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Growth Performance of Kuroiler (Rhode Island Red x White Leghorn) Chickens Fed Aflatoxin Contaminated Maize-Based Diets Treated with Different Amounts of Lemon (*Limon Citrus*) Juice



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ABSTRACT

Aflatoxin contamination in feed and feed ingredients is prevalent worldwide and poses serious risks to both livestock and humans. This study assessed the effects of feeding Aflatoxin (AF) contaminated maize-based diets treated with varying levels of lemon juice to Kuroiler chickens' growth performance. Lemon juice was extracted from fruits purchased from the local market and mixed with isocaloric and isonitrogenic diets (starter, grower and finisher) containing AF-contaminated maize, at 0, 250, 500 and 750ml lemon juice/Kg of feed. The diets were left to dry for 24 hours, after which 100g samples were collected from each diet to measure and compare the residual AF levels. Then, 100 day-old Kuroiler chicks were sourced from a local hatchery and divided into four treatment groups (n = 25). Each group was replicated four times. The treatment groups were randomly assigned to 0, 250, 500, and 750ml lemon juice/Kg feed-treated diets. These feeds and clean drinking water were supplied to the

birds *ad libitum* for 49 days. Feed intake was monitored daily, and body weights, weight gains, and feed conversion ratios were assessed weekly. It was observed that the amount of residual AF in each diet depended on the amount of lemon juice used to treat it. The untreated diet had the highest AF levels at 36 ppb, followed by that which was treated with 250, 500, and 750 ml lemon juice/Kg feed at 28.1, 21.3, and 7.4 ppb, respectively. Growth performance variables assessed were also influenced by the amounts of residual AF levels in the diet. Birds fed diets with the lowest residual AF levels at 7.4 ppb had the best ($p < 0.05$) growth performance in all the variables, while the least ($p < 0.05$) was observed in birds fed untreated diet, followed by those that were fed diets treated with 250 and 500ml Lemon juice/kg feed. It was concluded that lemon juice was able to degrade AF in diets that had maize contaminated with AF, but the extent to which it inactivated it, depended on the amount of juiced used. Similarly, growth performance of birds fed diets with AF-contaminated maize treated with lemon

juice was also dependent on the levels of residual AF in feeds after treatment, with the best performance observed in chickens fed diets treated with 750ml lemon juice per kg feed.

Keywords: Aflatoxin contaminated maize-based diet, Kuroiler chickens, improved village chickens.

INTRODUCTION

In Zambia, like in many other parts of the world, Maize and its milling by-products are the major and important ingredients in poultry diets. Maize forms the bulk (40-60%) of the compounded feed supplied to the birds. However, in Zambia, most of the locally supplied maize is often contaminated with mycotoxins (Kachapulula, et al., 2017). This is due to a lack of capacity in terms of good agricultural practices related to the production, harvesting and post-harvest handling of the crop, which promotes the proliferation of moulds responsible for the production of mycotoxins. Among the mycotoxins of the greatest concern are Aflatoxins (AF), which are secondary metabolites produced by various *Aspergillus* species, including *A. flavus*, *A. parasiticus* and *A. nominus* that invade maize plants during their growth and remain in the grains even after harvest (Manafi et al., 2011; Chibanga et al., 2014).

From the chemistry point of view, Aflatoxins are difuranocoumarin compounds that include AFB₁, AFB₂, AFG₁, AFG₂, AFM₁ and AFM₂, depending on their molecular structures. Among these, AFB₁ is the most commonly encountered and the most toxic and carcinogenic mycotoxin in both humans and livestock (Yunus et al., 2011; Chibanga et al., 2014).

The greatest economic losses in poultry associated with the consumption of AF are not the occasional flock mortalities but rather, the decreased production performance resulting from reduced feed intake and weight gains (Lee et al., 2012; Chen et al., 2014), poor feed utilisation efficiency (Chen et al., 2014), lower egg production, hatchability levels (Lee et al., 2012; Khan et al., 2014), and increased vulnerability of the birds to diseases (Hoerr, 2010; Chen et al., 2014; Chen, 2016). Additionally, residues of AF in food animals raised on diets contaminated with AF can appear in the meat and eggs (Sager et al., 2014), and consumption of AF-contaminated food products causes acute necrosis of the liver and hyperplasia of the bile duct, which reduces digestibility of fats and proteins (Lee et al., 2012; Yunus et al., 2013; Chen et al., 2014; Khan et al., 2014). This, therefore, raises a serious public health concern and prompts scientists to urgently identify measures that can eliminate, prevent, or reduce AF levels in poultry diets, especially those measures that can easily be adopted by the local farmers.

A number of chemical products that can convert AF into a less harmful compound with less mutagenic effects in animals have been studied and identified (Méndez-Albores et al., 2007; Safara et al., 2010). These are mainly acids, bases, oxidising agents, bisulfites and gases. However, most of these compounds are beyond the reach of most resource-poor small-scale farmers involved in small-scale poultry farming due to their high cost and need for the use of sophisticated equipment. Additionally, they are also unsafe for them because these compounds produce toxic residues during the treatment process, which may compromise the nutritional,

sensory and functional properties of the treated product (Méndez-Albores et al., 2007). Therefore, there is a need to identify a cheaper, safer to handle, and easy-to-use chemical compound that resource-poor small-scale farmers can use to detoxify AF-contaminated poultry feed ingredients.

One such compound with such attributes is citric acid. This compound, often used as a preservative, pH controller, and flavouring agent, as well as an antioxidant in many food products, has been observed to have not only the ability to inactivate AF in contacted feeds and ameliorate its chronic AF-toxicity effects in animals (Méndez-Albores et al., 2007), but also improve growth performance in chickens (Nourmohammadi et al., 2012).

However, commercially prepared citric acid is not readily available in Zambia, especially in the rural areas where small-scale poultry production occurs. Moreover, most resource-poor small-scale farmers may not have the financial ability to afford it.

Based on this understanding, it was hypothesised that lemon (*Limon citrus*) juice, which contains citric acid (Pham et al., 2020; Marta Klimek-Szczykutowicz et al., 2020), maybe a cheaper and readily available resource to use for inactivation of AF in contaminated feeds and ameliorate its harmful effects in chickens. However, scientific evidence to support this assertion is currently unavailable in the literature. Thus, this study aimed at assessing the effects of treating AF-contaminated poultry feeds with varying levels of lemon juice on residual AF levels in the treated diets, and also evaluate the response of Kuroiler chickens in terms of growth performance when they are fed on such feeds.

MATERIALS AND METHODS

Lemon fruits were sourced from a local fruit/vegetable market in Petauke District in the Eastern Province of Zambia and washed with clean running tap water before extracting the juice. The juice was extracted using a domestic kitchen fruit juice extractor and stored in a fridge till the commencement of the study.

Maize contaminated with Aflatoxin (AF) was also obtained from a local maize storage shed in the same district. It was ground to pass through a 3mm sieve and used to compound three isonitrogenic and isocaloric diets (i.e., starter, grower, and finisher diets) according to the Zambian feeding standards for broilers. A 100g sample from each diet was taken to analyse AF levels before treating the diets with lemon juice. Then, each diet was divided into four portions and treated by mixing each with 0, 250, 500, and 750mls of lemon juice per Kg of feed in a plastic container and left to dry under a shade for at least 24 hours. Thereafter, 100g samples were taken again from each treated portion to analyse residual AF levels.

Then, 100-day old Kuroiler chicks were purchased from a local hatchery within the district and randomly divided into four treatment groups. These groups were randomly assigned to 0, 250, 500, or 750-ml lemon juice per Kg feed-treated diets. Each treatment group was subdivided into four subgroups as replicates ($n \geq 6$). Treatment diets, as well as fresh, clean drinking water, were offered daily to birds in each treatment *ad libitum* until 56 days of age of the birds. All routine management tasks, such as brooding and vaccination, were the same for all treatment groups and

carried out simultaneously. Feed intake was monitored daily by first weighing the feed residues from the previous 24 hours feeding before putting in fresh weighed feed. Body weights were taken at 0, 7, 14, 21, 28, 35, 42, 49 and 56 days of age of the birds. Weight gains were calculated weekly as the difference between the weight of birds at one weighing age and the next. Feed conversion ratios were also calculated weekly by dividing the total feed consumed in a week (g of feed) by the total weight gained during the same week (g of weight gained).

All data collected was analyzed by the general linear model (GLM) procedure of SAS (2002), with lemon juice used to treat the ration as a factor based on the mathematical model;

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where: Y_{ij} is the observation; μ is the overall mean, T_i is the fixed effect of Lemon juice ($i = 0, 250, 500$ and 750ml), and ε_{ij} is the random error'

Means were compared using the Tukey test, and differences among means with $p < 0.05$ were accepted as representing statistical differences.

RESULTS AND DISCUSSION

Before treating the feeds with lemon juice (0 ml lemon juice per Kg feed), AF concentration in the diets was 36 ppb (Table 2). This value was higher than the 20 ppb deemed safe by FAO (2004). This observation was not without precedent because results from surveys conducted by Kachapulula et al., (2017) in the same district where maize used in this study was sourced also found higher AF contamination than the safe levels

set by FAO (2004). They attributed this to maize crop infection by one or more species of aflatoxin-producing fungi that disperse from soil, organic matter and alternative hosts to developing crops when conditions are hot and dry during crop development and warm and humid after crop maturation and/or harvest.

However, when the diets were treated with lemon juice at 250, 500 and 750ml lemon juice per Kg feed, AF levels decreased to 28.1, 21.3 and 7.4 ppb, respectively, from 36 ppb in untreated diets. This observation was in agreement with Méndez-Albores et al., (2005) and Méndez-Albores et al., (2007), who also observed that citric acid changed the molecular structure of AFB₁ that is usually detected in maize, by opening its Lactone ring to form a β -keto acid structure that, under acidic conditions, is hydrolysed to AFD₁ molecule. Thus, the citric acid in the lemon juice used in this study also altered the molecular structure of most of the AFB₁ in untreated diets. It was postulated that the detected AF was likely the AFB₁, as this is the most commonly found mycotoxin in maize (Yunus et al., 2013; Chibanga et al., 2014).

Treating the diets with lemon juice had a significant effect on the growth of the chickens (Table 3). Growth performance of the chickens fed on treated diets (250, 500, and 750ml lemon juice per Kg feed) was significantly better than those fed untreated diets (0ml lemon juice per Kg feed). Total feed intakes in birds fed 250, 500, and 750-ml lemon juice per Kg feed-treated diets were 3869.9, 4615.3 and 5370.9g per bird, respectively, which was 22.4, 46.0 and 69.8% higher than in birds that consumed untreated diets. These

observations were in agreement with the reports of Chibanga et al., (2014), Yunus et al., (2013), Daneshyar et al., (2014), and Alharthi et al., (2022), who also observed that Aflatoxicosis negatively affected feed intake and weight gains, which negatively impacted on body weights attained by the birds at the end of the rearing period. According to Pasha et al., (2007) Aflatoxicosis suppresses the appetite of the birds. Therefore, the low feed intakes observed in chickens that were fed untreated diets were attributed to suppressed appetite caused by high AF levels in their diets.

An increase in feed intake in birds fed treated diets also improved ($p < 0.05$) their weight gains and final body weights attained at 56 days of age. Chickens fed on treated diets with 250, 500 and 750ml lemon juice per Kg feed had gained 1164.1, 1484.9 and 2074.3g per bird, respectively, by the end of the feeding trial. The improvements in weight gain were 19.4, 52.3 and 112.2% compared to chickens that received the untreated diets. These improvements had a significant effect on the final body weights attained at 56 days of age. Birds that received untreated diets were the lightest ($p < 0.05$) at 1013.9g per bird, followed by those that were fed diets treated with 250, 500 and 750ml lemon juice per Kg feed at 1203.0, 1523.7 and 2112.9g, respectively. For feed conversion ratios, however, birds that consumed diets treated with 750ml of lemon juice per Kg of feed had a significantly better ratio of 2.6, compared to 3.2, 3.3 and 31, for chickens that received, respectively, diets treated with 0, 250 and 500ml lemon juice per Kg feed.

There is now a general consensus among

livestock nutritionists (Saleemi et al., 2020; Mogadam and Azizpour, 2011; Magnoli et al., 2011; Yunus et al., 2011; Bryden, 2012; Peng et al., 2014; Peng et al., 2017) that consumption of AF contaminated feeds causes Aflatoxicosis, and this disease has deleterious effects on growth performance in poultry. According to Fouad et al., (2019) the productive and reproductive performance of the birds can deleteriously be affected by even low concentration levels of AF in the feed. This, therefore, calls for measures that can either eliminate, minimise or ameliorate the negative effects of this undesired condition in birds. Our approach was to ameliorate the negative effects of AF in Kuroiler chickens by treating their diets with naturally occurring citric acid found in citrus fruits such as lemons. Extracted lemon juice was simply mixed with diets formulated using maize contaminated with AF.

Treating AF-contaminated maize-based diets with lemon juice had significant effects on feed intake, weight gained, and final body weights attained by the chickens at the end of the feeding trial. These variables were positively affected in four ways. Firstly, the lemon juice, due to the presence of citric acid in it, was able to reduce the AF in the diet from 36ppb in untreated diets to 28.1, 21.3 and 7.4 ppb in the 250, 500, 750 ml lemon juice per Kg feed-treated diets, respectively. This reduction in residual AF levels increasingly stimulated the birds' appetite, as explained by Pasha et al.. (2007). Secondly, the presence of citric acid in the juice reduced the pH of digesta in the crop and gizzard after ingesting treated diets. Atapattu and Nelligswatta (2005) observed that the nerve endings

that control appetite in birds are located in their crops. Therefore, the presence of citric acid in the juice and/or low pH environment created in these parts of the digestive system was able to stimulate their appetite, increasing their feed intakes. Thirdly, the low pH in the gizzard provided a better environment for digestion and higher uptake of nutrients from the gastrointestinal tract. This was evidenced by the significantly higher weight gains, better feed conversion ratios, and higher body weights attained at the end of the trial period. Improvement in these parameters indicated the availability of extra nutrients owing to acidification of the diet by the citric acid in the lemon juice. Finally, it was postulated that the lemon juice caused a shift in microbial ecology, favouring beneficial microbes that increased retention and absorption of nutrients activating the homeostatic mechanisms resulting in increased body weight gain in the birds offered organic acids in the diet as observed by Shivani et al., (2023).

CONCLUSION

This study has shown that lemon juice was able to deactivate AF in diets that contained maize contaminated with AF. However, the extent to which it inactivated AF depended on the amount of lemon juice applied to the feed. The more lemon juice was mixed with the AF-contaminated maize-based feeds, the more AF was deactivated. Similarly, the growth performance of birds fed diets with AF-contaminated maize treated with lemon juice was also dependent on the levels of residual AF in feed after treatment, with the best performance observed in chickens that were fed diets

treated with 750ml lemon juice per Kg feed.

CONFLICT OF INTEREST

The authors certify that there is no conflict of interest with any financial organisation regarding the material discussed in this manuscript.

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Table 1: Ingredient Composition (Kg) of Aflatoxin Contaminated Maize-based Diets Treated with 0, 250, 500, and 750 ml of Lemon (Lemon Citrus) Juice Per Kg of Fed, Offered to Kuroiler Chickens

Feed Ingredient	Diets		
	Starter	Grower	Finisher
AF contaminated Maize meal	53.3	63.6	68.5
Soybean meal (solvent extract)	40.5	30.9	26.5
Soybean oil	2.9	2.4	2.1
Dicalcium phosphate	0.23	0.24	0.26
Limestone flour	2.04	1.95	1.93
Lysine	0.1	0.12	0.10
DL-Methionine	0.12	0.1	0.07
Broiler premix	0.42	0.4	0.3
Salt (Sodium chloride)	0.3	0.3	0.3
Total	100	100	100
Calculated analysis			
Crude protein (%)	22.1	19.7	18.2
Metabolisabe energy (Kcal/Kg)	2869.8	2999.6	3099.8

Table 2: Residual Aflatoxin Concentration in Diets Treated with Varying Amounts of Lemon (Lemon Citrus) Juice

Treatment/Amount (ml Lemon juice per Kg feed)	Residual Aflatoxin Level (ppb)
0	36.0
250	28.1
500	21.3
750	7.4

Table 3: Growth Performance of Kuroiler Chickens Fed Aflatoxins Contaminated Maize-based Diets Treated with 0, 250, 500 and 750 ml Lemon (Limon citrus) Juice Per Kg Feed

Variable	0	250	500	750
Initial body weights (g/b)	38.7 ± 1.7	38.9 ± 1.4	38.8 ± 1.8	38.6 ± 1.9
Final body weight (g/b)	1013.9 ± 70.1 ^a	1203 ± 71.3 ^b	1523.7 ± 258.8 ^c	2112.9 ± 346.7 ^d
Total weight gained (g/b)	975.2 ± 70.4 ^a	1164.1 ± 0.9 ^b	1484.9 ± 148.9 ^c	2074.3 ± 307.3 ^d
Daily weight gain (g/b/d)	17.4 ± 1.4 ^a	20.8 ± 1.4 ^b	26.5 ± 5.3 ^c	37.0 ± 7.1 ^d
Total feed intake (g/b)	3162.3 ± 210.3 ^a	3869.9 ± 226.7 ^b	4615.3 ± 294.7 ^c	5370.9 ± 334.8 ^d
Daily feed intake (g/b/d)	64.5 ± 13 ^a	78.9 ± 36.2 ^b	94.2 ± 59.8 ^c	109.6 ± 6 7.7 ^d
Feed conversion ratio	3.2 ± 1.2 ^a	3.3 ± 1.2 ^a	3.1 ± 1.4 ^a	2.6 ± 1.1 ^b

Values are Means ± STD (n = 25)

^{a-d} Means within a row with different superscripts differ significantly ($p < 0.05$)