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Original Article

The effect of grower feed diet supplemented with *Ganoderma lucidum* against some enteric zoonotic parasites of pigeons (*Columba livia*)

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ABSTRACT: We report a preliminary study on the effect of grower feed diet supplemented with mashed *Ganoderma lucidum* against some enteric zoonotic parasites of wild rock pigeons (*Columba livia*) in Benin City, Nigeria. The pigeons were fed *ad libitum* with supplemented and non-supplemented grower feed diet in sawdust-floored squared wooden cages (45 cm x 30 cm x 20 cm) at room temperature for 21 days. They were sacrificed at days 14 and 21 for profiling of their zoonotic enteric parasites. High prevalence (77.7%) of Cestodes (tapeworms) and low prevalence (12.3%) of nematodes were recorded in the control pigeon fed non-supplemented grower feed compared to those on diet supplemented with *Ganoderma*. Cestodes (Helminths) such as *Hymenolepsis carioca, Amoebataenia cuneata, Raillietina tetragona, Raillietina serrata, Inermicapsifer spp., Hymenolepsis spp.*, and a species of nematode (*Ascaridia columbae*) were identified in the gastrointestinal tract of the experimental pigeons. A few species (< 8 %) of Trematodes yet to be fully identified were also observed. The low population of helminth parasites and overall weight decrease of pigeon fed with diet supplemented with *Ganderma lucidum* recorded during the study suggest improved removal of digestive microbes, intestinal health, and gastrointestinal motility. These findings were aimed at contributing to the already existing knowledge on the use of feed additives, probiotics and supplementations in poultry farming.

KEYWORDS: *Ganoderma lucidum*, pigeon *(Columba livia)*, helminth, zoonotic parasite, cestode, nematode

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INTRODUCTION

Fungi represent a collective group of biologically and morphologically diverse achlorophyllous organisms with versatile ecosystem services and functions that are central to healthy ecological balance of biological communities. Saprotrophic fungi are notable biodegraders that ensure recycling of dead vegetations and release of locked-up elements necessary for plant growth and development while the pathogenic fungi model the course of evolution by checking rampant monoculture or exerting pressure on biodiversity (Lange *et al*., 2012). The symbiotic interaction of fungi with herbaceous and woody plants i.e. mychorrhizae, endophytes or animals and humans have been reported to improve biological plasticity of non-fugal symbionts against survival constraints. Mushroom-forming fungi are a ubiquitous group of under-documented and untapped powerful source of diverse natural products of great economic values to humans. Majority of these macrofungi contain many biologically potent substances including polysaccharides, glucan, glycoprotein, lectin, lactones and alkaloids in their fruit bodies, myceliated bodies or cultured broth. They are also recently linked to studies on biomedicinal products with immunomodulating, antitumour, antidiabetic, antibiotic or antiparasitic, hypercholesterolemia, hepatoprotective, antipathogenic, detoxicant and antioxidant potentials (Wasser, 2010; Guo *et al*., 2004). Numerous edible mushrooms speciose have health-promoting potentials and represent a suitable choice as additives in this present study.

Ganoderma spp (Aphyllophoromycetideae) are polyporus, white-rot, wood degrading, bracket shaped macrofungi with cosmopolitan and tropical distributions. They are a rich source of novel drugs and are famous in traditional orient medicine (Wang *et al*., 2009; Stamets, 2002). Conservative estimate of about 80 species have been identified and are popular as shelf mushrooms or conks because of their non-perishable nature and/or woody texture (Chang & Buswell, 1999). Many species are not directly consumed as food due to their texture but were reported to possess novel health and economic benefits (Okhuoya *et al*., 2010). Although these *Ganoderma* mushrooms are well exploited as alternative remedies to diverse medical conditions, documentations on their use as food or food supplements in animal production (or livestock farming) especially in poultry are dearth and limited compared to the use of fleshy (gilled) macrofungi (Ogbe *et al*., 2009a; Guo *et al*., 2003).

Global demands for animal protein have soared over the past five decades in Nigeria and many other African countries. The overwhelming growth of poultry in many developing nations is attributed to its multidimensional benefits e.g. hypocholesterol and affordable meat, quick and high profit turnover, ease of establishment of the business and production of eggs which constitute another good source of nutrients. The most recent emergence of probiotics, prebiotics, toxin binders, oligosaccharides, organic minerals and other feed additives as alternative dietary supplements to therapeutic (curative and prophylactic) and non-therapeutic (growth promoters) antiobiotics, and other chemical e.g. vitamins also accounted for the persistent growth of poultry industries in many nations of the world (Castanon, 2007; Willis *et al*., 2007; Aarestrup, 1999). The concept behind the long term objective application of antibiotic as growth promoters and therapeutics in veterinary and livestock production is subverted by inherent biological plasticity which resulted over time in the natural selection of resistant species. It could have also compromised the quality of animal proteins reaching the end-users with biologically harmful residues (Montagne *et al*., 2003). Products that are used exclusively or in combination as feed additives (supplements), probiotics, prebiotics or phytobiotics in animal production may be derived from plants (phytogenic), fungi, algae (mostly sea weeds) or bacteria (Elijah & Ofongo, 2012; El-Deek *et al*., 2011; Hashemi and Davoodi, 2010). They usually serve to improve the health and growth performance of livestock, quality of feed and food from animal origin (Adu *et al*., 2009). Phytogenic feed additives are linked to diverse medicinal plants representing spices, vines, herbs, shrubs and tree. Their exploitation in livestock production anteceded additives derived from non-phytobiotic sources (Hashemi & Davoodi, 2010; Symeon *et al*., 2010). The experimental knowledge of their efficacy as exclusive feed or feed additive in poultry farming was fraught with incertitude vis-à-vis animal health and growth leading to the search for a more effective and suitable alternative option with better digestibility and growth performance feedbacks (Kevyani *et al*., 2012; Yang *et al*., 2009; Cross *et al*., 2007). This is consequently connected to the non-ruminant nature of birds and the anatomic integrity of their alimentary canal and/or gastrointestinal tract. Extracts from the sporophores (fruit bodies) of fleshy mushrooms such as *Agaricus bisporus*, *Lentinus edodes*, *Pleurotus ostreatus*, *Formitella fraxineo* and some microfungi have been studied as feed supplements and probiotics in the improvement of animal gastrointestinal performance, gut microbial ecology, and general health management, especially of poultry birds (Giannenas *et al*., 2010; Willis *et al*., 2011, 2007; Ogbe *et al*., 2009b; Guo *et al*., 2004). This concept is explored further in this study with the aim of determining the efficacy of a non-fleshy mushroom like *Ganoderma lucidum* in the growth and health performance of pigeons. In this study, we also sought to investigate the antihelminthic potentials of the fungus as feed additive for future poultry use.

MATERIALS AND METHODS

The fungus *Ganoderma lucidum* was collected in sufficient quantity from lowland forests around Benin City, Nigeria in aerated baskets. The mushrooms were brought to the Mushroom Biology Unit Laboratory of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria where the sporophores (visible fruit bodies) were air dried for four days, after which they were crushed using a wooden mortar and pestle.

Experimental Model

Six wild adult pigeons of mixed sexes were caught in Benin City environs using specialized traps. The average body weight of the birds was 0.24 kg and they were confined to a clean compartmentalized cage (45 cm x 30 cm x 20 cm) with sawdust beddings at the rate of two birds per compartment. These compartments represent treatments A, B and C respectively and the cages were kept in the Animal House of the Toxicology Unit of the Department of Animal and Environmental Biology, University of Benin. They were fed *ad libitum* with an experimental mixture of grower feed and *G. lucidum* at the ratio of 0.78 kg / 0.05 kg (B) and 1.60 kg / 0.05 kg (C) while treatment (A) served as the control (birds fed with only grower feed). The *Ganoderma lucidum* additive was mixed manually. The feeding protocol lasted for a 14-day acclimatization period of the experimental birds. Each bird was sacrificed using a killing gel, pinned to a dissecting board and dissected longitudinally from neck to the rear with a sterile surgical blade, followed by the careful removal of the zoonotic enteric parasites for further study (Ash and Orihel, 1991). This process was repeated 10 days later in a 24-day study experiment. Samples of each feed (10 g) were taken and refrigerated at -4 ^{0}C for chemical analysis.

Helminth Parasites Isolation

The intestine was separated and incised under a dissecting microscope in a petri dish containing 10% normal saline. The walls were carefully scratched to ensure thorough dislodge of the helminths. The helminths were collected, fixed using ethanol and formaldehyde in tandem, and cleared or stained according to the protocol described by Farahnak *et al*. (2004). The collected parasites were identified as nematodes and cestodes *ab initio* and preserved separately in 4% and 70% ethanol respectively for further identification to the genus level. In this study, prevalence was measured by the number of species across the helminth groups i.e. Cestode, Nematode or Trematode. Species identification was done with the aid of pictorial monographs containing diverse morphological descriptions of previously published studies (Baker, 2007; Spencer and Monroe, 1982). Microphotographs of fixed slides of wholesome (undamaged) species of helminthes were produced using a light microscope equipped with a camera lucida (Mirror type). In this study, infestation is also categorized as heavy (>7 different representative parasites), mild (3-4 representative parasites) and low (< 2).

Chemical Screening

Basic qualitative chemical analysis of the feeds used for this study was carried out to detect the presence or absence of selected secondary metabolites such as alkaloids, flavenoids, glycosides, steroids, polysaccharides and triterpenes. The screening was done in the Pharmaceutical Chemistry Research laboratory of the University of Benin, Benin City, Edo State, Nigeria according to the standard precept of Sofowora (1993) and AOAC (1990).

RESULTS

The study, although preliminary, recorded some enteric zoonotic parasites in the gut of wild pigeons (*Columba livia*) as presented in Table 1 and Figure 1. The species of Cestodes representing 77.7% total parasites identified from the study were *Hymenolepsis carioca*, *Amoebataenia cuneata*, *Raillietina tetragona*, *Raillietina serrata*, *Inermicapsifer sp*., *Hymenolepsis sp.* (Figure 1). They were all observed and represented in pigeons fed exclusively with grower feed (control). Nematode (12.3%) infestation in the gut of pigeons fed exclusive grower feed was however observed to be low and limited in diversity (*Ascaridia columbae*), and species richness (number observed per field of view). Pigeons on mixed diet treatment of grower feed and crushed fruit bodies of *G. lucidum* recorded mild to low Cestode infestation while there was no nematode infestation in pigeons on treatment B until day 14 and 21. The gut of pigeons on treatment B was observed to be constricted in diameter with tighter epithelia compared to those extracted from pigeons on treatments A and C. Low levels of unidentified species of Trematodes was also recorded during the study. Progressive weight gain of 0.03 kg (mean) was recorded for pigeons on treatment protocols A and C from day 14 to 24, while pigeons on treatment B showed weight loss (Table 2). The qualitative chemical analysis of *G. lucidum* used for this study showed the presence of triterpenoid, alkaloid, polysaccharides, saponins (Table 3).

DISCUSSION

It was reported in several literatures of animal production that poultry is one of the fastest growing livestock industries in Africa. Nigeria have witnessed sustainable improvement in the trial of diverse source of cheap renewable protein resource in compounding feeds and combating the decimating effect of economic diseases in poultry business (FAO, 2008; Dalloul *et al*., 2006). There were also reports on the use of many fleshy edible mushrooms either as exclusive supplement of poultry feeds or in combination with herbs of phytobiotic origin as growth and health promoters (Iyayi, 2008). In contrast, scanty data existed on the use of medicinally valuable polyporous (non-fleshy) mushrooms (Adu *et al*., 2009; Ahmad, 2006). The preliminary evaluation of the fruit body *G. lucidum* as a potential competitive feed additive (supplement) and alternative replacement option for phytogenics and antibiotic growth promoters in birds presented conflicting growth data.

Although poor growth performance of birds as measured by observed weight loss recorded in this study, the data indicated strong potential in the birds' agility and health. The exact reason (physiochemical or ecological) for this observations is not yet fully understood but may be linked to factors that include nutrient status of the feed, mechanism of digestibility and absorption, and/or the concentration of the mushroom additive used in the compounded feed. These findings should be taken into consideration when assessing the positive results from previous studies involving the use of extracts from *G.lucidum* as probiotics, feed additive or health promoter in poultry birds (Ogbe *et al*., 2009a and b). This invariably lead to the assumption that the weight decrease may be ascribed to high concentration of antinutritional factors (ANF) or the nature of inherent biochemical in the additive rather than the impact of ecological dynamics of helminthes on the occlusion of the bird's nutrient absorption process. Further study is necessary to gain insights into the digestion process and the dynamics of digestives (microbial population) to fully understand and reconcile the conflicting actions of the fungus as feed additive. The low prevalence of nematode and cestode recorded in the gut canal of these experimental birds concurred with previous studies by Adang *et al*. (2009), Baker (2007) and Farahnak *et al*. (2004). The various species of cestodes and nematodes recorded in this study were non-pathogenic inhabitants of birds' guts (Baker, 2007) with unclear ecological functions relative to the performance of the birds and their digestibility. This relationship may be connected to many years of convergent evolution in nutritional dynamics and/or divergent evolution of the host suppression response to gut helminth parasites of birds (Elijah and Ofongo, 2012; McCracken and Lorenz, 2001).

The observed decrease in the live weight of the experimental birds used for this study with increased *G. lucidum* supplementation may affect meat quality by strengthening/tightening the muscoskeletal muscles, decreasing the fat content (cholesterol) and elevating the fibre contents (Kavyani, *et al*., 2012; Giannenas *et al*., 2010; Willis *et al*., 2007; Ahmad, 2006). Etuk *et al*. (2012), Ogbe *et al*. (2009a), and Soetan and Oyewole (2009) reported that the classes of phytochemicals e.g. alkaloids, saponins, tannins, steroids and triterpenes likewise recorded in this study are capable of depressing the metabolic utilization of proteins, other nutrients (minerals and vitamins) and gastrointestinal microbiota observed in birds on feed protocol B. The observed depression of helminth population in the guts of birds with *G. lucidum* additives reaffirmed reports on their overall health (antihelminthetic) property. This however agrees with studies suggesting mushrooms as good pharmaceutical and/or therapeutics additives against various forms of infections (Willis *et al*., 2011; Wasser, 2010; Ogbe *et al*., 2009a and 2009b; Ogbe, 2008). In addition, the presence of antioxidants, glycosides, polysaccharides derivatives such as sugars and crude fibre fraction in *G. lucidum* facilitate increase in the population of non-pathogenic bacteria like *Bifidiobacterium* sp. which assist digestion of feeds (Chang and Mshigeni, 2001). Furthermore, these substances also offer receptor sites for binding of pathogens and facilitating their elimination along with digester from the gastrointestinal tracts of birds (Spring *et al*., 2000).

TABLE 1 Helminth parasites profile at days 14 and 24 respectively

Parasites	Day 14			Day 24		
Cestodes	A	B	C	A	B	C
Amoebataeni cuneata	+	-	-	+	-	+
Hymenolepsis carioca	+	-	+	+	+	+
Hymenolepsis sp.	+	-	+	+	-	-
Inermicapsifer sp.	+	-	-	+	-	-
Raillietina tetragona	+	-	+	+	+	+
Raillietina serrata	+	-	+	+	+	+
Nematodes						
Ascaridia columbae	+	-	+	+	-	-

+ = Present, - = Absent

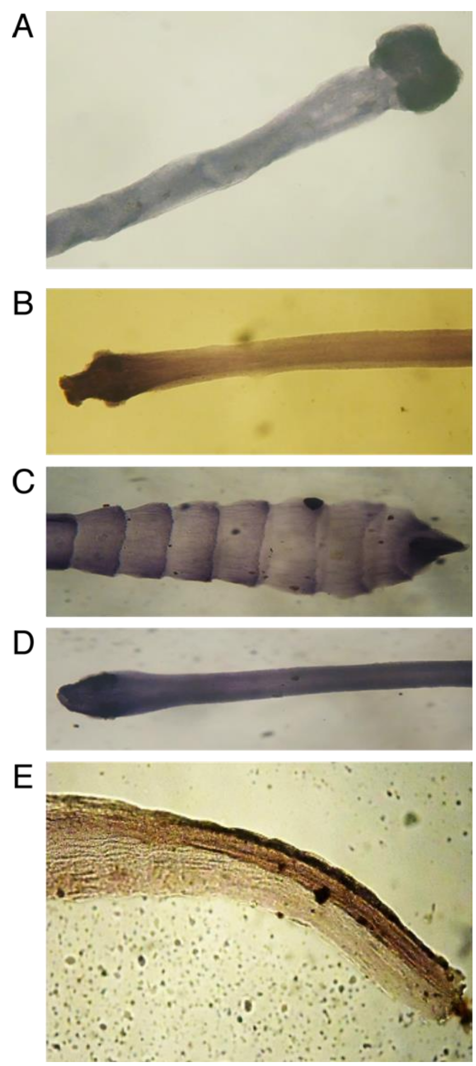

FIGURE 1 Some of the cestodal and nematodal symbionts in the gut canal of the experimental bird (A) *Raillietina tetragona* showing the scolex with sucker and neck region, (B) *Amoebataenia cuneata* with a triangular anterior end, (C and D) *Posterior and anterior ends of* adult *Hymenolepsis carioca* (E) gut canal *Ascaridia columbae*

TABLE 2 **Mean weight (kg) of pigeon from day 0 to day 24**

Diet Treatment	Weight (Kg) day zero	Weight (Kg) day 14	Weight (Kg) day 24
A	0.22	0.24	0.26
B	0.28	0.22	0.32
C	0.23	0.26	0.24

TABLE 3 **Result of chemical analysis of supplemented and unsupplemented feeds**

Parameter	*G.lucidum* supplemented feed	Unsupplemented feed
Alkaloids	+	-
Tannin	-	-
Glycoside	+	+
Saponin	+	-
Flavonoid	-	+
Steroid	+	-
Polysaccharides	+	+
Titerpenes	+	-

+ = present, - = absent

Diverse cestode (tapeworms) parasites representing *Hymenolepsis carioca*, *Amoebataenia cuneata*, *Raillietina tetragona*, *Raillietina serrata*, *Inermicapsifer sp.* and *Hymenolepsis sp.* were identified in the intestine of the experimental birds and this agreed with the findings of Audu *et al*. (2004) and Soulsby (1982). The wide species diversity of cestode parasites isolated from the intestine of the pigeons is a direct reflection of their cosmopolitan nature and diets which comprised ants of the genera *Pheidole* and *Tetramorium*, beetles, termites, flies, other arthropods, fruits and seeds (Adang, 1999). This diversity of helminthes is seldom reported in poultry birds. The arthropods are intermediate host or vectors of infectious microbes that can either coexist with the host in a symbiotic relationship or become opportunistic and cause harmful infection (Baker, 2007; Mush *et al*., 2000). Although, many helminthes parasites are non-pathogenic in most birds, the biochemical dynamics depressing their disease causing potential can be made clearer by further studies. *Ascaridia columbae* nematode identified in this study was commonly reported by a number of workers doing related study from different parts of the world (Farahnak *et al*., 2004). According to various studies, *Ascaridia columbae* along with *Capillaria, Dispharynx, Hadjelia truncate, Syngamus and Tetrameres sp.* were associated with pigeons (Yang *et al*., 2009).

This study while reaffirming previous knowledge concerning the health-modulating and pharmaceutic attributes of edible and medicinal mushrooms, the results obtained apparently confounded the choice of the fruit body of *G. lucidum* as a competitive, potentially sustainable probiotic or feed additive in the performance of the experimental birds. There is need for a more elaborate study on how best to harness fungal fruit body potentials in animal feed production, biochemical services and processes in enteric ecosystem and digestive health of the wild birds. The population variation of zoonotic parasites *vis-a-vis* gastrointestinal digestibility and synergic performance of *Ganoderma* sporophore with other feed additives also call for attention. Although the combination ratio of feed to additive is important and affects largely the outcome of results obtained from this study, it does not foreclose further trials of other medicinal polypores as effective alternate feed additive or probiotic in livestock productions. The mushroom however showed positive impact on the reduction of enteric zoonotic parasites (antihelminthetic) and health performance state of wild pigeons.

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