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# Effects of dietary sepiolite and mannanoligosaccharide supplementation on the performance, egg quality, blood and digestion characteristics of laying hens receiving aflatoxin in their feed

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**Summary:** In this experiment, sepiolite (% 1.5 and % 3) and mannanoligosaccharide (MOS, % 0.1) were fed to layers each receiving 120 ppb aflatoxin, and were compared to control (K) and negative control (NK) groups. The experiment began at 26 wk of hen age and continued for 12 wk. Each of the five dietary treatments was randomly assigned to six replicates each included six hens. There were no significant differences ($p>0.05$) in livability, live weight change, egg weight and feed intake between the groups. NK had worse feed conversion ratio than control ($P<0.05$). The addition of % 1.5 sepiolite resulted in an increase in egg production and egg mass in comparison to NK and MOS groups, and in an increase in feed efficiency ($P<0.05$). There were no differences between the groups in parameters characterizing egg quality, namely, in the ratio of cracked-broken eggs, albumen height, haugh unit, shape index, shell thickness, and shell resistance, moreover, in the colour (RYCF), shine (L) and yellowness (b) of egg yolk ($P>0.05$). Aflatoxin was not detected in eggs obtained from any of the groups. The pH of faeces of hens in the NK group was higher than that of birds in the K and the 3 % sepiolite-treated groups ($p<0.05$). The proportion of dry matter of the feces was the lowest in the NK group, nevertheless, the difference between the groups was not significant ($p>0.05$). The addition of MOS and sepiolite to the feed reduced the degree of digestion of aflatoxin by % 6-12. As a result, sepiolite supplementation to laying hen diets containing aflatoxins can be concluded that had the beneficial effects on hen performance.

Key words: Aflatoxin, mannanoligosaccharide, performance, sepiolite, laying hen.

## Aflatoksin içeren yumurta tavuğu yemlerine sepiolit ve mannanoligosakkarit ilavesinin performans, yumurta kalitesi, kan ve sindirim özelliklerine etkileri

**Özet:** Bu araştırmada, ortalama 120 ppb toplam aflatoksin içeren yumurta tavuğu yemlerine sepiolit (% 1.5 ve 3) ve mannanoligosakkarit (MOS, % 0.1) ilavesi yapılmış, bu gruplar, kontrol (K) ve negatif kontrol (NK) grupları ile karşılaştırılmıştır. Deneme tavuklar 26 haftalık yaşta iken başlamış ve 12 hafta devam etmiştir. 5 deneme grubunun her biri 6 tekerrürden ve her tekerrür de rastgele atılan 6 tavuktan oluşmuştur. Gruplar arasında yaşama gücü, canlı ağırlık değişimi, yumurta ağırlığı ve yem tüketimi gibi performans değerleri bakımından önemli farklılıklar gözlenmemiştir ($P>0.05$). NK grubu, kontrol grubuna göre yemi daha kötü değerlendirmiştir ($P<0.05$). Yeme % 1.5 düzeyinde sepiolit ilavesi yumurta verimi ve kütlesini NK ve MOS gruplarına göre önemli düzeyde artırmış, yem değerlendirmeyi iyileştirmiştir ($P<0.05$). Yumurta kalitesini belirleyen özelliklerden kırık-çatlak yumurta oranı, ak yüksekliği, haugh birimi, şekil indeksi, kabuk kalınlığı ve mukavemeti, yumurta sarısı RYCF, L, a ve b renk değerleri bakımından gruplar arasında farklılık bulunmamıştır ($P>0.05$). Hiçbir grubun yumurtasında aflatoksin tespit edilmemiştir. NK grubundaki tavukların dışkı pH'ının K ve % 3 sepiolit ilaveli gruplara göre yüksek olduğu belirlenmiştir ($P<0.05$). Dışkı kuru madde oranı bakımından gruplar arasında önemli farklılıklar belirlenmemesine rağmen ($P>0.05$), NK grubunun diğer gruplardan daha düşük dışkı kuru maddesine sahip olduğu görülmektedir. Yeme MOS ve sepiolit ilaveleri aflatoksinin sindirim derecesini % 6-12 oranında azaltmıştır. Sonuç olarak, aflatoksin içeren yumurta tavuğu yemlerine sepiolit ilavesinin tavukların performansı üzerine faydalı etkilerinin olabileceği söylenebilir.

Anahtar sözcükler: Aflatoksin, mannanoligosakkarit, performans, sepiolit, yumurta tavuğu.

## Introduction

The process of harvest and storage of plants used for feed, and the steps of feed production (silaging, transport, cooling, etc.), are all potential sources of microbial contamination. Consumption of feed contaminated either with microorganisms or their toxins is a major problem for the feed industry, animal growers and food producers as it causes considerable losses (6). In cases when prevention of microbial contamination had not been carried out and the release of toxins had not been

prevented, the removing of toxins from the feed or the elimination of their poisonous effect is of great economic importance. In recent years, in particular, effective results were seen with the adding of organic and inorganic additives to feeds containing toxins (11, 18, 22). Binding to them, these additives form compounds with the toxins thus preventing their absorption from the intestines (9, 15).

One of the inorganic materials used for such purpose is the natural mineral, sepiolite, a hydrous magnesium silicate belonging to the layer silicate (phyllosilicate) family. As a consequence of its molecular structure, sepiolite has high adsorption capacity. Because its fibrous character, sepiolite can be in the form of nano particles in water solutions or in other appropriate environments. This property, too, makes it an excellent adsorbent, rheological agent, drug-carrier substance and catalyst (19). Due to its magnesium ion in the octahedral layer, sepiolite is known to be a relatively good ion exchanger. It facilitates Mg ion discharge, especially at low pH values. In accordance with the above, it had been reported that, in the case of sepiolite from Turkish sources, Mg transfer to a water solution at neutral pH (approximately 8.5) is $10^{-4}$ mol/l, increasing to $10^{-2}$ at pH=3 (10).

The use of sepiolite in animal nutrition is based on its surface makeup (structure), and its cation-transfer and ammonium-bindig properties. In addition, it had been shown to increase carcass quality, have positive effect on performance by slowing the passing of feed through the digestive system. Thus it ensues a better consistency of the feces which, in return, positively affects the environment and house hygine control (7).

Lately, biological products are also widely used for disposing toxins in feeds. Some bacteria species (*Lactobasille*) and *Saccharomyces cerevisiae* type yeasts were investigated for this purpose, and good results were obtained (4). Similarly to this yeast, which can directly be added to the feed, glucomannan, obtainable from the cell wall of yeast, or its esterified forms (mannanoligosaccharide (MOS)) can also be used.

The objective of this study was to observe the possible adverse effects of AF (120 ppb) on performance, egg quality and blood and digestion characteristics of laying hens, and to evaluate the possible beneficial effects of dietary sepiolite (1.5 and 3 %) and MOS (0.1%) as a toxin-binder.

## Materials and Methods

*Animals:* The experiment was carried out on Barred Rock layers in the breeding sheds of the Poultry Research Institute, Ankara, in 2010. Prior to the experiment, hens were given standard layers' diet for one week. During this period, egg production and egg weight was monitored and 180 hens of similar body weight and egg production were selected. The experiment began at 26 wk of hen age and continued for 12 wk. Each of the five dietary treatments was randomly assigned to six replicates each included six hens. Cages were in three-storey rows, 25x47 cm in size, with manure band. The experiment during which feed and water was supplied *ad libitum*. Chicken houses were environmentally full-controlled with 14 hours lighting.

Table 1. Experimental design and aflatoxin composition of diets.
Tablo 1. Deneme deseni ve karma yemlerin aflatoksin içerikleri.

| Experimental groups | Sepiolite (%) | MOS (%) | Aflatoksin B1 (ppb) | Total aflatoxin (B1+B2+G1+G2) (ppb) |
|---|---|---|---|---|
| Control | 0 | 0 | 2.0 | 2.0 |
| Negative control | 0 | 0 | 107.7 | 122.4 |
| % 1.5 Sepiolite | 1.5 | 0 | 106.8 | 118.3 |
| % 3 Sepiolite | 3 | 0 | 106.5 | 119.6 |
| Mannan-oligosaccharide | 0 | 0.1 | 105.3 | 120.5 |

Table 2. Characteristics of sepiolite in this experiment.
Tablo 2. Araştırmada kullanılan sepiolitin özellikleri.

| Chemical composition, % | | Physical characteristics | |
|---|---|---|---|
| $SiO_2$ | 37.42 | Bulk density | 770 ± 20 g/lt |
| $AI_2O_3$ | 1.45 | $NH_3$ absorption efficiency, % | 96.8 |
| $Fe_2O_3$ | 0.76 | Humidity, % | 12 ± 2 |
| MnO | 0.007 | Sepiolite ratio, % | 40 |
| MgO | 23.27 | | |
| CaO | 13.21 | | |
| $K_2O$ | 0.23 | | |
| $TiO_2$ | 0.09 | | |
| LOI | 24.94 | | |

*Feed:* Sepiolite (% 1.5 and 3) and mannano-ligosaccharide (MOS, % 0.1) were supplemented to the feed of hens receiving 120 ppb aflatoxin, and they were compared to control (K) and negative control (NK) groups (Table 1). Mouldy corn was mixed to the feed in order to increase the amount of aflatoxin. For this purpose, corn of high aflatoxin content (100 ppb) was purchased and crushed coarsely. The corn was moistened and treated with *Aspergillus flavus*, aflatoxin B1 and B2, and kept at humidity of 80% in a room of 30-35 $^{o}$C temperature for 15 days. By the end of this period, aflatoxin level in the corn had inreased to 200 ppb. The mouldy corn was then mixed to the feed in a proportion of 60%. Diets were analysed (AOAC International, 2003) for aflatoxin B1 and total aflatoxin (B1+B2+G1+G2) composition (Table 1). Sepiolite (Anadolu Endüstri Mineralleri San. Tic. A.Ş, İstanbul) and mannanoligosaccharide (Bil-Yem A.Ş, Ankara) used in the experiment were provided from private companies and the characteristics of this sepiolite was given Table 2.

Table 3. Diet composition and chemical components.
Tablo 3. Karma yemler ve kimyasal bileşimleri.

| Diet composition | K | NK | S1 | S2 | MOS |
|---|---|---|---|---|---|
| Corn | 60 | 60 | 60 | 60 | 60 |
| Wheat | 7.94 | 6.4 | 3.06 | - | 6.13 |
| Soybean meal | 20.03 | 19.07 | 17.95 | 20.63 | 19.14 |
| Full fat soya | - | 2.5 | 5.39 | 3.02 | 2.6 |
| Vegetable oil | 0.5 | 0.5 | 0.5 | 1.71 | 0.5 |
| Ground limestone | 8.6 | 8.6 | 8.6 | 8.6 | 8.6 |
| Dicalcium phospahate | 1.7 | 1.7 | 1.7 | 1.7 | 1.7 |
| Salt | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| DL-Methionine | 0.28 | 0.28 | 0.29 | 0.30 | 0.28 |
| L-Lysine | 0.15 | 0.15 | 0.21 | 0.24 | 0.15 |
| Antioxidant | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Vitamin premix* | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Mineral premix** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Salmonella inhibitor | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Sepiolite | 0 | 0 | 1.5 | 3.0 | 0 |
| MOS | 0 | 0 | 0 | 0 | 0.1 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Crude protein (%) | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 |
| ME (kcal/kg) | 2779 | 2753 | 2745 | 2712 | 2770 |
| Dry matter (%) | 88.43 | 88.43 | 88.60 | 88.73 | 88.44 |
| Crude cellulose (%)[1] | 2.76 | 2.70 | 2.69 | 2.64 | 2.75 |
| Crude ash (%) | 12.36 | 12.32 | 12.40 | 12.31 | 12.35 |
| Ether extract (%) | 3.17 | 3.17 | 3.83 | 4.49 | 3.18 |
| Ca (%)[1] | 3.70 | 3.70 | 3.70 | 3.70 | 3.70 |
| Available P (%)[1] | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |
| Methionine (%)[1] | 0.51 | 0.51 | 0.52 | 0.52 | 0.51 |
| Methionine+cystine (%)[1] | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 |
| Lysine (%)[1] | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Tryptophan (%)[1] | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |

* Each kg of vitamin premix contains 15 000 000 IU A, 5 000 000 IU $D_3$, 50 000 mg E, 10 000 mg $K_3$, 4 000 mg $B_1$, 8 000 mg $B_2$, 5 000 mg $B_6$, 25 mg $B_{12}$, 50 000 mg niacin, 20 000 mg pentatonic acid, 2 000 mg folic acid, 250 mg biotin, 75 000 mg ascorbic acid, 175 000 mg colin.

** Each kg of mineral premix contains 35 000 mg Mg, 56 000 mg Mn, 140 000 mg Zn, 56 000 mg Fe, 10 500 mg Cu, 1 050 mg I, 280 mg Co, 280 mg Se, 700 mg Mo.

[1] Calculated values.

Feeds were formulated according to NRC requirements (13). Experimental diets were mash form and obtained using a cracker-mixer machine of 300 kg/hour capacity. Sugar and starch content of the diets was analyzed in accordance with AOAC standards (1). Metabolic energy was calculated according to the report by Vogt (23). The components and chemical composition of the experimental diets are given in Table 3.

*Performance parameters:* Live weight of the hens was measured individually at the beginning and at the end of the experiment. Mortality, egg production and number of broken eggs in the groups were recorded daily. In the laying period, feed intake was measured every 15 days, and eggs were weighed every other day. Egg production was expressed as %/hen/day. In addition, egg mass was calculated from egg weight and egg production, and feed conversion ratio was determined from egg mass and feed intake values.

*Egg quality parameters:* From the beginning of the experiment, 24 eggs from each treatment group were collected once in every 4 weeks, and their shape index, shell thickness, shell break resistance, albumen height, Haugh unit, RYCF (Roche yolk colour fan) value, L (brightness), a (redness) and b (yellowness) values were determined 24 hours after collection. At the end of the experiment, 6 eggs from each group were analyzed for aflatoxin B1 and total aflatoxin (B1+B2+G1+G2) content (2). Shape index was calculated by a tool determining the egg's width to length ratio. Shell thickness was calculated as the mean of measurements (by a Mitutoyo digital micrometer) taken after peeling off the membrane of the pointed, blunt and middle part.) Break resistance was measured by a Newton type Futura resistance meter. White height was measured electronically by Futura white and yellow height measuring unit. Haugh unit was calculated using albumen height and egg weight values

by Futura egg quality analyis program (8) using the following formula:
Haugh unit = 100 log (Albumen height + 7.57 - 1.7 Egg weight$^{0.37}$)

Values regarding egg yolk were determined by CR-10 Konica Minolta Colour Reader.

*Blood analysis:* At the end of the experiment, blood samples were taken from 10 hens per group, and total serum protein, albumin, bilirubin, total cholesterol, Ca, ALT ve AST values were determined. Blood was taken individually from the vein under the wing, with the help of a syringe. Analyses were carried out using Roche Cobas Integra original kits by Roche Cobas Integra 800 equipment.

*Digestibility:* At the end of the experiment, the feces of six hens from each group were examined for pH value and dry matter. Dry matter ratio of feces was determined according to AOAC (1), pH was measured by a digital pH meter set for 22 $^{o}$C. In addition, digestibility of aflatoxin B1 and total aflatoxin were determined. For this, chromium oxide was given in 0.3 % ratio to the feed of 6 hens from each group, and fed to them for three days. Feces from the last two days were collected and analyzed for chromium oxide and aflatoxin content (2). Digestibility of aflatoxin was calculated on the basis of the equation by (12):

$$\text{Nutrient digestibility (\%)} = \frac{\text{Indicator in feed (\%)}}{\text{Indicator in feces (\%)}} \times \frac{\text{Nutrient in feces (\%)}}{\text{Nutrient in feed (\%)}}$$

*Statistical analysis:* The results of all experiments were analyzed statistically using the analysis of variance procedures of the statistical program MİNİTAB 14. Significant differences were tested further using Duncan's test (5).

## Results

*Performance of laying hens:* No significant differences were observed in livability, live weights at the beginning and at the end of the experiment, change in live weight, egg weight and feed intake ($p>0.05$). The effect of the feeding of hens with feed-mixture containing aflatoxin (NK) was negative on egg production, egg mass and feed conversion ratio. The addition % 1.5 sepiolite resulted in increased egg production and egg mass compared to NK and MOS groups, and it improved feed efficiency ($p<0.05$). The group receiving % 1.5 sepiolite had values of these parameters similar to those of the control group ($p>0.05$). When % 3 sepiolite and MOS were supplemented, significant differences were not found ($p>0.05$), although there was an improvement in feed efficiency, egg production and egg mass (Tables 4 and 5). The positive effect of sepiolite supplementation on feed efficiency had been explained as a result of decreasing viscosity of the inside of *jejunum*, moreover, as a results of the increasing the digestibility of organic materials by reducing the speed of passaging through the intestine (16, 17).

*Egg quality:* There were no significant differences between the groups in internal and external qualities of eggs ($p>0.05$), such as in albumen height, haugh unit, cracked-broken eggs, shape index, shell resistance and shell thickness (Table 6). However, in NK group, the ratio of cracked-broken eggs was numerically higher and haugh unit was lower than in the other groups. In two separate experiments, % 1.5 sepiolite supplement to the feed increased Ca amount in the shell (21), whereas the addition of % 0.1 MOS increased the proportion of cracked-broken eggs (3).

While there was no difference found between the groups in RYCF, L and b parameters of egg yolk ($p>0.05$), value of NK was found to be numerically higher than that of the other groups (Table 7).

Analyses carried out at the end of the experiment showed no aflatoxin B1 and total aflatoxin (B1+B2+G1+G2) in the eggs from any of the groups. Thus it seems that aflatoxin given with the feed in this experiment had not passed into the eggs.

*Blood parameters:* There were no significant differences ($p>0.05$) between the test groups in their total serum protein, albumin, bilirubin, total cholesterol, calcium, phosphorus, AST and ALT values (Table 8).

*Digestion:* The pH of the faeces of hens in NK group was higher ($p<0.05$) than that of those in the K and S2 groups. The highest proportion of dry matter of the feces was found in the control group, although there was no significant difference between the groups ($P>0.05$) (Table 9).

Just about all of the aflatoxin, found in trace amount (2 ppb) in the control group, passed naturally from the digestive system through to the body. MOS and sepiolite supplementation decreased the digestibility of aflatoxin by 6-13% (Table 9). That is, in these groups, some of the aflatoxin received with the feed was bound and discarded with the feces. Therefore, since aflatoxin was taken up by the body in a lower quantity, its harmful effects were also less evident in these groups. It had been reported earlier that the micotoxin-binding capacity of silica minerals is determined by several factors such as chemical composition, particle size, surface acidity, toxin level. The *in vitro* micotoxin-binding capacity of various minerals had been reported to be between % 86 and % 97. However, the measurements taken under laboratory conditions had been difficult to reproduce in experiments with animals (*in vivo*), and the results were often not satisfactory (20).

Table 4. Livability, live weights at the beginning and end of the experiment, and changes in live body weight.
Tablo 4.Yaşama gücü, deneme başı ve sonu canlı ağırlık ve canlı ağırlık değişimleri.

| Groups | Livability (%) | Body weight at the beginning of the experiment (g) | Body weight at the end of the experiment (g) | Changes in live weight (g) |
|---|---|---|---|---|
| NK | 94.4±5.6 | 1680±47.4 | 1721±59.8 | 41.1±52.6 |
| K | 100±0.0 | 1678±43.0 | 1746±55.5 | 67.8±22.0 |
| MOS | 100±0.0 | 1618±42.4 | 1706±42.8 | 87.8±19.9 |
| S1 | 94.4±5.6 | 1642±25.8 | 1707±24.3 | 64.4±12.6 |
| S2 | 100±0.0 | 1648±31.4 | 1691±44.1 | 43.3±41.4 |
| P value | 0.580 | 0.771 | 0.941 | 0.852 |

Table 5. Performance parameters.
Tablo 5. Performans değerleri.

| Groups | Egg production (%/hen/day) | Feed intake (g/hen/day) | Egg weight (g/egg) | Egg mass (g/hen/day) | Feed conversion ratio (g feed/g egg) |
|---|---|---|---|---|---|
| NK | 77.8±1.06 $^{b}$ | 117.3±1.5 | 59.7±0.82 | 46.5±1.23$^{b}$ | 2.53±0.07$^{a}$ |
| K | 82.1±1.23$^{ab}$ | 114.3±1.2 | 60.4±0.94 | 49.6±1.46$^{ab}$ | 2.31±0.06$^{bc}$ |
| MOS | 79.6±1.03$^{b}$ | 117.3±2.3 | 60.7±1.19 | 48.3±1.03$^{b}$ | 2.43±0.10$^{ab}$ |
| S1 | 85.4±1.26$^{a}$ | 114.0±3.6 | 60.9±0.46 | 52.1±0.68$^{a}$ | 2.19±0.05$^{c}$ |
| S2 | 81.0±2.15$^{ab}$ | 117.0±2.1 | 60.4±0.86 | 48.8±0.58$^{ab}$ | 2.40±0.06$^{abc}$ |
| P value | 0.032 | 0.712 | 0.878 | 0.041 | 0.041 |

$^{a,b,c}$ Values within a column with no common superscripts differ significantly (P<0.05).

Table 6. Internal and external quality of eggs.
Tablo 6. Yumurta iç ve dış kalite özellikleri.

| Groups | Albumen height (mm) | Haugh unit | Cracked-broken eggs (%) | Shell resistance (N) | Shell thickness ($10^{-2}$ mm) | Shape index |
|---|---|---|---|---|---|---|
| NK | 6.24±0.14 | 76.8±0.76 | 2.43±0.52 | 38.3±0.91 | 32.25±5.80 | 78.3±0.3 |
| K | 6.29±0.13 | 79.1±1.20 | 1.55±1.13 | 39.7±0.81 | 32.57±3.01 | 77.8±0.3 |
| MOS | 6.53±0.13 | 79.7±0.92 | 1.86±0.93 | 38.3±0.61 | 32.26±3.38 | 77.2±0.4 |
| S1 | 6.31±0.18 | 79.4±0.99 | 1.07±0.45 | 39.8±0.92 | 32.28±2.08 | 78.5±0.3 |
| S2 | 6.53±0.14 | 79.5±1.17 | 1.75±0.91 | 40.1±0.57 | 32.89±2.32 | 78.6±0.3 |
| P value | 0.152 | 0.277 | 0.522 | 0.272 | 0.645 | 0.345 |

Table 7. Egg yolk parameters.
Tablo 7. Yumurta sarısı özellikleri.

| Groups | RYCF | L | a | b |
|---|---|---|---|---|
| NK | 12.1±0.05 | 40.52±0.09 | 5.30±0.18 | 13.51±0.12 |
| K | 12.3±0.06 | 40.43±0.10 | 5.72±0.08 | 13.66±0.14 |
| MOS | 12.2±0.06 | 40.38±0.13 | 5.32±0.12 | 13.22±0.17 |
| S1 | 12.2±0.09 | 40.56±0.08 | 5.31±0.11 | 13.61±0.17 |
| S2 | 12.3±0.09 | 40.58±0.18 | 5.48±0.13 | 13.37±0.18 |
| P value | 0.317 | 0.705 | 0.115 | 0.302 |

Table 8. Some blood parameters.
Tablo 8. Bazı kan parametreleri.

| Groups | Total protein (g/dL) | Albumin (g/dL) | Bilirubin (mg/dL) | AST (U/L) | ALT (U/L) | Total cholesterol (mg/dL) | Ca (mg/dL) | P (mg/dL) |
|---|---|---|---|---|---|---|---|---|
| NK | 5.7±0.2 | 2.23±0.07 | 0.03±0.007 | 164±6.3 | 1.50±0.17 | 164±12.8 | 33.0±2.0 | 6.6±0.5 |
| K | 5.5±0.1 | 2.25±0.05 | 0.02±0.004 | 163±5.9 | 1.80±0.20 | 194±17.4 | 36.1±2.1 | 6.6±0.4 |
| MOS | 5.7±0.2 | 2.22±0.04 | 0.03±0.007 | 168±10.6 | 1.89±0.31 | 166±23.9 | 33.9±1.5 | 6.5±0.3 |
| S1 | 5.6±0.2 | 2.15±0.05 | 0.02±0.005 | 165±7.2 | 1.70±0.21 | 164±16.4 | 34.3±1.7 | 6.1±0.3 |
| S2 | 5.9±0.2 | 2.22±0.05 | 0.04±0.006 | 176±8.5 | 1.80±0.42 | 174±26.2 | 35.1±1.9 | 6.2±0.3 |
| P value | 0.631 | 0.718 | 0.058 | 0.757 | 0.883 | 0.791 | 0.801 | 0.889 |

Table 9. Some digestion features.
Tablo 9. Bazı sindirim özellikleri.

| Groups | Faeces pH (22 $^0$C) | Faeces dry matter (%) | Digestibility* of aflatoxsin B1 (%) | Digestibility* of total aflatoxin (B1+B2+G1+G2) (%) |
|---|---|---|---|---|
| NK | 8.56±0.06$^a$ | 22.37±1.30 | 73.95 | 80.05 |
| K | 7.80±0.20$^b$ | 26.33±1.20 | 96.32 | 96.22 |
| MOS | 8.05±0.18$^{ab}$ | 23.07±1.35 | 67.17 | 67.71 |
| S1 | 8.16±0.24$^{ab}$ | 24.27±1.42 | 67.23 | 69.92 |
| S2 | 7.82±0.17$^b$ | 26.93±0.83 | 65.23 | 68.28 |
| P value | 0.033 | 0.101 | | |

* In the determination of the digestive degree of aflatoxin, samples taken from six hens were mixed for analysis without repetition, and statistical evaluation was not carried out.
$^{a,b}$ Values within a column with no common superscripts differ significantly (P<0.05).

## Discussion and Conclusion

The results showed that MOS and sepiolite added to the feed of layers receiving aflatoxin binds to a proportion of the toxin which, in turn, is eliminated with the feces. Although the binding capacity of MOS and sepiolite to the toxin is similar, sepiolite seems to be more beneficial in restoring the production parameters of hens; moreover, it seems to have other positive effects on the metabolism as well.

The added sepiolite reduced the losses, caused by aflatoxin, in the performance of the birds. On the other hand, MOS had no significant effect on these parameters, although it also caused numerical increases in them. In order to demonstrate more clearly the effects of sepiolite on restoring egg quality, further studies with higher doses of aflatoxin and longer experiment period should be carried out.

As a result, sepiolite supplementation to laying hen diets containing aflatoxins can be concluded that had the beneficial effects on hen performance.

## References


1. **AOAC** (1990): *Official Methods of Analysis (15th ed.),* Association of official analytical chemists, Washington, D. C.
2. **AOAC International** (2003): *Official methods of analysis of AOAC International*. 17th edition, 2nd revision. Gaithersburg, MD, USA, Association of Analytical Communities.
3. **Cabuk M., Bozkurt M, Alcicek A, Catlı AU, Baser KHC** (2006): *Effect of a dietary essential oil mixture on performance of laying hens in the summer season.* South Afr Anim Sci, **36**, 215-221.
4. **Dawson KA, Evans J, Kudupoje M (2001):** *Understanding the adsorption characteristics of yeast cell wall preparations associated with mycotoxin binding. In:* Science and Technology in the Feed Industry, Lyons, T.P. and K.A. Jacques (Eds.). Nottingham University Press, Nottingham, U.K., 169-181.
5. **Duncan DB** (1955): *Multiple Range and Multiple F Tests*, Biometrics, **11**, 1-42.
6. **Ergul M (2000):** *Feed harmful and effects.* Proceedings of the International Animal Nutrition Congress, September 4-6, Isparta, Turkey.
7. **Fernando AES** (2004): La *Sepiolite: A special clay in the field of food to animal,* http://www.racve.es/actividades/zootecnia/2004-04-14 FernandoEscribanoSaez.htm
8. **Haugh RR** (1937): *The Haugh Unit for measuring egg quality*. U. S. Egg Poultry Magazine, **43**, 522-555, 572-573.
9. **Huwig A, Freimund S, Kappeli O, Dutler H** (2001): *Mycotoxin detoxication of animal feed by different adsorbents.* Toxicol Lett, **122**, 179-188.
10. **Kara M, Sabah E, Yuzer H, Celik MS** (1998): *Sepiolite as an adsorbent for elimination of mine wastes.* Proceedings of the 5th International Symposium on Environmental Issues and Waste Management in Energy and Mineral Production, May 18-20, Ankara, Turkey, 717-721.
11. **Kubena LF, Harvey RB, Phillip TD, Clement BA** (1993): *Effect of hydrated sodium calcium aluminosilicate on aflatoxicosis in broiler chicks*. Poult Sci, **72**, 651-657.
12. **Maynard LA, Loosli, JK** (1969): *Animal Nutrition*. 5th Edn., Mc Graw-Hill Co., New York, 533.
13. **NRC** (1994): *Nutrient Requirements of Poultry.* NRC, Washington, D. C, USA
14. **Oguz H, Kurtoglu V** (2000): *Effect of clinoptilolite on fattening performance of broiler chickens during experimental aflatoxicosis*. Br Poult Sci, **41**, 512-517.
15. **Oguz H, Kurtoglu V, Ortatatli M** (2001): *Preventive efficiency of dietary zeolite (clinoptilolite) in broiler chickens during aflatoxicosis.* Proceedings XV European Symposium on the Quality of Poultry Meat, September 9-12, Kusadasi-Turkey, 145-150.
16. **Ouhida I, Perez JF, Gasa J, Puchal F** (2000a): *Enzymes (β-glucanase and arabinoxylanase) and/or sepiolite supplementation and the nutritive value of maize-barley-whet based diets for broiler chickens*. Br Poult Sci, **41**: 617-624.
17. **Ouhida I, Perez J F, Piedrafita J, Gasal J** (2000b): *The effects of sepiolite in broiler chicken diets of high, medium and low viscosity. Productive performance and nutritive value.* Anim Feed Sci Tech **85**, 183-194.

18. **Rajua MVLN, Devegowda G** (2000): *Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin).* Br Poult Sci, **41**, 640-650.
19. **Sabah E, Celik MS** (1999): *Sepiolite: Characteristics and using areas*. Proceedings of the 3rd Industrial Ingredients Symposium, October 14-15, Izmir, Turkey.
20. **Taylor D** (2000): Mycotoxin Binders: *What they are and how they work.* Proceedings of the 1st Annual OIL-DRI Animal Health Technical Symposium, August 25, Montreal, Canada.
21. **Tortuero F, Rioperez L, Martin L** (1993): *Effect of dietary sepiolite supplementation on the performance, egg composition and mineral metabolism in laying hens.* Arch Zootec, **42**, 347-360.
22. **Trevor KS, Macdonald EJ, Haladi S** (2001): *Current Concepts in Feed-Borne Mycotoxin and the Potential for Dietary Prevention of Mycotoxicoses. In:* Science and Technology in the Feed Industry, Lyons, T.P. and K.A. Jacques (Eds.). Nottingham University Press, Nottingham, UK.
23. **Vogt H** (1984): Kraftfutter 68; 327-328. In; Close S W, Menke K H (1986): Selected Topics in Animal Nutrition. 170+A 85. Wielinger Straβe 52, D-8133, feldafins.

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