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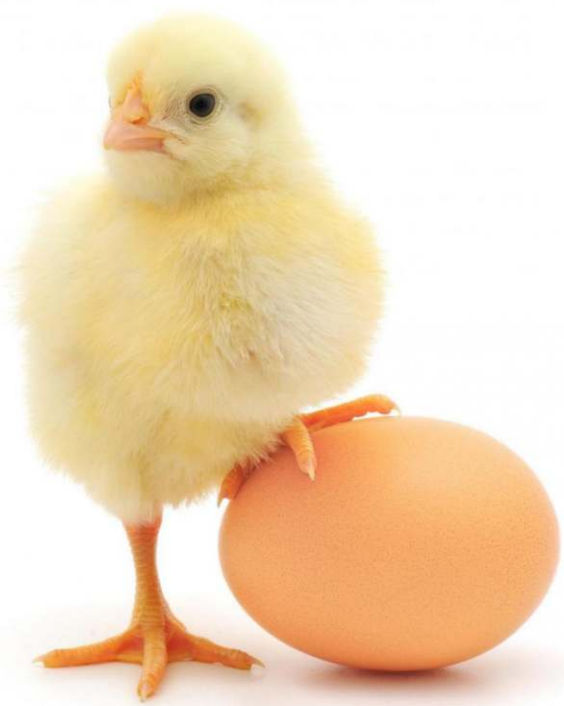

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Prevalence of Darkling Beetles (*Alphitobius diaperinus*) and Bacterial Load in Broiler Litters

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Abstract: The differently replaced litters (sand and wood shavings) on which broilers were raised for eight weeks were examined for bacterial loads and darkling beetle (*Alphitobius diaperinus*) population. The initial investigation into the litters revealed wood shavings to have higher bacterial load compared to sand. Litter replacement frequencies and litter type significantly affected ($p<0.05$) litter bacterial load, with wood shavings without replacement having the highest load ($3.09\times10^7\pm0.07$ mL^{-1}) and sand litters replaced seven times at weekly intervals and once at four-weekly intervals had the least loads of $1.32\times10^7\pm0.05$ mL^{-1} and $1.32\times10^7\pm0.13$ mL^{-1} respectively. The population of darkling beetle per m^2 were significantly affected ($p<0.05$) by the litter types, litter replacement frequencies. This showed that population of darkling beetle on wood shavings replaced seven times was significantly higher ($p<0.05$) than other replacement group while the rest were not significantly different from each other. In addition some pathogenic bacteria were identified among the organisms isolated in the used litters. Based on the litter type, wood shavings litter had significantly higher population of darkling beetle (1018.00 ± 290.00 m^{-2}) and bacteria load ($2.28\times10^7\pm0.11$ mL^{-1}). Hence sand is better than wood shavings as broiler litter.

Key words: Broiler litters, bacteria and darkling beetle

Introduction

The major management changes that revolutionized poultry production was the use of intensive housing (Smith, 2001). This is the transformation from the primitive free range system of poultry rearing to intensive rearing. This development prevented poultry based pollution and danger of disease outbreak, since birds' movement are restricted to a particular confinement. Under this intensive housing, the deep litter system was traditionally found suitable for broiler production (Oluyemi and Roberts, 1988). Hence, handling their accumulated wastes (dropping or faeces) with high moisture content became a great concern to poultry management experts. The moisture content of the dropping supports degradative action of bacteria, which releases a pungent smell of ammonia and other suffocating gases into the environment (Ritz *et al*., 2005). These gases are both hazardous to the birds and human health. Therefore, strategy to reduce the moisture level of the poultry droppings became an issue under intensive (deep litter system) management of broilers. This led to the discovery of many poultry dropping absorbents to enhance disposal with minimal impact on the environment. The absorbents or bedding materials are generally referred to as litter. Litter materials currently used include peanut hulls, sawdust, straw, corncobs, chopped newspaper, wood shavings but sand is currently under investigation. Trials with these materials have not show superiority over wood shavings. Wood shavings and other wood by-products have been used as litter materials of choice especially for broiler producers nearly as long as the commercial broiler industry has existed. The qualities of characteristics that must be possessed by any material for suitability of litter material include availability, inexpensiveness, free of deleterious compounds and have physical properties that do not compromise birds' health.

Many factors affect litter moisture, for instance, if new litter is not stored properly and becomes damp before it is spread in the broiler house, wet litter problems would likely be unavoidable. Nutrition also influence litter quality. Certain dietary ingredients (especially salt), when fed in excess, cause broilers to consume and excrete large amounts of water and result in wet litter conditions. Environmental conditions such as wet and humid weather condition or very cold temperatures can cause wet litter, if the broiler house ventilation system is not adequate for effective moisture content management. Drinker lines and evaporative cooling pads, if not managed and maintained carefully can contribute greatly to wet litter problems.

Livestock welfare and performance necessitate search for knowledge on the composition of the bacteria microflora present in broiler litter. Martin and McCann (1998) performed a survey of pathogenic

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Table 1: Total bacteria counts of sand and wood shavings

Samples	Bacteria Load
Sand	6.20×10^6 mL^{-1}
Wood Shavings	1.46×10^7 mL^{-1}

Table 2: Characteristics of isolates from Sand

	Isolates				
Characteristics	1	2	3	4	5
Gram's staining	-	+	+	-	+
Shape of cells	R	S	S	R	R
Motility	+	-	-	+	+
Catalase	+	+	+	+	+
Oxidase	+	-	-	-	-
Spores	-	-	-	-	+
Indole	-	-	-	+	-
Coagulase	-	-	+	-	-
Sugar fermentation					
Glucose	A	A	A	AG	A
Fructose	+	A	+	AG	AG
Galactose	-	A	AG	AG	AG
Manitol	+	A	A	AG	-
Lactose	+	A	A	AG	-
Sucrose	+	A	+	-	A
Oxidation Fermentation	O/-	OF	-/F	OF	OF

Key: R = Rod, S = Sphere, + = Positive, - = Negative; F = Fermentation, O = Oxidation, A = Acid, G = Gas, AG = Acid and Gas. Probable organisms: 1 = Pseudomonas aeruginosa; 4 = Aerobacter aerogenes, 2 = Micrococcus luteus, 5 = Bacillus cereus*, 3 = Staphylococcus aureus*, *Pathogenic

Table 3: Characteristics of isolates from wood shavings

	Isolates				
Characteristics	1	2	3	4	5
Gram's staining	+	-	-	-	+
Shape of cells	S	R	R	R	S
Motility	-	+	+	+	-
Catalase	+	+	+	+	+
Oxidase	-	+	-	-	-
Spores	-	-	-	-	-
Indole	-	-	-	+	-
Coagulase	+	-	-	-	-
Sugar fermentation					
Glucose	A	A	A	AG	A
Fructose	+	+	AG	AG	+
Galactose	AG	-	A	AG	AG
Manitol	A	+	A	AG	A
Lactose	A	+	-	AG	A
Sucrose	+	+	A	-	+
Oxidation Fermentation	-/F	O/-	-/F	OF	-/F

Key: R = Rod, S = Sphere, + = Positive, - = Negative; F = Fermentation, O = Oxidation, A = Acid, G = Gas, AG = Acid and Gas; Probable organisms: 1 = Staphylococcus Aureus*, 2= Pseudomonas aeruginosa; 3 = Serratia marcescens; 4 = Aerobacter aerogenes, 5 = Staphylococcus epidermides, *Pathogenic

microorganisms in poultry litter with selective medium and found *Staphylococcus xylosus* to be a predominant species.

Entomological study of the poultry litter by Campbell (1996) reported the presence of darkling beetles in poultry litter. Darkling beetles are migratory nocturnal insects that feed on poultry carcasses and also poultry may feed on them, hence regarded as mechanical vectors of several diseases, such as Marek's disease, avian influenza, Salmonella, fowl pox, Coccidiosis, botulism and Newcastle disease (Campbell, 1996). In a similar study conducted by Watson (2004), darkling beetle (*Alphitobius diaperinus*) and housefly (*Musca domestical*) were implicated in the transmission of Turkey Corona Viruses (TCV) in addition to other several poultry diseases.

This study was undertaken to determine and compare the relative darkling beetle population and bacteria load of wood shavings and sand litters.

Materials and Methods

Pre-study activity and experimental design: The different litters used for the study were sands of the sandy loam textural class and wood shavings. The sand was air-dried by spreading it in an open and well ventilated room. 500 *Anak* broilers were raised on the litters for eight weeks at five different litter replacement frequencies (7, 3, 2, 1 and 0 time within the eight week rearing period). Thus the litters were changed or replaced weekly, two weekly, three weekly and four weekly while zero weekly replacement serves as the control treatment. 2×5 factorial experiment in a Completely Randomized Design (CRD) with five replications was used for assigning the chicks on the litter treatments.

Litter sampling: Samples of the litter material and spent litters were for bacteria counting and characterization. The spent litter samples were collected from the four corners and centre position of each pen and thoroughly mixed. The samples were collected before each replacement of litters.

Darkling beetle counts: The beetles were earlier observed to be aggregated under the woody base carrying the plastic drinkers. The number of beetles (*Alphitobius diaperinus*) was counted out from the pens (1 m^2) within the litter by direct numerical counting at the time of litter replacement. The total number of beetles counted was based on 1 m^2 area of litter cover.

Isolation and counting of bacteria: One gram of each litter sample collect at different replacements was put into MacCartney bottle containing 5 mL of sterile saline solution (85%) and mixed properly. Serial dilutions up to 10^{-5} were made. 0.1 mL of each mixture was transferred into molten nutrient agar plates and incubated at 37°C for 24 hours. Individual colonies from each plate were sub-cultured until pure cultures were obtained, after which the resultant colonies were counted using a Gallen kamp colony counter (Olutiola *et al*., 1991).

Table 4: Characteristics of isolates from sand litter

Characteristics	Isolates 1	2	3	4	5	6	7	8	9	10
Gram's staining	-	-	+	-	-	+	+	-	+	+
Shape of cells	R	R	S	R	R	S	S	R	R	S
Motility	+	+	-	+	+	-	-	+	+	-
Catalase	+	+	+	-	+	+	-	+	+	+
Oxidase	-	+	-	-	-	-	-	-	-	-
Spores	-	-	-	-	-	-	-	-	+	-
Indole	+	-	-	-	+	-	-	+	-	-
Coagulase	-	-	-	-	-	+	-	-	-	-
Sugar fermentation										
Glucose	AG	A	A	AG	AG	A	A	AG	A	A
Fructose	-	+	-	-	-	+	-	AG	AG	+
Galactose		-	A	-	-	AG	A	AG	AG	AG
Manitol	A	+	A	A	-	A	+	AG	-	A
Lactose	+	+	A	-	-	A	+	AG	-	A
Sucrose	+	+	A	-	A	+	+	-	A	+
Oxidation	-/F	O/-	OF	-/F	-/F	-/F	-/F	OF	OF	-/F

fermentation, Key: R = Rod, S = Sphere, + = Positive, - = Negative; F = Fermentation, O = Oxidation, A = Acid, G = Gas, AG = Acid and Gas, Probable organisms: 1 = Escherichia coli, 2 = Pseudomonas aeruginosa, 3 = Micrococcus leteus, 4 = Salmonella sp.*, 5 = Proteus vulgaris*, 6 = Staphylococcus aureus*, 7 = Streptococcus faecalis*, 8 = Aerobacter aerogenes, 9 = Bacillus cereus*, 10 = Staphylococcus epidermidis, *Pathogenic

Table 5: Characteristics of isolates from wood shaving litter

Characteristics	Isolates 1	2	3	4	5	6	7	8
Gram's staining	-	-	-	+	+	-	+	-
Shape of cells	R	R	R	S	S	R	S	R
Motility	+	+	+	-	-	+	-	+
Catalase	+	+	+	-	+	+	+	-
Oxidase	-	-	-	-	-	+	-	-
Spores	-	-	-	-	-	-	-	-
Indole	-	+	+	-	-	-	-	-
Coagulase	-	-	-	-	-	-	+	-
Sugar fermentation								
Glucos	A	AG	AG	A	A	A	A	AG
Fructose	AG	-	AG	-	+	+	+	-
Galactose	A		AG	A	AG	-	AG	-
Manitol	A	A	AG	+	A	+	A	A
Lactose	-	+	AG	+	A	+	A	-
Sucrose	A	+	-	+	+	+	+	-
Oxidation	-/F	-/F	OF	-/F	-/F	O/-	-/F	-/F

fermentation, Key: R = Rod, S = Sphere, + = Positive, - = Negative; F = Fermentation, O = Oxidation, A = Acid, G = Gas, AG = Acid and Gas; Probable organisms: 1 = Serratia marcescens; 5 = Staphylococcus epidermides; 2 = Escherichia coli; 6 = Pseudomonas aeruginosa; 3 = Aerobacter aerogenes; 7 = Staphylococcus aureus*, 4 = Strptococcus faecalis*; 8 = Salmonella spp.*, *Pathogenic

Characterization of isolated bacteria: For characterization of bacterial isolates, the following biochemical tests were carried out; Catalase, motility, indole, oxidase, oxidative/fermentation, utilization of sugars and carbohydrates as described by Burchanan and Gibbins (1974).

Statistical analysis: Data on darkling beetles population and bacterial load were subjected to analysis of variance (ANOVA) and treatment means separated by Duncan's multiple range test according to Steel and Torrie (1980) using Minitab statistical package (v10.2 minitab Inc. USA).

Results

Table 1 presents the total bacterial counts of sand and wood shavings. The wood shavings had higher bacterial load than sand. The characteristics of various bacteria isolates from sand and wood shavings were as shown in Table 2 and 3 respectively.

Pseudomonas aeruginosa, *Staphylococcus aureus* and *Aerobacter aerogenes* bacteria were found to be common in both the sand and wood shavings. It was however observed that *Micrococcus luteus*, *Bacillus cereus*, *Serratia marcescens* and *Staphylococcus epidermidis* were separately found in them. *Staphylococcus aureus* and *Bacillus cereus* were

Table 6: Darkling beetles population and bacteria load in differently replaced wood shavings and sand litters

Litter type	Litter Replacement Frequency	Darkling beetles (No. m^{-2})	Bacterial load ($x10^7$ mL^{-1})
Wood shaving	0	426.00±326.00	3.09±0.07
	1	294.00±204.00	1.88±0.05
	2	886.60±193.60	2.20±0.07
	3	840.00±294.00	2.11±0.07
	7	2646.00±936.00	2.13±0.05
Sand	0	160.00±30.60	2.07±0.04
	1	20.00±20.00	1.32±0.13
	2	60.00±23.00	1.49±0.12
	3	40.00±30.60	1.45±0.02
	7	80.00±20.00	1.32±0.05
Statistical significance			
Litter type		*	*
Litter replacement freq.		*	*
Litter type×frequency		*	NS
Mean separation			
Litter type effect			
Wood shaving		1018.00±290.00[a]	2.28±0.11[a]
Sand		72.10±16.00[b]	1.53±0.08[b]
Litter replacement freq. effect	0	293.40±158.40[b]	2.58±0.23[a]
	1	156.60±110.00[b]	1.60±0.14[b]
	2	414.00±204.00[a]	1.85±0.17[b]
	3	440.00±222.00[ab]	1.78±0.15[b]
	7	1360.00±710.00[a]	1.72±0.18[b]

Mean+SEM; NS = Not significant (p>0.05), * = Significant (p<0.05); Means with different. Superscripts within the same column and for the same parameters are significantly different (p<0.05)

identified to be pathogenic.

The characteristics of bacteria isolates from sand and wood shavings litters are presented in Table 4 and 5 respectively. *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Aerobacter aerogenes*, *Bacillus cereus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Salmonella* species were the common bacteria isolated from both litters. *Serratia marcescens*, *Micrococcus luteus*, *Proteus vulgaris* and *Staphylococcus aureus* were separately isolated from wood shavings and sand litters. Apart from the fact that more pathogenic bacteria were identified in sand litter, the same litter was found to contain higher number of bacteria species. On the wood shavings and sand litters presented in Table 6, darkling beetles *(Alphitobius diaperinus)* population is significantly influenced (p<0.05) by litter type and litter replacement frequency. Wood shavings litter had significantly (p<0.05) higher population of darkling beetles (1018±290.00 m^{-2}) based on litter types while seven times litter replacement had the highest population (1360±710.00 m^{-2}) based on litter replacement frequency.

Also, litter type and litter replacement frequency significantly (p<0.05) affected bacterial load. Based litter type, wood shavings litter had significantly (p<0.05) higher bacterial load (2.28×10^7±0.11 mL^{-1}) than sand litter while zero replacement of litter had the highest bacterial load (2.58×10^7±0.23mL^{-1}) based on litter replacement frequency.

Discussion

The higher bacterial load of wood shavings can be attributed to its organic nature that is majorly cellulose. Such nature makes wood shavings to be potentially degradable and hence promotes bacterial growth. Also, isolation of various species of bacteria in sand and wood shavings was an indication that they were not naturally sterile. The pathogenic bacteria found in them showed that both had the potential to contain injurious organisms. These observations tend to show that sand and wood shavings contain bacteria at their natural state.

On the wood shavings and sand litters differently replaced for broilers, darkling beetle *(Alphitobius diaperinus)* was found to be of significantly (p<0.05) higher in wood shavings than in sand due to litter types and litter replacement frequency. This was in conformity with the report of Bilgili *et al.* (2000) that houses equipped with sand as a litter had lower darkling beetle levels, less caking and more beneficial temperatures.

The wood shavings litter replaced seven times had the highest darkling beetle population. The presence or higher population of darkling beetle *(Alphitobius diaperinus*) in wood shavings litter tends to show that looser litter promotes the survival of darkling beetles, which was an agreement with the findings of Townsend (1998), that darkling beetles congregate where there is adequate moisture or the litter is looser and deeper.

The bacterial load of the litters presented wood shavings litter to be of higher bacterial load than sand, with the wood shavings without replacement as the highest in bacterial load. This tends to show that even though both litters could be used continuously without replacement, sand litter seems to be safer bacteriologically. This agrees with the report of Bilgili *et al.* (2000), that moisture and ammonia levels were similar in pine shavings and sand, with significantly lower numbers of bacteria in the sand. Also corroborating Macklin *et al*. (2005), sand litter had lower bacterial counts, water activity and moisture level compared with pine shavings. Hence, bacteriologically, sand can be a viable litter alternative to wood shavings in Northern Nigeria where wood shavings is not readily available.

Wood shavings litter tend to contain higher load of bacteria but with relatively limited species compared to sand litter. This implies that wood shavings could promote better development and multiplication of limited species of bacteria than sand.

Some bacteria isolates from both litters are pathogenic but birds did not show symptoms of disease. This could be due to the absence of predisposing factors and possible lower bacterial load below pathogenic threshold (Awoniyi *et al*., 1995).

However, the non-pathogenicity of the few isolated pathogenic bacteria in both litters further support their relative safety to broilers' welfare.

Sand is very heavy and this could be disadvantageous, but there is a need for these positive qualities to be harnessed. Hence there is a need for further research to find out rate at which sand and wood shavings could be combined as litters to enjoy both the qualities of sand and wood shavings.

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