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The Effect of Fermented Aflatoxins Contaminated Feed on Digestibility and Performance of Broiler Chickens

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Abstract. Broiler chicken farming is a major sector of the poultry industry. Poultry is susceptible to mycotoxicoses caused by aflatoxins. The experiment was carried out, where 144 DOCs were allocated to six diets. The diets were diet1 (no aflatoxin and not fermented), diet2 (no aflatoxin and fermented without yeast), diet3 (no aflatoxin and fermented with yeast), diet4 (contained aflatoxin and not fermented), diet5 (contained aflatoxin and fermented without yeast) and diet6 (contained aflatoxin fermented with yeast). The aflatoxin level was 30.08 ppb. Each diet was assigned to 6 chicks, replicated 4 times for 21 days. Leftovers and mortalities were recorded daily and chicks were weighed on a weekly basis. Feed consumption and body weight gain were not different across treatments. However, gain to feed ratio was significantly (p=0.048) better in broilers fed diets fermented naturally. The mortality rate was 75.0% in chicks fed on a non-fermented aflatoxin diet. Therefore, natural fermentation is the best method of improving the quality of aflatoxin contaminated feed for broilers.

Keywords: aflatoxin, broiler chicken, fermentation, mycotoxins, poultry

Abstrak. Peternakan ayam broiler adalah sektor utama industri perunggasan. Unggas rentan terhadap mikotoksikosis yang disebabkan oleh aflatoksin. Percobaan dilakukan menggunakan 144 ayam broiler berumur satu hari yang diberi pakan enam jenis pakan. Pakan tersebut adalah P1 (tanpa aflatoksin dan tidak difermentasi), P2 (tanpa aflatoksin dan difermentasi tanpa ragi), P3 (tanpa aflatoksin dan difermentasi dengan ragi), P4 (mengandung aflatoksin dan tidak difermentasi), P5 (mengandung aflatoksin dan difermentasi tanpa ragi) dan P6 (mengandung aflatoksin yang difermentasi dengan ragi). Tingkat aflatoksin yang digunakan adalah 30,08 ppb. Setiap jenis pakan diberikan untuk 6 ekor anak ayam dan diulang 4 kali selama 21 hari. Sisa pakan dan kematian dicatat setiap hari dan anak-anak ayam ditimbang setiap minggu. Tidak ada perbedaan yang ditemukan dalam asupan pakan dan pertambahan bobot badan. Namun demikian rasio pertambahan terhadap rasio pakan menunjukkan perbedaan secara nyata (p = 0,048) lebih baik dibandingkan dengan ayam broiler yang diberi pakan fermentasi secara alami. Tingkat kematian adalah 75,0 % pada anak ayam yang diberi pakan aflatoksin non-fermentasi. Oleh karena itu, fermentasi alami adalah metode terbaik untuk meningkatkan kualitas pakan yang terkontaminasi aflatoksin untuk ayam pedaging.

Kata kunci: aflatoksin, ayam broiler, fermentasi, mikotoksin, unggas

Introduction

The consumption of poultry meat keeps growing across the globe in both first and third world countries (Kralik et al*.,* 2018). Broiler chicken farming is a major sector of the poultry industry. Additionally, chicken meat is a high biological value protein (Da Silva et al*.,* 2017) and a nutritious diet since it contains moderate energy (Marangoni et al., 2015). Having a lower fat content and higher PUFA than other meats, poultry meat is the recommended item in a balanced diet. (Riovanto et al*.,* 2012). The quality of feed may be compromized when it is exposed to any contamination (bacterial, fungal, and mold) during storage; consequently, the feed is spoilt and the poultry is more likely to get infected (Sugiharto and Ranjitkar, 2018). For example, broilers carrying aflatoxin B1 could make the economy and public health suffer tremendously (Yunus et al*.,* 2011). The negative effect of aflatoxin to birds is most significant in production aspects, such as weight gain, feed consumption, feed conversion ratio (FCR) and harvest (Hussain et al*.,* 2010). Aflatoxins were discovered in 1960 and are widely associated with maize, groundnuts, tree nuts, figs, dates and oil seeds, such as cotton seeds (Kanyi, 2018; Negash, 2018). Aflatoxin B1, B2, G1 and G2 are the most prevalent toxins to interfere with metabolism of carbohydrates, fats and nucleic acids in livestock (Negash, 2018). In maize, the most vigorous aflatoxin (Aflatoxin B1) is produced from an abundant amount of *A. flavus* and *parasiticus* (Zaki et al*.,* 2012).

According to many regulatory bodies on aflatoxins, the tolerable levels of total aflatoxins in foodstuffs and animal feeds are different. The World Health Organization (WHO) set aflatoxins limits of 5ppb for animals (Kajuna et al., 2013), while the United States Food and Drug Administration and European Commission set 20ppb as the maximum tolerable level of total aflatoxins for poultry feed (Syahidah et al*.,* 2017). The concentrations of AFB1 in poultry meat and the edible parts may be elevated irrespective of whether the aflatoxin levels in the diet is low (22ppb) or high (2500ppb) (Fouad et al*.,* 2019). Fermenting the feed could lower the non-nutritive substance, increase the overall nutritive value of the feed (Aljuobori et al*.,* 2014; Çabuk et al., 2018) and reduce total aflatoxin levels (Mukandungutse et al*.,* 2019). Additionally, digestive tract (crop and gizzard) may contain less Campylobacter and Salmonella due to consuming fermented feed. (Jawad et al*.,* 2016). Sugiharto et al. (2015) reported that grain fraction (not complete feed), which is fermented prior to mixing with compound feed may help avoid losing some essential nutrients in fermented feeds. The use of fermented liquid feed appears to be a cost-effective alternative to the use of antibiotics growth promoters (Missotten et al., 2013). This study evaluated the effect of fermented contaminated feed with aflatoxins on the digestibility and performance of broiler chickens.

Materials and Methods

Source of aflatoxin

Clean (tested, aflatoxin-free) maize kernels were inoculated with *Aspergillus flavus* obtained from contaminated samples of maize. The incubation of maize (31°C, 60 days) was conducted while retaining the moisture intermittently to allow fungi to grow well and aflatoxin to produce. ELSA technique was performed to obtain AF level following manufacturer's instructions.

Fermentation

After inoculation, the contaminated maize and clean maize was milled using a sieve of 0.8mm into flour from which the water was added to the maize flour in the ratio of 1:1.5w/v (weight of maize flour/volume of water). The fermentation was done either without or by adding *Saccharomyces cerevisiae* (NCYC 125) at room temperature (25°C) for 72hours, then sun dried. After drying, fermented maize flour was used to compound six experimental diets.

Experimental diets, animals, design and treatments

A total of 144 DOC (broilers) were purchased from a commercial breeding place (Kenchic). All vaccination procedures against Gumboro and New Castle diseases were carried out by the hatchery before supply. The chicks were then put in a room which was well ventilated and fitted with fluorescent lighting. It was cleaned with liquid soap and disinfected before occupation by the chicks. The chicks were weighed individually before feeding. During brooding, the room was warmed to 30-34°C using infrared bulbs and there was continuous lighting. In the poultry house, 24 partitions, with 1.2 x 1.2m each, were made. Each diet was assigned to 6 broiler chickens of similar body weights and replicated four times. Chickens were offered the six diets for 21 days. The six experimental diets were offered in clean disinfected feeders daily at 09:00hrs. The leftover feed was collected, weighed, recorded and fresh feed provided before the next feeding. The broilers were given clean, ad libitum drinking water. The diet composition was based on NRC standard (1994) for starter broilers (23% crude protein and about 3200Kcal/kg metabolizable energy). The experimental design was a "three by two factorial" arrangement where factor one was the type of fermentation (not fermented, fermented without yeast and fermented with yeast) and factor two was aflatoxin (0 and 30.08ppb). The six experimental diets are shown on Table 1. Every morning at 09:00hrs, the leftover feed was collected, weighed, and recorded before providing fresh feed. Daily feed consumption was calculated as total feed offered minus total leftover. The chicks were weighed once every week before feeding. Body weight gain (BWG) and average daily gain (ADG) of each chicken were calculated. BWG is the score after subtracting final weight by the initial weight, while ADG is BWG divided by seven days. Gain to Feed ratio (the total feed needed to gain one unit of weight) was obtained by dividing (ADG) by (ADFI).

All data were analyzed using an analysis of variance (ANOVA) in the General linear model (GLM) of Statistical Analysis Systems (SAS, 2009) software. An F-test at 5% probability level was employed to test for significance and means separation was done by Least Significant Difference Procedure

Table 1. Composition of experimental diets

Ingredients (g/100g)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Maize	69	69	69	69	69	69
Soy Bean Meal	6	6	6	6	6	6
Omena[1]	23	23	23	23	23	23
Dicalcium-Phosphate	1	1	1	1	1	1
Limestone	0.25	0.25	0.25	0.25	0.25	0.25
Iodized salt	0.25	0.25	0.25	0.25	0.25	0.25
Mineral and Vitamin Premix[2]	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100
Calculated ME (Kcal/kg)	3076	3076	3076	3076	3076	3076
Calculated CP (%)	22.6	22.6	22.6	22.6	22.6	22.6
Calculated AF in diet (ppb)	0	0	0	30.08	30.08	30.08

[1] Scientific name: Rastrineobola Argentea, common name; silver cyprinid, and it has also been called the Lake Victoria sardine or mukene.

[2] Supplied the following per Kg of diet: Vit. A = 10,000 IU; Vit. D = 2,800 IU; Vit. E = 24mg; Vit. K = 2mg; Vit. B1 = 1mg; Vit. B2 = 4mg; Vit. B6 = 4mg; Vit. B12 =16mg, Vit. C = 200mg; Niacin = 10mg; Folic acid =1.6mg; Biotin= 60mg; Mn = 80mg; Fe = 25mg; Cu = 5mg; Zn = 50mg; Se=200mg and KI=1.2mg.

Diet 1 (no aflatoxin and not fermented), Diet 2 (no aflatoxin and fermented without yeast), Diet 3 (no aflatoxin and fermented with yeast), Diet 4 (contained aflatoxin and not fermented), Diet 5 (contained aflatoxin and fermented without yeast) and Diet 6 (contained aflatoxin fermented with yeast).

Results and Discussion

The feed intake of the non-contaminated and contaminated diets was not improved by fermentation. Fermentation without yeast and fermentation with yeast had similar effects on feed intake whether diet is contaminated or not. The fermentation types did not significantly affect the feed consumption and body weight gain. The gain to feed ratio of chicken had been affected by fermentation types with *p*-

value=0.048. The total aflatoxin levels had no significant effect on the chickens' performance.

Table 2. The effect of fermentation and aflatoxins on feed intake (g/d), body weight gain (g/d) and gain: feed ratio of broiler chickens

	Fermentation types				
Nutrients	Not fermented	Fermented without yeast	Fermented with yeast	*SEM*	*p*-value
Intake	21.6	22.4	22.9	2.879	0.647
Body weight gain	10.8	13.5	9.8	3.209	0.083
Gain to feed ratio	0.51^{ab}	0.60^{a}	0.43^{b}	0.131	0.048
Total aflatoxin levels					
Nutrients	0ppb	30.08ppb	*SEM*	*p*-value	
Intake	22.4	22.2	2.87	0.849	
Body weight gain	11.7	11.1	3.516	0.706	
Gain to feed ratio	0.51	0.51	0.148	0.913	

$^{a, b}$ means in the same row with different superscripts are significantly different (*p*< 0.05)

Table 3. Dietary effect on mortality rate of broiler chickens at 21 days

Diet	Number of chicks per diet	Number of dead chicks	Mortality rate (%)
Diet 1	24	1	4.2
Diet 2	24	3	12.5
Diet 3	24	4	16.7
Diet 4	24	18	75.0
Diet 5	24	7	29.2
Diet 6	24	14	58.3

Diet 1: No aflatoxin and not fermented, Diet 2: No aflatoxin and fermented without yeast, Diet 3: No aflatoxin and fermented with yeast, Diet 4: Contained 30.08ppb aflatoxin and not fermented, Diet 5: Contained 30.08ppb aflatoxin and fermented without yeast, Diet 6: Contained 30.08ppb aflatoxin fermented with yeast.

The feed intake, body weight gain and gain to feed ratio were not significantly (*p*>0.05) different within the six diets (Table2). The types of fermentation did not improve feed consumption and body weight gain during 21-days of study, but improved the gain to feed ratio (Table2). The study done by Naji et al. (2016) indicated that fermented feed with probiotics was economically beneficial since it improved broiler feed conversion ratio. Broiler chickens fed on fermented moist feed showed a detrimental effect on early bird growth but affected beneficially feed efficiency (Missotten et al*.,* 2013). Pre-arranged probiotics for wet fermented feed may cause a significant improvement in chicken feed conversion ratio (Jawad et al*.,* 2016). The total aflatoxin level of 30.08ppb did not significantly affect intake, body weight gain and feed conversion ratio (Table2). Many studies reported decreased feed consumption and body weight gain as well as an increased feed conversion ratio when higher levels of aflatoxin were used in the feed (Table 4).

The mortality rate was 75.0% (Table 3) in the non-fermented feed with 30.08ppb total aflatoxin levels which was greater than the United States Food and Drug Administration (USFDA) and European Union Commission (EUC) maximum tolerable limit of 20ppb (Morrison et al*.,* 2017). Sobrane et al*.* (2016) observed the 20.13%±9.45 mortality rate of broilers fed contaminated feed with 2000ppb aflatoxin B1. During this study, some of the chicks fed on aflatoxins-contaminated feed were not able to stand on their feet.

Table 4. Summary of the effects of aflatoxin levels in broiler feed and their effects on body weight, feed intake and feed conversion ratio

Aflatoxin levels in the feed	Effects on body weight gain, feed intake and FCR	References
700, 1700 and 2800ppb AFs	Lowered body weight, depressed feed intake, increased FCR	(Marchioro et al., 2013)
1500ppb AFB1	Impaired growth	(Chen et al., 2016)
1000ppb AFB1	Lowered growth rate	(Ali Rajput et al., 2017)
200ppb and 400ppb AFs	Reduced body weight, daily weight gain, feed intake and increased FCR	(Valchev et al., 2017)

Conclusions

Fermentation with or without yeast did not affect feed intake and body weight gain but improved gain to feed ratio of broiler chickens. The mortality rate was high (75.0%) in non-fermented feed containing 30.08ppb aflatoxin.

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