

Inclusion of mineral fortified mushroom (*Pleurotus ostreatus*) in the broiler feed reduced the pathogenic microbial loads in the broiler fecal droppings

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ABSTRACT

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 fecal droppings of broilers fed with mushroom formulated feed. This experiment was performed for 42 days on broilers (*Gallus gallus domestica*). Dietary treatments included 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. Significant differences ($p > 0.05$) were observed in fecal total bacterial count (Cfu/g $\times 10^6$) and fungal loads of all broilers fed the treated mushroom feeds except 1.5% NFM bacterial count. A total of eleven bacterial and fungal isolates were isolated from the fecal droppings including *Micrococcus luteus*, *Proteus vulgaris*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. The fungal isolates, obtained from the fecal droppings were *Aspergillus* spp, *Fusarium* spp, *Rhizopus* spp and *Candida* spp. *Staphylococcus aureus* and *Staphylococcus epidermidis* were absent in fecal droppings of broilers fed with NFM and SeFM across treatments. *Micrococcus luteus* was also absent in the feces of broilers fed with FeFM across treatments. *Rhizopus stolonifer* was found absent in the fecal sample of broilers fed with 1.5% and 5% NFM, FeFM and SeFM. *Candida tropicalis* was also absent in fecal droppings of broilers fed with NFM (10%), FeFM (1.5% and 5%), and SeFM (1.5%, 5% and 10%). Feeding broilers with mushroom fortified feed at all inclusion levels had positive impacts on microbial loads and types of broilers fecal droppings. Fortification of broiler feed with mineral fortified and non fortified mushroom could reduce the level of pathogenic microorganisms present in poultry feces and this could minimize the shedding of pathogenic microorganisms into the environment.

Keywords: Mushroom, *Pleurotus ostreatus*, broilers, microbial loads mineral fortified mushroom, fecal droppings

Fecal microbiota may be related to broiler performance and health. It has been observed that the extent of damage done to birds by microorganisms depends largely on the type and population of microbes present in their feeds & their environment, the degree of virulence and duration of exposure to the birds (Chatterjee *et al.*, 2018). Presence of pathogens 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 (Alali *et al.*, 2012). Feed quality also determines how healthy the chicken will be and able to build immunity against

microorganisms that are not system friendly to its body system.

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 play important roles in poultry production by acting as immune enhancers or immune-modulators and showing antibacterial, antiviral, antiparasitic activities (Sohail *et al.*, 2018). Its phenolic compounds can act as antioxidants. Therefore, broiler's diet containing mushroom may be used as growth promoters as an alternative to antibiotics. This may improve egg and meat production and quality. *Pleurotus ostreatus*, one of the many species of mushrooms, is a natural non chlorophyllous medicinal fungus, known to have considerable health promoting properties (Ling *et al.*, 2017). It can bioaccumulate several minerals in its cell structure, has high nutritional properties and high yield potentials (Sandra *et al.*, 2014). There is need to investigate the use of poultry feed supplemented with *P.ostreatus* enriched with minerals and its effect gut microbiota by examining broilers fecal droppings. This study was therefore designed to investigate the microbial loads and types associated with fecal droppings of broilers fed non fortified, iron fortified and selenium fortified *Pleurotus ostreatus* mushroom feeds.

MATERIALS AND METHODS

Mineral fortification and mushroom substrate

Spawn of oyster mushroom (*Pleurotus ostreatus*) was purchased from Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria. The cultivation experiments were conducted at Afe Babalola University farm. The spawn was used for the inoculation of the substrates. The substrate used in the cultivation of *P. ostreatus* consisted of 60% sawdust and 40% rice bran and was sterilized in an autoclave at 121°C for 30 minutes. After sterilization,

the substrates were enriched with sodium selenite for selenium (Se) and ferrous sulphate for iron (Fe) into separate bags @ 50 mg/kg of Se and 50mg/kg of Fe. The salt solution of Se and Fe were sterilized using 0.22 µm milipore filter. Mineral supplementation into growth substrates was done according to method described by Oyetayo *et al.* (2021) and Ogidi *et al.* (2016). Non enriched mushroom served as the control. The enriched substrate already inoculated with *P.ostreatus* spawn was incubated at 26 °C in dark room until it fully ramified. After complete spawn run, it was transferred to fruiting room till it was ready for harvest.

Feed formulation

Experimental feeding of broilers with mushroom supplemented feed

The experiment was conducted at the Poultry Research and Training Center under the Department of Animal Husbandry, Federal University Technology Akure (FUTA). Standard feed was prepared and used throughout the experimental study. Composition of the experimental starter and finisher diets fed to broilers are shown in Table 1. *Pleurotus ostreatus* powders, enriched with minerals, were incorporated into the experimental diets with different inclusion levels (except control diet) at required amount according to each treatment. Three hundred (n=300) chicks were obtained from a commercial hatchery. The chicks were weighed and randomly distributed into 9 different treatment groups, replicated 3 times with 10 chicks per replication as per details: 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. Feed composition of the diet was used as

Table 1: Composition of Experimental diet and normal basal diet

Materials	Control	NFM			FeFM			SeFM		
		0	1.5%	5%	10%	1.5%	5%	10%	1.5%	5%
Mushrooms	0	1.5%	5%	10%	1.5%	5%	10%	1.5%	5%	10%
Maize	57	57	57	57	57	57	57	57	57	57
Wheat offal	5.55	5.55	5.55	5.55	5.55	5.55	5.55	5.55	5.55	5.55
G.N.C	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5
S.B.M	10	8.5	5	0	8.5	0	0	8.5	5	0
Fish Meal	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

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

previously described by Abro *et al.* (2016). Fecal samples were collected after 6 weeks of age.

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. Fecal samples were collected at day 40 from three (3) birds of the same treatment. Microbial examinations were carried out using standard methods for aerobic bacteria (Brown, 2005). Fecal droppings were collected in 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. 1.0ml of the dilution was plated aseptically on nutrient and potato dextrose agar for bacterial and fungal isolation, respectively in case of each poultry samples collected following the method of Kehinde *et al.* (2017).

Statistical Analysis

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

RESULTS AND DISCUSSION

Tables 2 show the microbial loads (bacterial and fungal isolates) from fecal droppings of poultry birds. No significant differences ($p > 0.05$) were observed in broilers fecal matters fed with 1.5% NFM (22.67×10^6 CFU/g) and fecal matters of broilers given the control diet (21.67×10^6 CFU/g) for bacterial count. Significant differences ($p > 0.05$) were observed in all the feces of broilers fed the fortified mushroom feeds for total bacterial and fungal count. At 5% and 10% NFM, 1.5%, 5% and 10% FeFM and SeFM fecal bacterial and fungal loads reduced drastically with value ranging from (0.67×10^6 CFU/g to 6.33×10^6 CFU/g) and (0.66×10^6 CFU/g to 8.00×10^6 CFU/

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Table 2: Microbial loads of broiler chicks fecal matters fed with enriched and non enriched *P.ostreatus* feeds

Fecal Samples	Total bacterial Counts (cfu/g x 10 ⁶)	Total fungal counts (sfu/g x 10 ⁴)
Control	21.67±5.49b	15.67±5.24b
Non fortified mushroom feed 1.5%	22.67±5.46b	5.00±2.08a
Non fortified mushroom feed 5%	6.33±2.33a	8.00±0.57ab
Non fortified mushroom feed 10%	2.33±1.86a	2.67±1.67a
Iron fortified mushroom feed 1.5%	1.67±0.06a	4.00±1.15a
Iron fortified mushroom feed 5%	5.00±1.53a	2.67±0.88a
Iron fortified mushroom feed 10%	2.33±0.67a	3.33±0.67a
Selenium fortified mushroom feed 1.5%	4.00±1.73a	0.66±0.67a
Selenium fortified mushroom feed 5%	2.67±1.67a	1.67±0.57a
Selenium fortified mushroom feed 10%	0.67±0.06a	1.00±1.20a

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

g) respectively. At 1.5% inclusion level, NFM had the highest bacterial count (22.67×10^6 CFU/g) as well as highest fungal count was observed in 5% NFM (5.00×10^4 CFU/g) but was significantly lesser than control (commercial diet).

The result obtained in this study reduced the bacterial and fungi loads from feces of broilers fed with mushroom feeds. Only the feces of broilers fed with 1.5% NFM (22.67×10^6 cfu/g) showed increased bacterial count when compared to control (21.67×10^6 cfu/g). SeFM feed at 10% inclusion level had lowest bacterial count (0.67×10^6 cfu/g) while the fungal load it was found lowest count at 1.5% SeFM feed given to broilers. Table 3 shows the microbial types associated with the fecal matters of broilers fed with the various treatments of mushroom feed and the standard basal diet (control). A total of eleven (11) microorganisms were isolated from the broilers fecal matters which included; *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Micrococcus luteus*, *Fusarium bacitiloides*, *Fusarium solarium*, *Aspergillblus flavus*, *Aspergillus niger*, *Candida tropicalis* and *Rhizopus stolonifer*.

Staphylococcus aureus and *Staphylococcus epidermidis* were found to be absent in the feces of broilers fed with the treated mushroom diets. Differences were observed in the fungi isolated from fecal matters of broilers fed with 1.5%, 5% and 10% NFM, FeFM and SeFM. *Fusarium solarium* was found absent in the faces of broilers fed with the NFM feed while 10% NFM was able to prevent the growth of *Candida tropicalis* in the feces of the broilers. From broilers administrered with FeFM feed, *Candida tropicalis* was found absent in their faeces. *Rhizopus stolonifer* was also absent in fecal of broilers fed with 1.5 and 5% FeFM. The absence of *Candida tropicalis* and *Rhizopus stolonifer* were noticed in the fecal samples of broilers fed with SeFM feed. 1.5% of SeFM was able to inhibit the growth of *Fusarium bacitiloides* in broiler feces. The use of mushroom as a natural antimicrobial is still being discussed. Alves *et al.* (2014) discussed the ability of wild mushroom extracts to inhibit bacterial biofilm production from Gram positive and Gram negative microorganisms which includes *Pseudomonas aeruginosa* and *Escherichia coli*. Hamad *et al.* (2022) reported that *Pleurotus ostreatus* extract inhibited *Candida albicans*, *Staphylococcus*

Table 3: Microorganisms Isolated from poultry fecal droppings

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

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

aureus, *Micrococcus luteus* and *Escherichia coli*. Its extract activity against *Fusarium oxysporum*, *Fusarium solani* and *Rhizoctonia solani* was also reported. Species belonging to *Pleurotus* (*P. eryngii*, *P. nerodensis* and *P. eryngii* var. *elaeoselini*) have been reported to have inhibitory effects against four pathogenic microorganisms *S. aureus*, *S. epidermidis*, *Escherichia coli* and *P. aeruginosa* (Schillaci *et al.*, 2013). Variation in the absence of some microorganisms observed in broilers feces due to different treatments of NFM, FeFM and SeFM could be attributed to membrane permeability of the microorganisms as well as their metabolism (Fakoya *et al.*, 2020). Mushrooms containing minerals had been reported to increase the antimicrobial potentials (Vetter *et al.*, 2004) and consequently improve the

immunity of animals that consume it. Polysaccharides extracted from mushrooms are revealed to have protective effects against infections (Arteel *et al.*, 2001). For, *P. ostreatus* that contains trace amount of selenium, the microbial reduction was distinct which may be due to high selenium content in the mushrooms (Vetter *et al.*, 2004). This activity can be linked to the effective introduction of glutathione, a synthetic enzyme. This surge can be explained by the enzyme's high level of selenium absorption or passive glutathione sparing through reduction of the oxidative load on the cells (Hassan *et al.*, 2020).

Conclusively, 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 on the microbial loads and types associated with broiler fecal matters, which is an evidence that fortification of feeds with mushroom *P.ostreatus* could be used to decrease the level of pathogenic microorganisms spread in poultry production.

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