Role of Chlorophyll Extract and Vitamin A to Reducing Negative Effects of Aflatoxin B1 to Diets on Productive Performance of Laying Hens

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Abstract

This study was conducted to determine the effect of adding vitamin A and different levels of chlorophyll extract to reducing negative effects of aflatoxin B1 on the production performance of Lohman Brown chickens at animal production farm belong to college of agriculture / University of Anbar from 1/2/2021 to 20/6/2021. Eighty-four laying hens were used, aged 24 weeks distributed randomly into seven treatments with four replicates per treatment (3 hens/replicate). The treatments of the experiment were as followed; T1 / control ration without any addition, T2 was treated with aflatoxin (B1) of 200 mg / kg of feed, T3 included addition vitamin A at 200 mg / kg, T4 addition chlorophyll extract 200 mg / kg of feed, T5 addition chlorophyll extract 100 mg / kg of feed + aflatoxin (B1) 200 mg / kg, T6 addition chlorophyll extract 200 mg / kg of feed + aflatoxin (B1) 200 mg / kg, T7 addition chlorophyll extract 300 mg / kg of feed + aflatoxin (B1) 200 mg / kg. The results showed the superiority of addition treatment (chlorophyll extract 300 mg / kg of feed) in productive traits during the production period (24-39) weeks compared with T1and T2. Also, results indicated that birds fed diets treated with aflatoxin B1 recorded lowest values in productive performance. Moreover, chlorophyll proved that it can enhanced productive performance for laying hens and decrease negative role aflatoxin B1 in feeds.

Key Words: Chlorophyll Extract, Aflatoxin B1, Laying Hens, Productive Performance.

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Introduction

Poultry nutrition is one of the most important episodes of the poultry industry, and on it depends the success of poultry production. Cook (1991), NRC (1994) appointed to the role of nutrition in raising the immune response of chickens and improving production performance by controlling nutrition requirements. Studies have shown that the use of medicinal plants has proven its ability to improve productive traits through possibility of using biological methods in linking toxins by using useful substances that are able to link them inside the small intestine and prevent their absorption (Lee *et al.,* 2003), as interest in poultry breeding projects in various parts of the world has increased as projects with good economic returns, which prompted researchers and breeders have to develop these projects to get the highest production with lowest costs, and that contamination of fodder crops or their industrial waste with mycotoxins is something that is difficult to avoid. In addition to its contents of vitamins and acids (E, A, BI, B2, B6, C, Niacine, Biotin, lnositol and Pantothenic acid) as well as vitamin A from Fat-soluble vitamins, one of the main antioxidants in the body and responsible for protect (PUFA) in cells membranes from oxidation by free radicals, as it prevents the reactions of free radical formation resulting from normal metabolic processes.

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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).

Materials and Methods

This study was conducted in the poultry farm which belonged to the Department of Animal Production/ College of Agriculture/University of Anbar from 1st February, 2021 to 20th June, 2021, to study effect of adding vitamin A, aflatoxin B1 and different levels from chlorophyll. 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 laying hens/ replicate). The experimental treatments were as follow: T1 / control without any addition, T2 was treated with aflatoxin (B1) of 200 mg / kg of feed , T3 included addition 200 mg + aflatoxin (B1) 200 mg / kg, T4 addition chlorophyll extract 200 mg / kg of feed , T5 addition chlorophyll extract 100 mg / kg of feed + aflatoxin (B1) 200 mg / kg, T6 addition chlorophyll extract 200 mg / kg of feed + aflatoxin (B1) 200 mg / kg, T7 addition chlorophyll extract 300 mg / kg of feed + aflatoxin (B1) 200 mg / kg. The birds were feed according to diet explained in Table (1). Water was given *ad libitum* according to the system of nipples water and the lighting period was calculated to be 15.5 hours per day. Chlorophyll extract and vitamin A, had been purchased from Guangzhou Ur, Trading. Co, Ltd, China. The percentage of egg production were calculated according to Hen Day (H.D%). The average weight of eggs and the feed consumed according to the equation mentioned by Al-Fayyad and Naji (1989). The measurement of eggs mass produced according to the equation mentioned by Rose (1997) and the feed conversion ratio, gm of feed / gm of eggs according to the equation mentioned by Ibrahim (2000). The 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) polynomial test.

Table 1. The composition (%), calculated and analyzed nutrients contents of the experimental diets fed during 24–39 weeks

Ingredients	**%**
Yellow corn	**35.4**
Wheat	**30**
Soy bean meal (48%)	**23**
Premix*	**2.5**
Vegetable Oil	**0.5**
Limestone	**7.5**
Salt	**0.1**
Di calcium phosphate	**1**
Total	**100**
Chemical analysis **	
ME kcal/kg	**2752**
CP %	**16.77**
Lysine %	**0.93**
Met. + Cys. %	**0.76**
Ca %	**3.75**
Available Phosphor %	**0.39**

*premix provided per kilogram of diet: 7.8 %crude protein, 29.3 kcal metabolizable energy, 23.1% Ca, 3.8% Ava. P %, 7.7% Methionine+ Cystin, 2.4% Lysine.

** Chemical analysis according to NRC (1994).

Results and Discussion

Egg Production

Percentage of the number of eggs produced on basis of Hen Day and accumulative total egg production was noticed in Table (2) that there is a significant superiority (P≤0.01) during first period (27-24) weeks between the different treatments of the experiment, as treatments T7, T5, T2 were significantly superior to treatments T4, T1, Treatments recorded an egg production rate of (91.07, 90.77, 89.88), while the treatments T1 and T4 (85.12, 87.20) were recorded on basis of H.D% without recording significant differences between them, as well as no significant differences were recorded between the treatments T6, T5, T3). As for the second period (28)-31 weeks, the experimental treatments showed significant differences at the level (P≤0.05), as treatment T5 (adding 100 mg chlorophyll + 200 mg aflatoxin B1) outperformed treatment T4 (adding 200 mg chlorophyll) without recording a significant difference between the aforementioned treatment and the treatments T1, T2, T3, T6 and T7. As for the third period, it was noticed that the highly significant superiority (P≤0.01) of T7 and T1 over the T6, T5, T3, and T2 treatments was noted. It is also noted from Table (2) that the treatments of adding chlorophyll T7, T6, T5, and T4 outperform the treatment T2 during the fourth period. It differs significantly with each of the treatments T1 and T3 (87.50, 87.80), respectively, either from the general average of experimental treatments on HD egg production. A highly significant (P≤0.01) was noted for treatment T7.

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

Treatments	Period 1 24-27 weeks	Period 2 28-31 weeks	Period 3 32-35 weeks	Period 4 36-39 weeks	Mean ± S.E
T1	C 1.03 ± 85.12	AB 0.84 ± 90.48	A 0.89 ± 91.96	AB 0.60 ± 87.50	AB 0.44 ± 88.67
T2	A 0.60 ± 89.88	AB 0.75 ± 89.58	D 0.34 ± 86.31	B 1.09 ± 85.71	B 0.14 ± 87.87
T3	AB 1.41±88.39	AB 0.75 ± 90.77	CD 1.13 ± 88.39	AB 0.57 ± 87.80	AB 0.35 ± 88.84
T4	BC 0.57 ± 87.20	B 0.84 ± 89.29	AB 1.03 ± 91.07	A 0.77 ± 89.88	B 0.56 ± 89.36
T5	A 0.30 ± 90.77	A 0.49 ± 91.67	CD 0.57 ± 87.80	A 0.75 ± 88.39	B 0.33 ± 89.66
T6	AB 0.89 ± 89.58	AB 0.57 ±90.77	BC 0.30 ± 88.99	A 0.49 ±89.29	B 0.14 ±89.66
T7	A 0.60 ± 91.07	AB 0.57 ±91.37	A 0.30 ± 91.96	A 0.77 ± 89.88	A 0.32 ±91.07
***P*-value**	**0.01**	**0.05**	**0.01**	**0.01**	**0.01**

T1: Control, T2: adding Aflatoxin B1 200 mg, T3: adding Vitamin A 200 mg + Aflatoxin B1 200 mg, T4: adding chlorophyll extract 200 mg, T5: adding chlorophyll extract 100 mg + Aflatoxin B1 200 mg, T6: adding chlorophyll extract 200 mg + Aflatoxin B1 200 mg, T7: adding chlorophyll extract 300 mg + Aflatoxin B1 200 mg.

***The different letters within the same columns are significant differences at (P<0.05).**

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The natural chlorophyll extract and synthetic vitamin A may contribute significantly and effectively to raising rate of egg production by increasing readiness of nutrients in the forage and increasing the digestion coefficient of nutrients by sequestering these elements in the digestive tract by linking nutrients to the cell wall of the small intestine (Antonio *et al.,* 2020). and thus achieving continuity in the production of eggs, which were mostly in the summer months when temperatures are high, or may be attributed to the additions containing important nutrients that meet the actual needs of the laying hens for energy, proteins and vitamins, in addition, it is a source of amino acids necessary to enrich the productive ration of laying hens, Reducing the harmful effects of aflatoxin in the ration, which affect the ability of the bird to produce eggs and thus increase metabolism or to the presence of an inhibitory effect on bacterial infection and thus enhance the health status of the bird, which is reflected on its productive performance. This is an evidence that negative effect of aflatoxin did not appear during that period, but as a result of accumulation of aflatoxin, especially the herd 2 ppm. of aflatoxin B1 on a daily basis. 1 kg of feed had a negative impact on herd and this is what data tables show at end of fourth period. The results also indicate a significant improvement in productive characteristics of chlorophyll treatments, which is one of the natural antioxidants, in proportions (100, 200, and 300 mg. kg-1 feed) compared with the treatment of industrial antioxidants A (200 mg. kg-1 feed). The reason may be due to the discrepancy in the content of these treatments from the active compounds and their synergistic effect in restricting the effect of free radicals, increasing the activity of antioxidants in the body, inhibiting lipid peroxidation and reducing oxidation stimuli. It is assumed that the antioxidants in poultry diets protect the lipoproteins and other fatty compounds Within the components of the yolk from oxidation, which leads to the presence of an abundance of these substances, and then the maturation of the ovarian follicles in a shorter time compared to chicken in control and chlorophyll-free treatments, and the role of chlorophyll may work to regulate the representation of body fat and encourage deposition of materials necessary for the growth of ovarian follicle and thus Leads to an increase in egg production (Panda *et al*., 2008). The positive effect of adding chlorophyll extract to chicken diets on egg production is related to an increase in protein concentration in the egg yolk The yolk contains three major macromolecules that make up the yolk protein, Lipovitelline, Phosvitin, derived from the intra-oocyticproteolytic component of Vitellogenim, as well as Livetin protein. Egg yolk contains VLDL (Burley *et al.,* 1993). Antioxidants encourage and accelerate the release of the yolk precursor vitellogenin from the liver to the yolk in the ovary and protect the membranes of hepatocytes from oxidative damage formed by free radicals. (OH^{0-}, O^{0-}_2) thus preventing damage and damage to the liver cell membranes from oxidation and maintaining regular cellular metabolic functions. Which increases the speed of the process of sedimentation of the yolk in the developing eggs, as it increases the mass of the egg and its maturation in a faster time and thus is reflected on the production of eggs (Panda *et al.,* 2008). Free radicals have the ability to break down cell membranes by lipid peroxidation or oxidation of double-linked polyunsaturated fatty acids (PUFA) in cell membranes, which have an opposite effect on the metabolic activity of hepatocytes, which leads to a reduction in the synthesis and release of egg yolk components from the liver (Puthpongsiriporn, 2001). The superiority of the birds of the natural chlorophyll extract treatment over the birds of the synthetic antioxidant (vitamin A) and control treatment in egg production. The reason may be due to its high efficiency as natural antioxidants because it contains more than one antioxidant vitamin, as it has a role in protecting fats and polyunsaturated fatty acids with double bonds (PUFA). From oxidation and rancidity in diets outside the body by inhibiting fat peroxide and curbing free radicals that attack and destroy fats and PUFA, causing a serious decrease in the nutritional value of fat and a significant decrease in the ability to use energy as it works to prevent the separation of hydrogen atoms from the site of the double bond in PUFA and thus prevents the occurrence of a process oxidation; The decrease in egg production in the T2 treatment may be due to the negative effect of aflatoxin B1 in the gastrointestinal tract, which reduces the work of digestive enzymes such as lipase and protezoamylase, which are necessary to digest the nutrients that the body needs to provide the components of the egg (Manafi, 2009). In addition, a significant deterioration of the T2 treatment was observed in the characteristics of productive performance in general, which appeared during the fourth period of the study due to the negative and harmful activity of aflatoxin B1, which led to a decrease in the number of eggs produced on the basis of H.D% and the percentage of cumulative egg production on the basis of H.D. Or the negative effect of mycotoxins may be due to the cumulative intake of aflatoxin B1 for a long time, which led to the accumulation of aflatoxin in the liver, causing damage to hepatocytes and the inability of chickens to get rid of it (Tilley *et al*. 2017). This was negatively reflected in the productive performance and the breakdown of the natural defense lines of the body, which has a direct relationship with the increase in the level of aflatoxin contamination, which may be due to liver tissue damage, causing a decrease in the production of yolk lipoproteins. Innate and improving productive performance traits during the fourth period, as it is a substance that absorbs mycotoxins and prevents its absorption by the villi in the intestines (Bantara, 2019). Studies have also confirmed that natural chlorophyll has the ability to remove toxins when it is absorbed and reduces the harmful effects of aflatoxin B1 (Bantara, 2019). Our results may be due to attributed that additions containing important nutrients that meet the actual needs of the laying hens for energy, proteins and vitamins, in addition, they are a source of amino acids necessary to enrich the productive ration of laying hens, and reduce the harmful effects of aflatoxin in the ration that affect ability of bird to produce eggs. Thus, increasing the metabolism or have an inhibitor effect on bacterial infection, thus enhancing health status of birds, which is reflected in its productive performance. This is evidence that the negative effect of aflatoxin did not appear during that period, but as a result of accumulation of aflatoxin, especially that the flock eats on a daily basis 2 parts per million aflatoxin B1. 1 kg of feed had a negative impact on herd and this what data tables show at end of the fourth period.

Egg Weight

From results in table (3), it can be said that the addition of natural chlorophyll extract at a concentration of 300 mg. kg of feed led to maintaining the egg weight rate superior to the rest of the treatments during the four production period despite the high temperatures of the experiment. Bantara, (2019) indicated that amino acids play a major role in increasing size of eggs, This may be due to fact that bird has benefited from the nutrients obtained from natural chlorophyll extract and vitamins that meet needs of laying hens, as they contribute in one way or another to compensate for deficiency metabolism, or perhaps this is due to the vitamins contained in natural extract of chlorophyll Which in turn led to reducing the negative effect of high temperatures on weight of eggs, so treatments of adding chlorophyll extract outperformed weight of eggs produced. This result is in agreement with obtained by other researchers (El-Sheikh *et al*., 1998) who confirmed that weight and size of egg increases with progression of the productive age of hen. Relatively large amounts of yolk are received, so the resulting eggs are larger (Qubih, 2012). This may be attributed to the fact that the bird has benefited from the nutrients obtained from the natural extract of chlorophyll from amino acids to improve egg production and egg weight. When calculating the general average weight of eggs, it is noted that productive performance of laying hens is affected by level and source of nutrients included in productive diets, in addition to other factors related to management of birds, so most important factor in its impact on production is nutrition, and the natural chlorophyll extract is rich in protein with Good quality, which contains essential amino acids, especially lysine, which plays a major role in increasing size of eggs (Scott *et al*. 1982), in addition composition of fatty acids that meet needs of laying hens contribute to metabolism.

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

Treatments	Period 1 24-27 weeks	Period 2 28-31 weeks	Period 3 32-35 weeks	Period 4 36-39 weeks	Mean ± S.E
T1	D 0.25 ±51.74	CD 0.50 ± 54.50	BC 0.50 ± 56.50	C 0.96 ± 56.50	C 0.28 ± 54.81
T2	B 0.00 ± 55.00	D 0.29 ± 53.50	D 1.00 ± 53.00	D 0.50 ± 50.50	E 0.42 ± 53.00
T3	D 0.18±51.18	D 0.00 ± 53.00	C 0.29 ± 55.50	C 1.80 ± 55.25	DE 0.43 ± 53.73
T4	C 0.48 ± 53.75	BC 1.19 ± 55.50	B 0.71 ± 58.00	B 0.25 ± 61.25	B 0.30 ± 57.13
T5	D 0.25±51.25	CD 0.48 ± 53.75	C 0.00 ± 55.00	C 0.25 ± 57.25	CD 0.19 ± 54.31
T6	B 0.25 ±54.75	B 0.63 ±56.75	B 0.63 ± 57.75	A 0.25 ±60.25	B 0.16 ±57.38
T7	A 0.00 ±60.00	A 0.48 ±62.25	A 0.48 ± 66.75	A 0.25 ± 70.25	A 0.06 ±64.81
***P*-value**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**

T1: Control, T2: adding Aflatoxin B1 200 mg, T3: adding Vitamin A 200 mg + Aflatoxin B1 200 mg, T4: adding chlorophyll extract 200 mg, T5: adding chlorophyll extract 100 mg + Aflatoxin B1 200 mg, T6: adding chlorophyll extract 200 mg + Aflatoxin B1 200 mg, T7: adding chlorophyll extract 300 mg + Aflatoxin B1 200 mg.
***The different letters within the same columns are significant differences at (P<0.01).**

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Egg Mass

Results in table (4) indicate that significant differences between the treatments were recorded in mass of eggs produced, as it is noted that birds in T7 outperformed compared with rest of experimental treatments during first period, followed by birds in T2 and T6 with a significant difference than T4 and T5 birds, which in turn outperformed birds in T4. As for second period, it is noted that birds in T7 continued to be significantly superior (P≤0.01) compared to different treatment birds in the characteristic of mass produced eggs, while birds in T3 and T2 recorded lowest significant values . It is also noted that third period included a significant superiority in favor of treatment birds T7 at the expense of the rest of the various experimental treatments, followed by the birds in treatments T1, T4 and T6 with a significant difference compare to birds in T2, T3, and T5. During fourth period, birds in treatment T7 showed a significant superiority in mass of eggs produced in comparison with the birds of other treatments, and this superiority continues to appear in the general average of periods, where birds of the treatment T7 outperformed the rest of the treatments in the mass of the eggs produced, followed by the birds of the treatments T4 and T6 with a significant difference than birds of the treatments T1,T2,T3 and T5. This result is natural and expected, as the mass of eggs is a natural reflection of weight and number of eggs produced, and weight of eggs increases linearly with age of the chicken, this directly affects mass of eggs

(Melissa *et al.*, 2016), number of eggs that reach stage of rapid growth will be reduced, so that fewer follicles receive relatively large amounts of yolk, and thus resulting eggs are larger in size.

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

Treatment	Period 1 24-27 weeks	Period 2 28-31 weeks	Period 3 32-35 weeks	Period 4 36-39 weeks	Mean ± S.E
T1	D 0.33 ± 44.04	BC 0.65 ± 49.31	B 0.85 ± 51.97	C 1.05 ± 49.45	C 0.44 ± 48.65
T2	B 0.33 ± 49.43	C 0.61 ± 47.93	D 0.98 ± 45.75	D 0.92 ± 43.30	D 0.41 ± 46.57
T3	D 0.58±45.23	C 0.40 ± 48.11	C 0.55± 49.05	C 1.38 ± 48.49	CD 0.53 ± 47.74
T4	C 0.32 ± 46.87	BC 1.21 ± 49.56	B 1.22 ± 52.84	B 0.33 ± 55.05	B 0.58 ± 51.05
T5	C 0.23±46.52	BC 0.60 ± 49.27	C 0.31 ± 48.29	C 0.45 ± 50.60	C 0.32 ± 48.70
T6	B 0.64 ± 49.05	B 0.39 ±51.51	B 0.57 ± 51.39	B 0.37 ±53.79	B 0.21 ±51.44
T7	A 0.36 ± 54.64	A 0.76 ±56.88	A 0.38 ± 61.38	A 0.64 ± 63.14	A 0.23 ±59.03
***P*-value**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**

T1: Control, T2: adding Aflatoxin B1 200 mg, T3: adding Vitamin A 200 mg + Aflatoxin B1 200 mg, T4: adding chlorophyll extract 200 mg, T5: adding chlorophyll extract 100 mg + Aflatoxin B1 200 mg, T6: adding chlorophyll extract 200 mg + Aflatoxin B1 200 mg, T7: adding chlorophyll extract 300 mg + Aflatoxin B1 200 mg.

***The different letters within the same columns are significant differences at (P<0.01).**

Feed Consumption

Table (5) shows effects of different experimental treatments on feed consumption by laying hens, as significant differences are noted during first time period, as birds in T6 recorded highest feed consumption rate 108.01 gm/bird and not differ significantly with birds in treatments T2, T3 and T5, which recorded (107.89, 105.65 and 107.00) gm/bird while birds in T4 recorded lowest feed consumption rate (102.32) gm/bird and not differ significantly with birds in T1 (104.45) gm /bird , as for second period, it is noted that the highest rate of feed consumption was in favor of birds in treatments T2, T5 and T6, with rates (108.97, 107.98 and 108.94) gm/ bird respectively, and lowest feed consumption rate was in favor of birds in T7 (103.92) gm/ bird which not change during third period, as there was a significant increase in feed consumption for birds in treatments T2, T4 and T6 (108.51, 108.92 and 108.77) gm/bird respectively. The lowest consumption rate was in favor of birds in T7 (106.90) gm/bird , As for fourth period and general average, it is noted that birds in T4 and T7 recorded lowest values with a significant difference than birds in T2 that treated with aflatoxin B1.

Table 5. Effect of adding different levels of chlorophyll, vitamin A to Laying Hens Diets treated with aflatoxin B1 on Feed Consumption

Treatment	Period 1 24-27 weeks	Period 2 28-31 weeks	Period 3 32-35 weeks	Period 4 36-39 weeks	Mean ± S.E
T1	C D 1.85 ± 104.45	BC 1.41 ± 105.86	AB 0.70 ± 108.07	BC 1.03 ± 106.91	BC 1.23 ± 106.32
T2	AB 0.61 ± 107.89	A 0.31 ± 108.97	A 0.22 ± 108.51	A 0.07 ± 108.75	A 0.27 ± 108.53
T3	ABC 1.12±105.65	AB 0.78 ± 107.05	AB 0.36 ± 107.94	AB 0.65 ± 107.69	ABC 0.66 ±107.08
T4	D 0.68 ± 102.32	BC 0.17 ± 105.37	A 0.39 ± 108.92	C 0.14 ± 105.96	C 0.23 ± 105.64
T5	ABC 0.64±107.00	A 0.52 ± 107.98	AB 0.31 ± 107.86	AB 0.14 ± 108.43	AB 0.36 ± 107.82
T6	A 0.12 ± 108.01	A 0.25 ± 108.94	A 0.10 ± 108.77	AB 0.16 ± 108.04	A 0.12 ± 108.44
T7	ABC 0.64 ±104.88	C 0.41 ± 103.92	B 0.32 ± 106.90	C 0.23 ± 106.17	C 0.25 ± 105.47
***P*-value**	0.01	0.01	0.01	0.01	0.01

T1: Control, T2: adding Aflatoxin B1 200 mg, T3: adding Vitamin A 200 mg + Aflatoxin B1 200 mg, T4: adding chlorophyll extract 200 mg, T5: adding chlorophyll extract 100 mg + Aflatoxin B1 200 mg, T6: adding chlorophyll extract 200 mg + Aflatoxin B1 200 mg, T7: adding chlorophyll extract 300 mg + Aflatoxin B1 200 mg.
*The different letters within the same columns are significant differences at (P<0.05).
**The different letters within the same columns are significant differences at (P<0.01).

Feed Conversion Ratio

Data in table (6) shows effect of adding chlorophyll and vitamin A levels on feed conversion ratio of laying hens. Birds in treatments T2, T4, T6 and T7 recorded best values during first period with a significant difference comparison with control T1 which did not differ significantly with birds in T3. As for second period of the experiment, results showed that birds in T7 recorded best feed conversion ratio with 1.83 gm feed/ gm of egg with a significant difference (P≤0.01) compared to birds of other experimental treatments, and birds of this treatment continue to show moral improvement during third and fourth periods. As for general average, birds in T7 recorded best feed conversion ratio, 1.79 gm feed / gm egg, followed by birds in T4 and T6 which recorded a feed conversion ratio 2.07 and 2.11 gm feed/egg gm respectively , while birds in T2 recorded a feed conversion ratio 2.33 gm feed/gm egg . Feed conversion ratio is an indicator of extent to bird benefits from the components of rations provided. The reason for improvement of feed conversion may be due to rations content of chlorophyll extract, vitamins and amino acids, which leads to stimulating functions of the bird's digestive system by increasing production Digestive enzymes by enhancing liver function of bile storage and concentration (Limantara, 2009). It is no importance in process of digesting feeds, as substances in chlorophyll extract play an important role to improve condition of intestines through its ability to improve content of microorganisms of digestive system, which makes use of feed better by bird as it is for the microorganisms that They live in that area in their natural state, thus creating a balance for micro-organisms within digestive system (Johnes and Ricket, 2003). As these cells absorption part of it and use it in process of manufacturing sugar from non-carbohydrate sources, gluconeogenesis. The exploitation of this extract by the intestinal cells as feed will lead to an increase in number of *microvilli* scattered on the surface of intestinal cells, as well as increasing length of intestinal villi, and this will increase coefficient of absorption of nutrients by those intestinal cells scattered on the inner lining of villi in small intestine (Bantara, 2019). On other hand, the superiority of treatments of adding chlorophyll extract, especially T7, in characteristic of feed conversion ratio may be due to superiority of treatments in mass of eggs produced as a result of vitamins A, E, C and a group B vitamins. It maintains lipoproteins and other fatty compounds that are included in composition of yolk, which leads to maturation of ovarian follicles relatively faster, and this leads to an increase rates of egg production and thus raising level of utilization of fatty compounds in feeds (Bollengier-Lee, 1998). Also, chlorophyll extract has ability to inhibit numbers of harmful bacteria in intestines, which use large amounts of energy of digested materials in intestine, which is reflected to improvement in general health of bird (Antonio *et al.*, 2020).

Table 6. Effect of adding different levels of chlorophyll, vitamin A to Laying Hens Diets treated with aflatoxin B1 on Feed Conversion Ratio (FCR)

Treatment	Period 1 24-27 weeks	Period 2 28-31 weeks	Period 3 32-35 weeks	Period 4 36-39 weeks	Mean ± S.E
T1	A 0.77 ± 3.15	B 0.05 ± 2.15	D 0.05 ± 2.08	B 0.04 ± 2.16	B 0.04 ± 2.19
T2	B 0.00 ± 2.18	A 0.03 ± 2.27	A 0.05 ± 2.38	A 0.05 ± 2.52	A 0.03 ± 2.33
T3	AB 0.03 ± 2.34	AB 0.03 ± 2.23	BC 0.03 ± 2.20	B 0.07 ± 2.23	B 0.03 ± 2.24
T4	B 0.00 ± 2.18	B 0.05 ± 2.13	D 0.05 ± 2.06	C 0.01 ± 1.93	C 0.03 ± 2.07
T5	AB 0.02 ± 2.30	AB 0.03 ± 2.19	B 0.02 ± 2.23	B 0.02 ± 2.14	B 0.02 ± 2.21
T6	B 0.03 ± 2.20	B 0.02 ± 2.12	CD 0.02 ± 2.12	C 0.01 ± 2.01	C 0.01 ± 2.11
T7	B 0.01 ± 1.92	C 0.03 ± 1.83	E 0.01 ± 1.74	D 0.01 ± 1.68	D 0.01 ± 1.79
***P*-value**	**0.05**	**0.01**	**0.01**	**0.01**	**0.01**

T1: Control, T2: adding Aflatoxin B1 200 mg, T3: adding Vitamin A 200 mg + Aflatoxin B1 200 mg, T4: adding chlorophyll extract 200 mg, T5: adding chlorophyll extract 100 mg + Aflatoxin B1 200 mg, T6: adding chlorophyll extract 200 mg + Aflatoxin B1 200 mg, T7: adding chlorophyll extract 300 mg + Aflatoxin B1 200 mg.
***The different letters within the same columns are significant differences at (P<0.05).**
****The different letters within the same columns are significant differences at (P<0.01).**

Conclusions

The negative effects of aflatoxin B1 at 200 mg.kg-1 feed added to laying hens' diets at the beginning of the experiment period did not appear until the third and fourth periods of the experiment. All feed additives in experiment showed excellent efficacy to reducing the negative effects caused by the mycotoxins added to laying hens' diets.

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