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# Efficacy of clay minerals for controlling aflatoxin B1 toxicity in commercial broilers

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Aflatoxin occurrence in poultry feed causes aflatoxicosis in birds and poses health hazards to the consumers. Aflatoxin B1 (AfB1) is the most predominant and toxic metabolite that is controlled through addition of argillaceous clays as non-nutritive additives. Non-smectitic indigenous clay reserves need testing as AfB1 adsorbent. With detailed mineral and adsorption characteristics, three indigenous non-smectitic clays: (i) palygorskite, (ii) palygorskite-smectite mix, and (iii) interstratified smectite with hydroxy interlayered smectite were tested against 250 µg $kg^{-1}$ AfB1 contamination in a poultry feeding trial with three replications at 1% and 2% (w/w) with positive and negative controls. A total of 330 Ross-308 male broiler chicks, initially reared on clean feed under controlled conditions of light and humidity, were distributed into eleven treatment combinations in a completely randomized design with 30 birds in each treatment on an equal weight basis with three replications at day 14 and fed on experimental feeds for three weeks. Three birds from each pen were randomly slaughtered at day 35 and for each replicated treatment, body weight gain, feed intake, and internal organs weight and morphology were recorded. AfB1 contamination reduced weight gain ($p$ 0.0001), feed conversion ratio ($p$ 0.0001) and feed intake ($p$ 0.0001) while clays addition in the toxin feed effectively controlled AfB1 toxicity as suggested by improved body weight, weight gain rate and feed consumption compared to the toxin fed birds. The liver morphology was comparatively better in palygorskite-smectite mix treatment when applied at 1% and the darkish colour was also improved with addition of the clay in the toxin feed. AfB1 feeding caused a 75% reduction in weight gain compared to the clean feed. Palygorskite and palygorskite-smectite mix clays were better than interstratified clay in increasing weight gain and caused a 60% and 65% increase over toxin feed treatment when applied at 1% and 2%, respectively. In conclusion, the indigenous clay sources overall and palygorskite-smectite mix in particular has the potential for use as a mycotoxin binder for controlling AfB1 incidence in poultry.
**Keywords:** Aflatoxin B1, non-smectitic clays, mycotoxin binder, feed contamination, poultry production.

## INTRODUCTION

Mould contamination through feed grains increases aflatoxins contamination that becomes a health hazard for poultry, dairy and consumer (Sana *et al.*, 2019). The AfB1 above 20 µg $kg^{-1}$ in the feed and 2 µg $kg^{-1}$ in body parts of birds (Saleemi *et al.*, 2020) affects liver and kidney and suppresses the cell growth in animals and humans. Poultry birds are exposed to aflatoxicosis through low quality contaminated feed (Kana *et al.*, 2010) that reduces feed conversion efficiency, feed intake, weight gain and egg production (Anjum *et al.*, 2012; Naseem *et al.*, 2018; Al-Ruwaili *et al.*, 2018). Incidences of feed contamination with AfB1 are reported widely in Pakistan with consequent economic losses (Khan *et al.*, 2013; Iqbal *et al.*, 2014). The addition of clay-based binders in the contaminated feed is an economical practice to control AfB1 toxicity (Barrientos-Velazquez and Deng, 2020).

Clays adsorb AfB1 (Arvide *et al.*, 2008), provide micronutrients (Tateo and Summa, 2007), increase enzymatic activities (Bhatti *et al.*, 2016), and has antibacterial effect (Haydel *et al.*, 2008; Mpuchane *et al.*, 2008). Palygorskite, sepiolite and smectite with their effective adsorption properties can be used as an additive in poultry feed for AfB1 control. The amendment rate and clay type for various animals and poultry have been reviewed (Dixon *et al.*, 2008). Binder with high adsorption kinetics without any dissociation in the gastrointestinal tract is an effective adsorbent (Li *et al.*, 2010).

Smectite based adsorbents are extensively studied and are effective for AfB1 adsorption but their non-selective behavior allows them to adsorb vitamins, protein and nutrients as well (Barrientos-Velazquez and Deng, 2020). Palygorskite-smectite mix has greater selectivity for AfB1 over nutrients (Zhou, 2016). Palygorskite addition at 2% in the feed improve

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broilers health and growth performance by increasing body weight gain and feed intake (Zhou *et al*., 2014). Palygorskite addition at 10% has no adverse effect on broilers health and mortality (Pappas *et al*., 2010). Palygorskite improves intestinal health by adsorbing toxins, bacteria and viruses (Galán, 1996), and act as a protective coating for the stomach and intestine in feed reared animals and birds.

Laboratory adsorption studies are mostly carried out in aqueous suspensions which do not emulate the real gut conditions and result in high variability during feeding trials (Kannewischer *et al.*, 2006). The AfB1 adsorption potential of the clay-based binder needs to be tested in feeding trials where the clay has to interact with multiple biomolecules and variable conditions of pH in a complex gut environment.

Understanding of smectites adsorption characteristics for AfB1 is extensively studied. While the effectiveness of non-smectitic clays need more investigation. Non-smectitic fibrous clays with specific tunneled structure and adsorption properties could control AfB1 toxicity in poultry. The study evaluates the potential of non-smectitic clays with detailed mineralogy and adsorption characteristics for use as an AfB1 adsorbent in a poultry feeding trial.

## MATERIALS AND METHODS

***Selection of clays:*** Three indigenous non-smectitic clays:(i) palygorskite (Pal) from Thano Bula Khan (Sindh) (25.369318, 67.852975), (ii) palygorskite-smectite mix (Pal-Sm) from Thatta, Sindh (24.768584, 67.899732) and (iii) interstratified smectite (ISS) from Dera Ghazi Khan, Punjab (30.695271, 70.678850) that had adsorbed higher AfB1 during initial screening in an in vitro adsorption trial were tested against 250 µg $kg^{-1}$ AfB1 contamination in eleven treatment combinations (Table 1). These clays adsorbed greater AfB1 than the reference clay, a commercial mycotoxin binder, Sorbatox.

**Table 1. Description of the treatment combinations for the feeding trial**

| Treatment | Description |
|---|---|
| CF | Clean feed (aflatoxin B1 < 20 µg $kg^{-1}$) |
| TF | Toxin feed (250 µg aflatoxin B1 $kg^{-1}$ feed) |
| CFISS | Clean feed + Interstratified smectite at 2% |
| CFPS | Clean feed + Palygorskite-smectite at 2% |
| CFP | Clean feed + Palygorskite at 2% |
| TFISS1 | Toxin feed + Interstratified smectite at 1% |
| TFISS2 | Toxin feed + Interstratified smectite at 2% |
| TFPS1 | Toxin feed + Palygorskite-smectite at 1% |
| TFPS2 | Toxin feed + Palygorskite-smectite at 2% |
| TFP1 | Toxin feed + Palygorskite at 1% |
| TFP2 | Toxin feed + Palygorskite at 2% |

***Experimental Details*:** Two feeds stocks: (i) clean feed and (ii) toxin feed, (Table 2) were homogenized in the feed mixer for 15 min and stored in controlled conditions of temperature and humidity. One-day-old Ross-308 male broilers were purchased from the local hatchery (K.K. Chicks Pvt. Ltd. Rawat, Pakistan) and kept under controlled conditions of temperature and humidity and were reared on the clean feed for two weeks during brooding at Avian Research Station, Department of Poultry Sciences, PMAS Arid Agriculture University, Rawalpindi. The shed temperature was maintained at 32°C for the first week and reduced 3°C per week until 22°C and was maintained thereafter (Aviagen, 2018). The relative humidity level was maintained between 65-70% throughout the experimental period. On day 14, the birds were distributed into different treatment combinations in a completely randomized design (CRD) on an equal weight basis and were fed on the experimental feed for the next three weeks. Each treatment was replicated three times, and each replicated pen had 10 birds making 30 birds per treatment and 330 birds in total. Birds were provided with ad libitum access to feed and water throughout the experiment. The lighting programme was used as follows: 23 h light: 1 h darkness from 0-7 days of bird age. After 7 days, darkness period was gradually increased up to 4 h for the rest of the experiment (Aviagen, 2018). The study protocol was approved by the Institutional Ethic Committee, PMAS-Arid Agriculture University Rawalpindi, Pakistan. The vaccination schedule as recommended by the National Disease Control Committee in Pakistan was followed. Daily growth, health and mortality status was monitored regularly during the experiment. Feed consumption and body weight of birds per pen were recorded on weekly basis. Three birds from each pen were randomly slaughtered at day 35 and were weighed for live body weight, dress weight and absolute weight of liver, heart and spleen. Apparent deformation of the internal organs was also recorded. The average body weight per bird was calculated by dividing the final weight per pen by the number of birds that survived, and the feed conversion ratio (FCR) was calculated by using the following equation.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Body weight gain (g)}}$$

**Table 2. Experimental poultry feed composition**

| Feed ingredients | Application rate (kg 100 $kg^{-1}$) |
|---|---|
| Corn | 45 |
| Soybean meal | 17 |
| Soybean grain | 5 |
| Wheat | 5 |
| Canola meal | 6 |
| Rice polish | 5 |
| Wheat bran | 5 |
| Di-calcium phosphate | 1 |
| Premix | 1 |
| Corn gluten 30 % | 4 |
| Corn gluten 60 % | 2 |
| Molasses | 3 |

***Statistical Analysis*:** Data on the live body weight, body weight gain, feed intake and feed conversion ratio were analyzed through multivariate analysis using GLM procedure of SAS/STAT® version 9.4. (SAS Institute Incorporation, 2003) and repeated for weeks. One-way ANOVA was used for the data on absolute and relative weight of the internal organs and dressing percentage. Treatment means were compared using Tukey's HSD test at $p \leq 0.05$.

## RESULTS

***Mineralogical details of the clay sources*:** Palygorskite from Thano Bula Khan, Sindh composed dominantly of palygorskite with minor smectite and kaolinite whereas, the palygorskite-smectite mix from Thatta, Sindh had palygorskite and smectite as major minerals with quartz and calcite in traces (Figure 1). The interstratified smectite with HIS from Dera Ghazi Khan, Punjab had interstratification of hydroxy interlayered smectite with moderate illite (mica) while kaolinite and quartz occurred as traces. Palygorskite had an intermediate structure between di- and tri-octahedral with AlMgOH octahedra. Interstratification of hydroxy interlayered smectite/vermiculite was evident with variable expansion properties. Palygorskite-smectite had 55% of particles in the size range of 0.2-20 µm, whereas, palygorskite and interstratified smectite with HIS had smaller particles and more than 90% volume fraction lied in the same size range. Palygorskite, palygorskite-smectite mix and interstratified clay had maximum AfB1 adsorption potential from *in vitro* trial of 400, 415 and 370 µg $kg^{-1}$, respectively that was far greater than that of a commercial mycotoxin binder, Sorbatox (150 µg $kg^{-1}$).

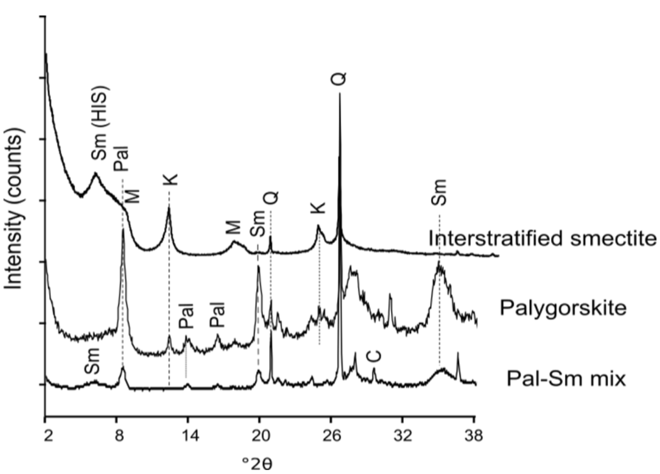


**Figure 1. X-ray diffraction of the clay sources for mineral composition indicating palygorskite (Pal), smectite (Sm), interstratified smectite with HIS, quartz (Q), calcite (C), kaolinite (K) and mica (M) occurrence with variable profusion**

***Body weight*:** The interactive effect of feed combinations and the week was significant and the hypothesis of no week × treatment effect for $F_{tab} > F_{cal}$ was rejected with Wilk's lambda $p$ 0.0004, (Figure 2). Body weight significantly changed ($p < 0.0001$) after first week on experimental diets (day 21), the highest body weight, 900 g $bird^{-1}$, was in the clean feed followed by the clean feed with palygorskite-smectite mix, 880 g $bird^{-1}$, and in the toxin feed with 2% interstratified smectite, 835 g $bird^{-1}$.

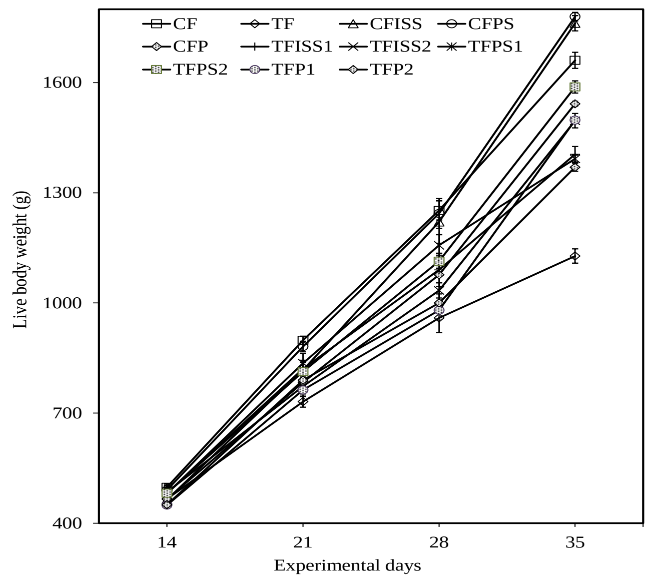


**Figure 2. Live body weight over the feeding weeks as affected by eleven treatment combinations:** CF=clean feed, TF=toxin feed, CFISS=clean feed with interstratified smectite, CFPS=clean feed with palygorskite-smectite, CFP=clean feed with palygorskite, TFISS1=toxin feed with interstratified smectite @ 1%, TFISS2=toxin feed with interstratified smectite @ 2%, TFPS1=toxin feed with palygorskite-smectite @ 1%, TFPS2=toxin feed with palygorskite-smectite @ 2%, TFP1=toxin feed with palygorskite @ 1%, TFP2=toxin feed with palygorskite @ 2%, indicating the clays addition to contaminated feed reduced AfB1 toxic effect. Graphical line plot represents mean values of three replications at each week. Error bars at each week indicate standard error.

The toxin feed had the lowest body weight, 730 g $bird^{-1}$, and was similar to the toxin feed with 1% palygorskite-smectite mix, the clean feed with palygorskite and the toxin feed with palygorskite at both application levels. On day 28, the highest body weight, 1250 g $bird^{-1}$, in the clean feed was similar to the clean feed with the palygorskite-smectite mix. The body weight was similar in the interstratified smectite and palygorskite-smectite at 2% in the toxin feed while the toxin fed birds had the lowest body weight, 960 g $bird^{-1}$. After three weeks on experimental diets, day 35, the highest body weight of 1780 g bird$^{-1}$ was in the clean feed with palygorskite-smectite mix followed by the clean feed with interstratified smectite, 1760 g bird$^{-1}$, which was significantly different to all the other treatment combinations. The palygorskite-smectite mix at 2% and palygorskite at 1% in the toxin feed were statistically at par. The body weight in the toxin feed with interstratified smectite at both applied levels was similar to the toxin feed with 2% palygorskite. The toxin feed had the lowest body weight, 1125 g bird$^{-1}$, which significantly differed from all the other treatment combinations. Overall, the addition of the clays in the clean feed either had higher or similar body weight as that of the clean feed suggesting no adverse effect of clays on broilers body weight. Palygorskite at 1% and palygorskite-smectite mix at 2% added in the toxin feed improved body weight of birds and reduced the toxic effect of AfB1.

***Weight gain rate*:** Weekly weight gain over the experimental feeding weeks changed significantly ($p < 0.001$) (Figure 3). On day 21, the clean feed had the highest body weight gain, 400 g bird$^{-1}$, which was similar to the clean feed with palygorskite-smectite mix, 390 g bird$^{-1}$. The toxin feed with 2% application of interstratified smectite had 355 g bird$^{-1}$ and was similar to the clean feed with the same clay addition. After the second week, day 28, the clean feed was similar for weight gain with the clean feed with added clays from any source. The toxin feed was similar to the toxin feed with added clays at both applied levels from any of the sources, except for 2% interstratified smectite. The weight gain differed significantly ($p < 0.001$) during the third week of experiment, and was highest, 540 g bird$^{-1}$, in the clean feed with interstratified smectite followed by the clean feed with palygorskite-smectite and the toxin feed with 1% palygorskite. The body weight gain in the clean feed was statistically the same as in the clean feed added with any of the clay source suggesting no harmful impact of the clay addition. The toxin feed had the lowest body weight gain, 168 g bird$^{-1}$, compared to all the other treatments suggesting a detrimental effect of AfB1 on broilers growth. Overall, the addition of the clays in the clean feed had no negative impact on weight gain rate and the clay sources added in the toxin feed reduced AfB1 toxicity with a better weight gain rate than the toxic feed alone.

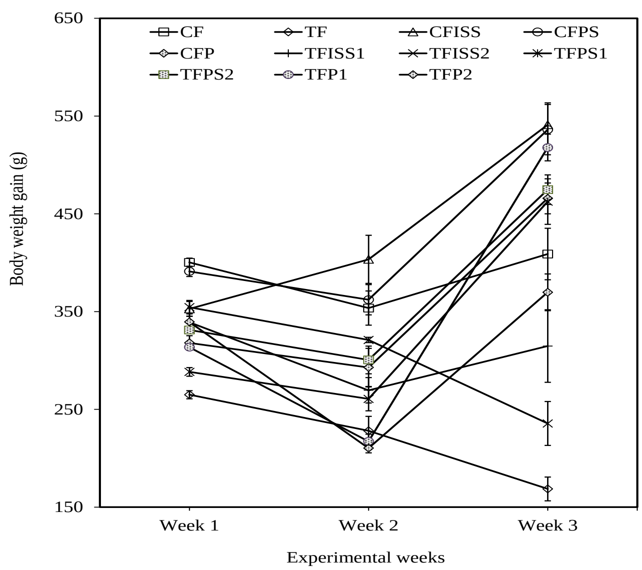


**Figure 3. Body weight gain rate over three experimental weeks affected by different treatment combinations:** CF=clean feed, TF=toxin feed, CFISS=clean feed with interstratified smectite, CFPS=clean feed with palygorskite-smectite, CFP=clean feed with palygorskite, TFISS1=toxin feed with interstratified smectite @ 1%, TFISS2=toxin feed with interstratified smectite @ 2%, TFPS1=toxin feed with palygorskite-smectite @ 1%, TFPS2=toxin feed with palygorskite-smectite @ 2%, TFP1=toxin feed with palygorskite @ 1%, TFP2=toxin feed with palygorskite @ 2%, indicating the lowest weight gain on the toxin feed. Graphical line plot represents mean values of three replications at each week. Error bars at each week indicate standard error.

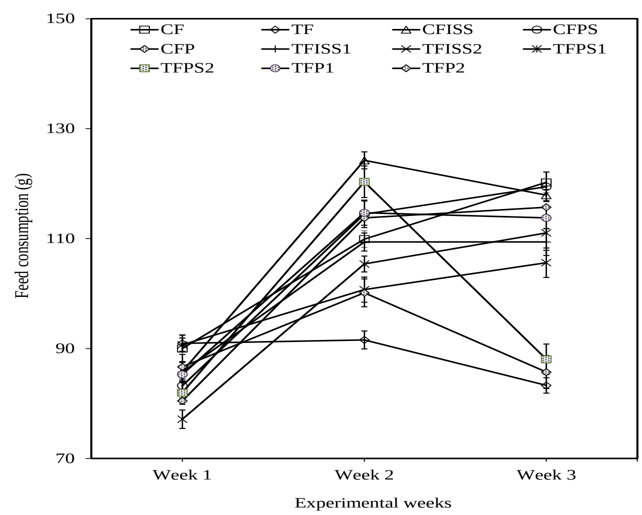


**Figure 4. Weekly feed consumption in response to treatment combinations:** CF=clean feed, TF=toxin feed, CFISS=clean feed with interstratified smectite, CFPS=clean feed with palygorskite-smectite, CFP=clean feed with palygorskite, TFISS1=toxin feed with interstratified smectite @ 1%, TFISS2=toxin feed with interstratified smectite @ 2%, TFPS1=toxin feed with palygorskite-smectite @ 1%, TFPS2=toxin feed with palygorskite-smectite @ 2%, TFP1=toxin feed with palygorskite @ 1%, TFP2=toxin feed with palygorskite @ 2%, showing the lowest feed intake with the toxin feed. Graphical line plot represents mean values of three replications at weekly interval. Error bars at each week indicate standard error.

***Feed intake*:** Average feed consumed per bird was recorded on weekly basis by subtracting the amount of feed retained from the feed offered. (Figure 4). The hypothesis of no week

× treatment effect was rejected through the MANOVA test criteria Wilks' Lambda, p 0.007. Feed intake at day 21 was non-significant for all the treatment combinations. However, at day 28 feed intake varied significantly ($p < 0.0001$). Feed intake in the clean feed with any of the clay source addition was higher and statistically at par to the toxin feed with palygorskite-smectite at 2% and the toxin feed with palygorskite at 1%. The lowest feed intake was recorded in the toxin feed. On day 35, the maximum feed intake was in the clean feed that was similar to the clean feed added with any of the clay and with the toxin feed with any of the three sources at 1% application level. The toxin feed with palygorskite-smectite at 2% and palygorskite at 2% were statistically similar to the toxin feed that had the lowest feed intake.

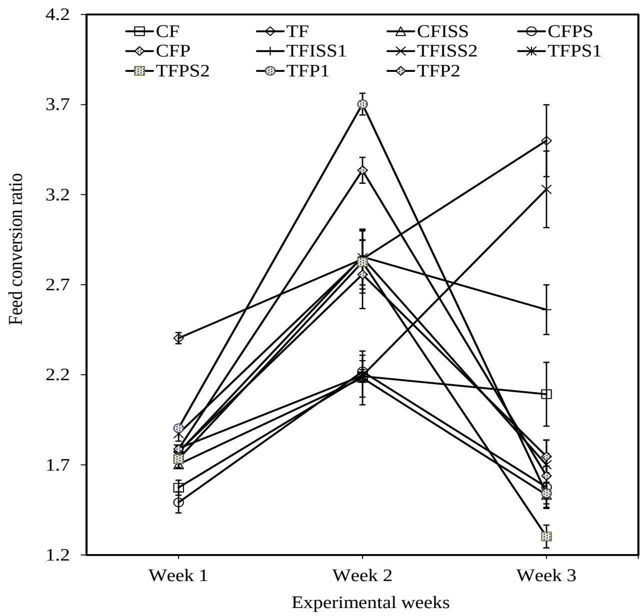


**Figure 5. Weekly feed conversion ratio influenced by experimental feed combinations:** CF=clean feed, TF=toxin feed, CFISS=clean feed with interstratified smectite, CFPS=clean feed with palygorskite-smectite, CFP=clean feed with palygorskite, TFISS1=toxin feed with interstratified smectite @ 1%, TFISS2=toxin feed with interstratified smectite @ 2%, TFPS1=toxin feed with palygorskite-smectite @ 1%, TFPS2=toxin feed with palygorskite-smectite @ 2%, TFP1=toxin feed with palygorskite @ 1%, TFP2=toxin feed with palygorskite @ 2%, indicating detrimental effect of the toxin feeding and improved FCR with the clays addition to the toxin feed. Graphical line plot represents mean values of three replications at each experimental week. Error bars at each week indicate standard error.

***Feed conversion ratio*:** Weekly FCR is presented in Figure 5. On day 21, the treatments differed significantly for the FCR ($p < 0.0001$) and was very poor, 2.40, in the toxin feed that significantly varied from all the other treatments suggesting high feed consumption with low poultry production. The addition of all the three clays in the toxin feed at 1% and 2% application rate in the toxin feed was similar during the first experimental week. The lowest FCR, 1.50, was recorded in the clean feed with palygorskite-smectite mix that was at par with the clean feed and the clean feed with interstratified smectite. The FCR generally increased during the second week of the experiment and the highest was in the toxin feed with palygorskite at both applied levels. For the third week, day 35, the toxin feed had the highest FCR, 3.50, followed by toxin feed with interstratified smectite at both levels of application. The lowest FCR was in the toxin feed with palygorskite-smectite at 2%. The FCR in the clean feed was similar to all other treatments except the toxin feed. Overall, the birds fed on AfB1 containing feed had poor FCR while the addition of the clays in the toxin feed improved FCR suggesting improved feed digestion and weight gain.

***Carcass yield percentage*:** Carcass yield percentage was significantly altered by the treatment combinations (F 2.62, *p* 0.0286) (Figure 6). The highest percentage, 64.5%, was in the clean feed with interstratified smectite at 2% followed by the clean feed with palygorskite-smectite while the toxin feed had the lowest carcass yield percentage of 57%. All the three clays addition at both application levels had a similar carcass yield in both feed types.

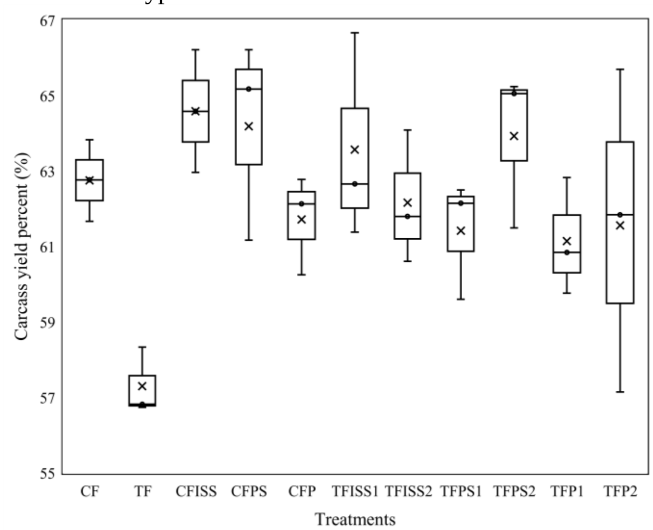


**Figure 6. Carcass yield percent as affected by different feed combinations:** CF=clean feed, TF=toxin feed, CFISS=clean feed with interstratified smectite, CFPS=clean feed with palygorskite-smectite, CFP=clean feed with palygorskite, TFISS1=toxin feed with interstratified smectite @ 1%, TFISS2=toxin feed with interstratified smectite @ 2%, TFPS1=toxin feed with palygorskite-smectite @ 1%, TFPS2=toxin feed with palygorskite-smectite @ 2%, TFP1=toxin feed with palygorskite @ 1%, TFP2=toxin feed with palygorskite @ 2%, indicating detrimental effect of the toxin feeding and improved FCR with the clays addition to the toxin feed.

**Table 3. The absolute and relative weight of internal organs**

| Treatments | Absolute weight (g) | | | Relative weight (%) | | |
|---|---|---|---|---|---|---|
| | Liver | Heart | Spleen | Liver | Heart | Spleen |
| | *p 0.0357* | *p 0.0836* | *p 0.4494* | *p 0.6457* | *p 0.1716* | *p 0.6168* |
| CF | 42.63±6.22 | 11.62±0.72 | 2.09±0.25 | 2.30±0.34 | 0.63±0.04 | 0.11±0.01 |
| TF | 30.90±2.69 | 9.40±0.59 | 1.67±0.32 | 2.29±0.13 | 0.70±0.06 | 0.13±0.02 |
| CFISS | 46.58±8.00 | 11.61±0.93 | 2.70±0.14 | 2.30±0.23 | 0.58±0.03 | 0.13±0.01 |
| CFPS | 47.76±3.75 | 11.57±1.58 | 2.22±0.30 | 2.34±0.06 | 0.57±0.05 | 0.11±0.01 |
| CFP | 38.65±1.41 | 11.38±0.46 | 1.99±0.21 | 2.27±0.03 | 0.67±0.03 | 0.12±0.01 |
| TFISS1 | 38.33±1.35 | 9.38±1.71 | 2.00±0.23 | 2.35±0.19 | 0.57±0.06 | 0.12±0.02 |
| TFISS2 | 36.48±1.05 | 11.68±0.53 | 2.77±0.94 | 2.09±0.13 | 0.67±0.05 | 0.16±0.05 |
| TFPS1 | 35.50±5.58 | 11.56±0.97 | 2.73±0.84 | 2.17±0.21 | 0.71±0.08 | 0.17±0.04 |
| TFPS2 | 49.71±7.40 | 12.51±0.87 | 2.56±0.41 | 2.60±0.19 | 0.66±0.09 | 0.14±0.03 |
| TFP1 | 40.48±7.86 | 12.03±0.63 | 2.34±0.76 | 2.29±0.29 | 0.69±0.06 | 0.13±0.04 |
| TFP2 | 35.45±5.31 | 10.81±1.11 | 2.04±0.26 | 2.25±0.19 | 0.69±0.09 | 0.13±0.01 |

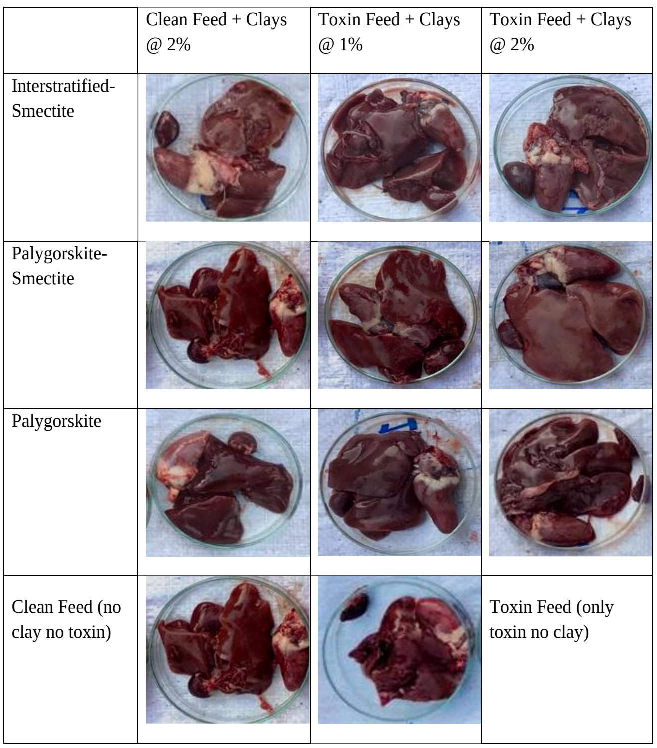


**Figure 7. Morphology of the internal organs influenced by different feed combinations:** clean feed, toxin feed (250 µg AfB1 $kg^{-1}$), clean feed with interstratified smectite, clean feed with palygorskite-smectite, clean feed with palygorskite, toxin feed with interstratified smectite @ 1%, toxin feed with interstratified smectite @ 2%, toxin feed with palygorskite-smectite @ 1%, toxin feed with palygorskite-smectite @ 2%, toxin feed with palygorskite @ 1%, toxin feed with palygorskite @ 2%, indicating detrimental effect of the toxin feeding.

***Internal organs morphology*:** The difference in morphology of the internal organs affected by different feed combinations is compared (Figure 7). The birds fed on AfB1 contaminated feed had smaller and darkish livers compared to the clean feed birds. Palygorskite resulted in a smaller liver size in both the clean and the toxin feed while the palygorskite-smectite mix at 1% application rate had better liver morphology than at 2%. Interstratified smectite clay added at a 2% level of application resulted in pale colouration and gross lesions of the liver.

The box and whisker plot represents carcass yield with stretched whiskers indicating lowest and maximum observed values. The box is divided in to quartiles with each quartile separating upper and lower 25% of the data, median line showing 50% of the data set on either side. The larger the width of box and whisker more is the variability in the data. The cross inside the box represents mean value.

***Internal organs weight*:** Absolute and relative weights of liver, heart and spleen were compared in response to different feed combinations (Table 3). Absolute liver weight significantly varied (F 2.49, *p* 0.0357) and the highest, 49.70 g, was in birds fed on the toxin feed with 2% palygorskite-smectite mix followed by the clean feed with palygorskite-smectite and the clean feed with interstratified smectite. The lowest absolute liver weight, 30.9 g, was in the toxin feed that varied significantly with all the other treatment combinations. However, the relative weight of the liver was non-significant in all the treatment combinations. The absolute and relative weight of the heart and spleen was non-significant indicating no impact of AfB1 or clays addition on internal organs weight.

## DISCUSSION

The toxicity of AfB1 at 250 µg $kg^{-1}$ of feed caused reduction in weight gain rate and poultry production compared to the clean feed treatment. The AfB1 contaminated feed reduces the weight of birds with adverse effects on health and growth and causes loss in livestock production through poor growth and feed conversion, increased mortality, decreased egg production and leg problems (Naseem *et al*., 2018; Bhatti *et al*., 2018). The AfB1 contamination above the permissible limit reduces production and induces economic losses in poultry (Kana *et al*., 2010) through immune-suppression, high feed intake and low feed conversion efficiency (Soliman *et al*., 2008; Yarru *et al*., 2009). In the current study AfB1 caused a 75% reduction in total weight gain compared to the clean feed treatments that was associated with the contaminated feedings (Saleemi *et al*., 2020). The AfB1 feeding resulted in lower body weight gain with poor FCR (Hussain *et al*., 2016). The consumption of AfB1 contaminated reduces all important production parameters in poultry such as weight gain, feed intake, yield and egg production (Hedayati *et al.*, 2014). Clays addition in the clean feed was similar to the clean feed treatment irrespective of the clay source in terms of live body weight, weight gain rate and feed intake. Clay can bind toxic compounds (Xu *et al*., 2004) and has antibacterial effects (Haydel *et al*., 2008; Mpuchane *et al*., 2008). The clay minerals such as smectite, palygorskite and sepiolite can adsorb AfB1 and effectively reduce its toxicity to feed reared animals and poultry (Wicklein *et al*., 2008; Barrientos-Velazquez and Deng, 2020). The AfB1 contaminated feeding resulted in reduced weight gain with higher feed intake and poor FCR each week and overall low production of poultry was related to aflatoxin-contaminated feeding (Saleemi *et al*., 2020). The reduction in weight gain is associated with extended exposure to AfB1 contamination (Mahmood *et al*., 2017). The toxicity of AfB1 adversely affect the body weight gain, feed intake and broilers health suggesting interference of AfB1 in the digestive system (Ahsan-ul-Haq *et al*., 2000). The inconsistency in the weight gain and reduction is related to exposure time of broilers to AfB1 toxicity (Tedesco *et al*., 2004; Denli *et al*., 2009; Zhao *et al*., 2010). Lower body weight, high mortality, poor FCR, immunosuppression and clinical disorders are related to AfB1 toxicity (Bhatti *et al*., 2018; Naseem *et al*., 2018). The contaminated diet reduces the feed intake in broilers (Rauber *et al*., 2007; Saleemi *et al*., 2020), with liver lesions as basic symptoms of AfB1 toxicity (Hussain *et al*., 2016).

The addition of the clays in the toxin feed and the clean feed significantly reduced AfB1 toxicity and had a lower FCR suggesting improved feed conversion efficiency in response to the addition of the clay. The consumption of clay has a protective effect against toxicity as the toxins are being adsorbed by the clays (Manafi *et al*., 2012). The palygorskite-smectite mix in the toxin feed improved FCR and weight gain rate that may be related to the higher AfB1 selectivity over nutrients due to its specific structure (Zhou, 2016). The addition of clay in the toxin feed improves feed intake by providing a protective coating to the digestive tract, balancing the pH buffering capacity and the dilution rate of food (Voros, 2001). Palygorskite improves the growth performance by increasing body weight gain and feed intake at 2% application rate (Zhou *et al*., 2014). Palygorskite added in the clean feed had no adverse effect on weight gain. In the current study palygorskite at 1% in the toxin feed increased the body weight, feed intake and FCR and was better than the 2 % application rate. Palygorskite inhibits the growth of bacteria, adsorb mycotoxin and harmful bacteria which respond to oxidative stress and free radicals which contribute to tissue damage (Zhou *et al*., 2014). A 60% increase for weight gain over toxin feed treatment was recorded with palygorskite addition at 1% in the toxin feed while palygorskite-smectite at 2% increased weight gain by 65% over the toxin feed.

The addition of fibrous clays in the feed is safe (Pappas *et al*., 2010), but the continuous use can lead to loss of enzymes, vitamins and other essential substances (Galán, 1996). The fibrous clays provide a protective cover to reduce gut damage (Zhang *et al*., 2013). The toxin fed birds had a darker, abnormal and smaller liver that may be attributed to severe immune suppression (Kubena *et al*., 1990). However, enlarged liver and kidney have also been reported due to AfB1 feedings (Miazzo *et al*., 2005; Wafaa *et al*., 2013). Addition of clay minerals in poultry feed ameliorates the adverse effect of AfB1 in commercial broilers (Bhatti *et al*., 2016, 2018; Mgbeahuruike *et al*., 2018). Aflatoxins are primarily hepatotoxic and cause liver damage in animals (Saleemi *et al*., 2020). The liver is the most targeted organ for AfB1 deposition in all birds (Khan *et al*., 2013; Iqbal *et al*., 2014). Better morphology of liver in the toxin feed with clays addition was associated with higher AfB1 adsorption by binders (Miazzo *et al*., 2005). The broilers fed on the toxin feed have abnormal, pale and friable liver, swollen kidneys and haemorrhages on different organs (Iqbal *et al*., 2014). Non-significant variation in the relative weight of the liver, heart and spleen may be associated with the low application levels of clay as the weight of the internal organs is non-sensitive at clay level below 5% (Barrientos-Velazquez and Deng, 2020).

***Conclusions*:** The contamination of AfB1 caused a reduction in weight gain rate with poor poultry production while the addition of the clay as a feed additive was safe and reduced the toxic effect of AfB1. The liver morphology was comparatively better in palygorskite-smectite mix treatment when applied at 1% application rate and the darkish colour of the liver was also improved with the clay addition in the toxin feed. Palygorskite and palygorskite-smectite mix clays were better than interstratified smectite in increasing poultry production and controlling AfB1 toxicity. The indigenous palygorskite and palygorskite-smectite mix clay may be promoted as AfB1 binders without any pretreatment or size fractionation for controlling AfB1 toxicity in poultry industry. Overall, the study has economic value and could result in the utilization of vast clay deposits of Pakistan in the poultry industry and reduce the risk of cancer in poultry consumers. Further research needs to be conducted to address the interaction of clays with nutrients.

***Conflict of Interest*:** The authors declare that there is no conflict of interest.

***Authors' Contribution Statement*:** SA and AK executed the feeding trial and completed writeup with the guidance of MSA and GJ, MSA and TA designed the poultry feeding trial and supervised the whole research work.

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