

Effect of African nutmeg (*Monodora myristica*) spice in aflatoxin-infected diets on growth performance of broilers

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Abstract

Fungal infected feedstuffs are denatured in nutrients and cause them to develop off flavors that often dampen the appetite of livestock and poultry. In this study, the effect of toasted African nutmeg (*Monodora myristica*) spice in *Aspergillus flavus*-infested diets on growth performance of broilers was investigated in a 2 x 3 factorial experiment that lasted for 42 days. Three out of 6 dietary treatments were infected with 1mg of pure culture of *Aspergillus flavus* and toasted *Monodora myristica* spice included at 0%, 0.5% and 1.0% while the other 3 diets were infection-free with 0%, 0.5% and 1.0% *Monodora myristica* inclusion respectively. Each treatment was replicated three times at 30 chickens per replicate. Infection status was significant ($P < 0.05$) for daily weight gain, feed conversion ratio (FCR) and protein efficiency ratio (PER). Daily weight gain, feed conversion ratio and protein efficiency ratio were 134.19g/bird/day, 39.23g/bird/day, 3.59g/bird/day and 1.10g/bird/day, respectively for the non-infected diets. The aflatoxin infection from *Aspergillus flavus* was observed to reduce ($P < 0.05$) feed intake to 130.51g/bird/day and weight gain (33.67g/bird/day). FCR and PER were also reduced ($P < 0.05$) by the presence of aflatoxin from *Aspergillus flavus* in the diet. Effect of *Monodora myristica* supplementation on feed intake, average daily weight gain, FCR and Protein efficiency ratio was significant ($P < 0.05$) for all the measured parameters. The Feed intake, average daily weight gain, FCR and protein efficiency increased ($P < 0.05$) as *Monodora myristica* inclusion level increased. Feed intake (119.8g/b/d), daily weight gain (28.90g/b/d), FCR (4.28) and PER (0.94) were observed in the *Monodora*-free (0%) diet. 0.5% and 1.0% levels of *Monodora* inclusion produced similar ($P > 0.05$) effects on all the growth parameters but were higher than the control. The interaction of aflatoxin infection and *Monodora* inclusion level were significant for all the growth parameters. The interaction of aflatoxin infection x *Monodora* inclusion levels recorded feed intake of 107.97g/b/d, 137.36 and 146.19g/b/d respectively for 0, 0.5 and 1.0% *Monodora*; 22.27g/b/d, 37.87g/b/d and 40.83g/b/d daily weight gain and 4.85%, 3.63% and 3.58% FCR. The interaction effect of *Monodora* inclusion level in infection-free diets on the birds feed intake were 131.65g/b/d, 147.24g/b/d and 123.67g/b/d respectively for 0%, 0.5% and 1.0%. The interaction effect at 0.5% inclusion level x non-infected diet was significantly higher (41.31g/b/d) than that at 0% inclusion level on daily gain. However, the daily weight gain of 40.83g/b/d observed in the interaction of 1.0% *Monodora* level x aflatoxin-free diet similar ($P < 0.05$) to that observed in the 0.5% inclusion level. The feed conversion ratio produced by the interactions at the three different levels (0, 0.5 and 1.0%) in the *Aspergillus*-free diets were ($P > 0.05$) 3.71, 3.57 and 3.49 respectively while PER were ($P < 0.05$) 1.05, 1.13 and 1.11 respectively. The *Monodora*-free diet recorded 9.26% drumstick, 2.26% liver 0.65% kidney and 2.43% gizzard, while the diet that had 0.5% *Monodora myristica* yielded 2.12% liver and 2.29% gizzard. Inclusion of *Monodora* up to 1.0% recorded 1.63% liver weight. *Aspergillus flavus* alone caused a reduction in live weight, dressed weight, thigh, kidney, breast meat, gizzard and an

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enlargement in the liver. *Monodora myristica* inclusion level also affected ($p < 0.05$) all the parameters except dressed weight, thigh and back cut. Kidney and liver were observed to decrease as the level of inclusion of *Monodora myristica* increased. The interaction of *Monodora myristica* and *Aspergillus flavus* on the parameters were significant ($p < 0.05$). From the results, *Monodora myristica* may be beneficial to poultry farmers, since about 90% of the poultry feed ingredients especially grains are constantly exposed to aflatoxin infection from *Aspergillus flavus* and other mycotoxin infections which hinder maximum nutrient utilization and affect the rate of conversion of feed.

Keywords: *Aflatoxin, Monodora Myristica, Aspergillus flavus*. growth performance, broiler birds

Introduction

Farmers in Nigeria are faced with an increasing challenge of poor storage facilities. Most often the feedstuffs under these poor storage conditions become mouldy. The moulds which are fungal in nature denature the nutrients of the feed and cause them to develop off flavour that dampen the appetite of the animals which are fed these feeds. Also, their presence in feed ingredients leads to the introduction of mycotoxin known as *Aflatoxin*. *Aflatoxins*, the most dangerous mycotoxins, are toxigenic secondary metabolites of fungi produced by certain strains of *Aspergillus species* - *A. flavus*, *A. parasiticus* and *A. nomis* (Fente *et al.*, 2001). These fungi can produce their toxic compounds on almost any food that will support growth. They often grow on agricultural products especially on grains such as groundnuts and maize. The metabolites produced by these fungi include AFB₁, AFB₂, AFG₁ and AFG₂, all of which occur naturally (Kozakiewicz and Smith, 1994). AFB₁ and AFB₂ are produced by *Aspergillus flavus* while *A. parasiticus* produces these same metabolites along with AFG₁ and AFG₂. Of the four metabolites, AFB₁ is found in highest concentrations followed by AFG₁. Due to their ability to grow on almost all food products, they can generate off flavours, produce toxins, and cause discolouration and proteolysis (Alharthi,

1997). *Aflatoxin* has been reported to have adverse effects on poultry. McDonald *et al.* (1995) reported high level of turkey poult and duckling poisoning as a result of groundnut infestation with *Aspergillus flavus*. The principal target organ of *Aflatoxin* is the liver (Alharthi, 1997). Animals which consume sub-lethal quantities of *aflatoxin* for several days or weeks develop a sub-acute toxicity syndrome which commonly includes moderate to severe liver damage with marked bile duct proliferation. Sonia *et al.* (1997) reported that even with low levels of *aflatoxin* in the animal feed, there would be a decrease in growth rate, lowered milk and egg production and suppression of immunity. Carcinogenicity has been attributed to *aflatoxin B₁*. Alharti (1997) reported that in chronic toxicity, liver becomes damaged and gall bladder becomes swollen. After the invasion of aflatoxin into the liver, lipids infiltrate the hepatocytes and lead to necrosis or liver cell death. He reported that the LD₅₀ of a day-old duckling is 0.3mg /kg body weight. The effect of aflatoxicosis can be compounded by stress and this can lead to induced haemorrhaging due to prolonged blood clotting time caused by lack of vitamin K utilization. The study was conducted to evaluate the effect of *Monodora myristica* on the growth performance parameters of birds fed diets infested with *Aspergillus*

flavus.

Materials and methods

Seeds of *Monodora myristica* were bought from Ndoro Market in Ikwuano Local Government Area of Abia State. They were cleaned and toasted at 65°C for one hour. Then they were milled into powder, bottled in an airtight container and then analyzed for its proximate composition according to the procedure of the A.O.A.C (1990). The Vitamin (ascorbic acid, thiamine, riboflavin and niacin) and Mineral compositions were also analyzed using the Johnson and Ulrich (1959) method and Barakat *et al.*, (1973) respectively as outlined by Okwu (2004).

The experiment lasted for forty-two days. The broiler birds were stabilized for fourteen days on standard broiler starter diet and thereafter randomly allocated to six treatment diets in a 2 x 3 factorial in Completely Randomized Design experiment. Factor A was *aflatoxin* infection status at two levels (infection and non-infection) and factor B was inclusion level at three levels (0%, 0.5% and 1.0%) of *Monodora* spice. A total of 540 unsexed Anak broiler birds were randomly allocated to six diets and replicated three times with thirty birds per replicate. The first three diets designated 1, 2 and 3 respectively were diets each infected with 1mL pure culture of *Aspergillus flavus* obtained from the Plant Pathology Laboratory of the National Root Crop Research Institute, Umudike in Abia State of Nigeria and supplemented with 0% (infected control), 0.5% and 1.0% milled *Monodora myristica*. The remaining three diets 4, 5 and 6 were not infected with *Aspergillus flavus*, but were also supplemented with 0% (non-infected control), 0.5% and 1.0% *M. myristica* spice. The broiler chicks were raised on deep litter floor. Routine management and vaccination procedures were followed. The diets in mash form and

water were provided *ad-libitum*. Records of feed consumption and liveweight were taken weekly on a group basis throughout the 49days duration of the experiment.

Statistical analysis

The data collected were subjected to Analysis of Variance (ANOVA) in a 2x3 factorial arrangement in a completely randomized design experiment and where significant differences were obtained, means were separated using Duncan's Multiple Range Test (Duncan, 1955) as packaged in SPSS (2006) for windows; version 16, SPSS Inc.

Results

The results of the average weight gain, feed intake and feed conversion ratio of broilers fed *Aspergillus flavus*-infected or non-infected diets with or without *Monodora myristica* additives are shown in Table 2.

Infection status effect was significant ($P<0.05$) for daily weight, feed conversion ratio and protein efficiency ratio. Daily weight gain, feed conversion ratio and protein efficiency ratio were 39.23g/b/d, 3.59 and 1.10 respectively for the non-infected diets while, the infected diets were lower ($P<0.05$) for daily gain (33.67g/b/d), FCR (4.02) and PER (1.05). There was no significant ($P>0.05$) infection status main effect on feed intake. Average feed intake was 130.51g/b/d and 134.19g/b/d for infected and non-infected diets, respectively.

Monodora myristica inclusion level significantly ($P<0.05$) affected all the growth parameters. Lowest ($P<0.05$) feed intake of 119.81g/b/d, daily weight gain (28.90g/b/d), feed conversion ratio (4.28) and PER (0.94) were observed in the *Monodora*-free (0%) diet. However, 0.5% recorded 142.24g/b/d feed intake, 39.59g/b/d daily weight gain, 3.60 FCR and 1.13 PER while 1.0% *Monodora* inclusion

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Table 1: Dietary composition of Aspergillus -infested and non -infested broiler diets supplemented with and without Monodora myristica

INFECTED DIETS <i>Monodora</i> level				NON-INFECTED DIETS		
	0.0%	0.5%	1.0%	0.0%	0.5%	1.0%
Maize	53.00	53.00	53.00	53.00	53.00	53.00
Soybean	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
G.N.C	12.00	11.50	11.00	12.00	11.50	11.00
P.K.C	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
<i>Monodora</i>	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
Methionine	0.10	0.10	0.10	0.10	0.10	0.10
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
CrudeProtein (%)	21.68	21.58	21.49	21.68	21.58	21.49
Calcium (%)	1.89	1.91	1.94	1.89	1.91	1.94
Phosphorus (%)	0.94	1.13	0.43	0.94	1.13	0.43
Lysine (%)	1.13	1.12	1.12	1.13	1.12	1.12
Methionine (%)	0.43	0.42	0.42	0.43	0.42	0.42
Crude fibre (%)	3.37	3.71	3.71	3.37	3.71	3.71

Vitamin/mineral Premix supplying the following per kg diet: Vit. A (1500 I.U); Vit D₃ (1600 I.U); Riboflavin (9.0mg); vit. B₆ (0.3); vit₁₂ (8.0mg); Nicotinic acid (8.0mg); iron (5.0mg); Selenium (0.01mg); Magnesium (10.0mg); Zinc (4.5mg); cobalt (0.02).

Results

Table 2 : Effect of *Monodora myristica* Level of inclusion and *Aspergillus flavus* on Growth performance of Broilers chickens

Inf. Status	Level of Inc. (%)	Feed intake (g)	Daily weight gain (g/b/d)	FCR (g feed/g gain)	PER
Inf.	0	107.97 ^c	22.27 ^c	4.85 ^c	0.84 ^c
N.Inf	0	131.65 ^b	35.54 ^b	3.71 ^b	1.05 ^b
Inf.	0.5	137.36 ^{ab}	37.87 ^b	3.63 ^b	1.12 ^a
N.Inf	0.5	147.24 ^a	41.31 ^a	3.57 ^b	1.13 ^a
Inf.	1.0	146.19 ^a	40.88 ^a	3.58 ^b	1.18 ^a
N.Inf	1.0	123.67 ^c	40.83 ^a	3.49 ^b	1.11 ^{ab}
	SEM	6.31	0.80	0.08	0.02
Infection Status					
Infected		130.51	33.67 ^b	4.02 ^a	1.05 ^b
Non-infected		134.19	39.23 ^a	3.59 ^b	1.10 ^a
	SEM	3.64	0.46	0.04	0.01
<i>Monodora</i> level main effect					
	0%	119.81 ^b	28.90 ^b	4.28 ^a	0.94 ^b
	0.5%	142.24 ^a	39.59 ^a	3.60 ^b	1.13 ^a
	1.0%	144.43 ^a	40.85 ^a	3.54 ^b	1.14 ^a
	SEM	4.458	0.564	0.054	0.016

abc- means in the same column with the same superscript are not significantly (P>0.05) different from one another.

Inf. and Non-infected represent infected and non-infected respectively.

levels yielded a daily feed intake of 144.43g/b/d, daily weight gain (40.85g/b/d), FCR (3.54) and PER (1.14).

The interactions effect of aflatoxin infection from *Aspergillus flavus* x *Monodora* inclusion level were significant ($P < 0.05$) for all the parameters. The infected control diet had 0% *Monodora* and was the least ($P < 0.05$) consumed (107.97g/b/d), and also recorded the least daily weight gain (22.27g/b/d), feed conversion ratio (4.85) and PER (0.84). Average feed intake of the infected diet increased ($P < 0.05$) above the control to 137.36g/b/d and 146.19g/b/d respectively as *Monodora* was included at 0.5% and increased to 1.0%. The inclusion of *Monodora* in the infected diet at 1.0% level yielded feed conversion ratio and protein efficiency ratio of 3.58 and 1.18 respectively. Similar ($P > 0.05$) effect was recorded with those fed infected diets with 0.5% *Monodora* level which recorded FCR (3.63) and PER (1.12). For the non-infected diets, the 1.0% *Monodora* was least ($P < 0.05$) consumed (123.67g/b/d). However, it recorded 40.83g/b/d weight gain, 3.49 FCR and 1.11 PER. When *Monodora* was added to aflatoxin-free diet at 0.5% level, it recorded the highest ($P < 0.05$) feed intake (147.24g/b/d), with daily weight gain, FCR and PER of 41.31, 3.57 and 1.13, respectively. The aflatoxin-free diet with no *Monodora* supplementation recorded feed intake of 131.65g/b/d, 35.54g/b/d weight gain 3.71 FCR

Discussion

Average daily feed intake was observed to be depressed by *Aspergillus flavus* infection. Feed intake was positively affected by *Monodora* inclusion over the control. The average daily feed intake in the infected feed increased as the levels of *Monodora* supplementation increased. For

the non-infected diets, feed intake also increased as the *Monodora* level rose to 0.5% but further increase in the *Monodora* level beyond that, resulted in the decrease in feed consumption. The reduced feed intake recorded for the infected diet without *Monodora* could be attributed to the non-palatability of the diet due to off-flavour that may have been caused by the presence of lipase enzymes in *Aspergillus flavus*. These enzymes break down the lipid contents of the diets in the process of lipolysis leading to the release of taste that the birds found unacceptable (McDonald *et al.*, 1995). The observed increase in feed intake resulting from the *Monodora* supplementation was in agreement with the observation made for sanguinaria rhizome on piglets and poultry (Gerbert *et al.*, 1999) and turmeric on laying hens (Samarasinghe and Wenk, 2002). The low feed intake (123.67g/b/d) by 1.0% *Monodora* in the diet that was not infected suggests probably that the flavour from the spicemay have been too high thereby increasing the taste and characteristic pungent odour from the essential oil components. This may *not have been tolerated by the birds* and thus negatively affected feed intake. From the results, the average feed intake of the non-infected diets at 0%, 0.5% and 1.0% *Monodora* level appear to be higher than that obtained from the *Mucuna* bean-based broiler diet (Ukoha *et al.*, 2011). The lower feed intake of 82.73g/b/d, 84.95g/b/d and 89.67g/b/d by birds fed *Mucuna*-based diet with *Monodora* supplementation at 0%, 0.5% and 1.0% respectively could probably be as a result of the antinutritional components in the *Mucuna cochinchinensis*. The difference observed in the feed intake may be an indication that the birds have well developed taste buds as opposed to by the report of Holdas and May (1966).

Inclusion of *Monodora myristica* in

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Aspergillus flavus diet improved average daily weight gain of birds over the control-fed birds. The least weight gain (22.27g/b/d) and protein efficiency ratio (0.84) was observed in the *Monodora*-free infected diet. The weight gain realized by supplementing 0.5% and 1.0% *Monodora* to non-infected diets were not superior to that obtained in the infected diet having 1.0% *Monodora* supplementation. The feed conversion ratio in all the groups except the groups fed infected control were similar ($P<0.05$). *Monodora* supplementation improved the protein efficiency ratio in birds fed the infected diets. The low weight and poor utilization of the nutrients in the infected diets with 0% *Monodora* could be as a result of the aflatoxin produced by the *Aspergillus flavus* which may have bound them, making them unavailable for absorption. The poor protein efficiency ratio confirmed this assertion. *Monodora* at 0.5% and 1.0% levels of inclusion were able to neutralize the effect of the aflatoxin and enhance the utilization of the nutrients comparable to the non-infected diets. *Monodora* was also observed to improve the utilization of non-infected diets especially

at levels not exceeding 0.5%.

The observations above were in line with the findings of Alharthi (2006) who reported that aflatoxin at 0.25ppm in turkey poults and ducklings impaired growth and a dose of 1.5ppm in broilers and 4.0ppm in Japanese quail had negative effect on growth. *Aspergillus flavus* alone appears to impair the availability of bile salts which decrease vitamin D3 production, probably through increased levels of biliary acids in the gastric juice. This also leads to the decrease in the absorption of fat soluble vitamins. Moreover, *aflatoxin* is known to react negatively with different cell proteins and this leads to the inhibition of carbohydrate and lipid metabolism, protein synthesis and mitochondrial respiration (Sonia *et al*, 1997).

From the results, *Monodora myristica* tends to be beneficial to poultry farmers, since about 90% of the poultry feed ingredients especially grains are constantly exposed to aflatoxin infection from *Aspergillus flavus* and other mycotoxin infections which hinder maximum nutrient utilization and affect the rate of conversion of feed. *Monodora* may find a place in future as

Table 3: Effect of Monodoramyristica and Aspergillusflavus infection in broiler diet on organs and cut part weights

Inf. Status	Monodora Level (%)	L.W. (%)	DRW (%)	DS (%)	TH (%)	BM (%)	Liver (%)	Gizzard (%)	Back cut (%)	Kidney (%)
Inf.	0	1310.15 ^c	85.67 ^b	9.71 ^c	9.92 ^a	18.15 ^a	2.46 ^a	2.35 ^b	14.31 ^{ab}	0.62 ^c
N. Inf.	0	1866.67 ^b	94.33 ^a	8.81 ^d	9.07 ^b	15.02 ^{bcd}	2.06 ^c	2.51 ^a	14.81 ^a	0.69 ^a
Inf.	0.5	1966.67 ^b	92.31 ^a	9.92 ^{bc}	9.39 ^{ab}	14.70 ^{bc}	2.21 ^c	2.07 ^c	14.32 ^{ab}	0.59 ^c
N. Inf.	0.5	2104.63 ^a	91.87 ^a	10.46 ^a	9.16 ^{ab}	15.10 ^{bc}	2.05 ^c	2.50 ^a	13.99 ^b	0.64 ^{bc}
Inf.	1.0	2092.00 ^a	91.98 ^a	10.25 ^{ab}	9.53 ^{ab}	15.60 ^b	2.08 ^c	2.20 ^b	14.77 ^a	0.52 ^d
N. Inf.	1.0	2090.00 ^a	92.79 ^a	9.51 ^c	9.71 ^{ab}	14.31 ^d	1.18 ^d	2.51 ^a	14.34 ^{ab}	0.67 ^{ab}
	SEM	34.90	1.35	0.13	0.24	0.23	0.03	0.04	0.194	0.02
Infection status main effect										
Inf.		1789.61 ^b	92.99 ^b	9.96 ^a	9.61 ^a	14.81 ^b	2.25 ^a	2.23 ^b	14.47	0.58 ^b
N.		2020.43 ^a	93.36 ^a	9.59 ^b	9.51 ^b	16.15 ^a	1.76 ^b	2.51 ^a	14.38	0.67 ^a
Inf.										
	SEM	20.15	0.78	0.08	0.14	0.13	0.02	0.02	0.13	0.01
Monodora Level main effect										
	0%	1588.41 ^b	90.06	9.26 ^c	9.50	16.59 ^a	2.26 ^a	2.43 ^a	16.59 ^a	0.65 ^a
	0.5%	2035.65 ^a	92.09	10.19 ^a	9.58	14.90 ^b	2.12 ^a	2.29 ^b	14.90 ^b	0.62 ^b
	1.0%	2091.00 ^a	92.38	9.88 ^b	9.62	14.96 ^b	1.63 ^b	2.38 ^a	14.96 ^b	0.60 ^b
	SEM		0.95	0.09	0.17	0.16	0.02	0.02	0.16	0.01

poultry feed additive which can compete favorably with the harmful chemicals or synthetic additives that are not consumer friendly.

Table 3 summarizes the effects of *Aspergillus flavus* infection and *Monodora myristica* spice on the carcass characteristics of broilers fed aflatoxin-infected and non-infected diets. The *Monodora*-free diet recorded 9.26% drumstick, 2.26% liver 0.65% kidney and 2.43% gizzard, while the diet that had 0.5% *Monodora myristica* yielded 2.12% liver and 2.29% gizzard. Inclusion of *Monodora* up to 1.0% recorded 1.63% liver weight. *Aspergillus flavus* alone caused a reduction in live weight, dressed weight, thigh, kidney, breast meat, gizzard and an enlargement in the liver. *Monodora myristica* inclusion level also affected ($p < 0.05$) all the parameters except dressed weight, thigh and back cut. Kidney and liver were observed to decrease as the level of inclusion of *Monodora myristica* increased. The interaction of *Monodora myristica* and *Aspergillus flavus* on the parameters were significant ($p < 0.05$).

The live weight, dressed weight, thigh and liver weights were enhanced by the inclusion of *Monodora myristica*. Dressed weight and thigh weights were improved as the level of inclusion of *Monodora myristica* increased. Also the liver weight decreased as the level of inclusion of the spice increased suggesting that it could help to reduce stress on the liver. Largest liver weight was observed in the infected diet that lacked *Monodora*. Inclusion of 0.5% and 1.0% *Monodora myristica* in the diet caused a reduction ($p < 0.05$) in the liver weight. For the non-infected diets, 1.0% *M. myristica* inclusion level resulted in the lowest ($p < 0.05$) liver weight. However, 0.5% *Monodora* did not make any significant impact over the control on liver weight.

Liver weight was significantly ($p < 0.05$) enlarged by *Aspergillus flavus* in the absence of *Monodora myristica*. The highest ($p < 0.5$) weight of the kidney was observed in the group fed non-infected control diet. In the presence of *Aspergillus flavus* infection, Kidney weight decreased ($p < 0.05$) when *Monodora* was included at 1.0% the liver is the principal target of aflatoxin metabolite. The increase observed in liver size observed in the infected diet without *Monodora* could be due to increased pressure from detoxification of the *Aspergillus flavus* infection. The size of the kidney recorded in the infected *Monodora*-free diet could be due to the increased metabolic rate of the organ in an attempt to reduce the toxicity in the system arising from the presence of aflatoxin in the *Aspergillus flavus*.

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