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Bacterial Quality Assessment of Drinking Water for Layer Chicken Managed Under Battery Cage and Deep Litter Systems from Sokoto Metropolis, Nigeria

SARKINGOBIR, Y; SAMBO, S; TUKUR, U.

Department of Biology Shehu Shagari College of Education, Sokoto, Nigeria
**Corresponding Author Email: superoxidedismutase594@gmail.com; Tel: 09096266980*

ABSRACT: This study assessed the bacterial quality of drinking water for layer chicken managed under battery cage (BC) and deep litter (DL) systems in Sokoto Metropolis. A total of 18 samples were collected from the two systems. Serial dilution, spread plate innoculation, colony count, subculturing, gram staining and biochemical characterization were carried out according to standard methods .The mean count concentrations in BC (1.4×10^6, 7.2×10^6 and 3.4×10^6) were relatively higher than those recorded in DL (1.57×10^7,4.52×10^7, and 1.2×10^6) .The mean count (CFU/ml)for BC was 72.11111 and that of DL was 207.4444.The bacteria determined in BC were: : *Bacillus* species, *Micrococcus varians*, *Corynebacterium xerosis* and *Lactobacillus fermenti*; whereas, those determined in DL were *Micrococcus varians, Lactobacillus fermenti, E. coli,* and *Corynebacterium xerosis*; thus *E.coli* was only recorded in DC, but the rest were found in both BC and DL. In BC, the most frequent was *Corynebacterium xerosis*, then *Micrococcus varians*, and lastly *Bacillus* species and *Lactobacillus fermenti* ; whereas, in DL *Corynebacterium xerosis* was also most frequent, then *Micrococcus varians* ,then the rests. Thus, *C. xerosis* was the most overall prevalent, then *Micrococcus varians*, then the rests. This work depicted that water used in the BC and DL systems surveyed contains a higher and diverse concentration of bacteria .This portend of contamination and unsanitary outcome is capable of harming the health, production, and ultimately the public health. More water treatment innovative methods should be use, regular and proper cleaning of farm and drinkers are needed and farmers need to be educated.

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Poultry is a source of food that has been accepted worldwide through the ages. The consumption of poultry products is increasing every year and consumers want a safe product, thus it is pertinent that the poultry producers achieved this goal .Often, poultry products are involved in human foodborne poisoning/diseases posing a considerable cost and threat to public health (International Consultative Group on Food Irradiation, 1999; Ventura daSilva, 2013; Sule and Ilori, 2017). Increase in contacts of poultry with microbes lead to increased contact rates with humans and open new avenues for introduction, proliferation, and transmission of pathogens ; and ultimately more threats to public health (The PEW Charitable Trusts, 2016). Two major poultry systems in Nigeria and Sokoto in particular are the Deep litter (DL), where birds are reared in restricted houses; and Battery cage (BC), where birds are reared in cages(Adam, 2017). Therein, quality water is essential for proper production and safety of poultry health and consequently public health (Folorunso *et al*., 2004; Abbas *et al*., 2008).Water make up large proportion of the body of chicken, from 55-75 percent, they cannot thrive without it comparatively to the feed for a long time; that is why they consume circa 1.5-2 fold of water than feed (Abbas *et al.*, 2008). Water is used in electrolyte replacement therapy, treatment with drugs, and cleaning among others. But the quality of drinking water in poultry can be jeopardized as a result of diverse things .Parable, the source (well or pipe), poor cleaning and maintenance of drinkers, regurgitated feed by the birds, chicken feed, chicken conduct, rearing sites, faeces, antimicrobials or drugs ,and knowledge of rearers (Folorunso *et al*., 2014; Oviasogie *et al*., 2016). Consequently, the objective of this paper was to determine the bacterial quality of drinking water for layer chicken managed under deep litter (DL) and battery cage (BL) systems in Sokoto, Nigeria.

MATERIALS AND METHODS

Sample collection: A total of 18 samples were collected randomly from 3 farms of DL and BC in Sokoto.

Sterilization, and Preparation of media: All glass wares were sterilized using standard methods outlined in Oviasogie *et al* .,(2016).Nutrient agar was prepared according to the standard procedure outlined by Microbiology Society (2016).Simmons Citrate agar ,

Triple Sugar Iron agar and urea agar were prepared based on methods reported in HiMedia Laboratories (2019), HiMedia Laboratories (2015),and Downes (2001) respectively. Indole agar was prepared according to protocols stated by MacWilliams (2009).

Microbial Analysis: Serial dilution, inoculation (spread plate method), bacterial counting, gram staining, and subculturing were performed based on standard methods outlined by Folorunso *et al*., (2014), Cheesbrough (2009), and Microbiology Society (2016).Biochemical Characterization of microbes was carried out according to Cheesbrough (2009).

Statistical Analyses: Data was analyzed using descriptive statistics (percentage, range, means, and standard deviation).T-test was carried out to compare the 2 housing systems using Statistical Analysis Software (SAS, 2002).

RESULTS AND DISCUSSIONS

Table 1 has shown the bacterial count between weeks 1-3 from samples collected from water troughs in BC and DL systems. The mean count concentrations in BC (1.4×10^6, 7.2×10^6 and 3.4×106) were relatively higher than those recorded in DL (1.57×10^7,4.52×10^7, and 1.2×10^6) .This may be why the body weight of chicken from DL systems was higher as echoed by (Adam, 2017).The results contradicts reports from Folorunso *et al.,* (2014).All the values (concentrations) recorded were high ,similar to a Southeastern study reported by Folorunso *et al.,* (2014).This finding points to a contamination point(s) /(sources) that endanger the quality of drinking water in the study birds and can ultimately harm production and public health (ICG,1999;

Abbas *et al*., 2008; Food Standards Australia New Zealand, 2008). Microbes in water or other contacts with the bird enters eggs and kill them or make them unhealthy to consumers (Abbas *et al*., 2008).

Table 1: Total viable counts of bacteria in dilution 10^5 of DL and BC

Housing system	Farm	Weeks	No. of colonies	Mean count (cfu/ml)	Standard (cfu/ml)
	Farm 1	Week 1	15		1.5×10^6
		Week 2	12	1.4×10^6	1.2×10^6
		Week 3	15		1.5×10^6
Battery cage	Farm 2	Week 1	124		1.24×10^7
		Week 2	61	7.2×10^6	6.1×10^6
		Week 3	320		3.20×10^7
	Farm 3	Week 1	96		9.6×10^6
		Week 2	3	3.4×10^6	0.3×10^5
		Week 3	3		0.3×10^5
	Farm 1	Week 1	51		5.1×10^6
		Week 2	194	1.57×10^7	1.94×10^7
		Week 3	228		2.28×10^7
Deep litter	Farm 2	Week 1	640		6.40×10^7
		Week 2	111	4.52×10^7	1.11×10^7
		Week 3	606		6.05×10^7
	Farm 3	Week 1	8		0.8×10^5
		Week 2	22	1.2×10^6	2.2×10^6
		Week 3	7		0.7×10^5

In table 2, the bacterial species associated with BC and DL systems of this study were shown.The mean count (CFU/ml) for BC was 72.11111 and that of DL was 207.4444.The bacteria determined in BC envisaged : *Bacillus* species, *Micrococcus varians, Corynebacterium xerosis* and *Lactobacillus fermenti*; whereas, those determined in DL are *Micrococcus varians*, *Lactobacillus fermenti, E. coli*, and *Corynebacterium xerosis*; thus *E. coli* was only recorded in DC, but the rest were found in both BC and DL. Folorunso *et al.,* (2014), observed *E.coli*, *Bacillus* species, and *Corynebacterium species*. Table 3 depicted the frequency of occurrence of the bacterial species in BC and DL systems of this study. In BC, the most frequent was *Corynebacterium xerosis*, then *Micrococcus* varians, and lastly Bacillus species and Lactobacillus fermenti; whereas, in DL *Corynebacterium xerosis* was also most frequent, then *Micrococcus varians*, then the rests. Thus, *Corynebacterium xerosis* was the most overall prevalent, then *M. varians*, then the rests. Sule and Ilori (2017) determined *Micrococcus* species (more particularly *M.varians*) from poultry feed in Ilorin Nigeria. *Lactobacillus* bacteria are nonpathogenic microbes that naturally inhabits the mucous of humans and animals (including chickens) providing a protective barrier in the gut.

Table 2: Bacterial load (CFU/ml) of species associated with drinking water under BC and DL in Sokoto metropolis

Housing system	Farm	Weeks	Range count (cfu/ml)			*Bacteria species*
	Farm 1	Week 1	1.5×10^6			*Bacillus species*
		Week 2	1.5×10^6			*Micrococcus varians*
		Week 3	1.2×10^6			*Corynebacterium xerosis*
Battery cage	Farm 2	Week 1	3.20×10^7	72.11111	102.79	*Lactobacillus fermenti*
		Week 2	1.24×10^7			*Corynebacterium xerosis*
		Week 3	6.1×10^6			
	Farm 3	Week 1	9.6×10^6			*Corynebacterium xerosis*
		Week 2	0.3×10^5			*Micrococcus varians*
		Week 3	0.3×10^5			
	Farm 1	Week 1	2.28×10^7			*Corynebacterium xerosis*
		Week 2	1.97×10^7			*Micrococcus varians*
		Week 3	5.1×10^7			
Deep litter	Farm 2	Week 1	6.40×10^7	207.4444	248.67	*Micrococcus varians*
		Week 2	6.06×10^7			*Lactobacillus fermenti*
		Week 3	1.11×10^7			
	Farm 3	Week 1	2.2×10^6			*Escherichia coli*
		Week 2	0.8×10^5			*Bacillus species*
		Week 3	0.7×10^5			*Corynebacterium xerosis*

Table 3: Frequency of occurrence of bacterial species from BC and DL systems in Sokoto Metropolis

Housing system	Farm	Weeks	*Bacteria species*	Frequency	Percentage
	Farm 1	Week 1	*Bacillus species*	1	14.2
		Week 2	*Micrococcus varians*	2	28.5
		Week 3	*Corynebacterium xerosis*	3	42.8
Battery cage	Farm 2	Week 1	*Corynebacterium xerosis*		
		Week 2	*Lactobacillus fermenti*	1	14.2
		Week 3			
	Farm 3	Week 1	*Corynebacterium xerosis*		
		Week 2	*Micrococcus varians*		
		Week 3			
	Farm 1	Week 1	*Corynebacterium xerosis*	3	37.5
		Week 2	*Micrococcus varians*	2	25
		Week 3	*Corynebacterium xerosis*		
Deep litter	Farm 2	Week 1	*Lactobacillus fermenti*	1	12.5
		Week 2			
		Week 3	*Microbacterium varians*		
	Farm 3	Week 1	*Corynebacterium xerosis*		
		Week 2	*Escherichia coli*	1	12.5
		Week 3	*Bacillus species*	1	12.5

It eliminate unfavourable microflora through diverse mechanisms such as production of organic acids, hydrogen peroxide, etc as inhibitors; blocking adhesion sites of epithelial, competition for nutrients and triggering of immunity. Thus, it is administered as probiotic in chicken's feed. Therefore, it is not uncommon to determine it in drinking water in this study (Gusils *et al.,* 1998; Dec *et al*., 2018).Some *E. coli* (parable, Avian Pathogenic *E. coli*, APEC) causes collibacillosis, a major bacterial disease of poultry worldwide and it is communicable to humans .Some *E.coli* can traverse to all organs (in birds) and cause fatal disease (Ibrahim *et al*., 2019). *E. coli* commonly form biofilm, an assembly of microbial cells that is surrounded by a matrix of extraplomeric substance released by the cells. It can stay alone or attract other microbes. Growing in biofilms confers intrinsically more resistance to antimicrobials of about 1,000 fold; therefore need more drugs (Ugwoke, *et al*., 2019). *E .coli* reduces weight of poultry (Elsaidy *et al*., 2015). *E. coli in* poultry water was determined by past studies such as Ibitoye *et al*., (2013) from Sokoto. Aliyu *et al*., (2012) determined it in diverse poultry feeds in Sokoto. *Bacillus* species are responsible for food poisoning in many cases (Cunningham, 1982). Aliyu *et al*., (2013) observed them in poultry feed in Sokoto. *Corynebacterium xerosis* is part of the genus of *Corynebacterium species*, which have been reported in chicken and have been suspected for causing food poisoning and spoilage and it remained as an indicator of unsanitary food handling (Alibi *et al*., 2016).

Table 4: Biochemical characterization of bacterial species identified from farms at Sokoto metropolis

Sample type	Gram reaction	Catalase	SH	Lactose	Glucose	Sucrose	Citrate	Motility	Indole	Urease	MR	VP	H2S	Gas	Spore	*Confirmation*
BC1[1]	+rod	+	-	-	+	-	LR	-	+	-	+	-	-	-	+	*Bacillus species*
BC1[2]	+cocci	+	NA	+	-	-	+	+	+	+	-	+	-	-	-	*Micrococcus xerosis*
BC1[3]	-rod	+	-	+	-	-	-	+	+	SR	-	+	-	-	-	*Corynebacterium xerosis*
DL1[1]	+rod	+	-	+	-	+	SR	+	+	SR	+	-	-	-	-	*Corynebacterium xerosi*
DL1[2]	+cocci	+	NA	+	+	+	-	+	+	-	-	+	-	-	-	*Micrococcus varians*
BC2[1]	+rod	+	-	+	+	-	+	+	+	-	+	-	-	-	-	*Corynebacterium xerosis*
BC2[2]	+ rod	+	-	+	+	-	-	+	-	SR	-	+	-	-	+	*Lactobacillum fermenti*
DL1[3]	+rod	+	-	+	-	+	-	-	+	-	-	+	-	-	-	*Corynebacterium xerosis*
DL2[1]	+rod	+	-	+	+	+	-	+	+	SR	-	+	-	-	-	*Lactobacillus fermenti*
DL2[3]	+cocci	+	NA	+	+	+	-	+	+	+	-	+	-	-	-	*Micrococcus varians*
BC3[1]	+rod	+	-	-	+	-	-	+	+	-	+	-	-	-	+	*Corynebacterium xerosis*
BC3[2]	+cocci	+	NA	+	+	-	-	+	+	-	-	+	-	-	-	*Micrococcus varians*
DL3[1]	+rod	+	-	+	+	+	-	+	+	-	-	+	-	-	-	*Corynebacterium xerosis*
DL3[2]	-rod	+	NA	+	+	+	LR	+	+	+	-	+	+	-	-	*E.coli*
DL3[3]	+rod	+	-	-	-	-	-	-	+	+	-	+	-	-	+	*Bacillus species*

KEY: *MR=methyl red VP=vokes-proskeur NA = not applicable SR= slow reaction LR=low reaction BC=battery cage DL= deep litter*

This work illustrated that water used in the BC and DL systems surveyed contains a higher and diverse concentration of bacteria namely, *Bacillus species*, *Corynebacterium xerosis*, *Micrococcus varians, E.coli*, and *Bacillus species*. This is a portend of contamination and unsanitary outcome which is capable of harming the health, production, and ultimately the public health. Ideally, points of contamination in water are diverse. The source of water (e.g. well, pipeborne),improper cleaning and maintenance of drinkers or rearing place, feeds, drugs, faeces ,farmers awareness or education are among the factors that triggers water contamination. Therefore, farmers should be made aware ,and innovative systems of water treatment should be applied, proper cleaning of drinkers and cages or farms are mostly needed in order to safeguard poultry production and public health (Cunningham,1982; Amaral ,2005; Uwaezuoke and Ogbulie, 2008; Aliyu *et al* .,2013; Dhaka *et al* .,2013; Ibitoye *et al* ., 2013; Elsaidy *et al*., 2015; Sarkingobir and Sarkingobir, 2017; Sarkingobir *et al*., 2019).

Conclusion: The microbes determined in this study were in high concentration, therefore the affected waters were contaminated.

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