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Effects of *Monodora myristica* Spice and *Aspergillus flavus* in Broiler Diet on Chemical Composition, Feed cost and liver Injury in Broiler Chickens

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Abstract
A research work that lasted for 42 days was conducted to evaluate the hepatoprotective effect, feed cost and chemical composition of *Monodora myristica* in *Aspergillus flavus*-infected broiler diets on broiler chickens. It was a 2x3 factorial experiment in a completely randomized design to evaluate the cost implication and the hepatoprotective effects of *Monodora myristica* and *A. flavus* in broiler diets. Three out of six treatments designated as T_1, T_2 and T_3 were infected with 2mg of pure culture of *Aspergillus flavus* fungi, obtained from the plant pathology department of the National Root Crop Research Institute, Umudike. Toasted *M.myristica* was included at 0%, 0.5% and 1.0% to T_1, T_2 and T_3 respectively. The remaining three diets designated as T_4, T_5 and T_6 were *A.flavus*-free and also had 0%, 0.5% and 1.0% toasted *M.myristica* respectively. Each treatment was replicated 3 times with 30 chickens per replicate. Toasting improved ash and nitrogen-free extract of *M.myristica*. Vitamin B_2 tannins, flavonoids, phenol and saponin were reduced by toasting the spice, while, the minerals except copper, zinc, iron and lead were increased in the toasted *Monodora myristica*. A 1.0% *Monodora* increased the crude fibre content of the diets. Cost per kg weight gain was higher in birds fed infected diets, thus reducing the gross margin. *A.flavus* recorded high levels of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvic transaminase (SGPT). However, in the absence of infection, the impact of *Monodora myristica* was not effective ($P<0.05$) beyond 0.5%. *Monodora myristica* could be beneficial in *A. flavus*-infected broiler diets.

Keywords: Aspergillus flavus, Monodora myristica, serum glutamate oxaloacetate transaminase, serum glutamate pyruvic transaminase, alkaline phosphatase

Introduction

Poultry is the quickest source of meat for the teaming population such as ours. Its production is less hazardous than other livestock enterprises. Recently, there is a downturn in the production scale in Nigeria due to high cost of livestock feed and feed ingredients. Poultry farmers are frequently faced with an increasing challenge of poor feed storage facilities. Most often, feed ingredients spoil because of mould infestation and they develop off flavours which lead to reduction in feed intake or outright rejection by the birds. Such feeds, when consumed by birds pose serious health challenges such as Mycotoxicosis and attack on vital organs such as the liver. *Aspergillus flavus* is the most common fungal infestation producing mycotoxins which when consumed by birds become deleterious to their health. *Monodora myristica* is a spice belonging to the family-*myristicaceae*. It is planted locally in Nigeria. The use of spices such as *Monodora myristica* as feed additive have gained wide acceptance for cattle, sheep, pig, and other farm stock, but is yet to gain much popularity and acceptance in poultry production in Nigeria and beyond. Scientists have almost always been cautious or even reluctant in using *M. myristica* and other spices as flavours in poultry feeds because of the argument that chickens lack well developed taste buds (Holdas and May, 1966). But this assertion was refuted by the report of Esmail (2004) that chickens find the tannin in sorghum distasteful. This study was aimed at evaluating the chemical composition of the spice, diets containing the spice and *A. flavus*, the cost implications of supplementing *Monodora myristica* to *Aspergillus flavus* –infected broiler diets and hepatoprotective effect of the spice in *A.flavus* induced liver injury.

Materials and Methods

Location

The study was carried out at the Poultry Unit of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria (MOUAU). The area falls within the Tropical rain forest zone, it is located at latitude 05° 21ˈN and longitude 07° 33ˈE, its elevation is about 112m above sea level. It has an average Rainfall of about 2177mm/annum, Relative Humidity of about 50-90% and a monthly temperature range of 17°-36°C (Meteorological Station, NRCRI, Umudike, 2018).

Duration of study and Experimental Materials

The research work lasted for 42 days and aimed at evaluating the hepatoprotective effect of *Monodora myristica* in *Aspergillus flavus*-infected diets on broiler chickens and feed cost and chemical composition of the infected diet supplemented with *Monodora myristica.* The experiment was conducted in two broad phases. Phase 1 involved the evaluation of the chemical composition of raw and toasted *Monodora myristica* and the experimental diets. *Monodora* myristica was toasted at a temperature of 65°C for 4 hours. Phase 2 involved a 2x3 factorial experiment in a completely randomized design. It evaluated the cost implication and the hepatoprotective effects of *Monodora myristica* and *Aspergillus flavus* in broiler diets. Seeds of *M.myristica* were purchased from Ndoro market in Ikwuano Local Government Area (LGA) of Abia State. Some were toasted and milled, while others were milled raw and used for chemical composition. Proximate composition of both raw and toasted *Monodora myristica* were determined using the procedure of A.O.A.C. (1990). Minerals and vitamins were determined according to the outline by Okwu (2004), while anti nutritional factors were determined according to the procedure described by Obadori and Ochuko (2001). The toasted *Monodora* was used for formulation of the experimental diets. Three out of six treatments designated T_1, T_2 and T_3 were infected with 2mg of pure culture of *Aspergillus flavus* fungi, obtained from the plant pathology department of the National Root Crop Research Institute, Umudike. Toasted *M.myristica* was included at 0%, 0.5% and 1.0% to T_1, T_2 and T_3 respectively. The remaining three diets designated T_4, T_5 and T_6 were *A.flavus*-free and also had 0%, 0.5% and 1.0% respectively. Each treatment was replicated 3 times with 30 chickens per replicate. All routine vaccination and management practices were carried out. Cost analysis was carried out according to the outline by Ukachukwu and Anugwa (1995).

Statistical analysis and model

The data collected were subjected to analysis of variance (ANOVA) in a 2x3 factorial arrangement in a completely randomized design experiment. Significant differences were observed, and means further subjected to Duncan's multiple Range Test (Duncan, 1952) analysed with SPSS (2006) for windows, version 16, SPSS Inc.

$$Y_{ijkl} = \mu + A_i + M_j + (AM)_{ij} \sum_{ijk}$$

μ = population mean
A_i= effect of infection of *Aspergillus flavus*
M_j= effect of inclusion level of *Monodora myristica*
$(AM)_{ij}$ = interaction of infection of *Aspergillus flavus* and inclusion level of *Momondora myristica*
$\sum_{ijk}$ = Residual error estimate

Results and Discussion

Chemical composition

The proximate composition of raw and toasted *Monodora myristica* is presented in Table 2. Raw *M. myristica* had 89.90% dry matter, 15.75% CP, 28.60% ether extract, 9.64% crude fibre, 8.84% ash and 37.17% NFE, while toasted *M. myristica* recorded 91.76% dry matter, 25.38% CP, 21.54% ether extract, 6.24% crude fibre, 9.40% ash and 37.44% NFE. The results indicate that toasting *Monodora* reduced the moisture content, ether extract and crude fibre, while it improved the crude protein, ash and nitrogen free extract of the spice. The reduction in the ether extract could be due to the volatilization of oil due to the heat from toasting. The reduction in crude fibre of the toasted *Monodora* seeds indicate that toasting could reduce the fibrous and less digestive carbohydrate fraction of the spice from 9.64% to only 6.24%. The low ether extract due to volatilization of oil explains the low value of gross energy observed in the toasted *Monodora* since fats high specific energy contribute about two and half as much energy as carbohydrates, or protein in feeds according to McDonald *et al.* (1995). The crude protein, ash and NFE values observed in this study were higher than those reported by Okwu (2001). The difference could probably be from the processing method applied. The spice used in this experiment was toasted at a temperature of 65°C for 4 hours, while Okwu (2004) used sun drying method.

Vitamin and Mineral Composition of Monodora myritica

Table 3 summarizes the vitamins and mineral composition; raw *M.myristica* was observed to have 184.60g/100g ascorbic acid, 0.77mg/100g thiamin, 0.15mg/100g riboflavin and 18mg/100g niacin, while toasted *M.myristica* recorded 240.46g/100g ascorbic acid, 0.98, 0.14 and 16.44mg/100g thiamin, riboflavin and niacin respectively. Calcium, magnesium, sodium, potassium, phosphorus and nitrogen recorded 4.62%, 2.01, 0.45, 1.2, 0.61 and 2.52% respectively for the raw spice, while the toasted spice recorded 5.22%, 2.01, 0.33, 1.38, 0.76 and 4.06% for Calcium, magnesium, sodium, potassium, phosphorus and nitrogen in that order. There was an improvement in the ascorbic acid and thiamin due to toasting. Toasting also improved the calcium, potassium, nitrogen and phosphorus contents, while a reduction was observed in the zinc and iron contents. The high value of ascorbic acid in the toasted spice is an indication that the toasted spice can protect the birds from oxidative damage by scavenging free radicals more than the raw *Monodora myristica.* This can be an indication that the toasted spice could be useful in reduction of stress in birds. The high values of thiamin are an indication that the appetite of the birds

could be improved since thiamin deficiency has been reported to cause poor appetite (McDonald *et al.*, 1995).

Anti-nutritional Factors in Monodora myristica

Table 4 shows the anti-nutritional constituents of raw and toasted *Monodora myristica*. Raw *M.myristica* was observed to have 3.84% alkaloid, 12.88% flavonoid, 0.13% saponin, 0.32% tannin and 0.15% phenol, while the toasted spice recorded 4.14% alkaloid, 11.45% flavonoid, 0.29% saponin, 0.18% tannin and 0.05% phenol. The raw *M.myristica* had higher values of flavonoid, saponin, tannin and phenol. The higher level of flavonoid in the raw spice may imply higher anti-inflammatory activity than the toasted spice in line with the report of Okwu (2001). Flavonoids from spices have been reported to have been effectively used in the treatment of arthritis in herbal medicine (Okwu, 2001). Flavonoids from *Magnifera indica* has been reported to aid protection against allergies, inflammation and free radical scavengers (Okwu 2004). Saponin was reported to have the ability to lower plasma cholesterol according to Osagie (1998). Total phenol was higher in the raw *M.myristica* than in the toasted seeds of *M.myristica.* The value of the toasted *Monodora* was similar to that reported by Castelli (1998). The alkaloid and phytate contents were observed to be higher in the toasted *Monodora* seeds. It could be that toasting was not adequate to reduce the alkaloid as much as boiling as reported by Osagie *et al.* (1996). Alkaloids have been reported to be highly poisonous (McDonald *et al.*, 1995). The anti-nutritional contents observed in this experiment were higher than those reported by Okwu (2001; 2004) and Achinewhu *et al.* (1995).

Effect of Aspergillus flavus and Monodora myristica on the proximate composition of Broiler diets

The determined proximate composition of the *Aspergillus flavus*-infected *broiler* diets supplemented with toasted *Monodora myristica* and their interactions are summarized in Tables 5 and Table 6. The diet infected by *Aspergillus flavus* was observed to have 24.37%CP, 8.77% CF, 42.68% NFE and 2972.8gcal/kg G.E, while the non-infected diets recorded 25.42%CP, 9.68%CF, 45.97%NFE and 2996.98g cal/kg G.E. Crude protein, ether extract, ash, crude fibre, NFE and gross energy recorded 25.18%, 2.85%, 10.33%, 9.28%, 43.36% and 2997.40g cal/kg; 24.70%, 2.66%, 11.17%, 9.46%, 42.27% and 2936.28g cal/kg G.E and 24.80%, 3.62%, 10.64%, 8.94%, 42.85% and 2991.01g cal/kg for 0%, 0.5% and 1.0% *Monodora myristica* respectively. The interaction effects were significant ($P<0.05$) for crude protein, crude fibre and NFE. The infected diet with 0% , 0.5% and 1.0%*Monodora myristica* had crude protein content of 24.70%, 24.60% and 23.80% respectively, while supplementing 0%, 0.5% and 1.0% *M.myristica* to non-infected diets recorded crude protein content of 25.65%, 24.80% and 25.80% in that order.

Average crude protein, crude fibre, NFE and gross energy contents were reduced by *Aspergillus flavus* infection. The *Aspergillus flavus* infection was observed to increase the ether extract and ash contents of broiler diets. The effect due to inclusion level of *M.myristica* did not follow any definite pattern. A 0% *M.myristica* recorded highest values of crude protein, nitrogen free extract and gross energy. A 0.5% *M.myristica* enhanced crude fibre content of diets, while the highest value of ether extract was achieved with 1.0% *M.myristica*. From the interactions of *A.flavus* infection and varying levels of *M.myristica*, it was observed that inclusion of *M.myristica* in the infected diet did not increase the crude protein content except at 1.0%. The crude protein of the non-infected diet was reduced by adding 0.5% *M.myristica*, however, 1.0% *M.myristica* in the absence of *A.flavus* recorded a better crude protein content. The ether extract of infected diets increased ($P<0.05$) as *M.myristica* level increased, while in the non-infected diets, ether extract decreased with an increase in *M.myristica.* Infected control diet had lower ether extract than the non-infected control, but inclusion of 0.5% and 1.0% *M.myristica* to both the infected and non-infected enhanced the ether extract contents of the infected diet more than the non-infected diets. Crude fibre, in the non-infected diets seemed to decrease as the level of inclusion of *M.myristica* increased. The level of inclusion of *M.myristica* in the diets did not seem to cause any tangible changes on the crude protein of the diets as can be seen in the table. The low crude fibre and NFE observed in the *A.flavus*-infected diets without *M.myristica* could suggest low carbohydrate due to the fact the *A.flavus* may have been feeding much on the carbohydrates. From the results, the crude protein contents were reduced by *A.flavus* and could not be remedied by inclusion of *M.myristica.* However, the crude protein content of all the diets were higher than the range of 20 -23% and 20 -22% for broilers according to Oluyemi and Robert (2000) and Obioha (1992). The low values of the chemical components, especially crude protein in the infected diets could be as a result of proteolysis caused by *Aspergillus flavus* through the actions of some protease enzymes.

Effect of Aspergillus flavus and Monodora myristica on the vitamin composition of Broiler diets

Tables 7 and Table 8 show the vitamin composition of *Aspergillus flavus* – infected and non-infected diets supplemented with or without *Monodora myristica.* Vitamins C, B_1, B_2 and niacin were 4.77mg/100g, 0.037mg/100g, 0.060 and 0.029mg/100g for the infected diets respectively, while the non-infected diets recorded 6.58, 0.047, 0.054 and 0.028mg/100g in that order. The effect of different levels of inclusion of *M.myristica* resulted to diets with 4.82mg/100g Vitamin C, 0.024mg/100g Vitamin B_1, 0.06mg/100g vitamin B_2 and 0.028mg/100g niacin for the *M.myristica*-free diet. A 0.5% *M.myristica* diet had 5.06mg/100g vitamin C, 0.037mg/100g vitamin B_1, 0.053mg/100g vitamin B_2 and 0.032mg/100g niacin, while 1.0% *M.myristica* in the diet increased the vitamin C, vitamin B_1 and vitamin B_2 to 7.15mg/100g, 0.55mg/100g and 0.58mg/100g respectively. From the interaction results, infected diet without *M.myristica* had lower vitamin C and niacin of 4.34 and 0.024mg/100g respectively, than the non-

infected diet with 0%*M.myristica* which recorded 5.30 and 0.031mg/100g for same vitamin C and niacin in that order. Infected diet with 0.5% *M.myristica* recorded 5.60mg/100g vitamin C, 0.04mg/100g vitamin B_1, 0.029mg/100g vitamin B_2 and 0.036mg/100g niacin. Similar quantity of *M.myristica* in non-infected diet had 4.52mg/100g, 0.065mg/100g, 0.045 and 0.028mg/100g for vitamins C, $B_1$and B_2 and niacin respectively. Meanwhile, supplementing infected diet with 1.0% *M.myristica* resulted to 4.37mg/100g vitamin C, 0.071mg, 0.046 and 0.026mg/100g vitamins B_1, B_2 and niacin respectively. A 1.0% *M.myristica* in the non-infected diet had 9.93mg/100g vitamin C, 0.045mg/100g vitamin B_1, 0.063 and 0.025mg/100g vitamin B_2 and niacin respectively.

Vitamin C and Vitamin B_1 (thiamin) were higher in the non-infected diets than those observed in the infected diets. However, Vitamin B_2 (riboflavin) and niacin were higher in the fungal infected diets. Vitamins C and B_1 increased as the level of inclusion of *M.myristica* increased in the diets. The presence of *Aspergillus flavus* fungi in the diet caused a reduction in the ascorbic and thiamin levels, but did not affect riboflavin and niacin. Riboflavin can be synthesized by fungi and most bacteria (McDonald *et al.*, 1995); and the high level of riboflavin observed in the infected diet could be traced to the ability of these fungi to synthesize vitamin. This must have boosted their levels in the infected diets. Ordinarily, the vitamin C levels in the diets were influenced by the quantity of *Monodora* in the diet. Vitamin C was observed to increase as the inclusion level of *M.myristica* increased in the diet. In the presence of *Aspergillus flavus*, 0.5% was observed to boost the vitamin C content of the diet, beyond which there was a reduction. Thiamin decreased below the control at 0.5% inclusion level of the spice, but was elevated by increasing *M.myristica* to 1.0%. However, thiamin (vitamin B_1) increased to the highest level by supplementing the non-infected diet with 0.5% *M.myristica.* Riboflavin was higher in the infected control diet than in the non-infected control. At 0.5% and 1.0% levels of inclusion of *M.myristica*, non-infected diets recorded higher riboflavin than the infected diets at equal levels of *M.myristica*. The high level of riboflavin in the infected control as observed against the non-infected control could be attributed to the synthesis of the vitamin by *A.flavus* and this may have been interrupted by the presence of *M.myristica.* The interaction of *A.flavus* and *M.myristica* at two levels (0.5% and 1.0%) must have caused some reactions that brought about a reduction in the riboflavin as shown in Table 6. Niacin which was lower in the infected control diet than in the non-infected control was improved by 0.5% *M.myristica* beyond which no further improvement was realized. In the non-infected diets, niacin decreased as the level of inclusion of *M.myristica* increased in the diet.

Cost implication of Monodora myristica supplementation in Aspergillus flavus infected and non-infected Broiler Diets

The infection, inclusion level of *M.myristica* and their interactions were significant (P<0.05) with respect to cost per kilogram weight gain and gross margin as shown in Tables 9 and 10. The cost per kilogram weight gained and gross margin for the infected diets were **N**357.54 and **N**1,301.84 respectively, while the non-infected diets were observed to have **N**306.14 and **N**1518.91 cost per kilogram weight gain and gross margin in that order. *M.myristica*-free diet had the highest (P<0.05) cost per kilogram weight gain of **N**380.70. This decreased (P<0.05) as the level of inclusion of *M.myristica* increased from 0.5% to 1.0%. Gross margin followed similar trend with **N**23.54, 25.77 and **N**31.74 for 0%, 0.5% and 1.0%. *M.myristica* respectively. The interactions revealed that the infected diet supplemented with 0% *M.myristica* significantly (P<0.05) recorded highest cost per kilogram weight gained (**N**431.43) by the bird. Increasing the inclusion level in the infected diets reduced the cost to **N**322.76 and **N**318.40 and increased gross margin up from **N**1,453.27 to **N**1,545.30 for 0.5% and 1.0% *M.myristica* respectively. For birds fed the non-infected diet, highest cost per kilogram weight gained was recorded in the diet with 0% *M.myristica.* The lowest cost per kg weight gain of **N**270.66 was observed in the diet that had 1.0% *Monodora myristica.* The high cost per kilogram weight gain observed in the infected control diet could be attributed to poor feed conversion ratio due to poor utilization of the nutrients. This must have resulted in poor conversion of feed to tissue mass. It implies that much feed was consumed for the bird to gain 1kg body tissue. Cost of feed contributes about 80% of total production cost as reported by Igboeli (2000). Efficiency of the infected diet without *M.myristica* was poor. The feed was not properly utilized probably due to poor digestion that resulted in low availability of nutrients to the birds. Thus, *Aspergillus flavus*-infected diet with 0% *M.myristicai* increased cost per kilogram gain in weight in line with the report by Ukachukwu and Anugwa (1995). The result indicate that supplementing infected diet with *M.myristica* at 0.5% and 1.0% could improve the economic value of such diet, thereby making it comparable to non-infected diet.

Effect of Monodora myristica in Aspergillus flavus-infected Broiler diet on Liver injury

The hepatoprotective activity of *Monodora myristica* in *Aspergillus flavus*-induced liver injury is shown in Table 8. The Serum enzymes evaluated for this include serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvic transaminase (SGPT) and alkaline phosphatase. The main effects of infection status were significant (P<0.05) on SGOT and SGPT only. Alkaline phosphatase was not affected (P>0.05) by *Aspergillus flavus,* level of *Monodora myristica* inclusion nor their interactions. The SGOT and SGPT levels were 241.83iμ/l and 12.51 iμ/l respectively, for the birds fed the *A.flavus*-infected diets. In the non-infected diets, 222.00iμ/l SGOT and 10.95iμ/l SGPT were observed. A

0%, 0.5% and 1.0% level of *Monodora myristica* recorded 289iμ/l, 204iμ/l and 202.76iμ/l SGOT and 13.25iμ/l, 11.25iμ/l and 10.68iμ/l SGPT respectively. The infected control group with 0% *M.myristica* had highest (P<0.05) levels of 312iμ/l SGOT and 14.00iμ/l SGPT. A 0.5% *M.myristica* produced 208.50iμ/l SGOT and 12.50iμ/l SGPT. Levels above 0.5% did not make any significant difference (P>0.05). Also, in the birds fed non-infected diets, 0.5% *M.myristica* recorded significantly (P<0.05) lower SGPT (10.00iμ/l) than the control having 12.50iμ/l. SGOT and SGPT were elevated in the *A.flavus*-infected diets more than the non-infected diets. These parameters were observed to decrease as level of inclusion of *M.myristica* increased above 0% in the diets. The reduction in SGOT and SGPT both in infected and non-infected diets was in agreement with the findings of Tatsuya *et al.* (2003) on *Myristica fragrans* which prohibited the elevation of these enzymes in mice with liver injury induced by intravenous administration of LSP/D-GaIN. Femandez *et al.* (1994) also reported an increase in serum glutamate oxaloacetate transaminase due to induced liver damage by aflatoxin in laying birds and reduction by turmeric spice powder. Akila *et al.* (1998) reported an increase in SGOT in chickens fed turmeric for 21 days. Geeson *et al.* (1990) reported that curcumin was able to reduce Liver Thiobarbituric Acid Reactive Substances (TBARS) caused by chronic administration of carbon chloride which caused hepatic damage. The high levels of SGOT and SGPT in the infected diets and the *Monodora*-free diets could suggest a skeletal or cardiac muscular injury implicating necrosis and myocardial infarctions from the *A.flavus*. They were reduced by *M.myristica* supplementation to the diet. In summary, toasting *Monodora myristica* improved the chemical constituents. Supplementing *M.myristica* also enhanced the cost of production of broiler birds and improved the activities of serum enzymes related to liver injury.

Conclusion

From the experiments, the inclusion of Monodora myristica to broiler diets especially fungi-infested diets between 0.5% up to 1.0% could be beneficial to the birds and the farmer. From the results, highly *Aspergillus flavus*-infected diets could need higher levels of *M.myristica* than just 1.0%.

Table 1: Composition of *Aspergillus flavus*-infected Broiler diet supplemented with *Monodora myristica*

	Infected diets			Non-infected diets		
Ingredients	0%	0.5%	1.0%	0%	0.5%	1.05
Maize	53.00	53.00	53.00	53.00	53.00	53.00
Soybean meal	20.00	20.00	20.00	20.00	20.00	20.00
Fishmeal	2.00	2.00	2.00	2.00	2.00	2.00
GNC	12.00	11.50	11.00	12.00	11.50	11.00
PKC	3.00	3.00	3.00	3.00	3.00	3.00
Wheat offal	4.25	4.25	4.25	4.25	4.25	4.25
Monodora myristica	0.00	0.50	1.00	0.00	0.50	1.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00
Oyster	2.00	2.00	2.00	2.00	2.00	2.00
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Vit/min premix	0.25	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**	**100**
Calc ME (kcal/kg)	2912.35	2901.37	2890.40	2912.35	2901.37	2890.40
CP (%)	21.68	21.58	21.49	21.68	21.58	21.49
Ca (%)	1.89	1.91	1.94	1.89	1.91	1.94
P (%)	0.94	1.13	0.43	0.94	1.13	0.43
Lys (%)	1.13	1.12	1.12	1.13	1.12	1.12
Meth (%)	0.43	0.42	0.42	0.43	0.42	0.42
CF (%)	3.70	3.71	3.71	3.70	3.71	3.71

Vitamin/mineral premix supplying the following per kg diet: vit A (1599 I.U.), vit D_3 (1600.U), Riboflavin (9.0mg), Biotin (0.25mg), Pantothenic acid (11.0mg), vit K (3.0mg), vit B_2 (2.5mg), vit B_6 (0.3mg), vit B_{12} (8.0mg), Nicotinic acid (8.0mg), iron (5.0mg), selenium (0.01mg), Magnesium (10.0mg), zinc (4.5mg), Cobalt (0.02mg)

Table 2: Proximate composition of *Monodora myristica* (%DM)

Nutrient	Raw	Toasted
Moisture Content (%)	10.10	8.24
Dry matter (%)	89.90	91.76
Ash (%)	8.84	9.40
Crude fibre (%)	9.64	6.24
Ether Extract **(%)**	28.60	21.54
Crude protein (%)	15.75	25.38
NFE (%)	37.17	37.44
Gross energy (g cal/kg)	469.08	445.14

Values in the table are means of triplicate determinations

Table 3: Vitamin and mineral composition of *Monodora myristica*

Parameter	Raw	Toasted
Ascorbic acid (g/100g)	184.60	240.46
Thiamin (mg/100g)	0.77	0.98
Riboflavin (mg/100g)	0.15	0.14
Niacin (mg/100g)	18.00	16.44
Mineral Composition		
Ca (%)	4.16	5.22
Mg (%)	2.01	2.01
Na (%)	0.45	0.33
K (%)	1.20	1.38
P (%)	0.61	0.78
N (%)	2.52	4.06
Cu (%)	23.24	19.15
Zn (%)	98.20	25.26
Fe (%)	158.24	145.10
Pb (%)	0.31	0.26

Values are means of triplicate determinations

Table 4: Anti-nutritional constituents of *Monodora myristica*

Parameter	Raw	Toasted
Alkaloids (%)	3.84	4.14
Flavonoid (%)	12.88	11.45
Saponin (%)	0.32	0.29
Phenol (%)	0.15	0.05
Tannin (%)	0.32	0.18

Values in the table are means of triplicate determinations

Table 5: Effect of *Aspergillus flavus* and *Monodora myristica* on Proximate composition of broiler diet

Factor	CP (%)	EE (%)	Ash (%)	CF (%)	NFE (%)	G.E (g cal/kg)
Infection status						
Infected	24.37^{b}	3.53	11.05	8.77^{b}	42.68^{b}	2972.81^{b}
Non-infected	25.42^{a}	2.55	10.37	9.68^{a}	42.97^{a}	2976.98^{a}
SEM	0.01	0.11	1.15	1.21	0.41	2.17
***Monodora myristica* level (%)**						
0	25.18	2.85	10.33	9.28	43.36	2997.40
0.5	24.70	2.66	11.17	9.46	42.27	2936.28
1.0	24.80	3.62	10.64	8.94	42.85	2991.01
SEM	1.01	0.11	1.15	1.21	5.41	20.17

Values are means of triplicate determinations. CP, EE, CF, NFE and G.E represent crude protein, ether extract, crude fibre, nitrogen-free extract and gross energy respectively

Table 6: Interaction of *Aspergillus flavus* and *Monodora myristica* on proximate composition of broiler diet

Inf.Status	*M.m* Level (%)	CP (%)	EE (%)	Ash (%)	CF (%)	NFE (%)	G.E (g cal/kg)
Inf	0	24.70^{b}	2.81^{c}	10.82^{b}	8.74^{b}	43.19^{c}	2968.50^{ab}
N.Inf	0	25.65^{a}	2.89^{b}	9.84^{c}	9.82^{a}	43.52^{a}	3026.30^{a}
Inf	0.5	24.60^{b}	2.95^{b}	11.71^{a}	9.16^{a}	41.51^{ab}	2909.90^{c}
N.Inf	0.5	24.80^{ab}	2.37^{d}	10.62^{b}	9.76^{a}	43.03^{c}	2962.65^{ab}
Inf	1.0	23.80^{c}	4.84^{a}	10.61^{b}	8.42^{b}	43.34^{a}	2972.81^{b}
N.Inf	1.0	25.80^{a}	2.40^{d}	10.66^{b}	9.45^{a}	42.35^{d}	2976.98^{a}
	SEM	1.15	1.49	2.01	0.21	1.12	11.05

Values are means of triplicate determinations.CP, EE, CF, NFE and G.E represent crude protein, ether extract, crude fibre, nitrogen-free extract and gross energy respectively

Table 7: Effect of *Aspergillus flavus* and *Monodora myristica* on vitamin composition of Broiler diets

Inf. status	*M.m* Level	Vit.C (mg/100g)	Vit.B_1 (mg/100g)	VIT.B_2 (mg/100g)	Niacin (mg/100g)
	Infection main effect				
Inf		4.77^{b}	0.037^{b}	0.060	0.029
N.Inf		6.58^{a}	0.047^{a}	0.054	0.028
	SEM	0.80	0.11	0.06	0.03
	Monodora main effect				
	0	4.82^{c}	0.034^{c}	0.060	0.028
	0.5	5.06^{b}	0.037^{b}	0.053	0.032
	1.0	7.15^{a}	0.055^{a}	0.058	0.026
	SEM	0.13	0.01	0.19	0.07

a,b,c means in a column with different superscripts are significantly different from one another

Table 8: Interaction of *Aspergillus flavus* and *Monodora myristica* on broiler diets

Inf. status	*M.m* Level	Vit.C (mg/100g)	Vit.B_1 (mg/100g)	VIT.B_2 (mg/100g)	Niacin (mg/100g)
Inf	0	4.34^{c}	0.068^{b}	0.036	0.024
N.Inf	0	5.30^{b}	0.051^{c}	0.032	0.031
Inf	0.5	5.60^{b}	0.040^{d}	0.029	0.036
N.Inf	0.5	4.52^{bc}	0.065^{b}	0.045	0.028
Inf	1.0	4.37^{c}	0.071^{a}	0.046	0.026
N.Inf	1.0	9.93^{a}	0.045^{d}	0.063	0.025
	SEM	4.10	0.18	0.17	0.09

a,b,c means in a column with different superscripts are significantly different from one another

Table 9: Effect of *Aspergillus flavus* and *Monodora myristica* in Broiler diet on feed cost

Factors	Cost/kg feed (₦)	Cost/kg gain (₦)	Gross margin	(₦)
Infection effect				
Infected	88.90	357.54^{a}	1301.84^{b}	
Non-infected	88.90	306.14^{b}	1518.91^{a}	
SEM	0.00	15.87	46.81	
***Monodora* Level**				
0	88.96	380.70^{a}	23.54^{b}	
0.5	88.99	320.30^{b}	25.77^{a}	
1.0	89.01	294.53^{c}	31.74^{a}	
SEM	0.00	21.29	4.96	

a, b,c-means in the same column with differet superscripts are significantly (P<0.05) different from on another

Table 10: Interaction of *Aspergillus flavus* and in Broiler diet on feed cost

Inf. Status	*M.m* Level	Cost/kg feed (₦)	Cost/kg gain (₦)	Gross margin	(₦)
Inf	0	88.96	431.43^{a}	906.67^{c}	
N.Inf	0	88.96	329.66^{b}	1375.09^{b}	
Inf	0.5	88.99	322.76^{b}	1453.27^{ab}	
N.Inf	0.5	88.99	317.84^{b}	1554.30^{ab}	
Inf	1.0	89.01	318.40^{b}	1545.48^{a}	
N.Inf	1.0	89.01	270.66^{c}	1627.69^{a}	
	SEM	***0.01***	***17.38***	***49.71***	

a, b,c-means in the same column with differet superscripts are significantly (P<0.05) different from on another

Table 11: Hepatoprotective Effect of *Monodora myristica* on *Aspergillus flavus*-induced liver injury

M.m Level	A.P (iμ/l)	SGOT(iμ/l)	SGPT(iμ/l)
Factors			
Infection	77.67	241.83^{a}	12.51^{a}
Non-infection	73.33	222.00^{b}	10.95^{b}
SEM	14.97	5.33	0.39
***Monodora* Level**			
0	93.83	289.50^{a}	13.25^{a}
0.5	47.50	204.50^{b}	11.25^{b}
1.0	75.50	202.76^{b}	10.68^{b}
SEM	18.33	6.53	0.47

a,b,c-means in the same column with same superscripts are not significantly (P>0.05) from one another. A.P., SGOT and SGPT represent alkaline phosphatase, serum glutamate oxaloacetate transaminase and serum glutamate pyruvic transaminase respectively

Table 12: Interaction effect of *Monodora myristica* and *Aspergillus flavus* on liver injury

Inf. Status	*M.m* Level	A.P (iμ/l)	SGOT(iμ/l)	SGPT(iμ/l)
Inf	0	89.00	312.50^{a}	14.00^{a}
N.Inf	0	98.67	266.50^{b}	12.50^{ab}
Inf	0.5	61.33	208.50^{c}	12.50^{ab}
N.Inf	0.5	33.67	200.50^{c}	10.00^{c}
Inf	1.0	77.67	204.50^{c}	11.02^{bc}
N.Inf	1.0	73.33	201.00^{c}	10.35^{bc}
	SEM	25.93	9.24	0.67

a,b,c-means in the same column with same superscripts are not significantly (P>0.05) from one another. A.P., SGOT and SGPT represent alkaline phosphatase, serum glutamate oxaloacetate transaminase and serum glutamate pyruvic transaminase respectively

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