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Isolation and Identification of Microorganisms Associated with Faecal Droppings of Broiler Fed with Feed Compounded with Mineral-Fortified *Pleurotus ostreatus*

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Abstract**:** *Health and performance are important aspects in the broiler industry. The promotion of good, healthy products and environmental friendly wastes from poultry is the target of sustainable development goals. The effects of feed supplemented with mushroom enriched with minerals in broiler faeces have not been investigated. This experiment was conducted to study the potential effects of iron and Selenium fortified P.ostreatus on microbial load and types associated with faecal droppings of broilers fed mushroom-formulated feed. This experiment was conducted to study the potential effects of non-fortified, Iron fortified and Selenium fortified P.ostreatus on microbial load and types associated with faecal droppings of broilers fed mushroom formulated feed. This experiment was performed for 42 days on broilers. Dietary treatments include standard basal diet as control, non-fortified mushroom feed group (NFM), Iron fortified mushroom feed group (FeFM) and Selenium fortified mushroom feed group (SeFM) at 1.5%, 5% and 10% inclusion levels. Faecal droppings of broilers fed 1.5% NFM feed had the highest bacterial count (2.25 × 107 CFU/g). A total of eleven (11) bacterial and fungal isolates were obtained from the faecal droppings and these are: Micrococcus luteus, Proteus vulgaris, Bacillus subtilis, Staphylococcus epidermidis and Staphylococcus aureus. The fungal isolates incudes: Aspergillus sp, Fusarium sp, Rhizopus sp and Candida sp. Staphylococcus aureus and Staphylococcus epidermidis were observed to be absent in the faeces of broilers fed 1.5% NFM and SeFM dietary treatments. Fusarium solarium was absent in the faecal sample of broilers fed NFM, Candida tropicalis was not present in the faeces of broilers fed FeFM while Candida tropicalis and Rhizopus stolonifer were observed to be absent in SeFM (1.5%, 5% and 10%) respectively. Feeding broilers with mushroom fortified feed at all inclusion levels 1.5%, 5% and 10% had observable decrease on microbial loads and types of broiler faecal droppings. Fortification of mushroom with minerals could reduce the level of microorganisms released into the ecosystem through poultry droppings that finds its way to the final consumers, humans.*

Keywords: Broilers, faecal, iron fortified, mushroom, *pleurotus ostreatus* and selenium fortified.

1. INTRODUCTION

Faecal microbiota may be related to broiler performance and health. It has been noted that the types and population of bacteria present in the birds' feeds, as well as their habitats, their level of virulence, and the length of time the birds were exposed to the microbes, all play a significant role in the quantity of harm caused by microorganisms to the birds. Also, presence of microbes usually results in the production of toxins and ingestion of such toxins could lead to feed refusal which may culminate into retarded growth, increased susceptibility to diseases, reduced vaccination efficacy as well as damage to liver (Wielogorska *et al*., 2016; Pinotti *et al*., 2016). The quality of feed therefore determines the productivity of birds, how healthy the chicken will be able to build immunity against microorganisms that are not system friendly to its body system (Alali *et al*., 2012).

Feed supplements with natural medicinal properties such as mushroom is now in use. It has been reported from different studies that mushrooms and their polysaccharides

plays important roles such as acting as immune enhancers or immunomodulators and also showing antibacterial, antiviral, antiparasitic activities (Oyetayo, 2023; Ogidi *et al*., 2019, Fasoranti *et al*., 2019; Sohail *et al*., 2018). Its phenolic compounds can act as antioxidants, broilers' diet containing mushroom may therefore be used as growth promoters as an alternative to antibiotics. This may improve egg and meat production and quality (Sohail *et al*., 2018).

Pleurotus ostreatus is one of the edible species of mushrooms. Like other microfungi, it is a natural non chlorophyllous medicinal fungi, - known to have considerable health promoting properties (Ling *et al*., 2017). It is a known fact that Pleurotus ostreatus can absorb minerals from the substrate they are cultivated upon and bio-accumulate them as functional organic compounds during growth. Some of these absorbable minerals are Selenium, Iron, zinc and so on. Iron plays important roles in oxygen and electron transport as well as DNA synthesis (Zhang *et al*., 2015; Puig *et al*., 2017). Dietary Selenium on the other hand is an antioxidant (Milovanovic *et al*., 2014; Fernandes *et al*., 2015, Brown and Authur 2001). The use of mushroom as a vehicle to supply these minerals through feeding could be a way out to solving the problem of microorganism contamination of poultry products from faecal source that could lead to infections in consumers (animal and man). There is need to investigate the use of poultry feed supplemented with P. ostreatus enriched with minerals on broilers faecal droppings. This study was therefore designed to investigate the microbial loads and types associated with faecal droppings of broilers fed non fortified, Iron fortified and Selenium fortified P. ostreatus mushroom feeds.

2. MATERIAL AND METHODS

2.1 Mineral Fortification and Mushroom Substrate

Spawn of oyster mushroom strain P. ostreatus was purchased from Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria. It was thereafter conveyed to Afe Babalola University farm for cultivation. The spawn was used for the inoculation of the substrates. The substrate used in the cultivation of P. ostreatus consist of 60% sawdust and 40% rice bran and was sterilized in an autoclave at 1210C for 30 minutes. Before sterilization, the substrates were enriched with Sodium selenite for Selenium and Ferrous sulphate for iron into separate bags (SIGMA-ALDRICH CHEMIE GmbH. Pcode:1002841399). The salt solution of Selenium (Se) and Iron (Fe) were sterilized using 0.22 µm milipore filter. Mineral supplementation into growth substrates was done according to method described (Ogidi *et al*., 2016). After sterilization of substrate 50 mg/kg of Selenium (Se) and 50 mg/kg of Iron (Fe) were added to the substrates. Non-enriched mushroom serves as the control. The enriched substrates already inoculated with P. ostreatus spawn was incubated at 260 ^{o}C in the dark room until it fully ramifies. It was transferred to fruiting room till it was ready for harvest.

2.2 Feed Formulation

Experimental feeding of broilers with mushroom supplemented feed

The experiment was conducted at the Poultry Research and Training Centre under the Department of Animal Husbandry, Federal University of Technology Akure (FUTA). Standard feed was prepared and used throughout the experimental study. Composition of the experimental diets fed to broilers is shown in Table 1. Pleurotus ostreatus powders that was enriched with minerals were incorporated into the experimental diets with different inclusion levels. Mixing was done manually, Mushroom powder, mineral enriched and non-enriched mushrooms were added to the experimental diets (except control diet) at required amount according to each treatment. Three Hundred (n = 300) day old chicks were obtained from a commercial hatchery. The chicks were weighed and randomly distributed into nine (9) different treatment groups, replicated three (3) times with 10 chicks per pen as follows; control – Non fortified mushroom (NFM 1.5%, 5% and 10%), Iron fortified mushroom (FeFM 1.5%, 5% and 10%) and Selenium fortified mushroom (SeFM 1.5%, 5% and 10%). All chicks were vaccinated against infectious diseases. Each pen contained one drinker and one hanging feeder. The bedding used was wood shavings while feed composition of the diet was used as previously described by Abro *et al*. (2016). At six (6) weeks of the feeding experiment the faecal samples were collected into a sterile container and transported immediately to the laboratory for analyses.

2.3 Microbial Analysis

The microbiological analysis of the non-fortified, Iron fortified and Selenium fortified mushrooms were carried out using Nutrient agar and Potato dextrose agar. Faecal samples were collected from three birds out of each of the treatment groups at day 40 of the feeding experiment. Microbial examinations were carried out using standard methods for aerobic bacteria (Brown, 2005). Faecal droppings were collected in sterile - MacCartney bottles, gently rocked and stirred with sterile glass rod until the dung mixed thoroughly. Aliquot (1.0 mL) was transferred into the test tube containing 9.0 mL of sterile distilled water and diluted serially. One (1.0 ml) of the dilution was plated aseptically on nutrient and potato dextrose agar respectively for each poultry sample collected following the method of Kehinde *et al*. (2017). The nutrient agar plates were incubated at 350 ^{o}C for 24 hrs while the potato dextrose agar plates were incubated at room temperature for 7 days.

Morphological and Biochemical Characterization of Isolates

Discreet colonies of the isolates were sub cultured to obtain a pure culture. The isolates were examined macroscopically for the colonial characteristics and microscopically for cells morphological appearance. A battery of tests such as Gram reaction, catalase, oxidase, indole, nitrate reduction and the ability of the isolates to utilize different sugars were investigated (Brown, 2005)

2.4 Statistical Analysis

Data collected were subjected to Analysis of variance (ANOVA), and tests of significance carried out by Duncan's multiple range tests at $p \leqslant 0.05$.

Table 1: Composition of Experimental Diet and Normal Basal Diet

Materials	Control		NFM		FeFM			SeFM		
Maize	57	57	57	57	57	57	57	57	57	57
Wheat offal	4	4	4	4	4	4	4	4	4	4
G.N.C.	18	18	18	18	18	18	18	18	18	18
S.B.M.	10	7.5	5	0	7.5	5	0	7.5	5	0
Fish Meal	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Bone Meal	2	2	2	2	2	2	2	2	2	2
Lime stone	1	1	1	1	1	1	1	1	1	1
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Priemix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100	100	100
Mushroom	0	1.5	5	10	1.5	5	10	1.5	5	10
Protein	22.4	21.3	2.0	18	22.9	20	18	22.9	20	18
M.E	2936	2876	2815	2694	2936	2815	2694	2936	2815	2694
Fat/oils	4.80	4.71	4.62	4.45	4.80	4.62	4.45	4.80	4.62	4.45
CF	3.06	2.90	2.73	2.41	3.06	2.73	2.41	3.06	2.72	2.49
Calcium	1.92	1.92	1.91	1.90	1.92	1.91	1.92	1.92	1.91	2.73
Phosphorus	0.97	0.95	0.94	0.91	0.97	0.44	0.94	0.97	0.94	1.91
Lysine	1.18	1.11	0.94	0.91	1.18	0.94	0.94	1.18	1.18	0.94
Methionine	0.63	0.62	0.6	0.57	0.63	0.6	0.6	0.63	0.63	0.63

Key: S.B.M. – Soya beans meal, G.N.C. – Groundnut cake, M.E – Metabolizable Energy, CF- Crude protein, CF- Crude fibre, NFM- Non-Fortified, FeFM-Iron Fortified, SeFM-Selenium-Fortified Mushrooms

3. DISCUSSION OF RESULTS AND CONCLUDING REMARKS

Tables 2 shows the microbial loads, for bacterial and fungal isolates observed in faecal droppings of broilers fed the supplemented feeds. Significant differences ($p<0.05$) were observed in the broilers faecal fed with the treated mushroom feed. Broilers fed with 1.5% NFM had the highest bacterial count (22.67× 106 CFU/g) when compared to faeces of broilers fed the basal diet (21.67 × 106 CFU/g).

Table 2: The Microbial Loads of Broiler Chicks Faecal Fed with Enriched and Non-Enriched *P.Ostreatus* Feeds

Faecal Samples	Total bacterial Counts (cfu/g x 10^6)	Total fungal counts (sfu/g x 10^4)
Control	21.67 ± 5.49^{b}	15.67 ± 5.24^{b}
Non-fortified mushroom feed 1.5%	22.67 ± 5.46^{b}	8.00 ± 0.57^{ab}
Non-fortified mushroom feed 5%	16.33 ± 2.33^{a}	5.00 ± 2.08^{a}
Non-fortified mushroom feed 10%	12.33 ± 1.86^{a}	2.67 ± 1.67^{a}
Iron-fortified mushroom feed 1.5%	11.67 ± 0.06^{a}	4.00 ± 1.15^{a}
Iron-fortified mushroom feed 5%	15.00 ± 1.53^{a}	2.67 ± 0.88^{a}
Iron-fortified mushroom feed 10%	12.33 ± 0.67^{a}	3.33 ± 0.67^{a}
Selenium-fortified mushroom feed 1.5%	14.00 ± 1.73^{a}	0.66 ± 0.67^{a}
Selenium-fortified mushroom feed 5%	12.67 ± 1.67^{a}	1.67 ± 0.57^{a}
Selenium-fortified mushroom feed 10%	10.67 ± 0.06^{a}	1.00 ± 1.20^{a}

Values are presented as mean ± standard error, values in the same column carrying the same superscript are not significantly different at $p<0.05$

Table 3 shows the microbial types associated with the faecal of broilers fed the various treatments of mushroom feed and the standard basal diet (control). A total of eleven (11) microorganisms were isolated from the broilers faecal which included; *Bacillus subtilis, Staphylococcus aureus, Staphylocccus epidermidis, Proteus vulgaris, Micrococcus luteus, Fusarium bacitiloides, Fusarium solarium, Aspergillus flavus, Aspergillus niger, Candida tropicalis* and *Rhizopus stolonifer*. These microorganisms were similar with what was reported by Adegunloye (2006) who isolated bacteria and fungi from broiler faecal droppings in Ilorin, Kwara State, Nigeria. They were *Bacillus cereus, Staphylococcus aureus, S. epidermidis, Escherichia coli, Aspergillus* sp and *Rhizopus* sp. Also, Mohammad *et al*. (2014) reported the presence of *Staphylococcus aureus* (33.33%) and *Staphylococcus albus* (16.66%) in poultry faecal.

Table 3: Microorganisms isolated from poultry faecal droppings

Sample Treatments	Bacterial Isolates	Fungi Isolates
Control	*Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus aureus, Proteus vulgaris, and Micrococcus luteus*	*Fusarium solarium, Fusarium bacitiloides Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer* and *Candida tropicalis*
NFM (1.5%)	*Bacillus subtilis, Proteus vulgaris* and *Micrococcus luteus*	*Aspergillus flavus, Fusarium bacitiloides Aspergillu niger, Rhizopus stolonifer* and *Candida tropicalis*
NFM (5%)	*Bacillus subtilis, Proteus vulgaris* and *Micrococcus luteus*	*Fusarium bacitiloides, Aspergillus flavus., Rhizopus stolonifer* and *Candida tropicalis*
NFM (10%)	*Bacillus subtilis,* and *Proteus vulgaris*	*Rhizopus stolonifer, Fusarium bacitiloides, Aspergillus niger* and *Aspergillus flavus*
FeFM (1.5%)	*Bacillus subtilis, Proteus vulgaris, Staphylococcus epidermidis* and *Staphylococcus aureus*	*Fusarium solarium, Fusarium bacitiloides, Aspergillus niger* and *Aspergillus flavus.*
FeFM (5%)	*Proteus vulgaris, Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus subtilis*	*Fusarium solarium, Fusarium bacitiloides, Aspergillus niger* and *Aspergillus flavus*
FeFM (10%)	*Staphylococcus epidermidis* and *Bacillus subtilis, Proteus vulgaris*	*Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus, Fusarium solarium and Fusarium bacitiloides.*
SeFM (1.5%)	*Bacillus subtilis, Proteus vulgaris* and *Micrococcus luteus*	*Aspergillus flavus, Aspergillus niger* and *Fusarium solarium.*
SeFM (5%)	*Bacillus subtilis* and *Proteus vulgaris*	*Aspergillus flavus, Fusarium solarium, Fusarium bacitiloides* and *Aspergillus niger*
SeFM (10%)	*Bacillus subtilis* and *Proteus vulgaris*	*Fusarium solarium, Fusarium bacitiloides, Aspergillus flavus* and *Aspergillus niger*.

Note: Control – Normal Basal broiler feed, NFM- Non fortified mushroom, FeFM- Iron fortified mushroom and SeFM- Selenium fortified mushroom

Differences were observed in the bacterial and fungi isolated from the faeces of broilers fed commercial diets and faecal of broilers fed 1.5%, 5% and 10% NFM, FeFM and SeFM diets. In broilers fed NFM and SeFM feeds, *Staphylococcus aureus* and *Staphylococuss epidermidis* were observed to be absent in their faeces across the different inclusion levels. *Micrococuss luteus* was not present in the faeces of broilers fed 5% and 10% SeFM diet. *Fusarium solarium* and *Aspergillus niger* were absent in the faeces of broilers fed NFM diet. *Candida tropicalis* also was observed to be absent in the faeces of broilers fed 10% NFM diet. *Rhizopus stolonifer* and *Candida tropicalis* were observed to be absent in the faeces of broiler fed 1.5% and 5% FeFM. Broilers fed 1.5% and 10% SeFM diets were observed to prevent the growth of *Rhizopus stolonifer* and *Candida tropicalis. Fusarium bacitiloides* was absent in the faeces of broilers fed 1.5% SeFM. Fasoranti *et al*. (2019) had earlier reported the antimicrobial activities of ethanol extracts of *P.ostreatus* against eleven (11) bacteria which are *Pseudomonas* sp, *Staphylococcus* sp, *Klebsiella* sp, *Proteus mirabilis*, *Escherichia coli*, *Bacillus* sp, *Enterobacter* sp, *Streptococcus* sp, *Salmonella* sp, *Seratia* sp. Settle *et al*. (2014) also reported the antimicrobial activities of *P.ostreatus* possible effects on intestinal microflora which aid nutrient absorption in the absence of health challenge. Also, Hamad *et al*. (2022) reported *Pleurotus ostreatus* extract inhibition of *Candida albicans, Staphylococuss aureus, Micrococuss luteus* and *Escherichia coli.* Its extract activity against *Fusarium oxyporium*, *Fusarium solani* as well as *Rhizoctonia solani* were reported. The ability of non-fortified, Iron and Selenium fortified mushroom feed to inhibit the growth of certain bacteria and fungi could be attributed to the presence of certain bioactive compounds present in them which had

been earlier reported by Fasoranti *et al*. (2019) who studied the phytochemical composition of cultivated *P.ostreatus* and observed the presence of alkaloids, phenols, tannins, saponnins and flavonoids. The fortification with Iron and Selenium could have enriched the bioactive components when incorporated into the broilers feed. The presence of secondary metabolites and bioactive compounds in mushrooms have previously been related to their antimicrobial activities. Differences observed in isolated microorganisms from broilers faeces as a result of supplemented feed NFM, FeFM and SeFM given to them could be attributed to membrane permeability of the microorganisms as well as their metabolic activities (Fakoya *et al*., 2020). Mushrooms containing minerals had been reported to increase the antioxidant potentials (Vetter *et al.,* 2004) and consequently improves the immunity of animals that consume it. In conclusion, this research suggests that feeding non fortified and mineral fortified *Pleurotus ostreatus* feed to broiler chicken at inclusion levels 1.5%, 5% and 10% had positive impact in reducing the microbial loads and types associated with broiler faecal which is an evidence that fortification of feeds with mushroom *P.ostreatus* enriched with Iron and Selenium could be used to decrease the level of microorganisms spread in poultry production which are capable of causing infections in both animal and man.

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