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Research Article

Effects of Local Sodium Bentonite as Aflatoxin Binder and Its Effects on Production Performance of Laying Hens

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Abstract

The study was designed to examine the effect of sodium bentonite (SB) as mycotoxin binder in poultry feed and its effects on productions performance of laying hens. A total of forty-five production hens white leghorn aged 34 weeks were caged in a naturally aired laying house into five different groups. Group A was kept as control group, Group B was fed with higher level 170 ppb aflatoxin without binders, and Group C feed contained 170 ppb aflatoxin with 1.5% SB binder, Group D contained 170 ppb aflatoxin with 2% SB and Group E feed contained 170 ppb aflatoxin with 2.5% SB. Aflatoxin contamination adversely affected the egg production and feed consumption. Addition of 2% and 2.5% SB increased ($P<0.05$) in feed intake and egg productions. SB appears to be useful as toxin binding additive and counteracting the deleterious effects of aflatoxin. However there were no obvious effects observed among groups in respect to egg yolk and albumin content. Significant effect was observed among the groups for feed consumption, egg production, weight, shell weight and thickness and for FCR value. The addition of 2% SB showed better feed and protein utilizations leading an increased egg production and reduced eggs defects in layer hens.

Keywords: *Mycotoxins binder, Sodium bentonite, Poultry feed, Laying hen, Layer*

Aflatoksin-Bağlayıcı Lokal Sodyum Bentonitin'in Yumurtacı Tavukların Üretim Performansı Üzerine Etkileri

Özet

Çalışma, kanatlı yemlerinde sodyum bentonit (SB)'in mikotoksin bağlayıcı olarak etkisi ve yumurta tavuklarında üretim performansı üzerine etkilerini incelemek için tasarlandı. Toplam kırk beş adet 34-haftalık beyaz leghorn üretim tavuğu beş farklı grup tarzında doğal havalandırmalı kafesler içine alındı. Grup A: Kontrol grubu olarak tutuldu; Grup B: Bağlayıcılı üst düzey 170 ppb aflatoksin ile beslendi; Grup C: Yem %1.5'lik SB bağlayıcılı 170 ppb aflatoksin içerdi; Grup D: %2'lik SB bağlayıcılı 170 ppb aflatoksin içerdi; Grup E: Yemler %2.5'lik SB bağlayıcılı 170 ppb aflatoksin içerdi. Aflatoksin kontaminasyonu yumurta üretimi ve yem tüketimini olumsuz yönde etkiledi. SB'nin %2 ve %2.5'lik ilavesi yem tüketimi ve yumurta üretimlerini artırdı ($P<0.05$). SB'nin toksin-bağlayıcı katkı özelliği sayesinde aflatoksinin zararlı etkilerine karşı faydalı olduğu gözükmektedir. Ancak, gruplar arasında yumurta sarısı ve albümin içeriği yönünden gözlenen belirgin bir etkisi yoktu. Gruplar arasında yem tüketimi, yumurta verimi, ağırlığı, kabuk ağırlığı ve kalınlığı ile FCR değeri için ise önemli etki gözlendi. SB'nin %2'lik ilavesinin, yumurta tavuklarında daha iyi yem ve protein kullanımı sayesinde yumurta üretiminde artışa ve yumurta kusurlarında düşmeye yol açtığı gözlendi.

Anahtar sözcükler: *Mikotoksin bağlayıcı, Sodyum bentonit, Kanatlı yemi, Yumurtacı tavuk, Yumurtacı*

INTRODUCTION

The demand in egg and broiler has increased, which put tremendous pressure and compression on apposite feeding of poultry. In order to sustain and uphold poultry industry a good management and proper feeding is indispensable. So far the notable problem in poultry feed is contamination of different toxin that not only reduces income but also impairing farm operations. Mycotoxin is odorless and invisible compound that can not be detected by

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smell or taste. The main types of mycotoxins are aflatoxins, trichothecene, zearalenone and fumonisin. Even a small quantity of these mycotoxins is extremely dangerous and their presence in poultry feeds lead to a poor performance of different poultry products [1]. Aflatoxins as major mycotoxins are produced by fungi such as *Aspergillus* and *Penicillium* species. These filamentous fungi contaminate cereals and grains during harvesting, storage, transportation and processing. Aflatoxicosis, severely affected the development of broiler as well egg production in layers and produced symptoms like anemia, enlargement of hemorrhagic liver, fat accumulation, kidney paleness and reduced feed uptake [2,3]. Another important mycotoxin in poultry diet is Ochratoxin A (OTA), which is produced by *Aspergillus and Penicillium* species of fungi, and have carcinogenic effects especially on kidney and liver [4-6]. Low-level exposure to OTA leads to reduced feed consumption and increase susceptibility to diseases [7]. These naturally occurring toxin vary through different climatic and geographical positions; however their occurrence and exposure in poultry feed is worldwide. The well-known carcinogenic mycotoxins are aflatoxins which includes AF B1, B2, G1, G2 and M1 but aflatoxin B1 is considered the major hepato-carcinogenic, which effects differently with age, species, sex and nutritional conditions in animals.

Poultry are considered sensitive to aflatoxins that severely reduce growth, feed intake and cause kidney and liver damage and eventually leads to death. Their feed consists of several types of agro industrial byproducts and cereal grains i.e., corn grain which can be easily contaminated by fungi under favorable temperature and humidity [8]. In this way, fungus contaminates feed and produces mycotoxin affecting animal health and nourishment and ultimately influence human health [9].

Different strategies have been used for detoxification of aflatoxin-contaminated feedstuff such as thermal inactivation, physical separation, microbial degradation, irradiation and chemicals treatment. Ammonia treatment is being used to decontaminate aflatoxin in feeds [10]. Chemical detoxification is considered the best method for aflatoxin containing poultry diet and reported more effective than physical and biological methods [11]. These chemicals are used as feed additives and also mixed in animal feed. These compounds have no side effects and are clay minerals that can immediately bind to aflatoxin because of opposite charge. Different aflatoxin binders like zeolites, calcium and sodium alumino-silicate have been tested for binding capability. In turkey and chicken feeds, phyliosilicate clay and hydrated sodium-calcium silicates have been reported to efficiently absorb the aflatoxin [1].

Bentonite is a hydrophilic compound, colloidal alumino-hydrosilicate in nature and has high swelling ability and act as enterosorbant in animal diet and can bind and reduce aflatoxin absorption [12-14]. It has capability to form complex with aflatoxin and prevents absorption through intestinal epithelium. It is also used as lubricating; binding agents improve food uptake efficiency and growth rate in broilers. Apart from this it also improves protein utilization and prolonged the foodstuff passage time. Nowadays, there are different commercial adsorbents available on the market and can be used as feed additives to control or neutralize the toxic effects. The present strategy was to bind the aflatoxins with sodium bentonite (SB) and inhibit absorption in digestive tract of layers and excrete the complex in feces. SB is a three-layer structure compound of aluminum silicate that can absorb a variety of compounds selected for aflatoxins binding in broiler feed. This study was planned to evaluate the ability of indigenous SB as aflatoxins binder, to observe its effects on production performance of layer and also to determine the most appropriate level of SB. To the best of author's knowledge the only well known naturally occurring deposit SB in Northwestern part of Pakistan.

MATERIAL and METHODS

Birds were handled with proper care and kept in cages at the poultry unit of the University poultry Farm, the University of Agriculture Peshawar-Pakistan from August to September 2014 inclusive. The bird care protocol used in this study was reviewed and approved by the Ethics Committee headed by the Chairman Department Live Management & Animal Breeding and Genetics with three members each from Department of Poultry Science, Department of Animal Nutrition and Department of Animal Health, Faculty of Animal Husbandry and Veterinary Sciences, the University of Agriculture Peshawar-Pakistan. Daily requirement including light, water, air and feed was given according to the bird's standard protocols during the experiment period including 1st week of adaptation period. Daily observation of bird's health was regularly monitored and problems were recorded. The feed testing for aflatoxins was performed at the Veterinary Research Institute (VRI) Peshawar-Pakistan.

Experimental Design

A total of forty-five hens of white leghorn breed (RIR and Fyoumi) aged 34 weeks and divided into five different treatment groups from the day 1st, while each group consisted of 9 birds. Group A was kept as control group and was given feed (F-1) with least level 15 ppb of aflatoxins without binders, Group B was given feed (F-2) with higher level 170 ppb aflatoxins without binders, and Group C feed (F-3) contained 170 ppb aflatoxins with 1.5% SB binder, Group D feed (F-4) contained 170 ppb aflatoxins with 2.0% SB and Group E feed (F-5) contained 170 ppb aflatoxins with 2.5% SB.

Selection Feed and Chemical Analysis

Feed samples were randomly collected from different feed sellers in the capital city of Khyber Pakhtonkhwa province of North-western part of Pakistan and tested for aflatoxins load (Feed-1: aflatoxins B1 (AFB1)=35 ppb, AFB2=10 ppb; Feed-2: AFB1=15 ppb; Feed-3: AFB1=40 ppb, AFB2=10 ppb; Feed-4: AFB1=95 ppb, AFB2=05 ppb, AFG1, 5.5 ppb; Feed-5: AFB1=150 ppb, AFB2=11.11 ppb, AFG1, 8.9 ppb). The well-known carcinogenic mycotoxins are aflatoxins including AF B1, B2, G1, G2 and M1 but aflatoxin B1 is considered the major hepatocarcinogen which effects differently with age, species, sex, and nutritional conditions in animals.

Feed samples from different feed dealers from the local market were randomly collected and tested for mycotoxins load using Thin Layer Chromatography (TLC) and feed containing the highest load of total mycotoxins was selected. TLC procedure carried according the standard protocol described briefly below:

A 25 g dry feed sample was mixed with 100 mL of acetone: Distilled water (81 mL: 19 mL) solution and was shaken for 40 min. The feed sample was filtered and mixed with 85 mL (0.2 N NaOH), 15 mL (6.6% $FeCl_3$), 2.5 g cellite powder and 1.5 g $CuCO_3$. The mixture was shaken for 20 min and then filtered. The filtrate was then mixed with 75mL of 0.03% of H_2SO_4 and 10 mL of 35% of chloroform and then shaked vigorously for 5 min. The solution was then suspended in a separating funnel for 30 min and the lower layer (precipitate) in flask was collected and 50 mL of KOH + KCl solution in the ratio of 1:10 was added and was suspended for 30 min again. Then again the lower layer in china dish was collected and it was dried and dissolved in 1 mL chloroform and spotted on TLC plates along with standard spots. The plates were then developed in developing tank having chloroform + acetone as 90:10 solution and air dried and observed under ultra violet lamp.

Feed Preparation

Sodium bentonite clay samples were collected from different parts of the Northwestern part of Pakistan. SB samples were crushed and grinded and submitted to the Pakistan Council of Scientific and Industrial Research (PCSIR) lab Peshawar-Pakistan for chemical analysis (Silica, 52.61; Iron, 13.30; Aluminum, 10.78; Calcium, 3.07; Magnesium, 0.43; Sodium, 3.56; Potassium, 2.50, Moisture, 5.69; loss on ignition, 8.00). After aflatoxins quantification, three rations were prepared i.e., 1.5%, 2% and 2.5% of SB and mix with commercial layers ration obtained from the local market.

Egg Production and Feed Intake

Egg were collected at 2 pm daily form each replicate of all groups to calculate egg production. Egg were collected and counted daily. Hen day egg production for all the groups was determined using the following formula.

Average Hen Day Egg production (%) = No of egg produced on 1 day/No of live birds in that group at that day) multiply 100.

Similarly feed intake was calculated on daily bases. It was measured by subtracting the feed rejected from the total feed given,

Feed consumption = Feed Offered-Feed Refused

Feed Conversion Ratio

Feed conversion ratio (FCR) was calculated on weekly basis. FCR was calculated for each replicate by the following formula;

$$\text{FCR per dozen of egg} = \frac{\text{Weekly feed consumption per replicate}}{\text{Weekly number of egg produced per replicate}} \times \frac{1}{12}$$

Egg Quality and Quantity Parameters

Two eggs from each group were taken and weighed individually, and then their mean was calculated. Weight eggs contents (*i.e.,* yolk, albumen, shell) were recorded on weekly basis. Two eggs per group were broken individually. The yolk was separated with help of egg yolk separator (Kitchen Craft, UK).

Yolk weight = Egg weight-(Albumin weight + Shell weight)

Albumen weight was calculated by subtracting yolk weight and shell weight from total egg weight. Similarly eggshell weight was determined after removing eggshell membranes. Eggshell thickness was determined with help of digital micrometer screw gauge. Shell thickness was determined without inner and outer membranes at three different regions to get the average value.

Statistical Analysis

Statistical analysis was performed with a commercially available software program SPSS version 18, SPSS Inc., Chicago, IL, USA. The data were analyzed using one-way analysis of a variance (ANOVA) between treatments. Least Significant Difference (LSD) test was applied when significant differences were found. The value of $P<0.05$ was considered to be significant.

RESULTS

Feed Intake

Laying birds that were fed with 2% and 2.5% SB level consumed higher ($P<0.05$) feed than other treatments groups *Table 1*. Feed consumption was significantly ($P<0.05$) affected by aflatoxins in treatment B. It was also cleared that lower feed intake was shown by group B, while high feed intake were observed by group D, and E as recorded in our experiment. It is also clear that SB increases efficiently uptake of feed in layers. Although group C, do not show any effects (P>0.05) as compared to group D and E. From these results we observed that SB neutralized the effect of aflatoxins in layer bird efficiently.

Egg Productions

The average number of eggs production in group D and E showed uniformity while in group B there was low eggs production recorded. It is clear that aflatoxins adversely affected egg production in layers. The addition of 2% & 2.5% SB level effectively increased (P<0.05) production of eggs *Table 2*. Because SB neutralized the major effects of aflatoxins in groups D, and E when compared to group B.

Feed Conversion Ratio

In 1st week our results showed that in groups D and E have significantly higher FCR when compared to other experimental groups. However in week 2nd, high FCR was recorded in group C which is followed by group B, and groups D and E, while groups B and C showed similarity results. In 3rd week, B and C showed significant (P<0.05) increase FCR followed by D and E. In 4th week again groups B and C had highest FCR when compared to groups A, D and E. From these results the best FCR was noticed in group A, followed by groups D and E during our observation period *Table 3*.

Egg Quality and Metric Parameters

Our data showed that egg weight was significantly (P<0.05) heavier in group D and E than B, C and in groups A *Table 4*. Eggs weight in group A and C showed uniformity while in group B were lighter in weight, however eggs in group D was significantly heavier.

Eggshell weight was significantly affected by SB level. Eggshell weight was higher (P<0.05) recorded in group D while lowest was found in group B. However 2% SB level do not only increase egg weight but also increase shell thickness. Our results also indicated that the addition of SB could not increase (P>0.05) in weight of yolk and albumin level. Egg yolk weight was not significantly (P>0.05) affected by different level of SB. So it is clear that SB couldn't affect egg yolk weight and there is no interaction between yolk, Albumen and SB concentration. However, eggshell

Table 1. *Total feed intake (Kg) by five groups of laying hen with different diet (Mean ± SE)*
Tablo 1. *Farklı rasyon verilen beş farklı gruptaki yumurta tavuklarının toplam yem tüketimi (Kg) (Ortalama ± SE)*

Groups	Week 1st	Week 2nd	Week 3rd	Week 4th	Total Feed Intake
A	0.61±0.03^{bc}	0.71±0.04^{bc}	0.75±0.02^{bc}	0.80±0.04^{bc}	2.87±0.04^{bc}
B	0.53±0.03^{c}	0.62±0.04^{c}	0.66±0.02^{c}	0.72±0.06^{c}	2.53±0.03^{c}
C	0.62±0.02^{b}	0.73±0.02^{b}	0.80±0.02^{b}	0.82±0.05^{b}	2.97±0.04^{b}
D	0.68±0.01^{a}	0.83±0.01^{a}	0.88±0.05^{a}	0.88±0.06^{a}	3.27±0.04^{a}
E	0.69±0.01^{a}	0.84±0.01^{a}	0.88±0.02^{a}	0.89±0.07^{a}	3.30±0.05^{a}

Mean values within the same column with the different superscripts are significantly different at (P<0.05). Where capital alphabet A refer to control group, B, 170 ppb aflatoxin added, C, 170 ppb aflatoxin plus 1.5% SB added, D, 170 ppb aflatoxin plus 2% SB added, and E,170 ppb aflatoxin plus 2.5% SB added. The letters in the following tables have the same meanings

Table 2. *Weekly egg production by laying hens used in the experiment*
Tablo 2. *Çalışmada kullanılan yumurta tavuklarındaki haftalık yumurta üretimi*

Groups	Week 1st (Mean ± SE)	Week 2nd (Mean ± SE)	Week 3rd (Mean ± SE)	Week 4th (Mean ± SE)	Total Egg in % (Mean ± SE)
A	2.28±0.01^{b}	2.32±0.01^{bc}	2.35±0.02^{bc}	2.36±0.01^{bc}	2.32±0.01^{bc}
B	2.20±0.02^{c}	2.27±0.01^{c}	2.28±0.02^{c}	2.30±0.02^{c}	2.26±0.02^{c}
C	2.24±0.01^{bc}	2.30±0.01^{bc}	2.37±0.02^{bc}	2.37±0.02^{bc}	2.34±0.02^{bc}
D	2.30±0.00^{a}	2.35±0.01^{a}	2.42±0.01^{a}	2.44±0.01^{a}	2.40±0.02^{a}
E	2.31±0.02^{a}	2.35±0.02^{a}	2.41±0.02^{a}	2.41±0.02^{a}	2.37±0.02^{a}

Mean values within the same column with the different superscripts are significantly different at (P<0.05)

Table 3. *Weekly FCR (Ratio) in laying hens fed on different diets (Mean ± SE)*
Tablo 3. *Farklı rasyonlarla beslenen tavuklarda haftalık FCR (Oran) (Ortalama ± SE)*

Groups	Week 1st	Week 2nd	Week 3rd	Week 4th	Total FCR
A	2.60±0.02	2.75±0.03	2.75±0.03	2.80±0.05	2.72±0.04
B	2.58±0.02	2.85±0.05	2.97±0.05	2.99±0.06	2.85±0.09
C	2.61±0.02	2.88±0.02	2.90±0.04	2.92±0.04	2.82±0.07
D	2.67±0.04	2.75±0.04	2.76±0.05	2.82±0.06	2.76±0.03
E	2.68±0.04	2.73±0.04	2.79±0.05	2.80±0.06	2.75±0.02

***Table 4.** Effects of SB supplementation on production performances of laying hens fed on aflatoxins contaminated diets (Mean ± SE)*
***Tablo 4.** SB ilavesinin aflatoksinlerle kontamine rasyonlarla beslenen tavukların üretim performanslarına etkileri (Ortalama ± SE)*

Groups	Egg Production %	Egg Weight (g)	Albumin (g)	Yolk (g)	Shell (g)	Shell Thickness (mm)
A	80.64±0.03[bc]	59.10±0.01[bc]	27.95±0.08	14.99±0.07	5.81±0.08[b]	0.376±0.0023[b]
B	76.72±0.04[c]	58.10±0.03[c]	27.66±0.19	14.91±0.12	5.34±0.05[c]	0.368±0.0007[c]
C	80.92±0.03[bc]	59.10±0.01[bc]	27.47±0.20	14.82±0.10	5.72±0.10[bc]	0.375±0.0021[bc]
D	84.28±0.03[a]	59.50±0.01[a]	27.67±0.19	14.87±0.13	6.07±0.14[a]	0.381±0.0024[a]
E	82.60±0.02[a]	59.25±0.01[a]	27.47±0.23	14.86±0.14	5.85±0.09[b]	0.377±0.0027[b]
P value	0.032	0.002	0.63	0.87	0.002	0.024

Mean values within the same column with the different superscripts are significantly different at (P<0.05)

***Table 5.** Economic cost (US $) of different groups and their egg production performances*
***Tablo 5.** Farklı grupların ekonomik maliyeti ve yumurta üretim performansı (Amerikan Doları)*

Items	Groups				
	A	B	C	D	E
Feed consumption in kg	28	28	28	28	28
Total feed cost	10.66	10.66	10.66	10.66	10.66
Medication cost	1.9	1.9	1.9	1.9	1.9
Sodium bentonite cost @ one-half of a US dollar/kg	0	0	0.20	0.26	0.33
Total cost	12.57	12.57	12.57	12.57	12.57
Total egg (For Selling)	1.52	1.26	1.47	1.61	1.59
Per egg price	0.07	0.09	0.08	0.07	0.07
Cost per dozen eggs	0.94	1.14	0.98	0.9	0.91

thickness was significantly affected by SB levels. Highest value (P<0.05) was measured in group D, while group A and E show uniformity and Group B has the lowest value followed by group C and A.

Economic Coast

The cost of feeding was calculated according to the rates of feed, eggs, medication, additives and selling prevailed during the experiment. From economic point of view, only group D & E were profitable and cheap in price while group A and C were satisfactory, but in group B showed high price per dozen of egg and no increase in production parameters in contrast to others experimental groups *Table 5*.

DISCUSSION

Sodium bentonite supplemented diets had highest amount of feed intake when compared to aflatoxin containing negative control group. This may be disturbance in the normal metabolism that results in decreased appetite and hepatic degeneration [15-17]. Adsorbent like SB mixed in feed has high economical competence value for ducks by adding 0.5 to 1% SB in feed, which enhance (P<0.05) feed consumption [18]. From the results of our study, we assumed that SB clay possibly has absorptive and selective character that improves digestion. Pervious study also considers SB, as strong colloidal material [19]. In another report, it was described that SB as good additive in feed intake of laying hens [20-22]. In studies, SB, as feed additives 1% and 1.5% found significant increase in feed intake [19,22]. However aflatoxin contaminated feed treatment showed significant decrease in feed efficiency when compared to positive control group [23]. From our results, feeding efficiency was enhanced by adding either 2% or 2.5% SB as compared to positive and negative control diets. These results indicate that 2% SB supplementation is productive in layers to maximize feed intake. Our results are in agreement with another study that ascertains 1.5% SB as effective for higher feed consumption in laying hens [24]. The reason behind the increase in feed consumption could be possibly prolonged passage time of feed in the intestine and energy and protein utilization in the presence of SB [25-27].

The present findings were different from the reports of other studies, who reported a non-significant on overall performance of white leghorn on aflatoxin level and sodium bentonite, which slightly decreased in feed intake after addition of 5% SB to the diet [26]. The difference with their results may be due to the lower level of aflatoxins i.e., 50 ppb and SB composition as well as it concentration. SB containing diets work well in egg production and maintaining good health condition when compared to negative control group. Egg production per layer hen during experimental study was significantly improved and the average increase of total egg production was 3.9% for 2% SB and 3% for 2.5% SB as compared to negative control group. Another study found that hen day egg production and egg mass boost when feed mix with sodium bentonite containing 10 g/kg [28,29]. The enhancement in egg production can be attributed to SB clay content possibly containing some minerals, vitamins, salts and other beneficial additives that helps eggs production and aflatoxins neutralization. Our results are also in agreement with other reports, indicating that a significant increase in egg number with addition of 1 to 2% of SB contents [18,21].

Dietary level of SB has showed better FCR as compared to toxin containing diets. From our results, highest FCR was shown by B group that consist 170 ppb aflatoxins only. The feeds containing SB 2.0% and 2.5% have good FCR. The results of FCR are substantiated by the findings of others [24]. They concluded that 1.5-3.0% of SB as supportive in FCR. Moreover, another suggested that feed with 2-8% SB showed significantly improved FCR [20]. Study also reported 1% SB containing feed as supportive for FCR [18]. Literature reports higher FCR for aflatoxin containing diets while overall best FCR for 2% SB containing feed in laying hens [23]. In our results, overall FCR was better in groups A and D than B and C. SB groups significantly ($P<0.05$) reduced the toxicity of aflatoxin on FCR in groups E and D when we compared to B although 1.5% SB was not as effective as 2% and 2.5% SB. The reason could be better digestibility of nutrients that is achieved at 2% and 2.5% SB level. Our results were also in agreement with the reports of others, who found improvement in FCR upon supplementation of toxin binders in poultry feeds [30-33].

In our study egg weight was significantly ($P<0.05$) increased during the whole experimental period. These results were also in accordance with the study that found increase in egg weight by use of dietary SB. According to this observation, SB significance on increasing in egg weight. These findings could be attributed to SB composition that can efficiently increase nutrient digestibility [26]. Our results were also in agreement with another study, who reporting increase in egg weight, when fed SB feed as compared to the toxin supplemented feed diet [20].

The most striking results in our egg traits were increase in eggshell weight and thickness. Maximum egg shell weight and thickness were observed in treatments D followed by E while lowest was observed in group B. Overall the result of treatments groups were significant ($P<0.05$) when compared to control treatment groups. The eggshell thickness of group three was almost comparable to group one that is without aflatoxin. Study has reported significant increase in relative ducks egg shell weight and thickness but to date no reports are available about shell thickness increase in layer [18]. Another group of observers reported that significances of sodium bentonite may be due to some minerals of bentonite and its natural values as well as the bentonite can be a natural anti-contamination of poultry feed used [25]. Egg trait parameters such as yolk and albumin weights were not significant ($P>0.05$) even within SB supplementation groups, although this level increase most of egg traits significantly. Yolk weight was higher in a group followed by group B, D and E. The lowest weight was shown by C in comparison to treatments groups with 1.5% SB but was not significant i.e. $P>0.05$. Our results are in agreement with a study, has reported that weight of yolk and albumen were not significantly affected [18]. Another study, suggested that egg quality parameters were not affected by adding sodium bentonite at any level to the different diet [19]. From different egg quality traits, egg weight, shell weight and thickness were increased ($P<0.05$) the entire experimental period.

The maximum egg production, feed conversion, feed consumption and minimum cracked and shell less egg were observed in treatments D and E. The highly affected group with minimum egg production and feed intake was treatment B that has not only produced less numbers of eggs but also showed reduced egg weight. Thus we conclude that the reason of significance can be the SB neutralizing effect and also aflatoxin binding abilities and has high mineral and vitamins content. The most important content in SB clay is calcium and ion exchange capability that helps in improvements of various traits [34]. In our study we observed significant difference among groups for egg production, feed consumption, egg weight, eggshell weight and thickness and FCR. Overall performance including egg production, feed intake and FCR were prominently affected in the birds feed on aflatoxin containing feed. These birds also showed better feed and protein utilizations having effect on increasing egg weight and reduced most of the soft, cracked and abnormal egg. These disastrous effects were efficiently diminished by the addition of SB at levels of 1.5, 2 and 2.5% in treated groups.

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