative effects of mycotoxins in exposed animals [15, 4]. The addition of mycotoxins-degrading agents to animal feed is one of the most suitable approaches [16]. These agents can be classified into two groups: binders and modifiers. Binder agents can bind to mycotoxins, lowering their bioavailability through the formation of a complex (binder-toxin) in the digestive tract, which is then secreted from the body (faeces) [17]. Clay is widely used as a mycotoxin binder due to its higher adsorption capacity and greater effectiveness in the removal of toxins, resulting in lowering the toxicity of toxins [18]. The adsorption process is the interaction between the surface of the mycotoxin binder (clay) and the toxins. In addition, the absorption of clay is not only confined to the clay particle surface but also extends to interlayer spaces, which can be increased with the swelling of clay particles, consequently generating more binding sites. The adsorption potential is greatly influenced by the physiochemical properties of clay [19]. These properties can be influenced by various treatments, including thermal treatment (calcination), cation and anion exchange, organic modification (polymer), and acid activation [20]. The calcination of clay is carried out by heating it to different temperature ranges, changing the structure and reactivity of the calcined clay [21]. Calcined clay has been used as an adsorbent for the removal of pollutants (heavy metals, dyes) in different mediums [22]. Various investigation on the effect of calcination on the mycotoxins AF and OTA binding potential of clay was done. Balochistan is a mineral-rich area of Pakistan that has been characterized by large deposits of clay. The purpose of the current study was to investigate the mycotoxins binding potential of indigenous clay of Balochistan for the removal of Aflatoxins B1 (AFB_1) and Ochratoxins A (OTA) and also to assess the effect of calcination on the adsorption capacity of clay soil.

Materials and Methods

Source and Texture Determination

The indigenous clay was procured from five different mountainous districts including Quetta, Pishin, Bolan, Killa

Saifullah and Barkhan of Balochistan Province, Pakistan (Table 1).

Preparation of Clay

The crude clay samples were ground and sieved at 360 mesh with the help of an electromagnetic sieve shaker (Matest S.p.A., Triviolo, Italy) to remove all the impurities and get fine powder [23]. All the collected soil samples were subjected to analysis (courtesy of the Agriculture Research Institute Quetta) for confirmation of soil type and texture. The texture of the soil was determined following the reported methodology [24].

Physio-chemical Characterization of Clay

An initial physical and mineralogical characterization was performed to investigate the properties of all the samples. The prescreening consisted of the following procedures:

X-Ray Diffraction (XRD)

The XRD technique was used to check the types of minerals present in the clay and crystalline phases. It was conducted at the Pakistan Geological Survey Department in Islamabad, Pakistan (PGSD). Sample preparation and analysis were carried out according to the method of Srodon *et al.* [25].

Determination of Cation Exchange Capacity (CEC)

The CEC of the sampled clay was evaluated using the methodology previously adopted [26], with slight modifications. Briefly, weigh a 1 g clay sample, thereby adding 8.25 mL of 1 N sodium acetate trihydrate solution, and reciprocally shake at 150 rpm for 15 min. The samples were then centrifuged at 3000 rpm for 5 min, decanted with the clear supernatant solution, and discarded as completely as possible. This was repeated four times. Thereafter, added 8.25 mL of ethanol (95%), which was reciprocally shaken for 15 min, centrifuged at 3000 rpm for 5 min, decanted the clear supernatant, and repeated the process three times. The electrical conductivity (EC) of the supernatant liquid from the third washing was <400 µS/cm. Extracted washed clay with three 8.25 mL portions of 1 N ammonium acetate solution, decanted, and saved into a 25 mL volumetric flask. Each time, the slurry was mixed for 5 min and thereafter centrifuged (each time, centrifugation made the supernatant liquid clear). Brought the volume of the contents of 25 mL flasks up to 25 mL by adding 1 N ammonium acetate, mixed well, and measured the sodium concentration according to the calibration curve by taking the emission readings at 768 nm by using a flame photometer.

CEC (meq/100 g) = meq /L Na (from calibration curve) x V/wt x 100/1000

V = Total volume of the soil extract (mL)
Wt. = Weight of air-dry soil (g)

Calcination of Clay

The clay samples were finely ground (360 mesh) with the help of a Pestel and motor and thereafter calcined using a muffle furnace (Daihan wise therm, muffle furnace, F-14, Italy) in such a way that initially maintained the temperature at 200 °C for 30 min and then raised the temperature to 800 °C and maintained it for 30 min [22].

Production of Mycotoxins

Laboratory-scale production of mycotoxins (AFB_1 and OTA) was carried out as per the needs of the assay. For this purpose, the methodology previously adopted elsewhere [23], was followed with minor

changes. Aflatoxin was produced by growing *Aspergillus flavus* strain NRRL 2999 on the substrate rice, and fermentations were carried out in batches as per the method described by Bhatti *et al.* [27]. The extraction and estimation of aflatoxin were done as per the procedure [27]. Lyophilized spores of OTA producing fungus *Aspergillus Ochraceous* (CECT 2948) were used for the production of OTA on broken wheat grains. Mycotoxin contents were finally quantified using an ELISA kit.

The mycotoxin adsorption potential of indigenous clay for AFB1 and OTA was determined following the reported strategy [28], with slight modifications. In brief, 1 g of each clay sample was added to 9 mL of AFB1 and OTA solutions (each with a 200 ppb concentration) in a 15 mL test tube, vortexed for 40 seconds, and then centrifuged at 3000 rpm for 3 min. Carefully drawn 1 mL supernatant aliquot and quantified the total aflatoxins and OTA concentrations using ELISA as per the kit manufacturer's advice. The rate of adsorption was calculated by using the following formula:

Binding capacity (%) = 100 x $(Ci - C_f)/Ci$

Ci is the initial concentration of mycotoxin

C_f is the concentration of unbound mycotoxin after an incubation period.

Statistical Data Analysis

Data were analyzed using the statistical software SPSS version 20 (IBM, New York, NY, USA), and results were presented as means ± SD. All analyses were performed in triplicate, and based on the significance of the mean value, the results were considered statistically significant at $P \leq 0.05$.

Results and Discussion

One of the most important preventive measures for animal feeds from mycotoxin contamination is the addition of clay additives [29]. To enhance animal production, clay minerals are usually used as binders in the preparation of pelleted feed and also act as adsorbents for mycotoxins [30]. Indeed, the addition of clay as a supplement to the animal diet is a known efficient way of mitigating the hazardous effects of mycotoxins in monogastric species [31]. However, each type of clay has a unique ability to bind, and even clays from the same family might behave differently depending on the component to bind [32]. The XRD technique was used for the determination of the mineral composition of the analyzed clay samples (Table 1). In Quetta, clay minerals like quartz, calcite-magnesium, and Palygorskite were found; in Pishin, quartz, and montmorillonite were found; and in Bolan, quartz, calcite, and montmorillonite were observed. Furthermore, Killa Saifullah and Barkhan clay contained quartz, calcite-magnesium, illite, and quartz, calcite-magnesium, illite, and Clinochlore were found, respectively. Clay minerals might be used for the removal of mycotoxins from various parts of animals due to their excellent properties [33]. The presence of certain minerals like quartz, montmorillonite, and Palygorskite in the clay improved the binding potential of mycotoxins [34]. Mycotoxins can bind to interlayers, edges, and external surfaces of minerals. Montmorillonite (aluminium silicate) has negatively charged surfaces and exchangeable cations. Subsequently, montmorillonite has excellent potential for binding to polar mycotoxins (Aflatoxins) thereby lowering their risk of toxicity [35].

Table 1. **Physico-chemical characterization of indigenous clays.**

Indigenous clay	Mineral composition	Texture
Quetta	Quartz, Calcite-magnesium, Palygorskite	Loam
Pishin	Quartz, Montmorillonite	Clay
Bolan	Quartz, Calcite, Montmorillonite	Clay
Killa Saifullah	Quartz, Calcite-magnesium, Illite	Loam
Barkhan	Quartz, Calcite-magnesium, Illite, Clinochlore	Clay

The CEC (Meq/100 g) values of indigenous clay are shown in Table 2. The CEC of the crude clay (CC) soil ranged from 59.40 ± 1.27 to 92.0 ± 1.0 while the CEC values of the thermally treated clay (TTC) ranged from 57.52 ± 1.24 to 70.36 ± 0.90. The CEC was significantly ($P<0.05$) reduced after heat treatment. Low CEC of TTC might be due to changes in crystalline structure due to the application of heat and can also be due to dehydroxylation and dehydration, resulting in the collapse of the interlayer of clays [36]. The result of our study is in agreement with the other study [37], which reported that heat treatment reduced the CEC of the clay.

Table 2. **CEC (Meq/100 g) of crude clay (CC) and thermally treated clay (TTC) (Mean ± SD).**

Indigenous clay	CC	TTC
Quetta	59.40 ± 1.27	57.52 ± 1.24
Pishin	92.0 ± 1.0	61.94 ± 1.36
Bolan	89.20 ± 1.25	64.61 ± 1.17
Killa Saifullah	71.34 ± 1.07	70.36 ± 0.90
Barkhan	76.75 ± 1.15	68.35 ± 0.68

An in vitro approach was used to assess the adsorbing potential of (mycotoxins) AFB_1 and OTA in the indigenous clay of Balochistan (Table 3). According to our results of the study, the highest binding capacity (%) for AFB_1 was shown by the CC of Bolan, and the lowest capacity was shown by the CC of Quetta. The residual concentration (µg/kg) of AFB_1 was 185.0 ± 5.0 (Quetta), 65.66 ± 3.05 (Pishin), 98.0 ± 3.61 (Bolan), 175.0 ± 3.61 (Killa Saifullah), and 166.0 ± 6.0 (Barkhan), respectively. Similarly, the binding capacity of clay was significantly ($P<0.05$) enhanced with thermal treatment.

Table 3. **Binding capacity of AFB1 of crude clay (CC) and thermally treated clay (TTC) (Mean ± SD).**

Indigenous clay	CC		TTC	
	Binding Capacity (%)	Residual concentration µg/kg	Binding Capacity (%)	Residual concentration µg/kg
Quetta	7.5	185.0± 5.0	9.5	181.00± 3.61
Pishin	67.17	65.66± 3.05	79.17	41.66± 3.51
Bolan	51.0	98.0± 3.61	62	76.00± 2.65
Killa Saifullah	12.5	175.0± 3.61	16.5	167.00± 2.0
Barkhan	17.0	166.0± 6.0	20.17	159.66± 6.03

The highest binding capacity (%) was reported by Pishin TTC (79.17), while the lowest binding capacity (%) was shown by Quetta clay. The residual concentration of AFB1 in the TTC clay was 181.0 ± 3.61 (Quetta), 41.66 ± 3.51 (Pishin), 76.0 ± 2.65 (Bolan), 167.0 ± 2.0 (Killa Saifullah), and 159.66 ± 6.03 (Barkhan). The highest binding capacity (%) for OTA was shown by the CC of Pishin (52.5), whereas, the CC of Quetta had the lowest binding capacity (2.84%) (Table 4). The residual concentration of OTA was 194.33 ± 04.51 (Quetta), 95.00 ± 04.00 (Pishin), 117.66 ± 02.52 (Bolan), 182.00 ± 02.64 (Killa Saifullah), and 173.00 ± 03.00 (Barkhan), respectively, The highest binding capacity (%) for OTA after thermal treatment was shown by the TTC of Pishin (79.17%), whereas the lowest binding capacity (%) was shown by the TTC of Quetta.

Table 4. **Binding capacity of OTA of crude clay (CC) and thermally treated clay (TTC).**

Indigenous clay	CC		TTC	
	Binding Capacity (%)	Residual concentration (µg/kg)	Binding Capacity (%)	Residual concentration (µg/kg)
Quetta	2.84	194.33 ± 04.51	4.83	190.33 ± 5.51
Pishin	52.50	95.0 ± 4.0	67.34	65.33 ± 4.73
Bolan	41.50	117.66 ± 02.52	50.17	99.66 ± 2.52
Killa Saifullah	9.0	182.0 ± 2.64	10.84	178.33 ± 3.51
Barkhan	13.50	173.0 ± 3.0	15.83	168.33 ± 6.03

Overall, the results showed that the binding capacity (%) of OTA was significantly ($P < 0.05$) increased after calcination. The residual concentration (µg/kg) of OTA was 181.0 ± 3.61 (QC), 41.66 ± 3.51 (PC), 76.0 ± 2.65 (BC), 167.0 ± 2.0 (KC) and 159.66 ± 6.03 (BRC), respectively. The residual concentration decreased after calcination, and this trend was shown for both AFB_1 and OTA. The findings of this research study demonstrated that TTC had a higher adsorption efficiency for both AFB_1 and OTA than CC. It has been reported that clay is modified to increase the adsorption capacity, consequently increasing the space between layers by providing greater space for mycotoxin attachment [38]. It was reported that modified clays have shown fewer desorption rates and a higher rate of adsorption than pure clay. The increase in the adsorption of AFB_1 and OTA after calcination in the current study might be due to increased pore size and decreased CEC values. The results in our study are in accordance with the study [22], which reported that the adsorption rate of aflatoxins and zearalenone was found to be higher after the calcination of clay [39]. Delineated that high-temperature calcination of Palygorskite increases the dye's adsorption effectiveness, which is related to changes in the pore size of the mineral. CEC has a vital role in adsorption besides the pore size. Exchangeable cations enhance the binding of aflatoxins by balancing the interlayer chargers of phyllosilicates [40]. It has been reported that thermal treatment has improved the bentonite clay adsorption affinity for AFB_1, primarily because of the loss of CEC.

To evaluate the adsorbent potential of the Balochistan clay in the removal of mycotoxins, the results were compared with those of studies carried out in another part of the world. Several research studies have been conducted to assess the adsorbing capacity of the clay for the removal of mycotoxins (Table 5). The comparison showed that the soil of Balochistan has excellent mycotoxin binding capacity (%).

Table 5. **Comparison in the binding capacity of mycotoxins of clay in different regions of the world.**

Location	AFB1 Con	Binding Capacity (%)	OTA Con	Binding capacity (%)	Reference
Republic of Serbia	0.2 µg/mL	95	2.0 µg/mL	66.67	Stancic et al. [41]
Mexico	0.15-0.67 µg/mL	12-70	-	-	Cardona et al. [42]
China	0.1-19.8 µg/mL	80-90	-	-	Desheng et al. [43]
Current study	200 ppb	9.5-79.17	200 ppb	4.83-67.34	-

Conclusion

The current study assessed the binding potential of indigenous Balochistan clay for mycotoxins (AFB_1 and OTA). Similarly, the effect of calcination on the binding capacity of clay was also determined. The results of the study showed that the clay of the study area was dominated by quartz, Palygorskite, montmorillonite, and calcite-magnesium minerals. The highest binding potential of mycotoxins was shown by the clay of Pishin 79.17% (AFB_1) and 67.39% (OTA), and the lowest binding potential was shown by the TTC of Quetta 9.5% (AFB_1), and 4.83% (OTA), respectively. The CEC values

decreased after calcination. Moreover, the binding capacity of AFB_1 and OTA was increased after calcination. Overall, the results of the study demonstrated that the clay from the indigenous area of Balochistan efficiently absorbed the analyzed mycotoxins and calcination also improved the binding capacity of clay. From the findings of the study, it is recommended that the indigenous clay of Balochistan can be used as a promising adsorbent for the removal of mycotoxins from poultry feeds.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Q. Wu, L. You, W. Wu, M. Long and K. Kuca, *Food Chem. Toxicol*., 174 (2023) 113673. https://doi.org/10.1016/j.fct.2023.113673
2. A. O. Aasa, F. F. Fru, O. A. Adelusi, S. A. Oyeyinka and P. B. Njobeh, *World Mycotoxin. J.,* 16 (2023) 33. https://doi.org/10.3920/WMJ2021.2766
3. F. Dong, X. Chen, X. Lei, D. Wu, Y. Zhang, Y. W. Lee and J. R. Shi. *Plant Dise.,* 107 (2023) 1060. https://doi.org/10.1094/PDIS-01-22-0024-RE
4. S. Roy, D. Shaw, T. Sarkar and L. Choudhury, *Novel Res. Microbiol. J.,* 7 (2023) 1897. https://doi.org/10.21608/NRMJ.2023.293196
5. J. L. Richard, *Intl. J. Food Microbiol.,* 119 (2007) 3. https://doi.org/10.1016/j.ijfoodmicro.2007.07.019
6. K. Bhardwaj, J. P. Meneely, S. A. Haughey, M. Dean, P. Wall, G. Zhang and C. T. Elliott, Food. Control. 3 (2023) 109675. https://doi.org/10.1016/j.foodcont.2023.109687
7. M. A. Klich, *Mol. Plant. Pathol*., 8 (2007) 713. https://doi.org/10.1111/j.1364-3703.2007.00436.x
8. F. Alvarez-Dias, B. Torres-Parga, A. G. Valdivia-Flores, T. Quezada-Tristán, J.I. Alejos-De La Fuente, J. Sosa-Ramírez and E. J. Rangel-Muñoz, Toxins, 14 (2022) 292. https://doi.org/10.3390/toxins14050292
9. I. S. BoueletNtsama, C. Frazzoli, G. B. Pouokam and V. Colizzi. Foods, 12 (2023) 1713. https://doi.org/10.3390/foods12081713
10. M. C. Priya, A. Jagadeeswaran, M. J. Raja, A. Natarajan and P. Srinivasan, A. *The Pharm. Innov. J.,* 12 (2023) 4056. https://www.thepharmajournal.com/archives/?year=2023&vol=12&issue=3&ArticleI d=19391
11. T. R. Bui-Klimke and F. Wu, *Crit. Rev. Food Sci. Nutri.,* 55 (2015) 1860. https://doi.org/10.1080/10408398.2012.724480
12. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Ochratoxin A. In Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins. International Agency for Research on Cancer (1993). https://www.cabidigtallibrary.org/doi/full/10.5555/19952006807
13. D. Urbani, D. Centonze, G. Varchi, M. Evangelisti, M. D. Parenti and A. Del Rio. *EFSA. Supporting. Publication*., 20 (2023) 7938. https://doi.org/10.2903/sp.efsa.2023.EN-7938
14. J. Valadas, A. Sachett, M. Marcon, L. M. Bastos and A. Piato, *Environ. Sci. Pollut. Res.,* 30 (2023) 21144. https://www.springer.com/article/10.1007/s11356-022-23692-4

15. L. Sun, R. Li, B. Tai, S. Hussain, G. Wang, X. Liu and F. Xing, *ACS Food Sci Technol.,* 3 (2023) 231. https://doi.org/10.1021/acsfoodscitech.2c00331
16. D. Greco, V. Dascanio, M. Abbasciano, E. Santovito, A. Garbetta, A. F. Logrieco and G. Avantaggiato, *Toxins,* 14 (2022) 393. https://doi.org/10.3390/toxins14060393
17. H. Shanakhat, A. Sorrentino, A. Raiola, A. Romano, P. Masi and S. Cavella, *J. Sci. Food Agric.,* 98 (2018) 4003. https://doi.org/10.1002/jsfa.8933
18. P.S. Bettiol, M. A. P. Cechinel, C. M. Oliveira, K. Goulart, A. G. Dol-Bo, A. M. Bernardin and M. Peterson, *Adv. Powder Technol.,* 33 (2022) 103682. https://doi.org/10.1016/j.apt.2022.103682
19. N. Zhao, F. Ju, Q. Song, H. Pan and H. Ling, *J. Hazard. Mater.,* 429 (2022) 127849. https://doi.org/10.1016/j.jhazmat.2021.127849
20. R. Novikau and G. Lujaniene, *J. Environ. Manag..,* 309 (2022) 114685. https://doi.org/10.1016/j.jenvman.2022.114685
21. T. Hanein, K. C. Thienel, F. Zunino, A. T. Marsh, M. Maier, B. Wang and F. Martirena-Hernandez, *Mater. Struct.,* 55 (2022) 3. https://doi.org/10.1617/s11527-021-01807-6
22. R. Rejeb, G. Antonissen, M. De Boevre, C. L. Detavernier, M. Van de Velde, S. and S. A. De Saeger, *Toxins,* 11 (2019) 602. https://doi.org/10.3390/toxins 11100602
23. H. Zhang, W. Wang, X. Wang, J. Xu, Y. Lu and A. Wang, *Powder Technol.,* 396 (2022) 456. https://doi.org/10.1016/j.powtec.2021.10.052
24. D. Kroetsch and C. Wang. Particle size distribution. In: D.A. Angers and F. J. Larney (Eds), Soil Sampling and Methods of Analysis, CRC Press, Boca Raton, (2008) 713. https://www.scrip.org/reference/referencespaper?referencid=3095474
25. J. Srodon, V. A. Drits, D. K. McCarty, J. C. Hsieh and D. D. Eberl., clays and clay Miner., 49 (2001) 514. https://www.springer.com/article/10.1346/CCMN.2001.0490704
26. J. Ryan, G. Estefan and A. Rashid, Soil and Plant Analysis Laboratory Manualanl, ICARDA, (2001). https://www.researchgate.net/publication/236984396_soil--plant-AnalysisSoil and Plant Analysis Laboratory Manual
27. S. A. Bhatti, M. Z. Khan, M. K. Saleemi, M. Saqib, A. Khan and Z. Ul-Hassan, *J. Immunotoxicol.,* 14 (2017) 66. https://doi.org/10.1080/1547691X.2016.1264503
28. M. Sharma, A. B. Mandal and R. Singh, *Indian J. Poult. Sci.,* 51 (2016) 253. https://doi.org/10.5958/0974-8180.2016.00056.8
29. Y. Li, G. Tian, G. Dong, S. Bai, X. Han, J. Liang and H. Zhang, Applied. *Clay. Sci.,* 163 (2018) 299-311. https://doi.org/10.1016/j.clay.2018.07.32
30. M. Nadziakiewicza, S. Kehoe and P. Micek, *Animals,* 9 (2019) 714. https:doi.org/10.3390/ani9100714
31. C. T. Elliott, L. Connolly and O. Kolawole, *Mycotoxin Res.,* 36 (2020) 115. https://doi.org/10.1007/s12550-019-00375-7
32. J. A. Adegoke, B. Ogunsile and A. O. Adetoro, *The Pacific J. Sci. Technol.,* 11 (2010) 639. http://www.akamaiuniversity.us/PJST.htm (2010)

33. E. M. Ayo, A. Matemu, G.H. Laswai and M. E. Kimanya, Toxins, 10 (2018) 510. https://doi.org/10.3390/toxins10120510
34. Y. A. Soltan, A.S. Morsy, N.M. Hashem, M. A. Elazab, M. A. Sultan, A. El-Nile and S. Sallam, *BMC Vet. Res.,* 18 (2022) 1. https://doi.org/10.1186/s12917-022-03476-1
35. W. J. Sheng, C. Guo, Z Liu, Y. Ma, J. Liu and H. Zhang, *J. Sci. Food Agric.,* 103 (2023) 4017. https://doi.org/10.1002/jsfa.12360
36. Y. Wang, M. Yang, F. Ge, B. Jiang, R. Hu, X. Zhou and M. Liu, *Mol. Cell. Proteomics,* 22 (2023) 100490. https://doi.org/10.1016/j.mcpro.2022.100490
37. R. Antonelli, G. R. P. Malpass, M. G. C. da Silva and M. G. A. Vieira, *J. Environ. Chem. Eng.,* 8 (2020) 104553. https://doi.org/10.1016/j.jece.2020.104553
38. J. Feng, M. Shan, H. Du, X. Han and Z. Xu, *Micropor. Mesopor. Mater.,* 113 (2008) 99. https://doi.org/10.1016/j.micromeso.2007.11.007
39. H. Chen, J. Zhao, A. Zhong and Y. Jin, *Chem. Eng. J.,* 174 (2011) 143. https://doi.org/10.1016/j.cej.2011.08.062
40. J. Martinez, M. Hernandez-Rodríguez, A. Mendez-Albores, G. Tellez-Isaias, E. Mera Jimenez, M. I. Nicolas-Vazquez and R. Miranda-Ruvalcaba, Toxins, 15 (2023) 101. https://doi.org/10.3390/toxins15020135
41. A. Bocarov-Stancic, Z. R. Lopicic, M. I. Bodroza-Solarov, S. Stankoviz, S. Jankovic, J. V. Milojkovic and J. A. Krulj, *Food Feed Res.,* 45 (2018) 87. https://doi.org/10.5937/FFR1802087B
42. Marroquín-Cardona, Y. Deng, J. F. Garcia-Mazcorro, N. M. Johnson, N. J. Mitchell, L. Tang and T. D. Phillips, *Appl. Clay. Sci.,* 54 (2011) 248. https://doi.org/10.1016/j.clay.2011.09.009
43. Q. Desheng, L. Fan, Y. Yanhu and Z. Niya, *Poult. Sci.,* 84 (2005) 959. https://doi.org/10.1093/ps/84.6.959