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# Effect of Vitamins, Amino Acids and Phyto-Active Biomolecules on *Aspergillus flavus* in Poultry Production

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Additional information is available at the end of the chapter

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## 1. Introduction

*Aspergillus* is a genus of fungus consisting of several hundred mould species found in various climates world-wide. Taxonomically, they belong to kingdom Fungi, phylum Ascomycota, class Eurotiomycetes, order Eurotiales, family Trichocomaceae and genus *Aspergillus*. There are several hundreds of *Aspergillus* species, including *Aspergillus aculeatus*, *Aspergillus candidus*, *Aspergillus clavatus*, *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*, *Aspergillus ustus*, and *Aspergillus tamari*. [1]

*Aspergillus* species are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as moulds on the surface of a substrate, as a result of the high oxygen tension. Commonly, fungi grow on carbon-rich substrates such as monosaccharides (e.g., glucose) and polysaccharides (e.g., amylose). *Aspergillus* species are common contaminants of starchy foods and grow in or on many plants and trees. In addition to their growth on carbon sources, many species of *Aspergillus* demonstrate oligotrophy, whereby they are capable of growing in nutrients-depleted environments or environments in which there is a complete lack of key nutrients. *A. niger* gives a prime example of this oligotrophic tendency, as it is found growing on damp walls as a major component of mildew. Species of *Aspergillus* are important medically and commercially. Some species are known to cause infection in humans and other animals [2].

*Aspergillus flavus* is a major food-borne pathogen that produces aflatoxin, a toxin that is carcinogenic [3]. It is a leading cause of aflatoxicosis in poultry. Aflatoxicosis results from ingestion of aflatoxin in contaminated feed. Effects of the aflatoxicosis include toxicosis and

immunosuppression [4]. The toxins released during *Aspergillus* infection depress production parameters and, specifically, cause impaired growth in poultry, while the immunosuppressive effect predisposes the animals to many secondary infections from other pathogens, such as fungi, bacteria and viruses. The consumption of a mycotoxin-contaminated diet by broilers has been reported to induce haematological, biochemical and liver changes. Other documented effects include growth depression, economic losses, increased mortality, decrease blood cell count, lower egg production, lower feed consumption, reduced resistance to infectious disease and vaccination efficiency, gross and microscopic changes in the liver and other organs, such as hepatomegaly, paleness, and hydropic degeneration. Fatty changes in the adipocytes, bile duct, hyperplasia and periportal fibrosis are other effects of aflatoxicosis [5]. Depletion of lymphoid organs such as the thymus and bursa of fabricius [6], kidney and spleen lesion [9] unfavourable reproductive changes [10], impairment of the humoral and cellular immune response [9] are common symptoms of aflatoxin ingestion in poultry and other livestock. Another possible effect of aspergillosis is the possible transmission of fungal mycotoxin residues to meat and eggs from infected chickens, which is potentially hazardous to public health.

Heterocyclic metabolites of the genera *Aspergillus* are aflatoxin and ochratoxin. It has been reported that both lower and higher doses of AFB<sub>1</sub> affect the haematological parameters of broiler chicks. This observation is closely linked to depressed cellular immunity due to suppression of the phagocytic activity of macrophages and decrease in T-lymphocyte activities [10]. Liver damage and temporary dysfunction may further predispose to deficiency of humoral immunity.

Aflatoxins have been known to be toxic and reported to cause immune suppression in birds [11]. These immune suppression effects of aflatoxins predispose the animal to many secondary infections due to other fungi bacteria and viruses [12]. Earlier reports [6] opined that contamination of broiler ration with aflatoxin resulted in a drastic reduction in performance both from a growth and a feed-efficiency standpoint. The aflatoxin-producing fungus, *Aspergillus flavus*, is a causal agent of pre-harvested contamination of food commodities, which can result in serious economic hardship for producers and an adverse health impact on both humans and domestic animals. The liver has been reported as the primary target organ of aflatoxin in most animals and humans, where Aflatoxins B<sub>1</sub> is metabolized to the toxic and carcinogenic aflatoxins B<sub>1</sub>-epoxies are formed by cytochrome P450 enzymes [13]. The disease produces a hard nodular area in the lungs and infection of the air sacs. Sometimes the air sac lesions are similar to that produced by infectious sinusitis or CRD. In some birds, colonies of mould growth can be seen on the air sac membrane [14]. The mode of transmission involves contact with the organisms through contaminated feed, litter or premises (formites). The disease is not contagious and does not spread vertically. Most healthy birds can withstand repeated exposure to *Aspergillus*. Inhalation of large amounts of the infectious form of the mould leads to reduced disease resistance of the bird. In the acute form of aflatoxicosis in young birds, the main symptoms include gasping, sleepiness, loss of appetite and sometimes convulsions and death (brooder pneumonia). Occasionally, CNS signs may become apparent in the brain, causing paralysis or other forms of nervous symptoms. The more chronic form in older birds

usually results in loss of appetite, gasping or coughing, and a rapid loss of body weight. Mortality is usually low and only a few birds are affected at one time.

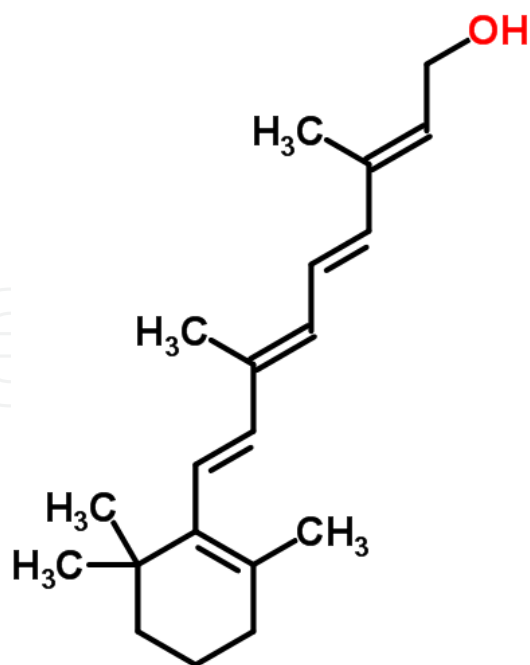
In broilers, a dose of 1.5 ppm of aflatoxin has been shown to impair bile salt availability, which causes a decrease in the absorption of fat soluble vitamins. In poultry, aflatoxicosis is characterized by restlessness, anorexia with decreased growth rate, poor nutrient utilization, decreased weight gain, decreased egg weight and production, increased susceptibility to environment and microbial stresses, and increased mortality. Post-mortem signs include yellowish caseous and hard nodular deposits in the infected air sacs. Sometimes the air sac lesions are similar to those produced by sinusitic or CRD infections. In some birds, colonies of mould growth can be seen on the air sac membrane (Figure 1).

Providing a diet containing high fat and high protein levels and augmenting the ration with vitamin supplements may be of value in mitigating the effects of *Aspergillus* infection [4]. In pigs, treatments with vitamins and protein supplementation have been shown to have some proactive effects [15]. Vitamins are an essential component of a well-balanced diet, and supplementation is aimed at optimizing the immune response in chickens [16]. Vitamin A is essential for the integrity of epithelial tissues, which represent a major defence against the entry of pathogens [16]. Vitamin A is a fat-soluble vitamin naturally occurring in plant and animal sources; these sources constitute the major forms in which the vitamin exists. In plants, the major form of vitamin A exists as a precursor (or provitamin) carotene, which can be converted to vitamin A during intraluminal absorption. The major sources of plant vitamin A are green vegetables, carrot, pawpaw, red palm oil, etc. In these sources, vitamin A exists as various types of carotene with varying vitamin A activities, e.g., all-trans- $\beta$ -carotene, neo- $\beta$ -carotene,  $\gamma$ -carotene and all-trans- $\alpha$ -carotene. Of all the feed ingredients used in animal production, only yellow maize has an ample amount of provitamin A. Vitamin A activity of carotenes is species dependent. In pigs, 5  $\mu\text{g}$   $\beta$ -carotene is equivalent to 1  $\mu\text{g}$  retinol or vitamin A activity, while in poultry the ratio is 2:1 of  $\beta$ -carotene to retinol or vitamin A activity.

RE = 1 mg all-trans retinol  
= 6 mg all-trans  $\beta$ -carotene  
= 12 mg other biologically active carotenoids  
= 3.33 IU retinol  
= 10.0 IU carotene

Vitamin A in food is found as retinol or as carotenes. Retinol is found exclusively in animal foods including eggs, milk, and milk products [18]. With the exception of fowl, meat products, including beef and pork, do not contain significant quantities of preformed vitamin A.

Carotenoids are found primarily in plant foods, whereas meats, fats, and dairy products are reportedly low in carotenoid content [18]. The richest known sources of provitamin A are palm oils. Red palm oil, a common cooking product in West Africa, is usually cited as having the highest concentration of provitamin A activity [19]. Vitamin A in the form of retinoic acid



Retinol source: [17]

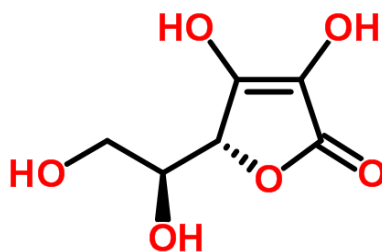
(tretinoin) has been reported to prevent acute promyelocytic leukaemia (APL) through the induction of terminal differentiation (anti-cancer), in which the leukaemic promyelocytes lose their ability to proliferate. It has also been reported to stabilize lysosomes, increase ribonucleic acid polymerase activity, increase prostaglandin F<sub>2c</sub>AMP, and cGMP levels, and increase the incorporation of thymidine into DNA [18].

In poultry, deficiency of vitamin A is manifested as impaired vision due to hyperkeratinization of the epithelial cells of the eye, drying of the cornea (xerosis) and irreversible drying of the cornea as a result of corneal hyperkeratinization and degeneration leading to blindness (keratomalacia). The impact of vitamin A deficiency on poultry productivity is linked to the use of sight for food seeking and consequently voluntary feed intake, as earlier itemized. Other deficiency symptoms are follicular keratosis observed in ruffled feathers, calcification of kidney lining, decreased bone growth, and central nervous syndrome (CNS) observed as paresis, unstable gait, etc. Deficiency of vitamin A also impacts negatively on poultry immunity by depressing cell-mediated immunity (CMI). In laying hens, early signs of deficiency are noticeable on epithelial tissues. Other consequences include eye conditions (xerophthalmia), predisposition to disease conditions, pale bird syndrome (PBS), renal dysfunction, ocular and nasal discharges, and reduction in egg production. In chicks, symptoms of vitamin A deficiency include post-hatch mortality, ataxia, poor growth and feathering. Vitamin A deficiency in neonate chicks may increase early embryonic mortality and failure to develop a neonate circulatory system [20].

Vitamin C (ascorbic acid) is a water-soluble vitamin, which is needed by the body to form collagen in bones, cartilage, muscle, and blood vessels, and which aids in the absorption of iron. Dietary sources of vitamin C include fruits and vegetables, particularly citrus fruits such

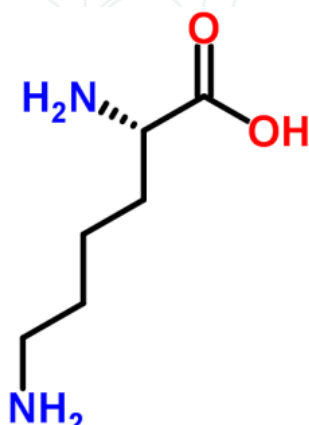
as oranges. Epidemiologic evidence suggests a role for vitamin C in hindering the development of cancer and heart disease, as well as a number of other diseases. Studies on CVD risk factors indicate that vitamin C may moderately decrease total serum cholesterol levels, increase HDL levels, and exert a hypotensive effect [21,22]. Chronic latent vitamin C deficiency leads to hypercholesterolaemia and the accumulation of cholesterol in certain tissues. Ascorbic acid supplementation of the diet of hypercholesterolaemic humans and animals generally results in a significant reduction in plasma cholesterol concentration [23]. Severe deficiency of vitamin C causes scurvy. Although rare, scurvy includes potentially severe consequences and can cause sudden death.

Vitamin C (ascorbic acid) has been reported as a non-essential nutrient for poultry, since birds are capable of synthesizing enough of the vitamin endogenously. This synthesis is attributed to the endogenous enzyme gulonolactone oxidase [24]. Studies have shown that exogenous ascorbic acid given in feed or drinking water or by injection improved performance of chickens during heat stress [25,26].

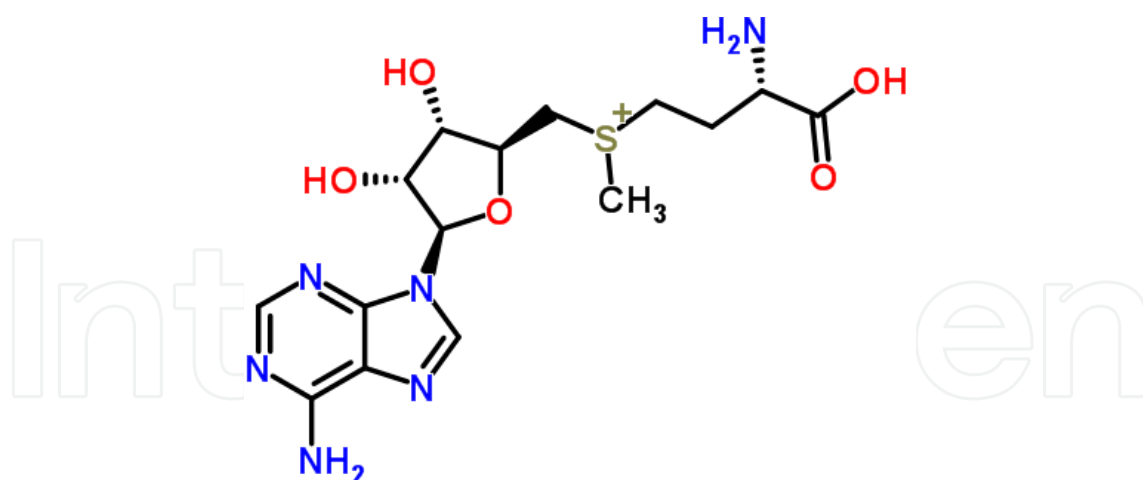


Vitamin C source: [17]

In *in vitro* testing, liquid methionine hydroxyl analogue has been observed to have an inhibiting effect on *Aspergillus flavus* [27]. According to [28], an approximate inclusion of 1.33% and 0.52% lysine and methionine, respectively, in diets of broilers subjected to aflatoxin-contaminated feed can give maximum performance.



Lysine source [17]

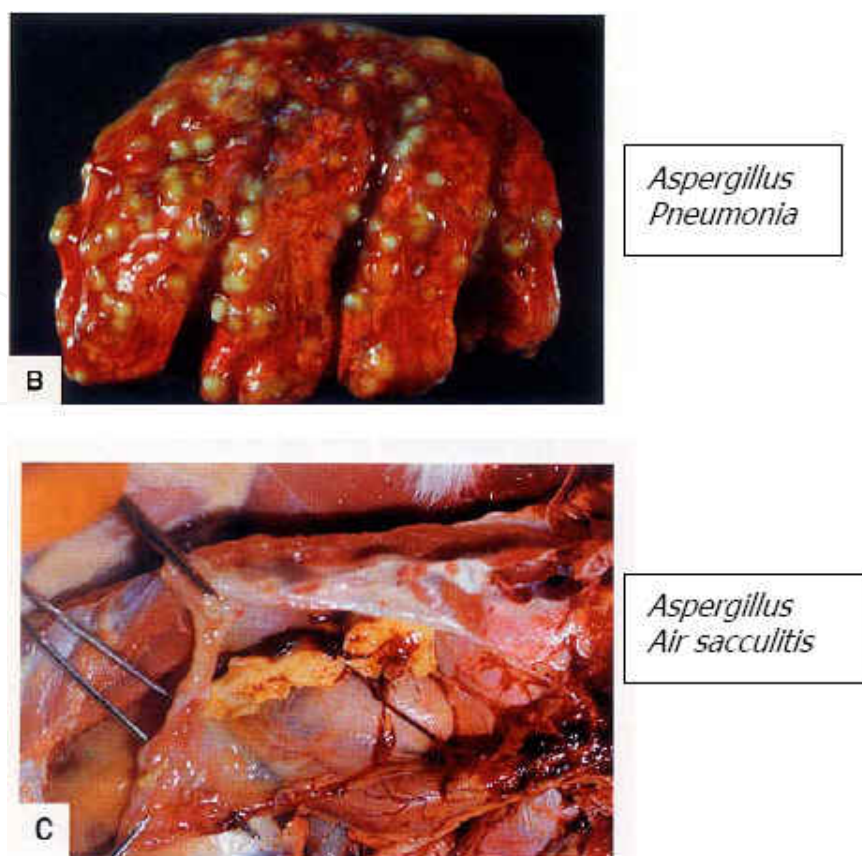


Methionine source: [17]

Feed refusal has also been reported to be a rapid and direct response to the presence of aflatoxin [29]. In an earlier report, contamination of broiler rations with aflatoxin resulted in a drastic reduction in performance both from a growth and a feed-efficiency standpoint [28]. Unfortunately, there is no treatment for aspergillosis once established in the flock. The common practice is to administer antibiotics to prevent secondary infections, while nutrient supplements are given *ad lib* to enhance tissue rejuvenation so as to hasten recovery. As a practical precautionary measure, sulpha drugs are administered on-farm to prevent *Aspergillus* infection [14].

Bark infusions of shea butter have medicinal and antimicrobial properties, e.g., against dysentery. They are applied in trado-medical practice as eyewash to counteract spitting-cobra venom. Shea butter is a suitable base for many medicines: its application relieves rheumatic and joint pains, and heals wounds, swellings, dermatitis, bruises and other skin problems. It is used traditionally to relieve inflammation of the nostrils. It is also administered to horses for the treatment of sores and galls. Extracts of the bark of *Vitellaria paradoxa* have been reported to possess antifungal properties against *Aspergillus niger*, *Aspergillus flavus*, *Epidermophyton floccosum*, *Microsporum audouinii* and *Trichophyton mentagrophytes* [31].

Gallic acid (trihydroxybenzoic acid or 3, 4, 5-trihydroxybenzoic acid) is a biologically active phenolic compound. It has been reported to show antioxidant and antimicrobial activities. It exists as free molecules or as part of tannin. Gallic acid is a trihydroxybenzoic acid, a type of organic acid. It is a colourless, crystalline organic powder. It is found in almost all plants. The chemical formula is  $C_6H_2(OH)_3COOH$  or  $C_7H_6O_5$  and the molecular weight is 170.12. Salts and esters of gallic acid are termed "Galletes". Despite its name, it does not contain gallium. Gallic acid is commonly used in the pharmaceutical industry. It is used in the synthesis of the psychedelic alkaloid mescaline as a starting material. It is used as a standard for determining the phenolic content of various analytes in the Folin-Ciocalteu assay; results are reported in gallic acid equivalents. It seems to have anti-fungal and anti-viral properties. Gallic acid acts as an antioxidant and it helps to protect human cells against oxidative damage. Gallic acid extracted from grape may also benefit diabetes patients by triggering the release of insulin by

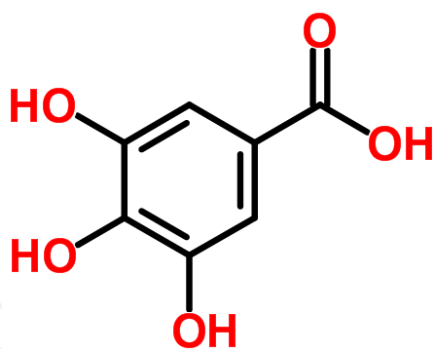


**Figure 1.** *Aspergillus* infection of the lung and air sacs [30]

the pancreatic cells. It exists in plant material in the form of free acids, esters, catechin derivatives and hydrolysable tannins. This ubiquitous chemical is one of the most biologically active phenolic compounds of plant origin. Antioxidant activity of gallic acid and its derivatives has been reported in several studies. Gallic acid has been shown to possess antimicrobial activity against human pathogens (*Staphylococcus aureus*, *Corynebacterium maccolans*) and plant pathogens (*Candida albicans*). The antifungal activity of gallic acid, isolated from *Oenothera biennis* roots, has been investigated [32]. Methylgallate has been demonstrated to show activity against a number of Gram-positive and Gram-negative bacteria and fungi. The cytotoxic effects of Triphala, an Indian herbal drug, on breast and prostate cancer cells have been attributed to gallic acid. Since gallic acid can act as a nucleophile, it can therefore scavenge electrophilic mutagens [33].

The poultry population of Nigeria is estimated at 140 million, the largest in Africa. The poultry industry is a major contributor to the economy. Production intensified steadily at a growth rate of 306.6% between 1999 and 2004 [34]. However, production within this sector is still below resource capacity. It is common to observe that, whereas broilers normally reach market weight at about six weeks in the West, in Nigeria market weights are rarely achieved before 10 weeks in intensive production. Mortality rates on a typical farm may also range between 10-15%, which further reduces farm profits [35]. Myriad factors have contributed to this under-utilization of capacity in this sector, e.g., disease, lack of technological know-how, etc.





Gallic acid source [17]

Currently, the most common methods of suppressing pathogens in animals have been treatment with antibiotics as a therapeutic agent and use of growth promoters, because these are readily available. However, the use of antibiotics in the treatment of animal disease is currently a subject of public health concern, as the development of resistant strains (superbugs) is a potential danger to humans. Furthermore, some of these antibiotics have recently been found to exhibit neurotoxic effects, while some others cause severe liver damage and bone marrow depression. Antibiotics are used mainly to protect poultry from pathogenic organisms and to enhance their growth and health. However, the emergence of antibiotic resistance in pathogenic bacteria has led to international reconsideration of the use of antibiotics in livestock [36]. Recommendations to ban sub-therapeutic use of antibiotics in animal feeds have been documented. Antibiotic resistance has been displayed by *Escherichia coli* isolates from commercial turkey farms, including resistance to Enrofloxacin, one of the most recently approved antibiotics for use in poultry [37]. With the recent ban on the use of sub-therapeutic antibiotics in the production of livestock by the EU [38], research attention has shifted towards the development of positive alternatives.

The use of plants for medicinal purposes predates the introduction of antibiotics and other modern drugs, and there has been renewed interest in natural products from higher plants which contain active ingredients of medicinal value. Scarcity and sale of fake and adulterated pharmaceutical drugs, which has been on the increase especially in the developing world, has made ethnoveterinary approaches even more attractive. The studies presented here present some alternative strategies to manage aspergillosis in poultry.

## 2. Study 1

### 2.1. Vitamins and amino acids

Broiler chicks were challenged with *Aspergillus flavus* via drinking water at the age of two weeks. Yellowish caseous deposits in the lung were established as confirmatory lesions of aspergillosis in the challenged birds [39]. The experiment included positive (non-challenged birds) and negative (birds challenged without dietary supplementations) control groups.

Dietary interventions of *Aspergillus*-challenged birds included vitamins A and C (A+C), methionine and lysine (METH+LYS), and vitamins A and C, lysine and methionine (A+C+METH+YS), which were incorporated into the basal diet formulated to meet the nutrient requirement [24] for broilers (Table 1).

## 2.2. Materials and methods

Commercial broilers of 120 days of age were used in this study. The chicks were weighed and randomly allotted to five treatment groups with three replicates of 24 chicks each. Birds were housed in an electrically heated metabolic battery cage. Routine management and vaccination procedures were followed. Feed and water were administered *ad libitum* for the 56-day feeding trial. Feed intake and weight gain were recorded weekly and used to determine the feed-to-gain ratio. Nutrient retention was determined at four weeks of age. Proximate analysis of the diet and faecal samples were determined according to the method given in [40]. At the end of the experiment, nine birds were selected for the treatments, denied feed overnight and slaughtered by severing the jugular vein. Blood samples were collected and used for haematological and serological indices according to [41], using Wintrob's microhaemometer improved Neubauer counter. Data obtained from the experimental trial were analysed using the completely randomized design [42]. Significant differences were subjected to the Duncan Multiple Range Test [43] at 0.05 probability.

Daily feed intake and feed conversion efficiency were influenced by the treatments (Table 1). The highest feed intake was observed for *Aspergillus*-challenged birds supplemented with A+C+METH+LYS, which compared favourably with the positive control birds. The lowest feed intake was observed for the negative control birds. Daily weight gain varied in response to dietary interventions of the challenged birds. The trend also followed the observation for feed consumption. Feed conversion efficiency was poorest for the negative control birds. Dietary interventions may have positively reduced the deleterious effects of aspergillosis on the performance of broilers. This effect was particularly pronounced for the *Aspergillus*-challenged broilers fed A+C+METH+LYS. It is also noteworthy that the performance of birds fed the supplemental combination A+C+METH+LYS compared favourably with the positive control groups. This diet may have stimulated the immune response of the broilers and thus enhanced their performance. According to [15], vitamins and protein supplementation in pigs has some proactive effect on the incidence of aflatoxicosis. In the same vein, [2] reported that providing a diet containing high protein and augmenting the ration with vitamin supplementation may be of value in mitigating the effects of *Aspergillus* infection. In the same vein, [42] reported the inability of vitamin supplements alone to totally prevent the negative effect of mycotoxin in broiler chicks.

The results of this study suggest that dietary vitamins A and C together with an increase in lysine and methionine can enhance the feed intake weight gain and feed conversion efficiency of broiler chickens infected with *Aspergillus flavus*. Thus, a combination of vitamin and protein supplementation may be an attractive alternative to on-farm use of vaccines. It may also serve as a contribution to the effective management of aspergillosis in poultry, since curative drugs have not been found to be effective in the control of the disease.

Diets	Feed intake	Weight gain (g/ bird/day)	Feed conversion ficiency (%)	Protein retention (%)	Fat retention (g/bird/ day)
Control	42.48 <sup>a</sup>	20.14 <sup>a</sup>	2.11 <sup>b</sup>	63.30 <sup>a</sup>	73.5 <sup>a</sup>
Control*	38.67 <sup>b</sup>	12.48 <sup>c</sup>	3.09 <sup>a</sup>	51.4 <sup>b</sup>	63.9 <sup>c</sup>
Vit. A+C	40.97 <sup>b</sup>	15.88 <sup>b</sup>	2.58 <sup>b</sup>	60.6 <sup>a</sup>	68.9 <sup>b</sup>
LYS +METH	39.08 <sup>b</sup>	15.14 <sup>b</sup>	2.58 <sup>b</sup>	53.9 <sup>b</sup>	66.6 <sup>b</sup>
Vit. A+C+ LYS + METH	42.81 <sup>a</sup>	18.37 <sup>a</sup>	2.33 <sup>b</sup>	61.1 <sup>a</sup>	72.0 <sup>a</sup>

a,b,c, values in the same column are similar (p>0.05)

\* challenged broilers without dietary intervention

**Table 1.** Dietary intervention and performance of *Aspergillus*-challenged broiler chicks

## 3. Study 2

### 3.1. *Vitellaria paradoxa*

*V. paradoxa* bark was collected and air dried for a period of two weeks. The extracts were pre-crushed in a mortar and later pulverized into fine powder. Extraction was done using cold water as the extraction liquid. Extraction was done in a rotary orbital shaker at 60 rpm for 24 hours. The mixture was further filtered through a sterile 0.45 µm Millipore filter. The filtrates were evaporated to semi-solid mass and subsequently dried to give a dark brown resinous mass. The dry extracts were later concentrated using a rotary evaporator. These dried extracts were reconstituted for antimicrobial activity evaluation.

The spore was established by growing a plate of *Aspergillus flavus* on a culture medium, which was left for three to seven days to sporogate. The spores were later scraped off the surface of the culture plate. The treatments were: Group 1, control; Group 2, infected and treated with antibiotics; Group 3, infected but no treatment (negative control); Group 4, infected and treated with 5 mg/ml of extract; Group 5, infected and treated with 10 mg/ml of extract.

### 3.2. Materials and methods

#### 3.2.1. Plant collection

The bark of the shea butter tree (*Vitellaria paradoxa*) was collected from a permanent site at the University of Ilorin. Ilorin is located at latitude 08 29'N and longitude 004 35'E. The elevation is 305 m 1001'. The annual temperature range is 22-34°C and the annual precipitation is 80-12 mm [44]. The plant was identified by experts from the University's Herbarium Unit. The bark was collected daily at 08:00 and air-dried to a constant weight. The samples were pre-crushed in a mortar, and then blended in an electric blender (Moulinex, Philips) to a fine particle (0.5 mm). 100 g of the sample was soaked in 500 ml of cold water for extraction. The mixture was

fitted to a rotary shaker and agitated at 60 rpm for four hours. The mixture was further filtered through a sterile 0.45 µm Millipore filter. The filtrates were evaporated to semi-solid mass and subsequently dried in a beaker on a water bath to give a dark brown resinous mass. The dry extracts were later concentrated using a rotatory evaporator (Model 349/2, Corning Limited) for the antimicrobial activity evaluation [45]. The extract was reconstituted to 5 mg/ml and 10 mg/ml and administered through drinking water.

### 3.2.2. Source of *A. flavus*

*A. flavus* spores were collected from the Department of Microbiology, University of Ilorin and grown on a plate on a culture of Potato Dextrose Agar (PDA), and incubated at 28°C for five to seven days. The spores were later scraped off the surface of the culture plate for inoculation.

### 3.3. Management of birds

Mixed-sex Hubbard broilers of 100 days of age were purchased from a commercial hatchery in Ilorin, Nigeria. The birds were brooded in an electrically heated metabolic cage and thereafter allotted randomly to five different treatments (Table 2). Each treatment was replicated in four pens containing five birds per replicate. The birds were given a basal diet (Table 1) and water *ad libitum* during the trial period. Routine vaccinations and medications were administered.

Ingredients	% Inclusion
Maize	37.0
Corn bran	6.0
Groundnut cake	24.0
Soyabean meal	24.0
Fishmeal	2.6
Bone meal	2.5
Oyster shell	1.5
Salt	0.2
*Vitamin/mineral premix	0.2
Total	100.0

Nutrient composition

Protein: 23.5%; Energy: 2700 kcal/kg;

\*Vitamin mineral premix contains antioxidant 125 mg, biotin 80 mg, choline chloride 500 mg, cobalt 240 g, copper 6 mg, folic acid 1000 mg, iodine 1.4 mg, selenium 240 mg, vitamin A 15,000 IU, vitamin B<sub>1</sub> 200 mg, vitamin B<sub>2</sub> 600 mg, vitamin B<sub>6</sub> 400 mg, vitamin D<sub>3</sub> 3000 IU, vitamin E 3000 IU, vitamin K 250 mg, zinc 60 mg, vitamin B<sub>12</sub> 20 mg.

**Table 2.** Composition of experimental diet (%DM)

Treatment	Infected with <i>A. flavus</i>	Supplemented with <i>V. paradoxa</i>	Supplemented with antifungal (furaprol)	Remark
1	-	-	-	Positive control
2	+	-	-	Negative control
3	+	-	+	Antifungal
4	+	+	-	5 mg/ml
5	+	+	-	10 mg/ml

**Table 3.** Composition of experimental treatments

### 3.4. Inoculation of chick feed with *A. flavus* spores

At the second week, bird feeds (except the positive control) were inoculated with the spores of *Aspergillus flavus*. The infected birds were placed under close observation for three to seven days, within which they would have manifested infections. Confirmation of infection was established by caseous yellow deposits in the lung.

### 3.5. Data collection

The experiment was conducted over six weeks. Body weights of broilers were determined weekly. Feed consumption and weight gain were recorded and feed conversion ratio (feed intake/weight gain) was calculated. Mortality was recorded daily. During the third week of the eight-week study, protein and fat nutrient retention were carried out for 72 hours.

Nutrient retention was calculated as follows:

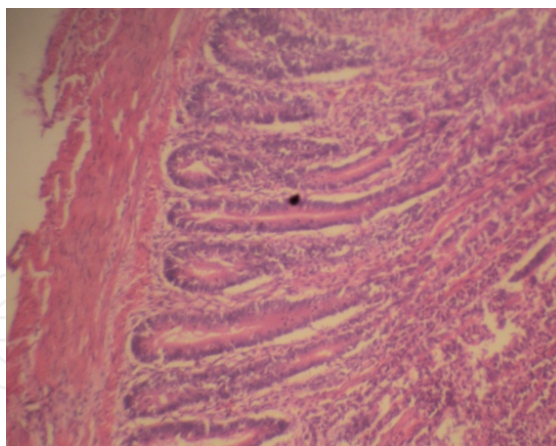
$$\text{Nutrient retention} = \frac{(\text{Nutrient consumed} - \text{Nutrient voided in faeces}) \times 100}{\text{Nutrient consumed}}$$

Items	Treatment groups				
	1	2	3	4	5
Av. Body weight gain (g/bird)	216.10 <sup>a</sup>	200.50 <sup>b</sup>	154.90 <sup>c</sup>	198.40 <sup>b</sup>	200.70 <sup>b</sup>
Feed intake (g/bird)	504.40 <sup>a</sup>	473.00 <sup>b</sup>	321.80 <sup>c</sup>	463.80 <sup>b</sup>	473.90 <sup>b</sup>
Feed:gain ratio	2.3 <sup>a</sup>	2.3 <sup>a</sup>	2.0 <sup>b</sup>	2.3 <sup>a</sup>	2.3 <sup>a</sup>

a,b,c values in the same column are similar (p>0.05)

**Table 4.** Effects of *Vitellaria paradoxa* on the performance of broiler chicks

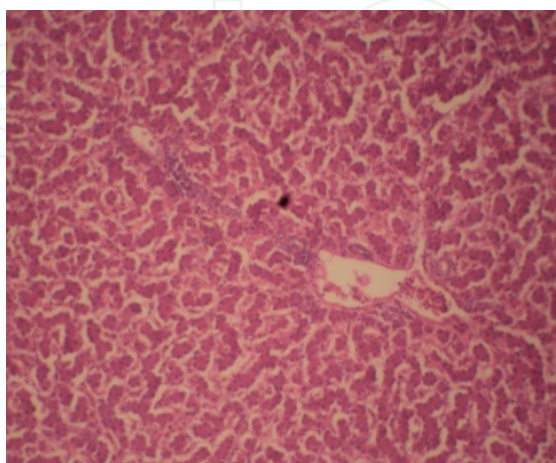
The production parameters average body weight gain, feed intake and feed:gain ratio were enhanced at the various levels of *V. paradoxa* interventions (Table 2). The negative groups showed the least performance. It was observed that the 10 mg extract (T5) was the most effective dose against *A. flavus*. At this level, histological architecture of specific organs (ileum, liver and breast muscle) was preserved (Figure 2).



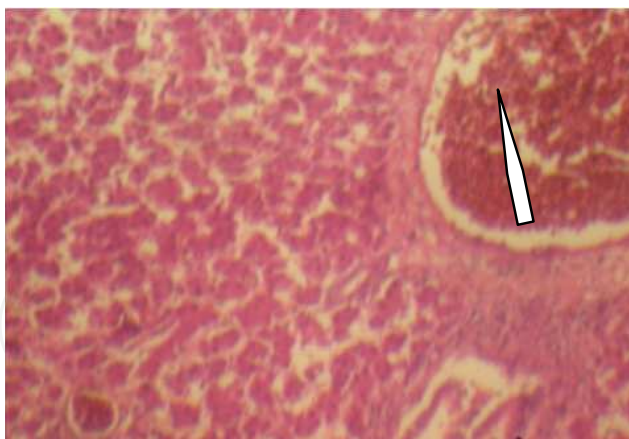
**Figure 2.** Micrographs of birds fed 10 mg extract of *V. paradoxa* showing normal ileal sections (X60)



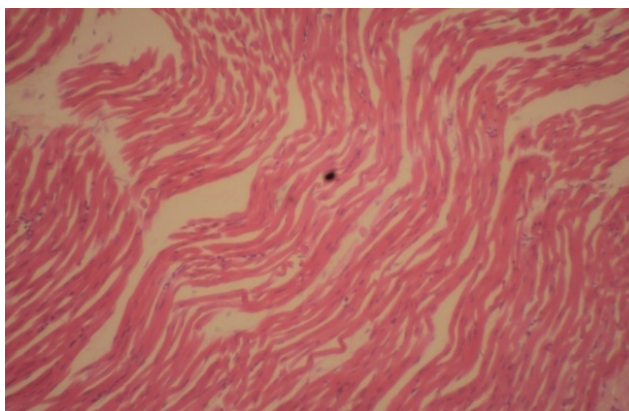
**Figure 3.** Micrographs of birds fed the negative control showing deranged ileal section (X60)



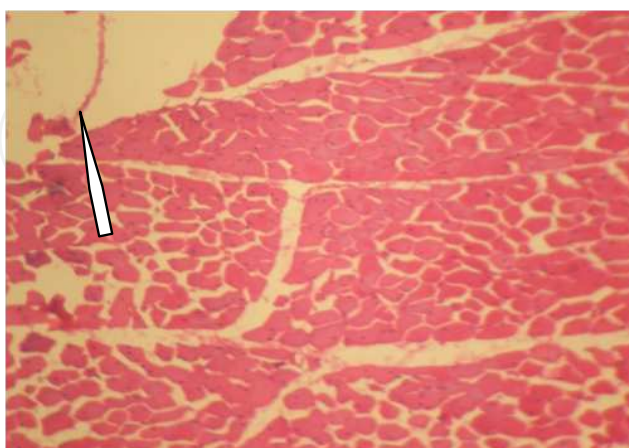
**Figure 4.** Micrographs of birds fed 10 mg extract of *V. paradoxa* showing normal liver sections(X40)



**Figure 5.** Micrographs of birds fed 10 mg extract of *V. paradoxa* showing abnormal liver sections (X60)



**Figure 6.** Micrographs of birds fed 10 mg extract of *V. paradoxa* showing normal breast muscles (X40)



**Figure 7.** Micrographs of birds fed 10 mg extract of *V. paradoxa* showing abnormal breast muscle sections (X60)

## 4. Study 3

### 4.1. Chloroform and butanol extracts of *Vitis vitifera* peel

The peeled rinds of *Vitis vitifera* were ground and mixed at 140 g/litre of water. Methanol was added and the mixture was decanted after 72 h and distilled. The distillates were further extracted using either chloroform or butanol. Gallic acid content of the extracts was separated with HPLC and concentrated. The concentration of gallic acid in the extract was determined. Pure gallic acid was dried out in a rotary evaporator. The extract was reconstituted and amended with the test extracts. The test fungus *Aspergillus fumigatus* was aseptically introduced into the growth medium and incubated at ambient temperature for seven days. The fungal growth was thereafter measured. Two controls (positive and negative) also constituted treatments. Chloroform and butanol extracts were administered to the inhibition test at 0, 50, 100, 150, 200 and 250 mg/ml

### 4.2. Materials and methods

#### 4.2.1. Source of Vine Grape

The vine grape used for the experiment is found growing naturally around the Tanke area in Ilorin, Kwara State, Nigeria. The fruits were picked carefully, selecting grapes free of physical damage and microbial attack.

#### 4.2.2. Preparation of crude extract of gallic acid

The fruits were peeled to remove the rind from the juicy part. The peels were ground manually using mortar and pestle. About 700 g of the ground peels was weighed into a 5 l container using an electronic balance. Two and half litres of methanol were added to the ground peel and left for 72 h to ferment. The sample was decanted into a flat-bottomed flask and distilled after the third day of fermentation using a water bath. Anti-bombing agent was added to the mixture during distillation to prevent bombing. The concentrate obtained was weighed.

For butanol extraction, a quantity of the concentrate obtained above (about 200 g) was mixed with 250 ml of butanol in a 500 ml conical flask. The mixture was shaken thoroughly manually for one hour and later allowed to settle in a separating funnel. The two liquids' layers were separated by gently running them off from the separating funnel. The butanol was distilled using a heater. This procedure was repeated for the chloroform extract but distillation was carried out using a water bath as the heating source. Phytochemical screening was carried out on the extracts to determine the presence of tannins, flavonoids and gallic acid.



#### 4.2.3. Test of anti-fungal property of the extracts

The fungal culture used (*Aspergillus fumigatus*) was obtained from the Department of Microbiology's laboratory at the University of Ilorin. It was routinely sub-cultured for purity during storage on a Potato Dextrose Agar (PDA) slant, and stored at 4°C until required for use.

#### 4.2.4. Reconstitution of the extract

The crude extracts were diluted with 12 ml of butanol and chloroform to obtain varying concentrations of 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, and 250 mg/ml. These were refrigerated until required for use.

#### 4.2.5. Preparation of PDA and its amendment with the extracts

Thirty-nine [39] grams of PDA powder was dissolved in 1000 ml of sterile distilled water in a conical flask. The suspension was heated to homogenize it and the flask was plugged with cotton wool, wrapped with aluminium foil and autoclaved at 121°C for 15 minutes. The medium was amended with the extracts at the designated concentrations, i.e., 50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml and 250 mg/ml. The control treatment was PDA only, without the extract.

#### 4.2.6. Determination of the growth of the test fungus

The test fungus was aseptically introduced into the growth medium in petri dishes (9 cm diameter). The dishes were incubated at ambient temperature for seven days, after which growth was determined by measuring the diameter of the fungus following two perpendicular lines passing through the centre of the dish.

#### 4.2.7. Results

The chloroform extract of *V. vitifera* peel had partial (26%) percentage inhibition on *A. fumigatus* (Table 5). The butanol extract of gallic acid was observed to completely suppress *A. fumigatus* growth, i.e., 100% inhibition (Figures 3-7) Gallic acid acts as an antioxidant and helps to protect human cells against oxidative damage. Gallic acid extracted from grapes may also benefit diabetes patients by triggering the release of insulin by the pancreatic cells. It exists in plant material in the form of free acids, esters, catechin derivatives and hydrolysable tannins.

Extract	Concentration mg/ml						% inhibition
	0	50	100	150	200	250	
Butanol	0.00	0.00	0.00	0.00	0.00	0.00	100
Chloroform	10.75	8.26	6.84	16.43	14.07	8.01	26

**Table 5.** Inhibition of *A. fumigatus* growth by the different gallic extracts



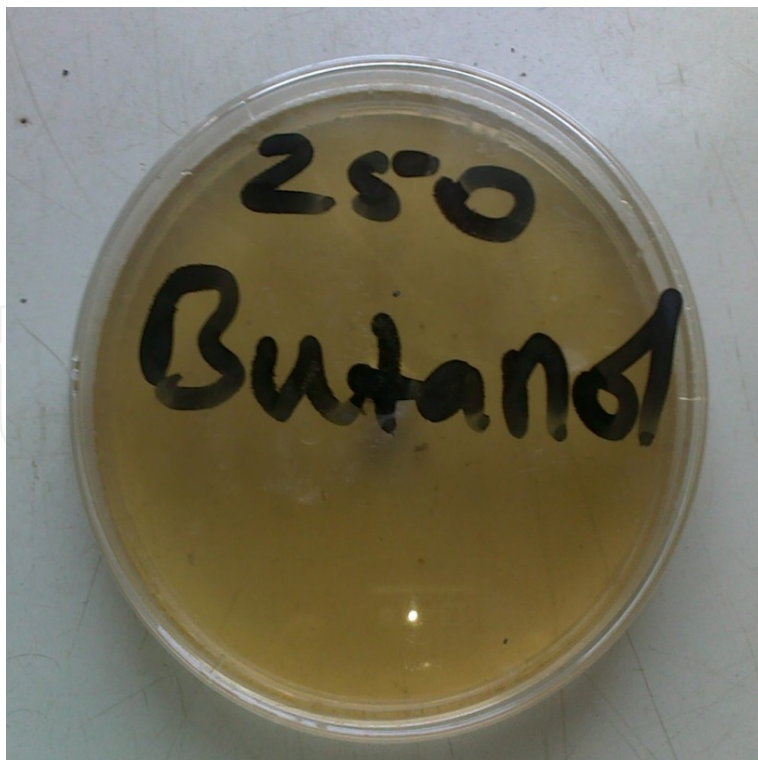
**Plate 1.** Control treatment



**Plate 2.** Negative control



**Plate 3.** Butanol extract (200 mg/ml) showing *Aspergillus* inhibition



**Plate 4.** Butanol extract (250 mg/ml) showing 100% inhibition



**Plate 5.** Chloroform extract (200 mg/ml) showing partial inhibition

## 5. Conclusion

Herbs and spices are known to exert antimicrobial actions *in vitro* against important pathogens including fungi [46]. The active substances are largely the same as those mentioned previously for antioxidative properties, with phenolic compounds being the principle active components [47].

The antimicrobial mode of action is considered to arise mainly from the potential of the hydrophobic essential oils to intrude into the bacterial cell membrane, disintegrate membrane structures, and cause ion leakage. High antibacterial activities are reported also from a variety of non-phenolic substances, for example, limonene and compounds from *Sanguinaria canadensis* [47]. Microbiological analysis of minimum inhibitory concentrations (MIC) of plant extracts from spices and herbs, as well as of pure active substances, revealed levels that considerably exceeded the dietary doses when used as phytogetic feed additives (Burt et al., 2004). This may indicate that antimicrobial action of phytoGENICS should not contribute significantly to the overall efficacy of this class of feed additives. On the other hand, some studies with broilers have demonstrated *in vivo* antimicrobial efficacy of essential oils against *E. coli* and *Clostridium perfringens* [48].

With the current emphasis on the use of alternatives to chemicals and antibiotics in the treatment of livestock diseases, potent materials of natural origin are becoming attractive. The strategies documented in this paper lend credence to the fact that livestock diseases can be

managed in a more robust manner than with non-biodegradable chemicals that are potentially a danger to public health. Generally, vitamins A and C combined with lysine and methionine act as an immune modulator, which can be adapted as an alternative to on-farm use of vaccines in poultry in the management of aspergillosis. Botanicals such as extracts of *Vitellaria paradoxa* and *Vitis vinifera* can be incorporated for robust control of aspergillosis in poultry production. Further studies on potential pharmacological, biochemical and physiological effects, for example in relation to safety limits, target organs of the active substances, side effects (especially on non-target organs), and so on, is imperative if the benefits of these botanicals are to be successfully harnessed.

## Author details

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