



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2026): 4.61

VET 2026; 11(5): 06-11

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www.veterinarypaper.com

Received: 05-02-2026

Accepted: 08-03-2026

Egidioh K Waititu

Department of Biochemistry and
Molecular Biology, Egerton
University, Kenya

Charles Mwendia

Department of Biochemistry and
Molecular Biology, Egerton
University, Kenya

Meshack A Obonyo

¹ Department of Biochemistry
and Molecular Biology, Egerton
University, Kenya

¹ Department of Biochemistry
and Molecular Biology, Egerton
University, Kenya, ²Inter-
University Council for East
Africa, Uganda

Effects of vitamin A, C, and E supplementation on growth performance of broilers fed on aflatoxin- contaminated feeds

Egidioh K Waititu, Charles Mwendia and Meshack A Obonyo

DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i5a.3234>

Abstract

Aflatoxins in poultry food adversely impacts broiler growth and health. This research assessed the role of vitamins (A, C, and E) in broilers fed aflatoxin-contaminated food. The chicks aged 72 days were subjected to six treatments, a negative control, a positive control (140 ppb aflatoxin), and four treatments with increasing vitamin supplementation. The average daily feed intake (ADFi), average daily gain (ADG) and feed conversion ratio (FCR) were measured and compared by one-way ANOVA with Tukey test ($p < 0.05$). Mortality was observed. There was no significant effect on ADFi ($P > 0.05$), which means it had no effect on the feed intake. Nevertheless, there were significant ($p < 0.05$) increases in ADG and FCR when vitamin levels were increased, and the highest (ADG: 32.25 g/day; FCR: 2.89) was achieved at the highest inclusions. There was no observed mortality. In general, vitamin A, C and E improved growth performance in a dose-dependent way.

Keywords: Aflatoxin, broilers, vitamin A, vitamin C, vitamin E, growth performance

1. Introduction

Aflatoxin (AF) is a potent mycotoxin produced mainly by the fungal species *Aspergillus flavus* and *Aspergillus parasiticus* [1]. These fungi can contaminate feed leading to aflatoxin buildup, a significant challenge for poultry farmers. In broilers chickens, ingestion of aflatoxin-contaminated feeds may result in aflatoxicosis, characterized by decreased productivity, poor growth, low feed intake, and mortality [2, 4]. One of manifestation of aflatoxicosis in broilers is deterioration of growth performance such as average daily gain (ADG), average daily intake (ADFi) and feed conversion ratio (FCR) [5, 6]. While feed intake may remain unaffected in some instances feed conversion is often compromised resulting in poor weight gain and efficient conversion ratio [7]. This underline the need for effective nutritional strategies to mitigate the adverse effect of aflatoxins in broilers diets [8]. Recent studies have suggested that vitamins can play an important role in aflatoxin detoxification [8, 9]. Some Vitamins act as important antioxidants that can help to alleviate the adverse effects of aflatoxins [10, 11]. These antioxidant vitamins function by neutralizing free radicals produced during detoxification of aflatoxins, thus preventing cell damage [12]. Several studies have demonstrated that Vitamins A, C, and E, act as important superoxide anion scavengers in the body [13, 15]. For instance, the supplementation of feed with vitamin A have been reported to ameliorate aflatoxin toxicity in poultry. In a 42-day study conducted in Zambia, Chibanga *et al.* (2014) reported that different levels of vitamin A supplementation in broiler chickens fed diets contaminated with aflatoxin B₁ (AFB₁) reduced aflatoxin toxicity. This resulted in improved growth performance, enhanced feed efficiency, improved liver function, and reduced oxidative damage associated with aflatoxin exposure. Furthermore, the supplementation of Vitamin E alongside selenium demonstrated an increased overall body and carcass weight, as well as internal organ weight, in broilers fed on aflatoxin-contaminated diet [16, 17]. In another study by Oloruntola *et al.* (2024), Vitamin C supplementation in broilers feed with aflatoxin B₁ contamination also demonstrated improved growth performance, carcass traits, serum

Corresponding Author:

Egidioh K Waititu

Department of Biochemistry and
Molecular Biology, Egerton
University, Kenya

biochemical indices, meat antioxidant status, and hematological parameters. Despite existing knowledge on the protective roles of these vitamins, there is limited information on their combined effects at different inclusion levels on growth performance of broilers fed aflatoxin-contaminated diets. Therefore, the present study was designed to evaluate the effects of dietary supplementation with vitamins A, C, and E at varying levels on average daily feed intake, average daily gain, feed conversion ratio in broiler chickens fed aflatoxin-contaminated diets.

2. Materials and Methods

2.1 Study area

Study was approved by National Commission for science, Technology and Innovation (NACOSTI/P/24/32444).

This *in vivo* study was conducted in the Department of Animal Science at Egerton University. The University is

located in the Njoro Sub-County, Nakuru County at coordinates 0° 23' S and 35° 55' N. The area is located 1800 meters above sea level and it receives an average rainfall of 900 to 1,140 mm per year. The region temperature ranges between 17 °C to 22 °C daily (Egerton University Department of Agriculture Engineering Metrological station, 2018). The

2.2 Study design and feeding

The study was conducted using a Completely Randomized Design (CRD) consisting of six treatments. Each treatment had three replicates, with four birds per replicate. Each replicate served as an experimental unit. The experimental feed rations were formulated as shown in Table 1 and were provided to the experimental units for twenty-one days. Fresh water was supplied to the bird's *ad libitum* throughout the experimental period.

Table 1: Experimental diet composition for broiler treatments

Component	Negative Control	Positive Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Basal Feed	Basal feed	Basal feed	Basal feed	Basal feed	Basal feed	Basal feed
Aflatoxin (ppb)	-	140 ppb	140 ppb	140 ppb	140 ppb	140 ppb
Vitamin A (IU/kg)	-	-	1000	1400	3000	4000
Vitamin E (mg/kg)	-	-	10	20	30	40
Vitamin C (mg/kg)	-	-	100	140	300	400

2.3 Experimental aflatoxin contaminated feed preparation

Clean (aflatoxin-free) feed samples were moistened using distilled water and inoculated with *A. flavus*, a laboratory strain isolated from maize samples collected earlier from Eastern Kenya [18]. The moist feeds were then incubated at 28 °C for 60 days [19]. Periodic moistening of the feeds was done to enable uninhibited growth of fungi and aflatoxin production. Afterward, the level of total AFs in the feeds was determined using the ELISA technique following the manufacturer's instructions where 52 kg of 470 ppb aflatoxin contaminated feed was obtained. This was used to contaminate a total of 175 kg of non-supplemented feed needed for treatments. The addition resulted in a final feed aflatoxin concentration of approximately 140 ppb. A control feed (basal feed), free of aflatoxins was also provided as the negative control. The chicks were also provided with water *ad libitum*.

2.4 Experimental Chicks and management

Seventy-two (72) unsexed, one day-old Arbo Acre broiler chicks that had been vaccinated against Gumboro and Newcastle were purchased from KENCHIC, a commercial hatchery in Kenya and transported to the Department of Animal Science, Egerton University. At the University, chicks were weighed and their initial weight recorded, brooded for 21 days, then randomly assigned to the six treatments with three replications, each consisting of four broilers for another 21 days. Artificial brooding was done using infrared bulbs, adjusted to 32 °C during the first week, and later reduced by 2 °C per week as described earlier [20]. A broiler starter ration was provided *ad libitum* to the one-day-old birds for up to three weeks.

2.5 Data collection after feeding with aflatoxin contaminated feeds

Feed intake and chick weight were computed weekly for each bird and weight gain was calculated as the weight difference of two consecutive weights. Daily feed intake was computed as the differences between feed offered and feed remaining before the next feeding as described [21]. Additionally, data on

mortality, stool consistency, and behavior towards food was taken daily.

2.6 Statistical analysis

All experimental data were compiled and summarized using Microsoft Excel computer software. Mortality was assessed descriptively while feed intake, weight gain and feed conversion ratio were expressed as means. Differences between group were computed using the one-way Analysis of Variance (ANOVA) under completely randomized design (CRD) at probability $P \leq 0.05$. Post-hoc analysis to determine the difference between the means of groups was done using the Tukey HSD method of multiple pairwise-comparison test. All the data was analyzed using the R statistical software.

3. Results

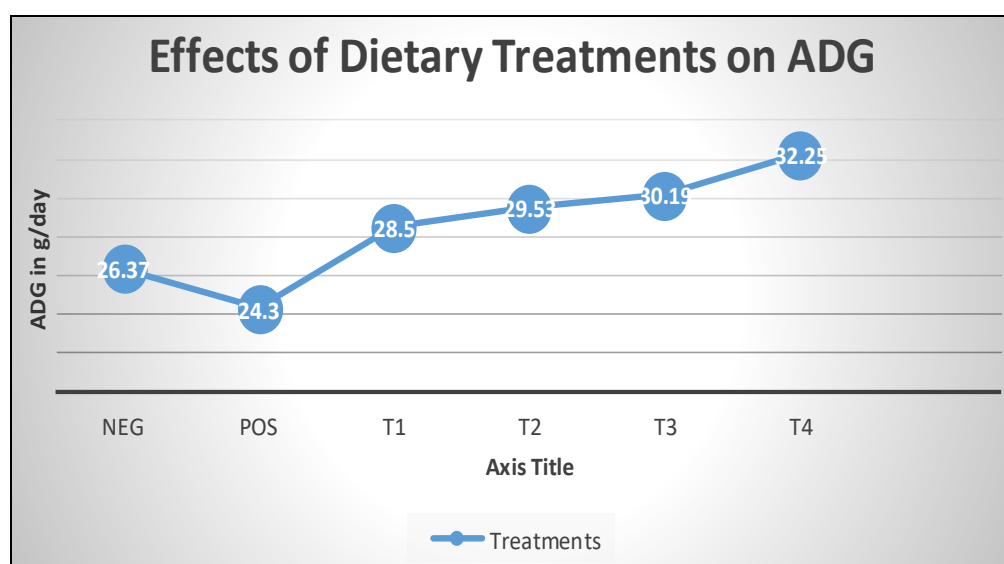
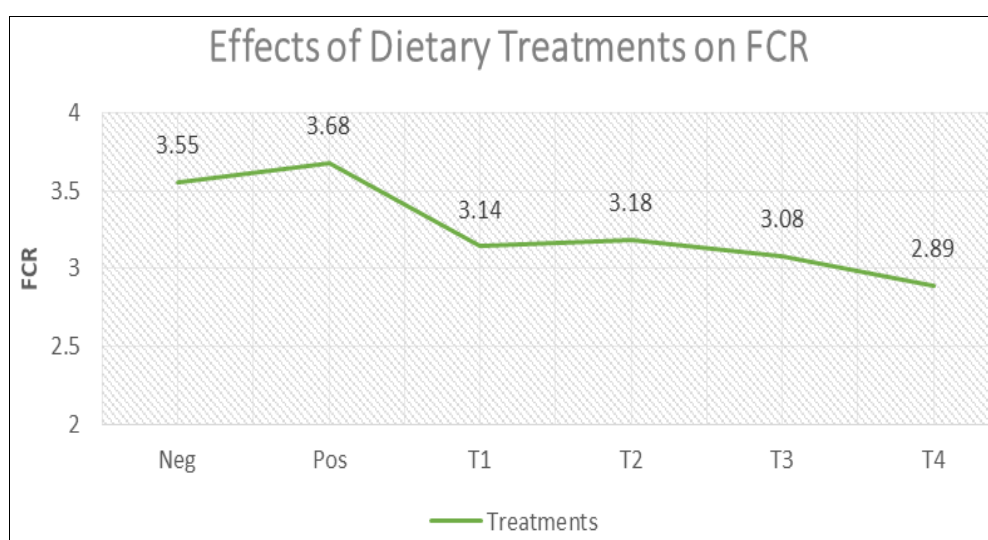
3.1 Effects of vitamins A, C, and E on the average daily gain (ADG), Average Daily feed intake (ADFi), Feed conversion ratio (FCR) and Mortality of Broilers

The effects of dietary treatments on growth performance of broilers are presented in Table 2 and figure 1 and 2, The average daily feed intake ADFI among the treatment groups, ranged from 89.29 to 93.26 g/day. There was no significant difference ($p > 0.05$) in ADFi. However, average daily gain (ADG) was significantly influenced by dietary treatments. Birds in T₄ recorded the highest ADG (32.25 g/day), which was significantly ($p < 0.05$) higher than those in the negative and positive control groups (26.37 and 24.30 g/day, respectively), but not significantly different from T₁, T₂, and T₃. Similarly, feed conversion ratio (FCR) differed significantly among treatments ($p < 0.05$). The lowest (best) FCR was observed in T₄ (2.89), followed by T₃ (3.08), T₁ (3.14), and T₂ (3.18), while the highest values were recorded in the control groups (3.55-3.68). Overall, supplementation with increasing levels of vitamins A, C, and E improved growth performance, as evidenced by higher ADG and improved FCR, with T₄ showing the most pronounced effect. Despite exposure to aflatoxins and varying levels of vitamin supplementation, the birds maintained normal feeding behavior and survived the entire study duration.

Table 2: Effects of supplementation with different levels of vitamins A, C, and E in aflatoxin-contaminated diets on average daily gain (ADG) and feed conversion ratio (FCR)

Dietary Treatment	Parameter		
	ADFi(g/day)	ADG(g/day)	FCR
Negative	93.26	26.37 ^b	3.55
Positive	89.29	24.30 ^b	3.68
T ₁	89.29	28.50 ^{ab}	3.14
T ₂	93.26	29.53 ^{ab}	3.18
T ₃	91.27	30.19 ^{ab}	3.08
T ₄	93.26	32.25 ^a	2.89
<i>p</i> <0.05	0.17 NS	0.0023*	0.0094*

*Different treatments with vitamins have a statistically significant effect on the average daily gain (ADG) and feed conversion ratio (FCR) of chicken fed with aflatoxin-contaminated feed by one-way ANOVA test. The ^{abc}-superscript Means within a column with different superscript letters differ significantly ($p \leq 0.05$) by Tukey's method. ADG; Average daily weight gain.

**Fig 1:** Effects of dietary treatments on average daily gain (ADG) in broilers**Fig 2:** Effect of dietary treatments on feed conversion ratio (FCR) in broilers

4. Discussion

The current study evaluated the effects of supplementation of vitamins A, C, and E in broilers fed aflatoxin-contaminated diets on growth performance and mortality rate. The findings demonstrated average daily feed intake (ADFi) was not significantly affected by the treatments. Both average daily weight gain (ADG) and feed conversion ratio (FCR) were significantly improved with increasing levels of vitamins supplementation. Dietary supplementation of vitamins A, C and E, in the current study, did not significantly alter average

daily feed intake and this implies that dietary inclusion of these vitamins in aflatoxin contaminated diets did not have any negative or positive impact on the feed intake. There was a relative stability in the ADFi values of the different treatment groups (89.29-93.26 g/day). Lack of significant differences in ADFi among treatments suggested that 140ppb aflatoxin did not impair feed appetite. This finding is consistent with the results of the past studies that have reported that low-to-moderate concentrations of aflatoxins do not necessarily reduce feed intake [22, 23]. The fact that the feed

intake was maintained across treatments also suggests that the gains in the ADG and the (FCR) were not brought about by a higher feed intake but rather gain in efficiency in the use of nutrients. Feed conversion ratio is essentially a measure of the efficiency of animals to convert ingested feed into body mass; therefore, an increase in FCR at a given feed intake indicates an increase in metabolic efficiency and nutrient allocation to growth, as opposed to maintenance^[24]. The effect of growth depression in aflatoxin-exposed broiler chicken has been observed in a study whereby depressed growth is in most cases accompanied by diminished or no difference in feed intake meaning that impaired performance is not only as a result of poor appetite but also as a result of poor nutrient utilization and metabolic imbalance^[25].

Average daily gain (ADG) showed some positive changes in birds fed with vitamin A, C and E supplements ($P<0.05$). Birds in T₄ (32.25 g/day) performed the best in gaining weight as compared to the positive and negative control groups (24.30 g/day and 26.37 g/day respectively). T₄ had a better performance but its ADG was not statistically different with T₁, T₂, and T₃ (Table 2 and Figure 1) but numerically different, indicating a dose-dependent reaction to the inclusion of vitamins^[26]. The same trends have been documented in a study involving broilers where graded doses of vitamins A, C and E had a step-by-step positive effect on ADG and feed efficiency^[27]. These vitamins are known for their antioxidant activity, which overcome oxidative stress that is caused by aflatoxins^[8]. Aflatoxin is reported to inhibit the protein production, cause hepatotoxicity, and cause oxidative damage, which all lead to slower growth in poultry^[28, 29]. The enhanced ADG of supplemented groups can hence be explained by the protective properties of these vitamins on cellular integrity and metabolic activities. This findings concur with^[30], who found that vitamins E and C showed synergistic antioxidative protection with improved performance of broilers when supplemented together. According to^[31], addition of both vitamins E and C, in aflatoxin-challenged broilers, showed a significant benefit in the improvement of ADG and FCR with the performance of the supplemented groups nearly matching that of the negative control, indicating a strong protective and growth-promoting effect of the two antioxidants. A systematic review by^[32] found that the use of feed additives, especially antioxidant vitamins, has a significant positive effect on reducing the adverse impacts of aflatoxins on growth performance.

Feed conversion ratio (FCR) is a critical indicator of production efficiency in broiler chickens, reflecting the ability of birds to convert feed into body mass^[33]. In the present study, dietary supplementation with vitamins A, C, and E significantly improved FCR ($P<0.05$) (Table 2 and Figure 2). Birds in treatment T₄ recording the lowest (best) FCR value (2.89), compared to higher values observed in the control groups (Negative 3.55 and Positive 3.68). Improved FCR in vitamin-supplemented groups suggests enhanced digestion, absorption, and utilization of nutrients despite the presence of aflatoxins^[34]. This observation is strongly supported by a study of^[35] who demonstrated the ability of vitamins and other dietary antioxidants to counteract the negative effects of aflatoxins on nutrient efficiency and growth performance. This findings could be attributed by vitamin A in maintaining epithelial integrity, vitamin E protecting cell membranes, and vitamin C enhancing immunity and reducing stress^[36-38]. These findings are consistent with previous studies that have reported improvements in feed efficiency following antioxidant vitamin supplementation in poultry diets exposed

to aflatoxins^[39, 41]. Together, these vitamins likely mitigated the deleterious effects of aflatoxins, thereby improving digestive efficiency and nutrient absorption, which ultimately translated into better FCR (Table 2 and Figure 2). FCR from T₁ to T₄ indicates a dose-dependent response to vitamin supplementation, with higher inclusion levels offering greater protective and performance-enhancing effects. According to^[31], broilers fed aflatoxin-contaminated feedstuffs supplemented with escalating amounts of vitamins C and E had significant increases in FCR, and concurrent high inclusion levels yielded performance similar to the control group. This is a clear indication that there is a dose-responsive relationship that had an increase in vitamin inclusion with an increase in nutrient utilization and growth performance.

The toxicity of aflatoxin contamination of poultry food has also been linked to an increased mortality rate and behavioral alterations related to feeding caused by the toxicity of this toxin to internal organs and metabolism^[42,43]. However, in the current study, there was no mortality noted in any of the treatments and the birds were able to feed normally throughout the experimental period. This is an indication that the concentration level of aflatoxin (140 ppb) was sub-hazardous and probably tolerable by broiler chicken under the circumstances in this study. Previous studies have reported 100 percent mortality in broilers at aflatoxin concentrations of 1000 ppb, whereas levels up to 200 ppb have been described as tolerable depending on exposure duration and experimental conditions^[44, 45]. The lack of mortality in the current study could hence be accredited to moderate level of toxin and controlled experimental conditions. However, additional research on the concentrations of aflatoxins (more than 200 ppb) and durations of exposure (more than 21 days) are required so as to establish the cut-offs at which aflatoxin starts to have a significant influence on the mortality and feeding-related behavior of broiler chickens.

5. Conclusion

Vitamin A, C, and E supplementation improved growth performance (ADG and FCR) in broilers fed aflatoxin-contaminated diets without affecting feed intake or mortality. The benefits appeared dose-dependent, likely due to the vitamins' antioxidant effects in reducing aflatoxin-related stress. The aflatoxin level used (140 ppb) was subclinical, impacting growth efficiency but not survival. Overall, combined vitamin supplementation is an effective strategy to mitigate moderate aflatoxin effects, though further research at higher toxin levels is recommended.

Availability of data and materials

Datasets used and analyzed during this study are available from the corresponding author on reasonable request.

Authors' contribution

EKW: Conceptualization of the idea, laboratory examination, collected data, analyzed and interpreted the data, drafted the original manuscript. CM and

MAO: Conceptualization of the idea, laboratory examination, study design, drafting the original manuscript, reviewing of the manuscript, and supervision. All authors reviewed and approved the final manuscript.

Acknowledgements

We thank the Department of Animal Science and CoELIB at Egerton University for support with brooding and treatments. We appreciate the Department of Biochemistry and Molecular

Biology (Mycotoxin Laboratory) for providing *Aspergillus flavus*, conducting ELISA analysis, and offering logistical support. We acknowledge the National Research Fund for financial support, Bahati Level Four Hospital Laboratory for biochemical analyses, Chiromo Histology Laboratory for tissue processing, and the Department of Human Anatomy Histology Laboratory at Egerton University for histopathological analysis.

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Waititu EK, Mwendia C, Obonyo MA. Effects of vitamin A, C, and E supplementation on growth performance of broilers fed on aflatoxin-contaminated feeds. *International Journal of Veterinary Sciences and Animal Husbandry*. 2026; 11(5): 06-11.