

Effect of Varying Levels of Aflatoxin, Ochratoxin and Their Combinations on the Performance and Egg Quality Characteristics in Laying Hens

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ABSTRACT : A 50 day feeding trial was conducted with White Leghorn (WL) laying hens, 42 weeks old, to determine if feeding of varying levels of aflatoxin (AF), ochratoxin A (OA) or their combinations has any effect on their performance and egg quality parameters. Feeding of T₄, T₇, T₈, T₉ and T₁₀ caused significant reduction in feed intake of hens. Hen day egg productions were significantly reduced at all the levels of toxins except 0.5 ppm of AF. Maximum reduction in egg production was noticed at 2 and 4 ppm of AF and OA, respectively. Average body weight and egg weight were not affected by toxin feeding. The feed efficiency in terms of net feed efficiency and feed consumed per dozen egg produced was significantly reduced at higher levels of both the toxins and their combinations. Feed consumption for production of 1 kg egg mass remained uninfluenced due to aflatoxin feeding whereas significant increase in the value of the same was noticed at 4 ppm level of OA and combination of 1 and 2 ppm of AF and 2 and 4 ppm of OA (T₉ and T₁₀), respectively. Various levels of OA (1-4 ppm) and all the combination of two toxins (T₈, T₉ and T₁₀) significantly altered the shape index of eggs in laying hens. The shell thickness was significantly reduced by higher level of AF (2 ppm), OA (2 and 4 ppm) and their combination. Albumen index, Haugh Unit and yolk index remained unchanged due to incorporation of toxins in the diet. It is concluded that AF, OA either singly or in combination at higher levels could depress the performance in terms of egg production and feed efficiency significantly. The egg quality parameters i.e. shape index and shell thickness were also significantly affected. (*Asian-Aust. J. Anim. Sci.* 2003. Vol 16, No. 7 : 1015-1019)

Key Words : Laying Hens, Toxin, Egg Production, Shape Index, Shell Thickness

INTRODUCTION

Aflatoxin at a level of 20 ppm when fed for 7 days resulted in impaired egg production by reducing liver synthesis and transport of yolk precursors (Garlich et al., 1973). Dietary aflatoxin decreased the mean egg weight and delayed the onset in the egg production whereas it had no effect on shell thickness or percent egg as shell (Hamilton and Garlich, 1971). Feeding of 0.5 and 1.0 ppm of aflatoxin to broiler breeder hens did not affect their fertility but the egg production and the hatchability were adversely affected (Howarth and Wyatt, 1976). Aflatoxicosis caused pathological changes in the chicken ovaries which has a detrimental effect on egg production (Hafez et al., 1982). Laying hens fed 1 ppm level of aflatoxin had significantly lower egg production whereas feed efficiency was adversely affected at 2 ppm level of aflatoxin (Iqbal et al., 1983). Feed consumption, body weight, egg weight, shell percentage, Haugh unit scores and serum protein levels were not affected by either toxin levels. There was significant decrease in egg production and egg weight in laying hens fed a diet containing 3,310 µg of AFB1 and 1,680 µg AFB2 per kg for a period of 28 days by third and fourth week, respectively (Wolzak et al., 1985). The transfer of the toxin into meat and eggs is influenced by the toxin

level as well as the period of exposure to toxin (Jacobson and Wiseman, 1974; Lotzsch et al., 1977). Iqbal et al. (1983) found that dietary toxin levels at 1-5 ppm promoted transfer of toxins to the eggs but it could not be measured because of very low levels. Delayed sexual maturity and lower egg production were observed in WL layers fed 1 ppm of OA (Chaudhury et al., 1971). Egg production of hens fed 2 and 4 ppm of OA reduced further with high morbidity and mortality. Prior and Sisodia (1978) reported that there was a significant reduction in egg production and feed consumption when laying hens were fed a diet containing as low as 0.5 mg OA/kg diet. Reduction in egg production was recorded in hens fed 0.5 and 1 ppm of OA for 3 weeks (Page et al., 1980). At a level of 3 ppm OA in laying hens diet, significant reduction in the egg production and increase in shell elasticity was observed (Haazele et al., 1993). The present experiment was conducted with white leghorn layers to study the effect of varying levels of aflatoxin, Ochratoxin and their combinations on the performance and egg quality characteristics.

MATERIALS AND METHODS

Experimental diet

A basal diet containing 18.2% CP and 2,720 kcal/kg ME was formulated (Table 1) based on the recommendations of Bureau of Indian Standards (BIS, 1992). Nine experimental diets were prepared by supplementing various levels of aflatoxin (AF), ochratoxin (OA) and their different

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Table 1. Gross and Chemical composition of the basal diet

| Ingredients | Composition (%) |
|------------------------------|-----------------|
| Maize, ground | 47.00 |
| Soybean meal | 15.00 |
| Fish meal | 11.00 |
| Rice kani | 17.93 |
| Limestone | 6.50 |
| DCP | 1.00 |
| Common salt | 0.30 |
| Mineral mixture ¹ | 1.00 |
| Vitamin mixture ² | 0.15 |
| B-complex | 0.02 |
| Mineral premix ² | 0.10 |
| Total | 100.00 |
| Chemical composition (%) | |
| Crude protein | 18.28 |
| Gross energy, Kcal/g | 3.652 |
| Ca | 3.25 |
| P (avail) | 0.50 |
| Aflatoxin | Traces |
| Ochratoxin | Nil |

Min. Mix²- MnSO₄, 19.5 g; FeSO₄, 18 g; ZnSO₄, 16 g; KI, 30 mg; Maize, 46.47g, Vit.Mix³- AB₂D₃K, 10 g; Maize, 90 g.

Min. Mix¹-Calcium, 30%; Phosphorous, 9.0%; Iron, 0.20%; Copper, 0.05%; KI, 0.01%; Mn, 0.40%; F, 0.05%, AIA, 3.0%.

combinations at the required levels to the basal diet.

Feeding regimen

One hundred twenty white leghorn (WL) hens of 42 weeks old were housed in individual cages and assigned to ten dietary treatment, T₁, control diet without toxins, T₂, 0.5 ppm AF, T₃, 1.0 ppm AF, T₄, 2.0 ppm AF, T₅, 1.0 ppm OA, T₆, 2.0 ppm OA, T₇, 4.0 ppm OA, T₈, 0.5 ppm AF+1.0 ppm OA, T₉, 1.0 ppm AF+2.0 ppm OA, T₁₀, 2.0 ppm AF+4.0 ppm OA. Each treatment consisted of 6 replications (each hen considered as a replicate). Birds were given the experimental diets for 50 days. Diets were presented in mash form and water was provided *ad libitum*.

Parameters of productivity and egg quality

Hen-day egg production was recorded daily. Feed consumption was recorded weekly and total feed consumption was calculated for the entire period for each treatment. The egg weight was recorded to the nearest of 1 g accuracy. The average weight of all the eggs laid in 50 days was taken as the weight of egg of each year. Feed efficiency was calculated as feed consumed per dozen of eggs or feed consumed per kg egg mass. Egg quality measured are shape index, albumen index, haugh unit (HU), yolk index and shell thickness. Net feed efficiency (NFE) was calculated as per the formula used by Reddy (1972). The feed consumed by each bird is utilized both for meat and egg production. Thus an estimate of NFE is required as a measure of feed utilization than feed efficiency ordinarily based on egg mass or body weight alone.

$$NFE = \frac{Fc}{Em + Bw}$$

Where, Fc = Feed consumed

Em = Egg mass produced

Bw = Body weight gain or loss

Statistical Analysis

Data were subjected to analysis of variance (Snedecor and Cochran, 1980) with toxins as main effect. Comparison among means were made by Duncan's multiple range test (Duncan, 1955) and significance was accepted at $p \leq 0.05$.

RESULTS

The results obtained from the feeding trial are presented in Table 2. There was no significant differences among the different treatments in the initial and final body weights of the layers. However, a numerical decline in the final body weight was observed in birds fed 2 ppm AF and 4 ppm of OA. Aflatoxin at a dietary level of 2 ppm caused significant

Table 2. Effect of different levels of aflatoxin, ochratoxin and their combinations on body weight, feed consumption, hen day egg production and egg weight in WL laying hens

| Treatments | Body weight (g) | | Total feed intake (g) | Hen day egg production (%) | Egg weight (g) |
|---|-----------------|---------|-------------------------|----------------------------|----------------|
| | Initial | Final | | | |
| T ₁ (control) | 1,320.8 | 1,473.3 | 4,678.38 ^a | 40.66 ^a | 51.57 |
| T ₂ (AF 0.5 ppm) | 1,371.3 | 1,400.3 | 4,458.36 ^{abc} | 36.83 ^{ab} | 50.88 |
| T ₃ (AF 1.0 ppm) | 1,358.1 | 1,407.1 | 4,632.24 ^{ab} | 30.00 ^{bcd} | 49.57 |
| T ₄ (AF 2.0 ppm) | 1,367.8 | 1,289.5 | 4,311.04 ^{cd} | 28.83 ^{cd} | 51.35 |
| T ₅ (OA 1.0 ppm) | 1,355.0 | 1,381.8 | 4,316.39 ^{cd} | 32.16 ^{de} | 49.27 |
| T ₆ (OA 2.0 ppm) | 1,395.1 | 1,407.0 | 4,129.14 ^d | 28.00 ^{cd} | 48.59 |
| T ₇ (OA 4.0 ppm) | 1,342.0 | 1,214.6 | 4,127.57 ^d | 23.66 ^{de} | 47.54 |
| T ₈ (AF 0.5 ppm+OA 1.0 ppm) | 1,382.0 | 1,331.9 | 4,358.53 ^{bcd} | 29.33 ^{cd} | 49.71 |
| T ₉ (AF 1.0 ppm+OA 2.0 ppm) | 1,389.3 | 1,279.1 | 4,262.12 ^{cd} | 23.16 ^{de} | 50.71 |
| T ₁₀ (AF 2.0 ppm+OA 4.0 ppm) | 1,372.8 | 1,265.1 | 4,196.75 ^d | 20.33 ^e | 49.61 |
| SEM | 66.51 | 75.13 | 122.39 | 2.49 | 1.30 |

Means having a similar superscript column wise differ significantly ($p \leq 0.05$).

Table 3. Effect of varying levels of aflatoxin, ochratoxin and their combinations on feed efficiency parameters in WL laying hens

| Treatments | Net feed efficiency (NFE) | Feed consumed per dozen egg (g) | Feed consumed per kg egg mass (g) |
|---|---------------------------|---------------------------------|-----------------------------------|
| T ₁ (control) | 2.120 ^c | 1377.74 ^l | 2241.48 ^c |
| T ₂ (AF 0.5 ppm) | 2.351 ^c | 1455.23 ^{ef} | 2383.14 ^c |
| T ₃ (AF 1.0 ppm) | 3.086 ^b | 1877.83 ^c | 3145.69 ^{bc} |
| T ₄ (AF 2.0 ppm) | 3.175 ^b | 1801.19 ^c | 2970.05 ^{bc} |
| T ₅ (OA 1.0 ppm) | 2.663 ^{bc} | 1606.14 ^{de} | 2700.05 ^{bc} |
| T ₆ (OA 2.0 ppm) | 3.019 ^b | 1763.06 ^{cd} | 3113.06 ^b |
| T ₇ (OA 4.0 ppm) | 4.269 ^a | 2067.06 ^b | 3622.41 ^{bc} |
| T ₈ (AF 0.5 ppm+OA 1.0 ppm) | 3.089 ^b | 1770.18 ^{cd} | 3003.21 ^{bc} |
| T ₉ (AF 1.0 ppm+OA 2.0 ppm) | 4.126 ^a | 2210.66 ^b | 3657.50 ^c |
| T ₁₀ (AF 2.0 ppm+OA 4.0 ppm) | 4.617 ^a | 2465.79 ^a | 4676.02 ^a |
| SEM | 0.204 | 63.89 | 328.3 |

Means having a similar superscript column wise do not differ significantly ($p \leq 0.05$).

Table 4. Effect of different levels of aflatoxin, ochratoxin A and their combinations on the egg quality parameters of WL layers

| Treatments | Shape index | Albumen index | Yolk index | Haugh unit | Shell thickness (mm) |
|---|----------------------|---------------|------------|------------|-----------------------|
| T ₁ (control) | 0.734 ^a | 0.0981 | 0.3731 | 83.821 | 0.2774 ^{ab} |
| T ₂ (AF 0.5 ppm) | 0.725 ^{ab} | 0.0981 | 0.3739 | 85.403 | 0.2826 ^{ab} |
| T ₃ (AF 1.0 ppm) | 0.715 ^{abc} | 0.0937 | 0.3757 | 79.977 | 0.2987 ^a |
| T ₄ (AF 2.0 ppm) | 0.703 ^{bc} | 0.0969 | 0.3919 | 84.077 | 0.2531 ^{bc} |
| T ₅ (OA 1.0 ppm) | 0.697 ^c | 0.1032 | 0.3864 | 86.255 | 0.2988 ^a |
| T ₆ (OA 2.0 ppm) | 0.716 ^{abc} | 0.0900 | 0.3898 | 82.154 | 0.2687 ^{bc} |
| T ₇ (OA 4.0 ppm) | 0.700 ^{bc} | 0.0910 | 0.3922 | 83.844 | 0.2455 ^c |
| T ₈ (AF 0.5 ppm+OA 1.0 ppm) | 0.693 ^c | 0.0851 | 0.3961 | 82.806 | 0.2835 ^{ab} |
| T ₉ (AF 1.0 ppm+OA 2.0 ppm) | 0.700 ^c | 0.0868 | 0.3960 | 80.951 | 0.2637 ^{bc} |
| T ₁₀ (AF 2.0 ppm+OA 4.0 ppm) | 0.698 ^c | 0.0895 | 0.3987 | 82.486 | 0.2714 ^{abc} |
| SEM | 0.007 | 0.0053 | 0.012 | 1.969 | 0.0096 |

Means possessing different superscripts column wise differ significantly ($p \leq 0.05$).

reduction in feed consumption whereas similar observations were made at all the three levels (1, 2 and 4 ppm) of OA. The feed intake of hens fed T₈, T₉ and T₁₀ differed significantly from that recorded on control group. However, the values of feed consumption did not differ amongst hens fed all the three levels of OA and the combinations of both toxins.

Hen day egg production was significantly reduced in birds fed 1 and 2 ppm of AF and all the three levels of OA and combination of toxins. However, maximum reduction in egg production was observed in layers fed 4 ppm of OA and combination of 1 ppm of AF and 2 ppm of OA and 2 ppm of AF and 4 ppm of OA compared to the egg production of the control hens. Either of the toxins or their combinations did not influence significantly the egg weight of the layers. Ochratoxin at highest level (4 ppm) produced numerically less egg weight compared to those recorded on all other groups.

The results on the net feed efficiency (NFE), feed consumed per dozen eggs and feed consumed per kg egg mass are presented in Table 3. NFE was significantly influenced by the varying levels of dietary AF, OA and their combinations. Significantly poorer feed efficiency values were recorded at a level of 4 ppm of OA and combination of 1 and 2 ppm of AF and OA, respectively. However, the

adverse effect of toxin was pronounced at combination of highest level of both the toxins (2 ppm AF and 4 ppm OA).

Dietary AF, OA and their combination resulted in a significant increase in the feed consumption for the production of a dozen egg. Birds fed combination of 2 ppm AF and 4 ppm OA consumed significantly more feed to produce 1 dozen of egg. Feed intake increased significantly in birds fed all the three levels of toxins except 0.5 ppm of AF. Feed consumed per kg egg mass significantly increased in birds fed 4 ppm of OA, combination of 1 ppm AF and 2 ppm OA (T₉) and 2 ppm AF and 4 ppm OA (T₁₀). Hens fed combination of highest levels of both the toxins (2 ppm AF and 4 ppm OA) consumed maximum feed to produce a kg of egg.

Results on the egg quality parameters are shown in Table 4. A significant reduction in shape index was noticed in hens fed 2 ppm of AF. Ochratoxins at all the 3 levels caused significant reduction in shape index. Further, Significant decrease in the shape index value was recorded at all the three levels of co-toxicity. Albumen index and HU are the tools to evaluate albumen quality of the eggs. Results of this study indicated no significant difference in the albumen index and HU among the different treatment groups. However, the combined toxicity due to AF and OA resulted in to numerical decrease in the albumen index

indicating deterioration of the albumen quality. Maximum HU value was recorded in the eggs laid by hens fed 1 ppm of AF. There was no significant differences in the yolk index values of eggs belonging to hens fed varying levels of toxins either singly or in combinations. Shell thickness of eggs was significantly reduced in hens fed OA at highest level (4 ppm) which was significantly different from those fed all levels of AF except 1 ppm level.

DISCUSSION

Dietary aflatoxin, ochratoxin and their combinations did not influence the initial and final body weight of laying hens. In agreement with the findings of present study, Iqbal et al. (1983) reported no appreciable change in the body weight of layers after feeding of 1-5 ppm of AF for 3 periods of 28 days each. Contrary to the present finding, loss in body weight of layers during aflatoxicosis was noticed by Sims et al. (1970). Ochratoxin at 1, 2 and 4 ppm level did not cause significant change in body weight of birds where as marked depression in body weight was reported by Chaudhury et al. (1971). However, low doses of ochratoxin (0.5, 1 and 2 ppm) did not influence the body weight (Chaudhury et al., 1971; Page et al., 1980) which supported the result of present study. Birds fed AF at a level of 2 ppm, ochratoxins at all the three levels and combination of the two toxins at all the 3 levels caused significant reduction in feed intake. In contrast, no change in feed intake has been reported with increasing levels of AF (1, 2, 3, 4 and 5 ppm) by Iqbal et al. (1983), Wolzak et al. (1985) and Garlich et al. (1973). Ochratoxins at levels of 0.5, 1, 2, and 4 mg/kg diet did not influence significantly the feed consumption of birds (Chaudhury et al., 1971; Page et al., 1980). Significant reduction in egg production was recorded in birds fed 1 and 2 ppm dietary level of AF in this study. In agreement with this, impairment of egg production in hens was observed due to feeding of 2 ppm of AF for 7 days (Garlich et al., 1973). A reduction in egg production by 50 percent was observed in hens fed 5 ppm AF (Iqbal et al., 1983). In accordance to the result of present study, a significant reduction in egg production was recorded in laying hens fed diet containing as low as 0.5 mg of OA/kg feed over a period of 6 weeks. Similarly Chaudhury et al. (1971) observed a significant reduction in egg production at a level of 4 ppm of OA. The pronounced effect of simultaneous feeding of the two toxins on the egg production in the present study might be attributed to the synergistic effect of the AF and OA. Feeding of either toxins or their combinations did not affect the egg weight of hens. In contrast, Garlich et al. (1973) and Huff et al. (1975) observed reduction in egg weights due to feeding of AF contaminated diets (20 ppm). These workers proposed that the production of smaller eggs by AF fed hens might be a

mechanism by which the hen compensates for decrease in plasma lipids and proteins. Hens fed a diet containing 3,310 µg of AFB₁ and 1,680 µg of AFB₂ per kg feed produced significantly smaller eggs during 4th week of feeding (Wolzak et al., 1985). In consistence with the results of this study, Iqbal et al. (1983) reported no change in egg weight in hens fed 1, 2, 3, 4 or 5 ppm AF. In the present study 1, 2 or 4 ppm of OA did not influence the egg weight significantly. Conversely, a decrease in egg weight was recorded in the birds fed diet having 1 and 2 ppm of OA for a period of 6 week (Prior and Sisodia, 1978).

Net feed efficiency (NFE) was significantly influenced due to varying levels of AF, OA and their combinations. AF at a level of 1 and 2 ppm resulted in significant reduction in NFE. Hens fed OA at a level of 2 and 4 ppm had significantly poor NFE compared to the control hens. Severe adverse effect of toxins on NFE was recorded in hens fed combination of two toxins (T₉ and T₁₀) at different levels. Feeding of toxins either singly or in combinations significantly influenced the feed consumed per dozen egg. Significant reduction in feed efficiency of laying hens was recorded at 1 and 2 ppm level of AF. Ochratoxin A significantly increased the feed consumption of hens for production of 1 dozen eggs at all the three levels (1, 2 and 4 ppm). At 4 ppm of OA the feed consumed to produce 1 dozen egg was 2,067 g which was significantly higher than values recorded at 1 and 2 ppm level. The feed consumption for production of 1 dozen egg recorded in T₁₀ group was maximum and significantly higher than those recorded in hens belonging to all other groups. Hens fed OA at a level of 4 ppm consumed significantly higher feed to produce 1 kg egg mass compared to those recorded in hens fed other levels of AF and OA. However, hens fed combination of AF and OA (T₉ and T₁₀) had significantly higher feed consumption for production of 1 kg egg mass. The feed consumption to produce 1 kg egg mass recorded on T₁₀ group was maximum and was significantly different from all other groups. In accordance with the result of present study, Iqbal et al. (1983) reported adverse effect of increasing doses of AF (1, 2, 3, 4 and 5 ppm) on the feed efficiency of laying hens in 3 periods of 28 days each. Similarly, a significant decrease in feed efficiency (feed consumed per dozen egg produced) was reported in hens fed higher levels of OA (Chaudhury et al., 1971). The effect of combined toxicity on the feed efficiency was maximum which might be due to the synergistic effect of toxins on the feed efficiency.

Results of present study indicated that aflatoxin at a level of 2 ppm, ochratoxin at a level of 1 and 4 ppm and combination of toxins at all the levels caused significant reduction in the shape index of eggs. Further, OA at highest level (4 ppm) resulted in maximum reduction of shell thickness. This decreased shell strength due to feeding of

AF, OA or their combinations might be due to the poor calcium and phosphorous absorption and interference in vitamin D₃ metabolism resulting in poor shell quality at higher level of toxins. Likewise, Huff et al. (1977) reported that aflatoxicosis caused poor availability of calcium and phosphorous. Further, it is reported that aflatoxin interferes with absorption of vitamin D₃ (Britton and Wyatt, 1978, 1979). Contrary to this, Washburn and Wyatt et al. (1978) could not find any detrimental change in the shell strength at a level of 5 ppm AF. Result of this study indicated no significant differences among the various treatments containing different toxin levels either singly or in combinations on the yolk index and HU of eggs. In accordance to this finding, Iqbal et al. (1983) did not find any effect of varying levels of AF (1, 2, 3, 4 and 5 ppm) on the egg quality parameters.

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