Effect of Adding different Levels of Chlorophyll Extract and Vitamin A to Laying Hens Rations for Reduce Negative Role of Aflatoxin B1 in Egg Shell Characteristics

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Abstract

This study carried out to study effect of additives vitamin A and chlorophyll extract to reduce the negative effects of aflatoxin B1 on external qualitative characteristics of eggs shell of Lohman Brown chickens. The experiment was at animal production farm in college of agriculture, University of Anbar from 1st February, 2021 to 20th June, 2021. Total of Eighty-four laying hens lohman brown were used, 24 weeks of age randomly distributed to seven treatments with four replicates (3 hens/replicate). Treatments of experiment were as follow: T1 control without any additives, T2 was treated with aflatoxin (B1) 200 mg. kg^{-1} feed, T3 addition vitamin A mg. kg^{-1} feed, T4 addition chlorophyll extract 200 mg. kg^{-1} feed , T5 addition chlorophyll extract 100 mg . kg^{-1} feed + aflatoxin (B1) 200 mg. kg^{-1} feed, T6 addition chlorophyll extract 200 mg. kg^{-1} feed + aflatoxin (B1) 200 mg. kg^{-1} feed, T7 addition chlorophyll extract 300 mg. kg^{-1} feed + aflatoxin (B1) 200 mg. kg^{-1} feed. The results showed the superiority of addition treatment (chlorophyll extract 300 mg. kg^{-1} feed) in egg shell traits during production period (24-39) weeks compared with T2. Also, results indicated that birds fed diets treated with aflatoxin B1 recorded lowest values in External characteristics of Egg shell,. Moreover, chlorophyll proved that it can enhanced characteristics of Egg shell for laying hens and decrease negative role aflatoxin B1 in feeds.

Key Words: Feed Additives, Chlorophyll Extract, Aflatoxin, Laying Hens, Characteristics of Shell.

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Introduction

Aflatoxins are considered a very dangerous substance due to the lack of an immune response to it and because of its physiological effects on the cells of the bird's body, as it is considered an immunosuppressive, mutagenic and carcinogenic substance (Althagafi *et al.,* 2019). In addition, the exposure of the gastrointestinal tract to mycotoxins leads to damage to the membranes lining the digestive system, which in turn affects the extent of digestion and absorption of nutrients by the intestine (Mousa *et al*., 2019). The most important kind of mycotoxins is aflatoxin, whose severity depends on the age and type of the bird, the amount of poison to which the bird's body was exposed, and the length of the exposure period (Erdélyi *et al*., 2018). In addition, exposure of the digestive tract to mycotoxins leads to damage to the lining membranes of the digestive system, which in turn affects the ability to digest and absorb nutrients by the intestine (Chunpeng *et al*., 2019). Many studies have been followed to find appropriate solutions to reduce the effects of mycotoxins on animal health, chlorophyll contain vitamins and amino acids such as (E, BI, B2, B6, C, Niacin, Biotin, lnositol and Pantothenic acid) as well as vitamin A which Fat-soluble vitamins, one of the

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main antioxidants in body and responsible for protect (PUFA) in cells membranes from oxidation by free radicals, as it prevents reactions of free radical formation resulting from normal metabolic processes. It is mainly produced from *Aspergillus flavus* and *Aspergillus parasiticus*, which is indicated by the presence of suitable humidity and high temperature (Rashid *et al*., 2008). Thus, this study conducted to study effect of adding chlorophyll extract with different levels and vitamin A to laying hens diets to reduce negative effect of aflatoxin B1.

Materials and Methods

An Experiment was conducted in poultry farm belonged to Animal Production Department, College of Agriculture/University of Anbar from 1st Feb. to 20th Jun., 2021, to study effect of adding vitamin A, aflatoxin B1 and different levels from chlorophyll extract. Eighty-four laying hens were used in this experiment of Lohmann Brown breed, 24 weeks were used. The laying hens were distributed randomly to seven treatments with four replicates per treatment (3 hens for each replicate). The experimental treatments were as follow: T1 control without any additives, T2 was treated with aflatoxin (B1) 200 mg. kg^{-1} feed, T3 addition vitamin A mg. kg^{-1} feed, T4 addition chlorophyll extract 200 mg. kg^{-1} feed, T5 addition chlorophyll extract 100 mg. kg^{-1} feed + aflatoxin (B1) 200 mg. kg^{-1} feed, T6 addition chlorophyll extract 200 mg. kg^{-1} feed + aflatoxin (B1) 200 mg. kg^{-1} feed, T7 addition chlorophyll extract 300 mg. kg^{-1} feed + aflatoxin (B1) 200 mg. kg^{-1} feed. The study continued for four periods (28 day per period). Diets were provided in fixed amounts for all cages. Water was given *ad libitum* according to system of nipples water and lighting period was calculated to be 15.5 hours per day. All diets were isocaloric and isonitrogenous content, as they contained all the required nutrients and according to recommendations of birds guide strain. Chlorophyll extract and vitamin A, had been purchased from Guangzhou Ur, Trading. Co, Ltd, China. At end of weeks 27, 31, 35 and 39 of age, three eggs collected from each replicate in each treatment. Egg shell characteristics were measured through breaking three eggs per replicate were taken randomly from each treatment and their quality traits were measured once weekly for 16 times (Al-Fayadh and Naji, 1989). Eggs collected from each bird in each treatment. Egg shell quality parameters including egg shell quality (shell weight and membranes), shell thickness determined by using a vernier caliper, relative egg shell and Shell Weight per Unit Surface Area (SWUSA) determined according to (Carter, 1975). Birds fed traditional diet as NRC (1994) which chemical composition was (16.77 % crude protein, 2752 metabolizable energy). Data were statistically analyzed using the statistical program of (SAS, 2012) to study the effect of different treatments in the studied traits under study according to the complete randomized design (CRD) and compare the significant differences between means with (Duncan, 1955).

Results and Discussion

Egg Shell Weight

Table (1) shows effect treatments of adding chlorophyll and vitamin A to diets treated with aflatoxin B1 on egg shell weight produced by Lohman Brown laying hens, results did not indicate significant differences between different experimental treatments at four study periods and general average.

Table 1. Effect of adding different levels of chlorophyll and vitamin A to Laying Hens Diets treated with aflatoxin B1 on Egg shell weight

Treatments	P 1 24-27 weeks	P 2 28-31 weeks	P 3 32-35 weeks	P 4 36-39 weeks	Mean ± S.E
T1	5.65 ± 0.27	5.98 ± 0.31	6.29 ± 0.10	5.99 ± 0.27	5.98 ± 0.13
T2	6.03 ± 0.55	6.46 ± 0.20	6.55 ± 0.17	6.44 ± 0.22	6.37 ± 0.19
T3	5.73 ± 0.59	6.39 ± 0.21	6.42 ± 0.31	5.94 ± 0.31	6.12 ± 0.29
T4	6.30 ± 0.41	6.33 ± 0.34	6.29 ± 0.26	6.05 ± 0.22	6.24 ± 0.22
T5	6.25 ± 0.12	6.67 ± 0.35	6.50 ± 0.33	6.30 ± 0.13	6.43 ± 0.04
T6	5.60 ± 0.31	6.31 ± 0.14	6.96 ± 0.06	5.98 ± 0.26	6.21 ± 0.12
T7	6.33 ± 0.17	6.66 ± 0.28	6.86 ± 0.21	6.28 ± 0.26	6.53 ± 0.18
***P*-value**	N.S	N.S	N.S	N.S	N.S

T1: Control, T2: Aflatoxin B1 200 mg, T3: Vitamin A 200 mg + Aflatoxin B1 200 mg, T4: chlorophyll 200 mg, T5: chlorophyll 100 mg + Aflatoxin B1 200 mg, T6: chlorophyll 200 mg + Aflatoxin B1 200 mg, T7: chlorophyll 300 mg + Aflatoxin B1 200 mg.
***N.S: Non significant.**

Egg Shell Thickness

The results of statistical analysis in table (2) indicate effect of feed additives to diets of laying hens treated with aflatoxin B1 in shell thickness.

The results during first period indicated that there were significant differences (P≤0.05) for thickness of eggshell produced by hens in T1 (control) compared to eggs produced by hens in treatments T2 and T5 while there were no significant differences between treatments T1, T3, T4, T6 and T7 in shell thickness. As for second period, results indicated a significant increase for treatments T6 and T7 compared to treatments T2, T3 and T5, while no significant differences recorded between treatments T1, T3, T4 and T5, During third period T7 recorded highest value of egg shell thickness compared with T1, T2, T3, T4 and T5 except for treatment T6, which did not differ with it significantly, reaching at fourth period eggs of birds in T7 continuous recorded high values compared with T1, T3 and T2 which recorded lowest value, while no significant differences between T7 and T4, T5 and T6. As for general average of effect of treatments, birds in T7 recorded best values of shell thickness compared with T1, T2, T3, T4 and T5.

Table 2. Effect of adding different levels of chlorophyll, vitamin A to Laying Hens Diets treated with aflatoxin B1 on Egg shell thickness

Treatments	P 1 24-27 weeks	P 2 28-31 weeks	P 3 32-35 weeks	P 4 36-39 weeks	Mean ± S.E
T1	0.48 ± 0.02 ^{A}	0.44 ± 0.01 ^{AB}	0.43 ± 0.01 ^{BC}	0.41 ± 0.00 ^{BC}	0.44 ± 0.01 ^{B}
T2	0.41 ± 0.01 ^{C}	0.39 ± 0.00 ^{C}	0.38 ± 0.00 ^{C}	0.39 ± 0.00 ^{C}	0.40 ± 0.00 ^{C}
T3	0.45 ± 0.01 ^{ABC}	0.42 ± 0.00 ^{B}	0.44 ± 0.00 ^{BC}	0.42 ± 0.01 ^{BC}	0.43 ± 0.01 ^{B}
T4	0.45 ± 0.01 ^{ABC}	0.44 ± 0.00 ^{AB}	0.44 ± 0.01 ^{BC}	0.44 ± 0.00 ^{AB}	0.44 ± 0.00 ^{B}
T5	0.43 ± 0.01 ^{BC}	0.42 ± 0.01 ^{B}	0.44 ± 0.01 ^{BC}	0.44 ± 0.02 ^{AB}	0.43 ± 0.01 ^{B}
T6	0.45 ± 0.01 ^{ABC}	0.46 ± 0.01 ^{A}	0.47 ± 0.01 ^{AB}	0.44 ± 0.01 ^{AB}	0.45 ± 0.00 ^{AB}
T7	0.46 ± 0.01 ^{AB}	0.46 ± 0.01 ^{A}	0.53 ± 0.05 ^{A}	0.46 ± 0.01 ^{A}	0.48 ± 0.02 ^{A}
***P*-value**	0.05	0.05	0.05	0.05	0.05

T1: Control, T2: Aflatoxin B1 200 mg, T3: Vitamin A 200 mg + Aflatoxin B1 200 mg, T4: chlorophyll 200 mg, T5: chlorophyll 100 mg + Aflatoxin B1 200 mg, T6: chlorophyll 200 mg + Aflatoxin B1 200 mg, T7: chlorophyll 300 mg + Aflatoxin B1 200 mg.
***Different letters within same columns are significant differences at (P<0.05).**

Relative Egg Shell Weight

Table (4) indicates the effect of different experimental treatments on the relative egg shell weight, as it is noted that first period 24 - 27 weeks showed a superiority at level (P≤0.05) in favor of the treatments T4, and T7 compared to treatment T6. At second period 28-31 weeks indicated that there were significant decrease for treatment T6 compared to treatments T5 and T7, while no significant differences between treatments T1, T2, T3, T4, and T6. As for third period, 32-35 weeks, a high superiority (P≤0.01) was observed for T7 compare to T1, T2 and T4, During fourth period birds in T7 recorded high significant differences (P≤0.01) in Relative egg shell weight compare with birds in all treatments except birds in T1. Also, results showed that general average of relative eggshell weight was a significant increase at level (P≤0.01) in favor of birds treatment T7, which recorded 12.12 compared to rest of experimental treatments, which did not recorded any significant differences between them (10.61, 9.74, 10.67, 10.68, 10.78 and 10.08) respectively.

Table 3. Effect of adding different levels of chlorophyll, vitamin A to Laying Hens Diets treated with aflatoxin B1 on relative egg shell weight

Treatment	P 1 24-27 weeks	P 2 28-31 weeks	P 3 32-35 weeks	P 4 36-39 weeks	Mean ± S.E
T1	10.51 ± 0.50 ^{AB}	10.47 ± 0.34 ^{BC}	10.94 ± 0.21 ^{BC}	10.51 ± 0.58 ^{AB}	10.61 ± 0.07 ^{B}
T2	10.21 ± 0.34 ^{AB}	10.33 ± 0.37 ^{BC}	9.60 ± 0.33 ^{C}	8.82 ± 0.44 ^{C}	9.74 ± 0.28 ^{B}
T3	10.51 ± 0.86 ^{AB}	10.82 ± 0.30 ^{BC}	11.05 ± 0.48 ^{ABC}	10.30 ± 0.67 ^{BC}	10.67 ± 0.46 ^{B}
T4	11.44 ± 0.50 ^{A}	10.87 ± 0.50 ^{BC}	10.15 ± 0.71 ^{BC}	10.28 ± 0.37 ^{BC}	10.68 ± 0.32 ^{B}
T5	10.48 ± 0.19 ^{AB}	11.56 ± 0.55 ^{AB}	11.03 ± 0.61 ^{ABC}	10.04 ± 0.17 ^{BC}	10.78 ± 0.05 ^{B}
T6	9.45 ± 0.44 ^{B}	9.95 ± 0.16 ^{C}	11.23 ± 0.14 ^{AB}	9.68 ± 0.57 ^{BC}	10.08 ± 0.15 ^{BC}
T7	11.76 ± 0.86 ^{A}	12.36 ± 0.68 ^{A}	12.52 ± 0.52 ^{A}	11.83 ± 0.40 ^{A}	12.12 ± 0.34 ^{A}
***P*-value**	0.05	0.05	0.01	0.01	0.01

T1: Control, T2: Aflatoxin B1 200 mg, T3: Vitamin A 200 mg + Aflatoxin B1 200 mg, T4: chlorophyll 200 mg, T5: chlorophyll 100 mg + Aflatoxin B1 200 mg, T6: chlorophyll 200 mg + Aflatoxin B1 200 mg, T7: chlorophyll 300 mg + Aflatoxin B1 200 mg.
***Different letters within same columns are significant differences at (P<0.05).**
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Shell Weight per Unit Surface Area (SWUSA)

Results shown in Table (5) indicated effect of additives on Shell Weight per Unit Surface Area

(SWUSA) which expresses the mineralization of shell in surface area. However, significant differences (P≤0.05) appeared in favor of birds in T7 during the second, third and fourth periods compared to treatment T2, while no significant differences appeared between treatments at first period. As for general average of periods, treatment T7 was significantly superior to treatments T1, T2, T3, T4, T5 and T6, while mentioned treatments did not show any significant difference between them in Shell Weight per Unit Surface Area (SWUSA).

Table 4. Effect of adding different levels of chlorophyll, vitamin A to Laying Hens Diets treated with aflatoxin B1 on SWUSA

Treatment	P 1 24-27 weeks	P 2 28-31 weeks	P 3 32-35 weeks	P 4 36-39 weeks	Mean ± S.E
T1	0.0853 ± 0.0037	0.0866 ± 0.0033 ^{B}	0.0906±0.0014 ^{AB}	0.0869±0.0045 ^{AB}	0.0873±0.0009 ^{B}
T2	0.0864 ± 0.0026	0.0885± 0.0032 ^{B}	0.0848±0.0028 ^{B}	0.0778±0.0037 ^{B}	0.0844±0.0024 ^{B}
T3	0.0857 ± 0.0075	0.0903 ± 0.0026 ^{AB}	0.0919±0.0042 ^{AB}	0.0854±0.0052 ^{AB}	0.0883±0.0039 ^{B}
T4	0.0935 ±0.0046	0.0904 ±0.0044 ^{AB}	0.0860±0.0053 ^{B}	0.0857±0.0030 ^{AB}	0.0889±0.0028 ^{B}
T5	0.0878 ± 0.0016	0.0959 ± 0.0046 ^{AB}	0.0920±0.0049 ^{AB}	0.0853±0.0009 ^{AB}	0.0902±0.0003 ^{B}
T6	0.0790 ± 0.0038	0.0848 ±0.0013 ^{B}	0.0951±0.0009 ^{AB}	0.0819±0.0043 ^{B}	0.0852±0.0012 ^{B}
T7	0.0942 ± 0.0074	0.0995 ± 0.0046 ^{A}	0.1009±0.0036 ^{A}	0.0964±0.0028 ^{A}	0.0978±0.0026 ^{A}
***P*-value**	**N.S**	**0.05**	**0.05**	**0.05**	**0.05**

T1: Control, T2: Aflatoxin B1 200 mg, T3: Vitamin A 200 mg + Aflatoxin B1 200 mg, T4: chlorophyll 200 mg, T5: chlorophyll 100 mg + Aflatoxin B1 200 mg, T6: chlorophyll 200 mg + Aflatoxin B1 200 mg, T7: chlorophyll 300 mg + Aflatoxin B1 200 mg.
***Different letters within same columns are significant differences at (P<0.05).**
****N.S: Non significant.**

Results of qualitative characteristics obtained from experiment show a significant decrease in thickness of shell, SWUSA and relative weight of shell for birds in T2. The reason for decrease of these characteristics may be due to negative effect of aflatoxin B1 which represented by absorption and metabolism of nutrients from intestinal villi, which causes a decrease in weight of shell and its internal components and is negatively reflected in performance of laying hens and decrease of external egg shell quality (Qubih, 2012). Chlorophyll extract is rich with nutrients and vitamins, including vitamin D3, which may have an important role by increasing thickness of shell. This reflects balance of calcium in all different treatments with concentrations of natural chlorophyll extract. As for decrease external egg shell quality, it may be due to a decrease in amount of feed consumed due to stress caused by aflatoxin B1, which leads to bird not getting its calcium needs and thus reducing its percentage in the blood. On other hand, it affects secretion of thyroxin hormone, which works to regulate calcium transported between blood and bones, as the decrease in secretion of this hormone led to a decrease in blood plasma calcium as a result for this, the egg shell quality decreased (Sturkie, 1986). The negative effect of mycotoxins may be due to prolonged intake of aflatoxin B1, which led to aflatoxin accumulation in the liver, causing damage to hepatocytes and the inability of the bird's body to eliminate them (Tilley *et al.*, 2017), which negatively affected the production performance. The breakdown of the body's natural defense lines has a positive relationship with the increased level of aflatoxin contamination, which is due to liver tissue damage, causing a decrease in the production of lipoproteins to the yolk, and the negative damage caused by aflatoxin B1 toxin has caused great economic losses in the poultry industry, including a decrease in the daily production rate of eggs as well as Spoilage of eggs due to contamination, breakage, and small size (Yin *et al.* 2017; Althagafi *et al*., 2019). The effectiveness of the anti-mycotoxins (chlorophyll) added to diets helped in reducing the effect of mycotoxins and improving egg shell characteristics during periods production as it is a substance that acts on binding of mycotoxins and prevents its absorption by intestinal *villi* (Schlumbohm *et al*., 2014). It may be due to the fact that mycotoxins cause fat oxidation, which increases free radicals, causing damage to those cells (Mousa *et al*., 2018), and feed additives like chlorophyll consider a binder played indirectly an effective role in preserving liver cells from damage caused by mycotoxins, or attributed to the role of chlorophyll and vitamin A in binding the aflatoxin toxins and preventing their negative damage inside the body and excreting them with the feces

to outside (Gul *et al*., 2017).

Conclusions

The appearance of negative effects of aflatoxin at the end of the experiment was clear, and it had a significant effect on the qualitative characteristics of eggs (egg shell). All the feed additives used in experiment showed positive effective for reducing negative effects caused by aflatoxins added to laying hens' diets.

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