Turk J Vet Anim Sci
29 (2005) 601-605

Research Article

The Effects of Aflatoxin and Sodium Bentonite Combined and Alone on Some Blood Electrolyte Levels in Broiler Chickens

Gökhan ERASLAN
Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Erciyes University, Kayseri - TURKEY
Dinç EŞSİZ
Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Kafkas University, Kars - TURKEY
Mehmet AKDOĞAN
Department of Biochemistry, Faculty of Medicine, Süleyman Demirel University, Isparta - TURKEY
Fatma ŞAHİNDOKUYUCU
Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Akdeniz University, Burdur - TURKEY
Levent ALTINTAŞ
Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ankara University, Ankara - TURKEY

Received: 21.11.2002

Abstract: The aim of the study is to determine the effects of aflatoxin and sodium bentonite given alone and in combination on some of blood electrolyte levels in broiler chicks. For this aim, 72 male one-day-old Avian broiler chicks were used. The animals were separated into 6 groups, as one control (Group 1) and the others trial (Groups 2 to 6). While commercial feed was given to Group 1 (control), the other groups received feed containing 0.25% sodium bentonite, 0.50% sodium bentonite, 1 ppm aflatoxin (approximately, 85% B_1, 10% B_2, 3% G_1 and 2% G_2), 1 ppm aflatoxin with 0.25% sodium bentonite and 1 ppm aflatoxin with 0.50% sodium bentonite (Groups 2-6), respectively, for 45 days. Blood samples were taken on the 15th, 30th and 45th days of the study and the sodium, potassium, chloride, phosphorus and calcium levels in serum were analyzed. At the end of the study, it was determined that aflatoxin caused a decrease in calcium and phosphorous levels. On the other hand, it was observed that sodium bentonite in the feed caused a decrease in both of these electrolyte levels, even though these decreases were higher in the groups that received aflatoxin alone.

Key Words: Aflatoxin, blood electrolyte, broiler chick, sodium bentonite.

Etçi Piliçlere Yalnız Başına ve Birlikte Verilen Aflatoksin ve Sodyum Bentonitin Bazı Kan Elektrolit Düzeyleri Üzerine Etkisi

Özet: Çalışmanın amacı, yem ile tek başına ve birlikte verilen sodyum bentonit ve aflatoksinin etçi piliçlerde bazı kan elektrolit düzeyleri üzerine etkisini incelemektir. Bunun için, Avian ırkı 72 adet, erkek, günlük civciv kullanılmıştır. Hayvanlar, biri kontrol (Grup 1) diğerleri deneme (Grup 2-6) olmak üzere toplam 6 gruba ayrılmıştır. Kırk beş gün süreyle, kontrol grubuna normal yem verilirken, deneme gruplarına sırasıyla % 0,25 sodyum bentonit (Grup 2), % 0,50 sodyum bentonit (Grup 3), 1 ppm aflatoksin (% 85 B_1, % 10 B_2, % 3 G_1, % 2 G_2) (Grup 4), 1 ppm aflatoksin + % 0,25 sodyum bentonit (Grup 5), 1 ppm aflatoksin + % 0,50 sodyum bentonit (Grup 6) içeren yem verilmiştir. Çalışmanın 15., 30. ve 45. gününde kan alınmış ve serum sodyum, potasyum, klor, fosfor ve kalsiyum düzeyleri ölçülmüştür. Çalışma sonucunda, aflatoksinin serum kalsiyum ve fosfor düzeyinde düşüşe sebep olduğu anlaşılmıştır. Ayrıca, yeme adsorban olarak katılan sodyum bentonitin de, yalnızca aflatosin verilen grup (Grup 4) kadar olmasa da bu iki elektrolit düzeyini düşürdüğü tespit edilmiştir.

Anahtar Sözcükler: Aflatoksin, kan elektrolit, etçi piliç, sodyum bentonit,

Introduction

Aflatoxins (AF) are toxic metabolites produced by *Aspergillus parasiticus* and *Aspergillus flavus* genus fungi (1). The 4 main compounds that have been described so far are AF B_1, B_2, G_1, G_2. However, the number of these compounds had already been reached to 17 with their metabolites, such as M_1, M_2, R_0 formed within the animal's body (2). The natural development of the optimum conditions (heat, moisture, oxygen and pH) during harvesting, drying and storing may led to the production of toxin by toxigenic fungi; therefore, the risk of contamination with AF increase in agricultural crops (2,3). There are hundreds of ongoing studies at present in order to minimize or antagonize their harmful effects due to high levels of toxicity even at low doses (4,5). In poultry, the effect takes place mainly in the liver and other organs (6,7). Various procedures have been implemented to minimize their ingestion by poultry (8,9). Of these, the most frequently used one is the irreversible binding of the AF, present in the feed by adding clay at certain rates in order to prevent the absorption of toxin by digestive tract (10,11). The aim of the present study is to define the impacts of AF (1 ppm) and sodium bentonite (0.25%, 0.50%) given alone and in combination on the blood levels of potassium, chloride, phosphorus and calcium levels over 45 days.

Materials and Methods

In the study, 72 male one-day-old Avian breed chicks were used. The animals were weighed individually and divided into 6 groups as one control and the others trial groups. While the control group was fed (Group 1) commercial feed, the trial groups were fed feeds containing 0.25% sodium bentonite (Group 2), 0.50% sodium bentonite (Group 3), 1 ppm AF (Group 4), 1 ppm AF with 0.25% sodium bentonite (Group 5), and 1 ppm AF with 0.50% sodium bentonite (Group 6) for 45 days. Blood was taken on the 15th, 30th, and 45th days of the study. The blood samples were centrifuged at 3500 rpm for 10 min to separate their sera. The sodium, potassium, chloride, calcium and phosphorus levels were determined in the sera. The AF production in rice was done according to the method reported by Demet et al. (12) and it was based on the method described by Shotwell et al. (13). The method of Nabney and Nesbit was used for detecting AF species and its proportion (14). The results obviously showed that total AF consisted of Af B_1, B_2, G_1 and G_2 at approximate rates of 85%, 10%, 3% and 2% respectively. The total AF level was measured on an Enzyme-Linked Immunosorbent Assay (ELISA) apparatus by using a Ridescreen® brand kit and the relevant method. As a result of the measurement, total AF amount detected in rice flour was 105.45 ppm. An Abbot brand kit was used for measuring the sodium, potassium, chloride, calcium and phosphorus levels in the serum. Measurements were performed by using Olympus AU 640 autoanalyzer. The data were presented as arithmetic means and standard deviation. One-way variance analyses were used and Duncan's test was performed to demonstrate the differences between groups (15).

Results

On the 15th day of the study, there were no statistically significant changes in the sodium, potassium and chloride levels of experimental groups (Groups 2-6). However, the calcium and phosphorus levels showed the meaningful decreases in all experimental groups (Groups 2-6). On day 30 of the study, while there were no statistically significant changes in the sodium levels of experimental groups (Groups 2-6) compared to control (Group 1), statistically significant decreases were determined in the calcium level of group 4, and in the phosphorus levels of groups 2 to 6. On the other hand, the chloride levels of groups 2 to 6 showed a statistically significant increase. On the 45th day of study, while there were no statistically significant differences in the levels of sodium, potassium or chloride, statistically significant increases were determined in the calcium level of group 2, compared to control. In addition, the phosphorus level of group 5 showed a promising decrease in comparison with control (Tables 1-3).

Discussion

There have been lots of studies about the effects of AFs involved in the various metabolisms and their effects that were investigated individually or in combination (16-20). The fungi that contaminate feeds (especially *Aspergillus parasiticus*) cause synthesis to form the 4 kinds of AF derivatives in a natural environment. The most toxic one is Af B_1, followed by B_2, G_1 and G_2. Again, the highly produced AF derivative is Af B_1, and the other 3 AF derivatives make only small portion of it (2,3,16,17,20). The regulation of blood calcium and phosphorus levels is directly related to renal, intestinal and PTH (18). Glahn et al. (18) investigated the relationship between calcium, phosphorus and vitamin D and concluded that it led to a decrease in the level of vitamin D, reduced the PTH synthesis, declined the feedback of kidneys and intestines to PTH, and eventually altered the blood calcium and phosphorus levels directly or indirectly by these mechanisms. There are statistically significant differences between the groups with respect to both parameters (calcium, phosphorus) for all periods

Table 1. Sodium, potassium, chloride, calcium and phosphorus levels in control and experimental groups on day 15.

Groups*	Electrolytes				
	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)	Calcium (mg/dl)	Phosphorus (mg/dl)
Group 1	151.80 ± 3.11	4.12 ± 0.08	103.20 ± 4.86	12.50 ± 0.79[a]	4.82 ± 0.08[a]
Group 2	149.20 ± 2.94	4.30 ± 0.21	108.60 ± 5.31	10.12 ± 0.27[c]	4. 28 ± 0.47[c]
Group 3	153.00 ± 3.93	4.32 ± 0.16	107.40 ± 5.02	10.92 ± 0.26[d]	4.76 ± 0.15[d]
Group 4	149.80 ± 2.16	4.28 ± 0.21	103.00 ± 2.34	8.84 ± 0.25[b]	3.88 ± 0.13[b]
Group 5	151.60 ± 1.67	4.28 ± 0.13	104.60 ± 1.67	9.40 ± 0.51[b]	3.34 ± 0.18[b]
Group 6	151.00 ± 2.12	4.18 ± 0.13	104.20 ± 2.04	9.60 ± 0.46[b]	3.08 ± 0.40[b]

[a, b, c, d] : Difference is statistically significant in groups having different letters in the same column (P < 0.05).

* Group 1, control; Group 2, 0.25% sodium bentonite; Group 3, 0.50% sodium bentonite; Group 4, aflatoxin; Group 5, aflatoxin + 0.25% sodium bentonite; Group 6, aflatoxin + 0.50% sodium bentonite.

Table 2. Sodium, potassium, chloride, calcium and phosphorus levels in control and experimental groups on day 30.

Groups	Electrolytes				
	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)	Calcium (mg/dl)	Phosphorus (mg/dl)
Group 1	152.60 ± 1.34[ab]	4.18 ± 0.10	99.40 ± 2.30[a]	11.36 ± 1.53[a]	4.04 ± 0.20[a]
Group 2	152.20 ± 5.63[ab]	4.18 ± 0.13	104.00 ± 1.87[b]	10.66 ± 0.66[ab]	3.20 ± 0.69[c]
Group 3	149.40 ± 1.34[a]	4.16 ± 0.11	102.20 ± 0.83[b]	11.16 ± 0.47[a]	3.10 ± 0.24[c]
Group 4	153.40 ± 2.07[ab]	4.26 ± 0.11	103.20 ± 1.64[b]	9.58 ± 0.25[c]	2.26 ± 0.16[b]
Group 5	150.00 ± 3.67[a]	4.14 ± 0.11	102.60 ± 1.81[b]	9.82 ± 0.35[abc]	2.76 ± 0.40[bc]
Group 6	155.00 ± 1.00[b]	4.22 ± 0.14	103.60 ± 1.67[b]	10.42 ± 0.48[abc]	2.64 ± 0.53[b]

[a, b, c] : Difference is statistically significant in groups having different letters in the same column (P < 0.05).

Table 3. Sodium, potassium, chloride, calcium and phosphorus levels in control and experimental groups on day 45.

Groups	Electrolytes				
	Sodium (mEq/l)	Potassium (mEq/l)	Chloride (mEq/l)	Calcium (mg/dl)	Phosphorus (mg/dl)
Group 1	148.60 ± 2.40	4.16 ± 0.11	101.80 ± 1.64	12.70 ± 2.00[ab]	2.98 ± 0.51[bc]
Group 2	150.80 ± 4.43	4.16 ± 0.11	104.40 ± 1.81	12.84 ± 0.97[c]	2.32 ± 0.30[c]
Group 3	151.80 ± 1.92	4.18 ± 0.13	102.00 ± 2.64	12.62 ± 0.66[bc]	2.06 ± 0.40[bc]
Group 4	151.60 ± 3.36	4.24 ± 0.11	102.20 ± 1.92	10.22 ± 1.12[a]	2.04 ± 0.28[bc]
Group 5	149.40 ± 2.88	4.20 ± 0.01	102.60 ± 2.30	12.30 ± 0.28[bc]	2.50 ± 0.12[a]
Group 6	150.00 ± 1.22	4.20 ± 0.10	104.20 ± 5.21	11.48 ± 0.96[ab]	2.76 ± 0.11[ab]

[a, b, c] : Difference is statistically significant in groups having different letters in the same column (P < 0.05).

(on days 15, 30 and 45). Concerning the blood calcium level, there is a tendency to decrease in all experimental groups (Groups 2-6) for 3 periods (except for Group 2 on day 45 in calcium level), compared to control (Group 1). These decreases were obvious in the group that received AF alone (Group 4). Many factors may play a role in this change. The calcium level in the groups that received AF and adsorbent (Groups 5 and 6) showed an increase, compared to the group that received only AF (Group 4) for all periods. However, that increase was not high enough in the levels of experimental groups, in order to reach to the level of control group. It was understood that the adsorbent compound bound to AF and blocked its absorption from the gastrointestinal tract. On the other hand, the changes in phosphorus and calcium levels seemed to be parallel. The decrease was seen in the phosphorus level in all experimental groups (Groups 2-6), compared to control (Group 1) for all periods (on days 15, 30 and 45). Interestingly, in the groups that received AF and adsorbent (Groups 5 and 6), the increase was observed in the blood phosphorus level, compared to the group that received only AF (Group 4) on days 30 and 45. However, that increase did not reach the level of control. It revealed that the adsorbent compound could not completely eliminate the effects of AF added into feed at specified proportions. The decrease was also observed in calcium and phosphorus levels in the groups that received only adsorbent agent in the feed (Groups 2 and 3) compared to the control (Group 1) in all periods (on days of 15, 30 and 45 except for Group 2 on day 45 in calcium level). This decrease was considered to be combined with high binding efficacy of sodium bentonite that was used as AF binder, because the binding efficacy of sodium bentonite is above 80-milliequivalent/100 g (21). Due to the high binding capacity of adsorbents, they do not bind to only AF, they may also bind to minerals. For this reason, it is considered that it irreversibly bound to calcium and phosphorus present in feed, and, in turn, the blood calcium and phosphorus levels decreased in the groups that received adsorbent agent (Groups 2 and 3), compared to the control (Group 1). Concerning the sodium, potassium and chloride levels, there were statistically significant changes in the sodium and chloride levels between the groups (Groups 1 to 6) on day 30 only. From these changes, the sodium level made an increase in the group that received only AF (Group 4). On the other hand, this level increased or decreased in the group that received AF and sodium bentonite (Groups 5 and 6) compared to control (Group 1) on day 30. For chloride level, increases existed in all groups (Groups 2-6), compared to control (Group 1) on day 30. No statistically significant differences were seen in these parameters (sodium and chloride) between the groups with respect to the values of the 15th and 45th days of the study. The changes in potassium levels were not statistically significant for all periods (days 15, 30 and 45).

Concerning this subject, Keçeci et al. (19) determined that the levels of calcium and phosphorus were decreased by AF in broiler chicks that received AF for 21 days and at a dose of 2.5 ppm. In the study by Oğuz et al. (16), AF and clinoptilolite were given to broiler chicks at the same doses and periods and decreases were observed in both electrolyte levels in the groups, which received only the compound alone (AF or clinoptilolite). The decrease in the phosphorus level was statistically significant in the group that received only AF. In the groups that received each of them in a combination both parameters were close to the level of control. On the other hand, Huff et al. (20) showed that AF caused a decrease in blood calcium level in broiler chicks for 3 weeks and at a dose of 2.5 ppm. Similarly, Smith et al. (22) observed that AF that was given at a dose of 3.5 ppm for 3 weeks caused a decrease in phosphorus level. Again, Giroir et al. (23) reported that AF led to a decrease in phosphorus level at the dose of 2.5 ppm over 3 weeks. It was understood that there was a correlation between the results, compared to previous studies.

In conclusion, the specified doses of AF (1 ppm) affected the blood calcium and phosphorus levels for all periods. Both electrolyte levels decreased in blood compared to the control. On the other hand, no statistically significant change was found in the blood potassium levels for all periods and in sodium and chloride level for most periods. This indicated that AF did not affect the relevant electrolytes at the specified dose and period exactly. A decrease was found in calcium and phosphorus levels in the groups that received only sodium bentonite and it could be concluded that the adsorbent agents added to feed might be irreversibly bound to some minerals like calcium and phosphorus that are present in feed and the body and therefore inhibit their absorbance from the digestive tract.

References

1. Huff, W.E., Kubena, L.F., Harvey, R.B., Hagler, W.M., Swanson, S.P., Phillips, T.D., Creger, C.R.: Individual and combined effects of aflatoxin and deoxynivalenol (DON, Vomitoxin) in broiler chickens. Poult. Sci., 1986; 65: 1291-1298.
2. Dalvi, R.R.: An overview of aflatoxicosis of poultry: Its characteristics, prevention and reduction. Vet. Res. Commun., 1986; 10: 429-443.
3. Kaya, S.: Küflenmeden şüpheli yem ve yem hammaddelerinde aflatoksinler. Ankara Üniv. Vet. Fak. Derg., 1985; 32: 1-12.
4. Oğuz, H., Kurtoğlu, F., Kurtoğlu, V., Birdane, Y.O.: Evaluation of biochemical characters of broiler chickens during dietary aflatoxin (50 and 100 ppb) and clinoptilolite exposure. Res. Vet. Sci., 2002; 73: 101-103.
5. Oğuz, H., Kurtoğlu, V., Coşkun, B.: Preventive efficacy of clinoptilolite in broilers during chronic aflatoxin (50 and 100 ppb) exposure. Res. Vet. Sci., 2000; 69: 197-201.
6. Quezeda, T., Cuellar, H., Jaramillo-Juarez, F., Valdivia, A.G., Reyes, J.L.: Effect of aflatoxin B_1 on the liver and kidney of broiler chickens during development. Comp. Biochem. Physiol. Part C., 2000; 125: 265-272.
7. Ortatatlı, M., Çiftçi, M.K., Tuzcu, M., Kaya, A.: The effects of aflatoxin on the reproductive system of roosters. Res. Vet. Sci., 2002; 72: 29-36.
8. Harvey, R.B., Kubena, L.F., Phillips, T.D.: Evaluation of aluminosilicate compounds to reduce aflatoxin residues and toxicity to poultry and livestock: a review report. Sci. Total. Environ., 1993; 2: 1453-1457.
9. Phillips, T.D., Clement, B.A., Kubena, L.F., Harvey, R.B.: Detection and detoxification of aflatoxins: prevention of aflatoxicosis and aflatoxin residues with hydrated sodium calcium aluminosilicate. Vet. Hum. Toxicol., 1990; 32: 15-19.
10. Schell, T.C., Lindemann, M.D., Kornegay, E.T., Blodgett, D.J.: Effects of feeding aflatoxin-contaminated diets with and without clay to weanling and growing pigs on performance, liver function, and mineral metabolism. J. Anim. Sci., 1993; 71: 1209-1218.
11. Diaz, D.E., Hagler, W.M. Jr., Hopkins, B.A., Whitlow, L.W.: Aflatoxin binders I: in vitro binding assay for aflatoxin B_1 by several potential sequestering agents. Mycopathologia, 2002; 156: 223-226.
12. Demet, Ö., Oğuz, H., Çelik, İ., Nizamlıoğlu, F.: Pirinçte aflatoksin üretilmesi. Vet. Bil. Derg., 1995; 11: 19-23.
13. Shotwell, O.L., Hesseltine, C.W., Stubblefield, R.D., Sorenson, W.G.: Production of aflatoxin on rice. Appl. Microbiol., 1966; 14: 425-428.
14. Nabney, J., Nesbit, B.F.: A spectrophotometric method for determination of the aflatoxins. Analyst, 1965; 3: 155-159.
15. Anon.: SPSS 9.05 for Windows Package Program.
16. Oğuz, H., Keçeci, T., Birdane, Y.O., Onder, F., Kurtoğlu V.: Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Res. Vet. Sci., 2000; 69: 89-93.
17. Oğuz, H., Kurtoğlu, V.: Effect of clinoptilolite on performance of broiler chickens during experimental aflatoxicosis. Br. Poult. Sci., 2000; 41: 512-517.
18. Glahn, R.P., Beers, K.W., Bottje, W.G., Wideman, R.F., Huff, W.E., Thomas, W.: Aflatoxicosis alters avian renal function, calcium, and vitamin D metabolism. J. Toxicol. Environ. Health., 1991; 34: 309-321.
19. Keçeci, T., Oğuz, H., Kurtoğlu, V.: Effects of polyvinylpolypyrrolidone, synthetic zeolite and bentonite on serum biochemical and hematological characters of broiler chickens during aflatoxicosis. Br. Poult. Sci., 1998; 39: 452-458.
20. Huff, W.E., Harvey, R.B., Kubena, L.F., Rottinghaus, G.E.: Toxic synergism between aflatoxin and T-2 toxin in broiler chickens. Poult. Sci., 1998; 67: 1418-1423.
21. Stul, M.S., Vliers, D.P., Uytterhoven, J.B.: In vitro adsorption-desorption of phenethylamines and phenylimidazoles by a bentonite and a resin. J. Pharm. Sci., 1984; 73: 1372-1375.
22. Smith, E.E., Kubena, L.F., Braithwaite, C.E., Harvey, R.B., Phillips, T.D., Reine, A.H.: Toxicological evaluation of aflatoxin and cyclopiazonic acid in broiler chickens. Poult. Sci., 1992; 71: 1136-1144.
23. Giroir, L.E., Huff, W.E., Kubena, L.F., Harvey, R.B., Elissalde, M.H., Witsel, D.A., Yersin, A.G., Ivie, G.W.: The individual and combined toxicity of kojic acid and aflatoxin in broiler chickens. Poult. Sci., 1991; 70: 1351-1356.