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Performance of weanling rabbits fed aflatoxin treated diets with sweet orange (*Citrus sinensis*) fruit peel

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The performance of twenty four 6-8 week old apparently healthy male and female weanling rabbits of mixed breed fed diets treated with aflatoxin, which contained 5% sweet orange (*Citrus sinensis*) fruit peel meal was evaluated in an 8 week feeding trial. Sweet orange peels were collected from orange sellers, sun-dried and milled. Fungal strain of *Aspergillus flavus* was cultured and inoculated into groundnut cake to produce aflatoxin using solid state fermentation method. Treated groundnut cake was incubated for seven days with incremental incubation temperature from 20-25 ºC The groundnut cake was autoclaved, milled and, aflatoxin extracted from 10 g sample of the milled cake with 50 ml chloroform, and its concentration quantified by Thin Layer Chromatography (TLC). Treated groundnut cake was included at 0, 50, 100 and 150 gram in grower rabbit diets to produce diets T_1, T_2, T_3 and T_4, having 0 ppb, 50 ppb, 100 ppb and 150 ppb aflatoxin, respectively. The rabbits were randomly allocated to four diets at the rate of six per diet, housed singly in rabbit hutches, fed and served water free choice. The result showed significant (P<0.05) negative effect of diets on final live weight, weight gain, feed intake, feed conversion ratio, water consumption, protein intake and protein efficiency ratio as the dietary aflatoxin increased from 0 ppb to 150 ppb. Also, diets had significant (P<0.05) negative effect on dressed weight and carcass length and, kidney. Total aflatoxin residue varied significantly (P<0.05) from 0 µg/kg - 2.76µg/kg, 0 µg/kg - 1.94µg/kg and 0 µg/kg - 0.85µg/kg for liver, kidney and meat tissue, respectively as the dietary aflatoxin increased from 0 ppb to 150 ppb. Performance response of rabbits was affected negatively by aflatoxin, thereby showing the inability of 5% dietary inclusion of sweet orange (*Citrus sinensis*) peel meal to mitigate the adverse consequences of aflatoxin intake by rabbit.

Key words: Growth responses, residual level, visceral organs, mitigate.

INTRODUCTION

Rabbit production has a considerable potential in developing countries for the supply of the much needed animal protein which is caused by protein deficit as a result of uncontrolled human population growth in most countries. Rabbit has low capital investment and space requirements, short generation interval, rapid growth rate and high reproductive potential.

Furthermore, rabbit has a functional caecum (Chiba,

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2014) and thus its inherent ability to utilize the abundant herbaceous plants and fibrous agricultural by-products as feed. The performance of rabbit is being threatened by aflatoxin contamination in feeds. In spite of occasional high profile incidents of acute poisoning outbreak, animals are considered the most highly vulnerable group, exposed to high accumulation of aflatoxin through consumption of contaminated feedstuffs, which develop into health problems with attendant huge economic losses. These losses have pronounced effect on the quality and quantity of meat and eggs due to contamination with aflatoxin residues (Bintvihok et al., 2002; Farombi, 2006). In chickens, the effects of aflatoxins include liver damage, impaired productivity and reproductive efficiency, decreased egg production, inferior eggshell quality, inferior carcass quality and increased susceptibility to disease (WHO, 2018). It also reported that pigs are also highly affected by aflatoxins, with the chronic effects largely apparent as liver damage. In cattle, the primary symptoms are reduced weight gain as well as liver and kidney damage; where milk production is also reduced. Different forms of the enzymes that metabolize aflatoxins (e.g. cytochrome P450s, glutathione S-transferases) are considered responsible for the different susceptibilities of different animals to the toxic effects of aflatoxins (WHO, 2018). Direct relationship between aflatoxin in the diet and the residue level in liver, muscle and eggs, and poor growth and feed conversion, increased mortality, leg problems and carcass condemnations are some of the economic losses associated with aflatoxin exposure in broilers (Makun et al., 2010), a non-ruminant animal like rabbit. Citrus peel contains essential oils (90% D-Limonene) which according to Sun (2007) are well known antimicrobial agents. It has been reported that 30% fermented sweet orange peel can be used as a replacement for maize in the diet of rabbits (Oluremi et al., 2018) and, that sweet orange peel is available throughout the year (Oluremi et al., 2007). Its utilisation will yield a significant reduction in the cost of rabbit feeding and total cost of production, thereby making cheaper animal protein available, in addition to the benefit of the antimicrobial property it possesses due to its intrinsic D-limonene. D-limonene in orange and lemon oil is inhibitory to mold growth and aflatoxin production (Esper et al., 2014; Dhanapal et al., 2014). This study was designed to assess the potential of sweet orange peel to mitigate the effect of aflatoxin contaminated diets on the growth response of grower rabbits.

MATERIALS AND METHODS

Sweet orange (Citrus sinensis) fruit peel was the test ingredient evaluated for its potential to mitigate the negative effect of dietary aflatoxin on rabbit (*Oryctolagus cuniculus*) performance. It was collected from sweet orange fruit retailers on the campus of the Federal University of Agriculture Makurdi, Nigeria, where the feeding trial was conducted in a wire net wall Rabbitary house of the Livestock unit of the Teaching and Research Farm. Makurdi is located between latitude 7° 44′ 1.5″N and longitude 8° 31′17″ E (Geodatos, 2021). It is in the Guinea Savanna Zone of West Africa, with annual rainfall of 508 mm to 1016 mm within a period lasting for 6-8 months (March-October) and, a minimum temperature of 24.20 ±1.4 °C and maximum temperature of 36.33 ±3.70 °C (Tageo, 2009). The relative humidity ranges between a minimum of 39.50 ± 2.20% and a maximum of 64.00 ± 4.80% (Tageo, 2009). The peels were sun-dried for about 48 h on a concrete floor until they attained approximately 10% moisture. These were stored in synthetic bags and, milled when the experimental diets were to be compounded. The proximate composition of sweet orange peel has been reported by Oluremi et al. (2020). Groundnut cake was inoculated with a spore suspension of fungal strain of Aspergillus flavus (NRRL, 1999) for aflatoxin production. The treated groundnut cake was incubated for 7 days with incremental increases in the incubation temperature from 20-25 °C. The groundnut cake was then autoclaved at 121 °C for 30 min to kill the mold. It was washed, dried, and ground to fine particles. Aflatoxin was extracted from 10 g weighed sample of the ground cake powder with 50 ml chloroform and its concentration quantified by Thin Layer Chromatography (TLC) at the Pathology unit, International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. Four (4) grower rabbit mash diets T_1, T_2, T_3 and T_4, each containing 5% sun dried sweet orange fruit peel meal were formulated to meet requirements for crude protein and metabolisable energy. Diet T_1 served as the control and had 0 g of groundnut cake while, diets T_2, T_3 and T_4 were derived from diet T_1 by adding the aflatoxin containing groundnut cake at levels of 50 g (+), 100 g (++) and 150 g (+++), respectively (Table 1). The levels of aflatoxin in T_1, T_2, T_3 and T_4 were 0 ppb, 50 ppb, 100 ppb and 150 ppb, respectively.

A total of twenty four (24) six to eight week-old apparently healthy male and female weanling rabbits of mixed breed with average initial body weight 815.50 g – 820.80 g were used. An adaptation period of seven (7) days was allowed for the experimental animals to acclimatize to the environment before the start of the feeding trial which lasted for eight (8) weeks. The rabbits were housed individually in 60 cm x 60 cm x 60 cm wooden frame hutches with metal frame supported wire mesh floor, which were randomly assigned the experimental unit treatment and replicate codes. The rabbits were randomly grouped into four (4) of similar weight. One group each of six (6) rabbits was then randomly allotted to a dietary treatment and, each rabbit served as a replicate. The experiment was a completely randomized design. Each rabbit was provided experimental diet and drinking water free choice for the experimental duration and, the performance of the rabbits evaluated using the following growth response and carcass characteristics:

i) Live body weight and body weight gain (BWG): Each rabbit was weighed at the beginning of the experiment and weekly thereafter to obtain the weekly weights. The growth rate (average body weight gain) was computed as the difference between the final weight (FW) and the initial weight (IW) divided by 56 days (experimental duration) that is

BWG =FW – IW / 56

ii) Daily feed intake (FI): This was determined by calculating the difference between the quantities of feed offered each rabbit and the left over weekly. The average daily feed intake was then computed as FI= FI1 + FI2 + -------- + 568 / 56
Where 1, 2 --- 8 are the no of weeks.

iii) Feed conversion ratio (FCR): This was calculated as the ratio of average daily feed intake to average daily body weight gain that is
FCR= $FI\ /\ BWG$

iv) Water consumption: 1000 ml calibrated plastic cylinder was used

Table 1. Gross composition of aflatoxin contaminated experimental diets (kg/100 kg).

Ingredient	Dietary treatments			
	T_1	T_2	T_3	T_4
Maize	30.00	30.00	30.00	30.00
Groundnut cake	10.00	10.00	10.00	10.00
Full fat soyabean	15.00	15.00	15.00	15.00
Sweet orange peel meal	5.00	5.00	5.00	5.00
Rice offal	20.00	20.00	20.00	20.00
Brewer's dried grain	16.00	16.00	16.00	16.00
Bone ash	3.50	3.50	3.50	3.50
Premix*	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25
Aflatoxin	-	+	++	+++
Total	100.00	100.00	100.00	100.00
Calculated nutrients				
Crude protein (%)	18.88	18.88	18.88	18.88
Crude fibre (%)	5.05	5.05	0.05	0.05
Ether extract	8.91	8.91	8.91	8.91
Ash (%)	6.46	6.46	6.46	6.46
Ca (%)	1.52	1.52	1.52	1.52
P (%)	1.02	1.02	1.02	1.02
ME** (kcal/kg)	2333.65	2333.65	2333.65	2333.65

*1kg of vitamin/mineral premix manufactured by BEAUTS Co. Inc. Man, U.S.A., contained: Vitamin A 220000 IU, Vitamin D 66000 IU, Vitamin K 88 mg; Vitamin B12 0.76 mg, Niacin 1122 mg, Calcium 27% Phosphorus 10%, Iron 0.6%, Zinc 0.35%, Manganese 0.25%, Copper 0.06%; Iodine 0.002%, Cobalt 26 ppm, Selenium 4 ppm; - = (0 ppb) aflatoxin, + = (50 ppb) aflatoxin, ++ = (100 ppb) aflatoxin, +++ = (150 ppb) aflatoxin.
ME = [37 x %CP] + [81.8 x %EE] + [35.5 x %NFE] Pauzenga (1985).

to measure drinking water to each rabbit and the left over determined 24 hourly to obtain water consumed by difference.
v) Water: feed ratio was obtained from water consumption and feed intake data.
vi) Protein intake (PI): This was computed as average daily feed intake x % crude protein in the diet that is PI= $[(\mathrm{FI})\ \mathrm{x}\ (\%\ \mathrm{CP})]$
vii) Protein efficiency ratio (PER): This was calculated with the protein intake and body weight gain data as PER= PI / BWG
viii) Mortality of rabbit was recorded.

Carcass investigation was done with randomly selected three rabbits per treatment. They were fasted for 12 h prior to slaughter at the termination of the feeding trial on the 56th day. The fasted rabbits were weighed before slaughter, slaughtered and weighed. Each rabbit carcass was eviscerated and weighed, singed and weighed to obtain the dressed weight. The carcass was cut into forelimbs, hind limbs, back/rib, loin and head, which were weighed with an electronic balance. Visceral organs namely; heart, lung, kidney, liver, pancrease, spleen and gall bladder were carefully removed and weighed. The weight of each of the carcass cuts was expressed as percentage of dressed weight and the visceral organ weight was expressed as percentage of the live weight. A total of nine (9) samples consisting of 3 livers, 3 kidneys and 3 tissues (muscle) samples were collected from three rabbits slaughtered rom each of T2, T3 and T4. The samples were preserved in 10% formaldehyde solution, and sent to the Animal Care Technical Laboratory, Ogere-Remo, Nigeria, to determine the total aflatoxin residue using the ELISA protocol (Engvall and Perlmann, 1972). Data collected were subjected to the analysis of variance (Minitab, 2012). The means of significantly different data was separated using Duncan's Multiple Range test as described by Duncan (1955).

RESULTS AND DISCUSSION

The proximate composition of sweet orange peel which has been reported by Oluremi et al. (2020) contained 8.15% crude protein, 13.88% crude fibre, 3.22% ether extract 7.67% ash, 67.06% nitrogen free extract and 2913.92 kcal ME/kg. While, the crude protein, crude fibre and nitrogen free extracts levels in the sweet orange peel were inferior to those of maize 8.90% CP, 2.70% CF and 83.1% NFE (Aduku,2012), the herbivorous nature of rabbits though a non-ruminant animal species showed the feed resource ability of the sweet orange peel. The calculated nutrients in the experimental diets were 18.88% crude protein, 5.05% crude fibre, 8.91% ether extract and 6.78% ash (minimum). The experimental diets caloric content of 2333.65 kcal ME/kg was adequate for grower rabbits.

The growth performance of the rabbits showing the weights, feed intake, water intake, water: feed ratio, feed

Table 2. Growth performance of rabbits fed aflatoxin contaminated diets containing sweet orange peel meal.

Parameter	Dietary treatments				
	T_1	T_2	T_3	T_4	SEM
Average initial weight (g)	818.20	820.20	820.80	815.50	27.36^{ns}
Average final weight (g)	1591.00^{a}	1431.00^{ab}	1374.80^{ab}	1141.00^{b}	48.76^{*}
Average daily feed intake (g/day)	51.44^{ab}	52.89^{a}	53.23^{a}	45.61^{b}	1.14^{*}
Average daily weight gain (g/day)	13.80^{a}	10.90^{ab}	9.89^{ab}	5.81^{b}	0.72^{*}
Average daily water intake (ml/day)	289.37^{a}	282.48^{ab}	253.29^{ab}	241.25^{b}	7.17^{*}
Water: Feed ratio (ml/g)	5.76	5.38	4.79	5.37	0.23^{ns}
Feed conversion ratio	3.72^{a}	4.85^{ab}	5.38^{ab}	7.85^{b}	2.45^{*}
Protein intake (g)	9.67^{a}	9.58^{a}	9.54^{ab}	8.38^{b}	0.31^{*}
Protein efficiency ratio	1.43^{a}	1.14^{ab}	1.04^{ab}	0.69^{b}	0.07^{*}
Mortality (%)	0	0	16.67	16.67	

^{a,b} Means on the same row with different superscripts are significantly different ($p<0.05$), SEM = Standard error of mean, *Significant difference ($p<0.05$), ^{ns}Not significant ($p>0.05$). T_1 contained 0 ppb aflatoxin; T_2 contained 50 ppb aflatoxin; T_3 contained 100 ppb aflatoxin; T_4 contained 150 ppb aflatoxin.

conversion ratio (FCR), protein intake, protein efficiency ratio (PER) and mortality are presented in Table 2. The effect of dietary treatment on these growth indices were significantly different ($P<0.05$) among treatments. The result revealed that the final body weight, body weight gain, feed intake, FCR protein intake and PER decreased with increase in aflatoxin presence in the diets. Reduction in feed intake, giving the plane of nutrition of the rabbits, may have been caused by the mold aflatoxin. Aflatoxin results in aflatoxicosis which is able to make nutrients unavailable for absorption in the stomach and caeca of rabbit with consequent effect on utilisation by the animal, thereby affecting growth. It has been reported by Oluremi et al. (2019) that 5% level of sweet orange peel in grower rabbit diets could not ameliorate the adverse effect of aflatoxin on nutrient digestibility. Aflatoxin has also been reported to affect the hypothalamic centre which controls feed intake and that it impairs the digestion and absorption of different nutrients in the rabbit (Ibrahim, 2000). Nutrient availability from feed is vital for animal growth hence, in this study the reduction in feed intake caused a decrease in live body weight, body weight gain, FCR water intake, water: feed ratio, protein intake and PER, which are associated growth physiological response. Therefore, it is evident that the 5% sweet orange fruit peel meal incorporated in the dietary treatments was unable to mitigate the effect of aflatoxin in the diets and thus, may not be adequate. Mortality of 16.67% which translated to one rabbit occurred only in T_3 and T_4. The rabbits were observed to exhibit rapid breathing, lack of vitality and interest.

The data on the effect of the aflatoxin contaminated diets on rabbits carcass characteristics yield is presented in Table 3. The diets had significant effect ($P<0.05$) on dressed weight and carcass length but had no significant effect ($P>0.05$) on dressing percentage, fore limbs, hind limbs, rack/rib, loin and head weight of the rabbits across the treatments. The significant decline ($P<0.05$) in the carcass length of rabbits also has a direct relationship with their growth. The significant decrease ($P<0.05$) in the dressed weight as the aflatoxin content increased from 0 ppb to 150 ppb is consistent with the depressed growth rate and final live body weight of the rabbits. The non-significant effect ($P>0.05$) on the dressing percent and other carcass cuts in the study showed that although, the dressed weight had a significant decrease, the experimental diets did not cause a disproportionate growth in any of these important carcass cuts. The effect of feeding aflatoxin contaminated diets on the relative weight of visceral organs is presented in Table 4. The weight of kidney expressed as percent of live weight of rabbit, varied significantly ($P<0.05$) from 0.60% to 0.93% across the dietary treatments however, the relative weights of the heart, liver, lungs, gall bladder, pancrease and spleen were not significantly different ($P>0.05$). The paired kidney weights of 0.60 - 0.93% in this study, were higher than 0.47% - 0.60% (Oluremi et al., 2005). Ibrahim (2000) also found that the relative weight of spleen, brain, liver and kidney were significantly affected and the gall bladder relative weight increased in rabbit treated with aflatoxin while, Abd El-Hamid et al. (2002) reported that liver, kidney and heart were significantly higher in rabbits which suffered from aflatoxicosis. The incidence of aflatoxicosis caused by the contamination of the diets by aflatoxin in this study probably caused the inflammation of the rabbit kidney and thus, the subsequent higher relative weights observed for diets T_2, T_3 and T_4.

The effect of aflatoxin contaminated diets on the resultant total aflatoxin residue deposited in internal organs and meat tissue of rabbits is presented in Table 5. Total residual aflatoxin levels in liver, kidney and rabbit tissue differed significantly ($P<0.05$) across the dietary

Table 3. Carcass yield of rabbits fed aflatoxin contaminated diets containing sweet orange peel meal.

Parameter	Dietary treatments				
	T_1	T_2	T_3	T_4	SEM
Dressed weight (g)	891.67^{ab}	943.33^{a}	758.33^{b}	595.00^{c}	18.62*
Dressing percent	47.81	47.76	49.96	46.95	1.61^{ns}
Fore limbs (% DW)	7.47	8.46	8.04	7.31	0.25^{ns}
Hind limbs (% DW)	14.98	16.43	15.46	15.77	0.57^{ns}
Rack/Rib (% DW)	7.29	7.70	7.04	7.90	0.32^{ns}
Loin (% DW)	11.54	12.51	10.84	11.90	0.60^{ns}
Head (% DW)	6.59	7.38	8.21	7.66	0.26^{ns}
Carcass length (cm)	31.93^{a}	31.50^{ab}	29.79^{b}	27.80^{c}	0.63*

^{a,b,c} Means on the same row with different superscripts are significantly different ($p<0.05$), SEM = Standard error of mean, *Significant difference ($p<0.05$), ^{ns} Not significant ($p>0.05$), DW = Dressed weight.T_1 contained 0 ppb aflatoxin; T_2 contained 50 ppb aflatoxin; T_3 contained 100 ppb aflatoxin; T_4 contained 150 ppb aflatoxin.

Table 4. Visceral organ weight of rabbits fed aflatoxin contaminated diets containing sweet orange peel meal (% live weight).

Parameter	Dietary treatments				
	T_1	T_2	T_3	T_4	SEM
Heart	0.29	0.35	0.28	0.32	0.01^{ns}
Liver	3.07	3.73	3.55	3.39	0.18^{ns}
Lungs	0.63	0.64	0.53	0.65	0.02^{ns}
Gall bladder	0.04	0.06	0.08	0.07	0.01$_{ns}$
Kidney	0.60^{b}	0.89^{a}	0.79^{a}	0.93^{a}	0.03*
Pancrease	0.50	0.60	0.60	0.60	0.01^{ns}
Spleen	0.06	0.08	0.05	0.08	0.01^{ns}

^{a,b} Means on the same row with different superscripts are significantly different ($p<0.05$), SEM = Standard error of mean, *Significant difference ($p<0.05$), ^{ns} Not significant ($p>0.05$). T_1 contained 0 ppb aflatoxin; T_2 contained 50 ppb aflatoxin; T_3 contained 100 ppb aflatoxin; T_4 contained 150 ppb aflatoxin.

Table 5. Total aflatoxin residue in rabbits fed aflatoxin contaminated diets containing sweet orange peel meal.

Parameter	Dietary treatments				
	T_1	T_2	T_3	T_4	SEM
Aflatoxin ingested (µg/kg)	0^{d}	8.01^{c}	24.33^{b}	34.67^{a}	0.10*
Liver (µg/kg)	ND	0.65^{c}	1.02^{b}	2.76^{a}	0.03*
Kidney (µg/kg)	ND	0.92^{b}	1.01^{b}	1.94^{a}	0.06*
Tissue (µg/kg)	ND	0.06^{bc}	0.07^{b}	0.85^{a}	0.01*

^{a,b,c,d}Means on the same row with different superscripts are significantly different ($p<0.05$), SEM = Standard error of mean, *Significant difference ($p<0.05$), ND = not detected, ppb = parts per billion. T_1 contained 0 ppb aflatoxin; T_2 contained 50 ppb aflatoxin; T_3 contained 100 ppb aflatoxin; T_4 contained 150 ppb aflatoxin;

treatments. The aflatoxin residues detected followed a particular trend whereby, there was a steady increase in its concentration with increase in the aflatoxin level in the diets from 0 ppb to 150 ppb and, aflatoxin ingested from 0 µg/kg to 34.67 µg/kg by the rabbits. No aflatoxin residue was found in the liver, kidney and meat tissue in dietary group T_1 (control). This trend is in agreement with the findings of Hussain et al. (2010) who reported a direct

relationship between aflatoxin in the diet and the residue level in the examined organs and muscle tissue. The highest level of aflatoxin residue in this study was 2.76 µg/kg (0.00267 mg/kg) in the liver, 1.94 µg/kg (0.00194 mg/kg) in the kidney and 0.85 µg/kg (0.00085 mg/kg) in muscle tissue, which showed that consumption of these rabbit carcass products will not be injurious to humans. This is because the level of consumption of food containing aflatoxin concentrations of 1 mg/kg or higher has been suspected to cause aflatoxicosis (WHO, 2018).

Conclusion

The study has revealed that aflatoxin contamination of grower rabbit diet affected rabbit growth, meat yield and health status negatively, thereby demonstrating the inability of 5% dietary inclusion of sweet orange (*Citrus sinensis*) peel meal to mitigate the adverse consequences of aflatoxin intake by rabbit. The residual aflatoxin level in the kidney, liver and muscle tissue of rabbits showed that there is a direct relationship between the level of aflatoxin consumed in feed and the residue deposited in the organs, which was not depressed by the inclusion of 5% sweet orange peel meal in the diets. Higher dietary inclusion levels of sweet orange peel meal is recommended for future studies.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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