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The effect of natural products in binding aflatoxins in chicken feed on growth of broilers

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Abstract
This experiment evaluated the effects of a commercial binder ((Novasil®) and two natural products with the potential to bind Aflatoxin B1 (AFB1) on the growth performance of broilers. There were eight dietary treatments; treatment 1 (control), treatment 2 (control + AFB1), treatment 3 (control + (Novasil®)), treatment 4 (control + (Novasil®) +AFB1), treatment 5 (control + RHA), treatment 6 (control + RHA+ AFB1), treatment 7 (control + Banana peels) and treatment 8 (control + Banana peels + AFB1). The two natural binders were banana peels and rice husk ash (RHA). Four treatments (1, 3, 5 and 7) were formulated without aflatoxins while the other four (2, 4, 6 and 8) contained AFB1. All dietary treatments were formulated to meet the National Research Council (NRC, 1994) requirements for broiler grower and finisher feeds. The AFB1 concentration in the feeds was 100 ppb. One hundred and forty four unsexed day-old Arbo Acre day-old broiler chicks were randomly assigned to the eight treatments with three replicates each consisting of six chickens. The experimental period was forty two days. During the first 21 days, broilers were fed a grower feed and from 22-42 days, a finisher feed. The experimental design was a CRD with a 2 by 4 factorial arrangement. Feed intake, feed efficiency and chicks' weight were calculated on weekly basis for each bird. Data were analyzed using the general linear model (GLM) procedure of Statistical Analysis System software (SAS, 2009). The results showed that diets containing AFB1 did not significantly affect all the performance parameters. Feed efficiency of diet with rice husk ash was significantly ($p<0.05$) higher than the commercial binder ((Novasil®) ((Novasil®)®)) and banana peels during the finisher phase. The overall feed efficiency for the whole period (0-42 days) however, showed that RHA and banana peels had feed efficiency that was significantly higher ($p<0.05$) than the commercial binder. In conclusion, the natural binders had a significant improvement on feed efficiency compared to the commercial binder implying that they are superior in binding capacity to the commercial binder.

Keywords: Aflatoxin B1, broilers, commercial binder, natural products

Introduction
Aflatoxins (AFs) contamination in food and feeds contributes to food insecurity [12]. Aflatoxins are produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* [12]. Of the four AFs naturally produced, only AFB1 is the most common in feeds and it causes oxidative stress, immunosuppression, decreased feed intake, and growth rate in poultry [13, 30]. Tropical and subtropical regions are characterized by warm and humid conditions which favors the growth of aflatoxins hence the reason for the high incidence of AFs reported in these regions [12]. Additionally, inferior quality grains are more often directed to the formulation of poultry feeds which predisposes them to fungal contamination especially if the grains are damaged [17]. Some of the negative effects of aflatoxins in chicken include; immunosuppression, low body body weight, unexpected mortality, and poor feed efficiency [27].
There have been safety concerns about aflatoxins residues in the meat, eggs and dairy products which are caused by frequent contamination of the animal feed ingredients with mycotoxins. Carryover of the toxins from the feedstuffs into animal tissues or/and biological fluids results in these residues in food products which is an end-user problem [27]. Moreover, the International Agency for Research on Cancer (IARC) classified aflatoxins as group one carcinogens [5].

The increased knowledge and awareness about potential of health hazards due to aflatoxins to both man and farm animals has pushed the producers, researchers and government institutions to look for more technologies which can reduce aflatoxins content in feeds and food [23]. Conventional strategies for reducing AFs that have been used includes; irradiation, bio-degradation and physical separation; adsorption/biosorption, filtration and extraction [24]. The most economical and feasible of these is adsorption which employs the use of inorganic clay minerals (mostly bentonites and zeolites) and organic materials (activated charcoals, processed plant fibers, yeast cell walls and polymeric organic compounds) which are an effective way of reducing AFs in poultry carcasses [24, 4].

Mycotoxin binders reduce AFs by decreasing their availability and absorption while synthetic polymers, vitamins and enzymes converts AFs to non-toxic metabolites [13]. Bentonites have been tested in many animal species including chicken, turkey poults, ducklings, pigs, lambs, mink, trout, tilapia fish, dairy cows and goats for its potential in binding mycotoxins [6]. Novasil, a commercial bentonites has shown promising results in reducing aflatoxin M1 (AFM1) and AFB1 concentration [6]. Agricultural by-products e.g. grape pomace, artichoke waste, almond hulls, plantain peels and grape seeds can be utilized as potential AFB1 biosorbents [10]. This strategy is simple, economic and ecofriendly. The efficacy of these biosorbents is attributed to the presence of soluble fibre contents, lignin, flavonoids and their high ability to bind water and oil [10]. They also have Omega-3 fatty acids and dietary phenols bioactive compounds which have a potential to bind mycotoxins [14]. Plantain peel, almond hull, carobos, grape seeds and Malvasia grape pomace were found to bind AFB1 at 67%, 87%, 100%, 83% and 94% respectively [10]. Biosorbents usually contains many functional groups (hydroxyl, carbonyl, sulfhydrl, phenolic, esters etc.) which help to eliminate mycotoxins using different modes of action including; hydrogen bonding, physiochemical interactions, and secondary bond forces [24]. An effective biosorbent should have a high binding efficacy at all pH ranges in the gastrointestinal tract to avoid dissociation of the bound aflatoxins [10]. Some binders can limit the bioavailability of amino acids and minerals [32]. Despite the fact that binders are good for combating aflatoxins problem, commercial binders are expensive which restrain their use by farmers. Therefore, there is a need to identify locally available materials with binding capacity which will help reduce economic burden and importation of commercial binders [27]. This experiment tested the efficacy of locally available materials in binding AFB1 in broiler feed and the performance of broilers.

Materials and Methods

Source of aflatoxins

Clean (tested, aflatoxin-free) maize grain were inoculated with *Aspergillus flavus* obtained from contaminated samples. The clean maize was incubated for 60 days at 31 °C as described by [19]. The level of AFB1 was determined by analyzing samples using the HPLC as per the (AOAC, 2008) guidelines. The concentration of AFB1 in the contaminated diets was 100ppb.

Experimental birds, Design, Management and Diets

This experiment was approved by Egerton University Research Ethic committee (Approval No. EUREC/APP/110/2021) and NACOSTI (License No. NACOSTI/P/20/6919). One hundred and forty four unsexed day-old Arbo Acre broiler chicks which had been vaccinated against Gumboro and Newcastle were purchased from KENCHIC, a commercial hatchery in Kenya. At Egerton University the chicks were weighed and their initial weight recorded, then randomly assigned to the eight treatments with 3 replications each consisting of six broilers. The experimental design was a CRD with 2 by 4 factorial arrangement, whereby factor one was aflatoxins level (0, 100 ppb) and the second one was binder (no binder, binder 1, binder 2 and commercial binder). The experimental period was forty two days. Artificial brooding was done using infrared bulbs, adjusted to 32 °C during the first week and later reduced by 2 °C per week [13]. Feed offered was *ad-libitum* and freshwater was given to the chicks at all times. The grower and finisher diets (Table 1) were formulated to meet or exceed the NRC (1994) requirements for Poultry. Eight dietary treatments were formulated to test binding capacity of the two natural binders (banana peels and rice husk ash (RHA). The dietary treatments included; T1 (control), treatment 2 (control + AFB1), treatment 3 (control + Novasil®), treatment 4 (control + Novasil® +AFB1), treatment 5 (control + RHA), treatment 6 (control + RHA+ AFB1), treatment 7 (control + Banana peels) and treatment 8 (control + Banana peels + AFB1). The basal diet was compounded using the locally available feed ingredients which were screened for AFB1 before using for feed formulation. All diets were formulated to contain approximately 22.03% CP and 3064 Kcal ME per kilogram for the grower phase while the finisher phase contained 18% CP and 3209 Kcal ME per kilogram.

Table 1: Composition of the experimental diets for the broiler chicks during the grower and finisher phase (g/100g)

Ingredient g/100g	Grower phase								Finisher phase							
	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Trt 7	Trt 8	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Trt 7	Trt 8
Maize grain	59.8	59.8	59.8	59.8	59.8	59.8	59.8	59.8	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6
Corn oil	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	1.96	1.96	1.96	1.96	1.96	1.96	1.96	1.96
Soya bean meal	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6
Omena1	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
Lysine	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Methionine	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
Dicalcium Phosphate	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	4.9	4.9	4.9	4.9	4.9
Limestone	1.96	1.96	1.96	1.96	1.96	1.96	1.96	1.96	1.96	1.96	1.96	1.96	1.96	1.96	1.96	1.96
Salt	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Premix2	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29	0.29
AFs	_	+	_	+	_	+	_	+	_	+	_	+	_	+	_	+
Binder	_	_	Novasil [3]	Novasil [3]	1	1	2	2	_	_	Novasil [3]	Novasil [3]	1	1	2	2

[1] Scientific name: *Rastrineobola argentea*, common name; silver cyprinid, and it's also called the Lake Victoria sardine or mukene, [2] Supplied the following per 2 Kg of diet: Each 2 Kg contains: Vitamin A, 8,500,000 IU; Vitamin D3, 1,600,000 IU; Vitamin E, 4,000 IU; Vitamin K3, 2,000 mg; Vitamin B2, 5,000 mg; Vitamin B3, 20,000 mg; Vitamin B5, 8,800 mg; Vitamin B6, 1,200 mg; Vitamin B9, 00 mg; Vitamin B12, 8 mg; Chlorine chloride, 200,000 mg; Antioxidant, 125,000 mg; Fe, 5,000 mg; Mn, 80,000 mg; Zn, 50,000 mg; Cu, 2,000 mg; I, 1,200 mg; Co, 200 mg; Se, 100 mg., [3] A patented Natural clay rich in calcium montmorillonite derived from bentonites, 2= Rice Husk Ash, 3= Banana Peels Binder inclusion at 1.96%

3.4.3 Data collection
Feed intake, feed efficiency and chicks' weight were computed weekly for each bird and weight gain was calculated as the weight difference of two consecutive weighing. Daily Feed Intake was computed as the difference between feed offered and feed remaining before the next feeding. Feed efficiency was calculated as weight gain divided by feed intake.

3.4.5 Statistical analysis
All the data from the experiment were analyzed using the GLM (General Linear Models) procedure of the Statistical Analysis System software (SAS, 2009). Overall data were analyzed using two-way ANOVA. The difference between the means was separated using Tukey's Range Test Procedure (HSD) and LSD. The statistical model was;

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where,
Y_{ijk} = Effect of response variable (feed intake, weight gain and feed conversion ratio)
μ= overall mean
α_i = effect of the ith binders (i=1, 2, 3, 4)
β_j = effect of jth aflatoxins level (j=1,2)
$(\alpha\beta)_{ij}$ = interaction effect of ith binder on jth level of aflatoxins
ε_{ijk} = random error term

Results and Discussions
The results of the effect of treatments on growth performance are presented in Tables 2 and 3.

Table 2: Dietary effect on Average Feed Intake (g/d), Average Daily Gain and Feed Efficiency of broilers during grower (g), finisher (f) and overall (o) periods

Dietary treatment	Parameters								
	ADFI			ADG			FE		
	ADFIg	ADFIf	ADFIo	ADGg	ADGf	ADGo	FEg	FEf	FEo
Trt 1	20.57	41.16	30.86	8.81	21.92	15.37	0.43	0.56	0.51
Trt 2	19.57	37.56	28.57	8.14	19.91	14.02	0.41	0.52	0.48
Trt 3	21.25	37.97	29.61	7.62	18.25	12.93	0.36	0.48	0.44
Trt 4	26.91	50.67	38.79	10.30	23.12	16.71	0.39	0.47	0.43
Trt 5	21.53	39.85	30.69	9.26	23.76	16.51	0.43	0.61	0.54
Trt 6	23.34	23.87	23.60	8.70	19.23	13.96	0.37	0.80	0.59
Trt 7	19.39	35.42	27.41	7.94	19.20	13.57	0.41	0.54	0.50
Trt 8	20.85	34.86	27.85	8.98	20.94	14.96	0.43	0.62	0.54
Pooled SEM	2.24	4.79	2.89	0.7755	2.5181	1.6376	0.0276	0.0567	0.0363
p<Value A x B	0.5389	0.061	0.0751	0.1483	0.2990	0.2586	0.3821	0.2037	0.6139

Means within row a without a superscript do not differ significantly ($p<0.05$) ADFI = average daily feed intake during grower phase, ADG = the average daily body weight gain, FE= represents the Feed Efficiency, g = during grower phase, f =during finisher phase, o= the overall period A = aflatoxins B1 effect, B = binder effects, A x B = interaction effects.

Table 3: Effect of type of binders and aflatoxins on Average Feed Intake, Average Daily Weight Gain of broilers during grower (g), finisher (f) and overall (o) periods

Parameter	Binder types					
	Control	Novasil	Rice Husk ash	Banana Peels	SEM	P-Value
ADFIg	20.07	24.08	24.43	20.12	2.24	0.26
ADFIf	39.36	44.32	31.86	35.14	4.79	0.12
ADFIo	29.72	34.20	27.15	27.63	2.89	0.12
ADGg	8.48	8.96	8.98	8.47	0.78	0.84
ADGf	20.91	20.68	21.50	20.07	2.52	0.95
ADGo	14.70	14.82	15.24	14.27	1.64	0.95
FEg	0.42	0.38	0.42	0.42	0.03	0.36
FEf	0.54^{b}	0.48^{b}	0.70^{a}	0.58^{b}	0.06	0.01
FEo	0.50^{ab}	0.44^{b}	0.57^{a}	0.52^{a}	0.04	0.02
	AFs Levels (ppb)				SEM	P-Value
	0		100			
ADFIg	20.68		22.67		2.24	0.23
ADFIf	38.60		36.74		4.79	0.60
ADFIo	29.64		29.70		2.89	0.98
ADGg	8.41		9.03		0.78	0.28
ADGf	20.78		20.80		2.52	0.99
ADGo	14.60		14.91		1.64	0.79
FEg	0.41		0.40		0.03	0.71
FEf	0.55		0.60		0.06	0.18
FEo	0.50		0.51		0.04	0.79

Means within a row without a superscript do not differ significantly ($p<0.05$) ADFI = average daily feed intake during grower phase, ADG = the average daily body weight gain, FE = represents the Feed Efficiency, g = during grower phase, f = during finisher phase, o = the overall period A = aflatoxins B1 effect, B = binder effects, A x B = interaction effects

Effects of aflatoxins on the performance of broilers

The results of this experiment show that broiler diets spiked with AFB1 (100 ppb) did not significantly ($p>0.05$) decrease daily feed intake, daily body weight gain and feed efficiency during the grower, finisher and overall periods. In agreement, [23], found no significant effect on feed intake, weight gain and feed conversion rate. Researchers who used higher levels of AFB1 (500 ppb and 170 ppb) in poultry diets still observed no decrease on the weight and feed intake [11, 15]. The insignificant AFB1 effect on feed intake could be explained by the fact that aflatoxins normally reduce energy availability which results to birds consuming more feed to meet their energy requirements [23]. Some collaborators found out that birds fed higher concentration of AFB1 had low weight compared to ones that consumed low AFB1 concentrations [14, 35, 29], which could behave resulted from a possible adaptation of the chronic AFB1 exposure. Additionally, the weight gain reductions at higher pharmacological AFB1 doses could be due to its biphasic nature that indirectly stimulates an overcompensation response to the initial disruption [35]. It is possible to lack a noticeable effect on the growth performance of the animals fed contaminated diets but detect the residues in the liver, [8], thus predisposing the consumers to their harmful effects. On the contrary, other studies found significant effect aflatoxins contaminated diets on body weight gain, feed intake and feed efficiency probably due difference in species and age [20, 32, 1, 28, 7, 26, 25, 21].

Effects of natural toxin binder inclusion on the growth performance of broilers fed diets with/without aflatoxins

The addition of the Banana peels and rice husk binders to the non-contaminated feed did not significantly ($p>0.05$) affect daily feed intake, daily weight gain and feed efficiency. This implies that the two natural products are safe for inclusion in broiler diets. In line with these findings, several authors working with binders found similar effects on broilers [20, 28, 7]. Of three inclusion levels (10%, 20% and 30%) of banana peels, a significant body weight increase was recorded only in the 30% inclusion level [34]. Maybe the low inclusion levels (1.9%) used in this study is the reason for no change observed in body weight gain. On the other hand, the addition of Novasil to the non-contaminated diet led to a non-significant decrease in average daily weight gain which was recorded other researchers who used Hydrated Sodium Calcium Aluminosilicates (HSCAS) [9]. This was contrary to the fact that clays are known to indiscriminately bind important minerals, vitamins and fatty acids and also release toxic compounds (heavy metals or dioxins) hence causing the decreased weight gain [36]. There was no significant increase in average daily feed intake and average daily weight gain observed when Novasil binder was added into the AFB1 contaminated diet. Similarly, some authors found no significant increase in body weight and feed intake when 0.5% HSCAS containing binder was added to AFB1 contaminated diets [21]. When different levels of sodium bentonites were added to AFB1 contaminated layer diets, 3% inclusion significantly increased feed intake while to 1.5% inclusion did not [11]. The inclusion levels in this study were 1.9%and thus it might have caused the non-significant increase in feed intake and body weight gain. Banana peels and rice husk binders added to AFB1 contaminated diets did not affect feed intake and weight gain significantly. The overall FE however, showed that broilers on diets with RHA and banana peels binder had feed efficiency that was significantly ($p<0.05$) different from the commercial binder. Phenols in the banana peel aid in the binding of aflatoxins. On the contrary, phenol-containing binder did result in higher feed efficiency compared to a clay binder [23]. Rice husk ash had a significantly higher feed efficiency compared to the commercial binder. This could be explained by the excellent aflatoxins binding capacity (85%) of RHA which is a result of its high cation exchange capacity [3]. On the contrary, a binder containing clay had no significant effect on performance parameters when compared to a binder containing activated charcoal [23].

Conclusions

The incorporation of Banana Peels and Rice hush ash natural binders in AFB1 contaminated feed had a significant improvement in overall feed efficiency compared to the commercial binder in broilers.

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