Low Egg Production Performances Associated With Multi-drug Resistant Bacteria Among Some Nigerian Commercial Layer Flocks

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Abstract

Bacteriological investigations were carried out on commercial layer flocks of 19 poultry farms in parts of Lagos and Ogun States of Nigeria, in the years 2005 and 2006. The layers totalling 21,450 (range 1,050 – 2,900) had records of low egg production performances. Cloacal swabs and blood from live birds, as well as liver, spleen and heart blood of dead birds were investigated for pathogenic bacteria. The isolated pathogenic bacteria include *Escherichia coli*, *Salmonella enterica* serotype Typhimurium, *Salmonella enterica* serotype Enteritidis, *Salmonella enterica* serotype Gallinarum, *Klebsiella azanae* and *Klebsiella aerogenes*. The bacteria were resistant to multiple of drugs. Following treatment of birds with appropriate antibiotics, egg productions increased significantly ($P < 0.05$). Administration of antibiotics following laboratory investigations, as well as regulations to the use of antibiotics and other medicaments are highly necessary for the growth of the Nigerian poultry industry.

Key words: Bacteria, drug resistance, layers, low egg production, poultry.

Introduction

Intensive animal production is associated with large scale antimicrobial use worldwide (Moulin, 2001). This often leads to a high level of colonization of animals with anti-microbial resistant bacteria (Corpet, 1988; Aubry-Damon *et al.*, 2004). Infections due to such drug resistant organisms are of grave consequences as they can lead to high economic loss in poultry (Poppe *et*

al., 1991; Ojo, 1991). Ojeniyi (1989), has long time reported higher incidence of drug resistant bacterial isolates obtained from commercially reared poultry than isolates from locally reared birds in Ibadan, Western Nigeria.

In Nigerian poultry industry, the use of antibiotics is without restriction. Often, farmers themselves and quacks administer drugs on flocks without supervision by veterinarians. Low egg

production performances among commercial layers could be due to factors such as infections, nutrition, stress and management lapses among others (Pattison, 1993; Speight, 1993). However, when there is drop in egg production most Nigerian farmers go for antibiotics. If he uses one antibiotic preparation without result, he changes to another until he is able to get result. The article presents a field report of investigations carried out on some commercial layer flocks with complaint of low egg production performances and measures taken to resolve the problems.

Materials and Methods

Ten commercial poultry farms located in some towns and villages in parts of Lagos and Ogun States, Nigeria, were investigated for the cause of drop in egg production among their layers flocks in the years 2005 and 2006. The commercial layers flocks of the ten poultry farms investigated for bacteriological cause of drops in egg production were 45.0 $\pm$ 18.3 weeks old (mean $\pm$ SD) and 21,450 (range: 1,050 – 2,900) in total.

The birds in the farms investigated had records of low egg production performances with occasional low mortalities. The farms' records were gone through, birds were closely examined, the buildings inspected and post-mortem examination carried out on dead birds. On the spot investigations eliminated some problems commonly associated with drop in egg productions such as: accommodation, feeding, watering, Newcastle disease (absence of typical clinical signs and pathognomonic lesions), as well as worm (based on examination of faecal

droppings and intestinal contents) and ectoparasite infestations (absence of fleas and lice).

Bacteriological Investigations

For each of the ten farms, cloacal swabs were obtained from 20 birds and blood samples (0.5–1.0ml) were collected via the wing veins with the aid of syringes and needles from another 20 birds. All the birds bled were randomly selected. Livers, spleens and hearts were harvested from dead birds obtained from the farms and taken to the laboratory in sterile bottles. A loopful of cloacal swab rinsed in sterile distilled water, blood samples, fluids from liver and spleen as well as blood from heart were streaked separately on freshly prepared MacConkey agar plates. The agar plates were incubated at 37°C for 18–24 hours. The bacterial isolates were sub-cultured in selective media, subjected to biochemical tests, characterized and identified according to Barrow and Feltham (1995).

Antibiotic susceptibility tests (AST) on the isolated bacteria were determined by the disk diffusion technique (NCCLS, 1997). The antibiotics and their concentrations in microgrammes (μ g) were as follows: Augmentin (30), Ofloxacin (10), Tetracycline (25), Gentamycin (10), Amoxicillin (20), Cloxacillin (20) and Nitrofurantoin (20). *Escherichia coli* (NCTC 10418) served as control. Based on the degree of clearance from the antibiotic disk, the results obtained were classified as highly susceptible ($\geq$ 10mm), intermediate susceptible ($<$ 10mm) or resistant (no clearance), which is the standard being used by the University of Benin Teaching Hospital.

Treatment of Birds

Flocks were administered drugs based on the outcome of the AST performed. Birds in farms A, E, F and G were treated with oral preparation of Coflox[®] (Concept Pharmaceutical Ltd., Mumbai, India), an enrofloxacin, for five days. Birds in farms B, C, D, H, J and I were treated with oral administration of pure Furaltadone[®], a nitrofurantoin, for a period of five days at a dosage of 0.5g/litre. Eggs production performances before medication and 3 weeks post medication with appropriate drugs were recorded. Average of one week production was considered for each flock.

Statistics

The results of AST were scored: highly susceptible = 2, intermediately susceptible = 1

and resistant = 0. Student's *t*-test was used to determine the statistical difference in percentage egg production performances of the flocks before and after medication.

Results

The bacteria isolated from the commercial laying flocks of the ten farms investigated for bacteriological cause of low egg production performances included, *Escherichia coli* (50%), *Salmonella enterica* serotype Typhimurium (8.33%), *Salmonella enterica* serotype Enteritidis (16.67%), *Salmonella enterica* serotype Gallinarum (8.33%), *Klebsiella ozae* (8.33%) and *Klebsiella aerogenes* (8.33%) [Table 1]. The distribution of the bacterial isolates in the investigated organs, harvested from

Table 1: Antibiotic susceptibility pattern of isolated bacteria

Farms	Isolated bacteria	Aug	Ofi	Tet	Gen	Amo	Cot	Nit	
A	<i>Escherichia coli</i>	2	2	1	1	2	0	2	
B	<i>Escherichia coli</i>	2	0	0	0	0	0	2	
	<i>Salmonella Enteritidis</i>		2	2	2	0	0	0	2
C	<i>Escherichia coli</i>	2	1	1	0	0	0	2	
	<i>Klebsiella pneumoniae</i>	2	2	0	0	2	0	2	
D	<i>Salmonella Typhimurium</i>	2	1	0	0	1	0	2	
E	<i>Escherichia coli</i>	2	2	2	1	2	0	2	
F	<i>Escherichia coli</i>	2	2	0	2	0	0	2	
G	<i>Klebsiella aerogenes</i>		2	2	1	0	2	2	2
H	<i>Escherichia coli</i>	2	0	0	0	0	0	2	
I	<i>Salmonella Enteritidis</i>		2	2	1	1	2	0	2
J	<i>Salmonella Gallinarum</i>	2	1	1	0	2	0	2	
Average score			2.00	1.42	0.75	0.41	1.08	0.17	2.00
Percentage susceptibility		100	70.1	37.5	20.8	14.2	08.3	100	
Percentage resistance			0	29.9	62.5	79.2	15.8	91.7	0
Key									
Aug-	Augmentin	Ofi-	Ofloxacin						
Tet-	Tetracycline	Gen-	Gentamycin						
Amo-	Amoxycillin	Cot-	Ceftriaxazole						
Nit-	Nitrofurantoin								

Table 2: Distribution of bacterial isolates in body organs.

Farms	Bacteria	Venous blood n=20	Liver n=30	Spleen n=10	Heart blood n=10	Cloacal swabs n=20
A	<i>Escherichia coli</i>	75	100	90	100	55
B	<i>Escherichia coli</i>	70	100	100	90	ND
	<i>Salmonella</i> Enteritidis	80	90	80	90	ND
C	<i>Escherichia coli</i>	ND	80	70	80	ND
	<i>Klebsiella ornax</i>	ND	70	70	70	ND
D	<i>Salmonella</i>					
	Typhimurium	65	90	80	70	50
E	<i>Escherichia coli</i>	100	100	100	100	ND
F	<i>Escherichia coli</i>	85	90	90	90	100
G	<i>Klebsiella aerogenes</i>	ND	60	70	70	ND
H	<i>Escherichia coli</i>	75	90	90	80	100
I	<i>Salmonella</i> Enteritidis	ND	90	ND	80	ND
J	<i>Salmonella</i> Gallinarum	50	70	30	50	ND

Values within table are in percentages.

* The bacteria indicated from cloacae were isolated along with many intestinal flora.

diseased birds, were as presented in Table II. The prevalence of pathogenic bacteria species among the laying flocks of the ten farms is illustrated in Figure 1; *Escherichia coli* being the most prevalent. The bacteria were resistant to multiple of drugs. All the bacterial isolates were highly susceptible to nitrofurantoin and augmentin, but with varied degrees of resistance to the remaining five antibiotics used (Table I).

Egg production performances before medication and 3 weeks post medication were as presented in Figure 2. Treatment with appropriate drugs improved egg productions significantly ($T = 2.41$, $P < 0.05$).

Discussion

One of the most common problems in brooders and commercial layers is investigating the cause of drop in egg production (Partison, 1993). Drop

in egg production is associated with anxiety on the part of the farmer, as he/she in being desperate to the return production to peak often abuse antibiotics usage.

In Nigeria, farmers having commercial layer flocks with stocking capacity over 3,000 birds are many. They may contribute up to 50% of total eggs produced in the country. Majority of such farmers do not engage the services of veterinary surgeons, as they consider such services too expensive. Some engage the services of veterinarians as consultants when commencing the poultry project, but after one or two years they believe they have acquired enough knowledge and terminate such services. In summary, most Nigerian farmers in this category administer drugs to flocks by themselves or patronize quacks whose services they considered less expensive. They seek for assistance from

Figure 1: Prevalence of multidrug resistant bacteria species among commercial laying flocks

Figure 2: Production performances of laying birds with records of low egg production before and after treatment with sensitive drugs ($P < 0.05$)

qualified veterinarians only when they might have tried their best without achieving the desired results (Okiki, unpublished field observations). All the farmers reported in this study had stocking capacities less than 3,000 commercial layers. The farmers admitted that they had used at least 2 different types of antibiotic preparations before our intervention. The resistance to many drugs by the isolated bacteria from these flocks (Table 1) attested to the fact that antibiotics had been abused in the farms. The significant increase in egg production (Figure 2), following treatment of flocks with sensitive drugs (drugs selected from AST results, Table 1), is an indication that inappropriate drugs were earlier administered. Earlier studies in these locations reported the prevalence of multi-drug resistant bacteria in poultry farms (Okiki *et al.*, 2006) and association of multidrug resistant gram-negative bacteria with high mortalities among commercial poultry flocks (Okiki and Ogburn, 2008).

The isolated bacteria in this study might have acquired resistance to multiple drugs as a result of usage of such drugs at a dosage below the curative level on the farms. The drugs used could have suppressed the bacterial infection from developing into disease state. This stage of infection with bacteraemia could produce stress in the birds which could be responsible for such drops in egg production.

Multidrug resistant gram-negative bacteria has been emerging worldwide (Yamane *et al.*, 2005) and resistance to antibiotics could be plasmid or chromosomal mediated (Mayer, 1988). Diagnostic uncertainty has however been reported as a key driver of drug misuse and overuse, which can lead to antimicrobial

selection pressure and increased rates of resistant microbes (Nuernberger and Bishai, 2004). Fischer *et al.* (2004) noted that the risks associated with untreated microbial infections and the lack of accurate clinical and laboratory prediction methods result in low threshold for initiating empirical antimicrobial drug therapy. In Nigerian poultry industry, laboratory investigations of aetiology of diseases are not yet popular as diagnosis of diseases are still based on physical observation of flocks and autopsy which are insufficient. The future of Nigerian poultry industry should depend on improved diagnostic techniques for microbial infections. Whenever there is drop in egg production, use of antibiotics should not be considered first, rather the cause of low performances should be determined. In investigating the cause of drop in egg production among breeders and commercial layer flocks, it is recommended that the under listed points should be checked as described by Partison (1997)

- (a) Water consumption. has there been a sudden interruption in water supply? Check water meters, if fitted in each house. Water samples should be taken for investigation of possible contamination (especially if water supply is uninterrupted).
- (b) Does the drop in production coincides with a new feed delivery and if so has this batch of feed been distributed to the birds in all the houses? A feed sample should be taken from current feed batch and should be analysed for any major abnormalities, initially to ensure it is the correct feed i.e. layer or breeder feed.

Then one can assay for protein, energy, calcium and mycotoxin levels

- (c) Are the poultry well ventilated? If not necessary correction should be done.
- (d) Do the birds look healthy? If not, are there signs of diseases or a rise in mortalities?
- (e) Are the eggs normal in shape and size? If not, are there soft shells or excess calcification?
- (f) Has there been a change in management or staff looking after the birds? Also, has there been visitors who could have frightened the birds e.g. a vaccination team?
- (g) Is the stocking density on the floor or cages appropriate to the breed and climate?
- (h) Check point of lay weights and rearing history and vaccination records.
- (i) In case of mortalities, take a selection of organs of organs to store in deep freezer for later bacterial isolation if necessary e.g. trachea, liver, spleen, caecal tonsil and gut contents
- (j) Take blood samples from affected houses to be tested for infectious diseases such as Newcastle disease, avian influenza, infectious bronchitis, etc.

Recommendations

In order to reduce the incidence of multi-drug resistant bacteria and their socio-economic effects in the poultry industry, the followings are hereby recommended:

1. Strict bio-security will reduce bacteria and other infectious microbes in the poultry confinements.
2. There should be regulations to the use of antibiotics in poultry so as to prevent further abuse of antibacterial agents.

References

- Aubry-Damon, H.; Grenet, K; Sall-Ndiaye, P.; Cho, D.; Gordeiro, E. and Bougnoux, M. 2004. Antibiotics resistance in commensal flora of pig farmers. *Emerging Infectious Diseases* 10 (5): 875-879.
- Barrow, G.I. and Feltham, R.K.A. 1995. *Cowan and Steel's Manual of Identification of Medical Bacteria*. Cambridge University Press U.K.
- Corpet, D.K. 1988. Antibiotic resistance from food. *New England Journal of Medicine* 318: 1206-1207.
- Fischer, J.E.; Harbarth, S.; Agthe, A.G.; Benn, A.; Ringer, S.A. and Goldman, D.A. 2004. Quantifying uncertainty: physicians estimate of infections in critical ill neonates and children. *Clinical Infectious Diseases* 38: 1383-1390.
- Mayer, L.W. 1988. Use of plasmid profile in epidemiological surveillance of disease outbreaks and in tracing the transmission of antibiotic resistance. *Microbiology Review* 1:228.
- Moulin, G. 2001. Surveillance of antimicrobial consumption activities in France (Agence Nationale du Medicament Veterinaire). In: *2nd International Conference of the Office International des Epizooties 2001, Paris*.
- National Committee for Clinical Laboratory Standards, NCCLS. 1997. *Methods for Dilution, Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. 4th Edition, Approved Standard M7-A4. NCCLS, Villanova P.A.
- Nuemberger, E.L. and Bishar W.R. 2004. Antibiotic resistance in *Streptococcus pneumoniae*. What does the future hold? *Clinical Infectious Diseases* 38 (supl. 4) S 363- S 371.
- Ojeniyi, A. A. 1989. Direct transmission of *Escherichia coli* from poultry to human. *Epidemiology and Infection* 103 (3) 513 - 522.
- Ojo, M. O. 1993. *Manual of Pathogenic Bacteria* Shaneson C. I. Limited, Ibadan, Nigeria. 412pp.
- Okiki, P.A.; Moro, D.D. and Fajana, O.O. 2006. Effect of storage conditions on physical, nutritional and bacteriological quality of eggs. *ASSET Series B* 5 (1): 29-38.

- Okiki, P.A. and Ogbimi, A.O. 2008. Atypical infectious bursal disease with MDR *Escherichia coli* co-infection among 16-week old pullets. *ASSET Series* 11 7 (2): 154-164.
- Pattison, M. 1993. Disease prevention and control in broiler breeders and layers. In: *The Health of Poultry* (Editor: Pattison M). Longman Singapore Publishers (Pte. Ltd.), Singapore. Pp 178-204.
- Poppe, C.; Irwin, R. J.; Forsberg, G. M.; Clarke, R. C. and Oggel, J. 1991. Prevalence of *Salmonella enteritidis* and other *Salmonella* spp among Canadian registered commercial layer flocks. *Epidemiology and Infection* 106:259-270.
- Speight, D. 1993. Influence of nutrition on health control. In: *The Health of Poultry* (Editor: Pattison, M.). Longman Singapore Publishers (Pte. Ltd.), Singapore. Pp 101-139.
- Yamane, K.; Wachiho, J.; Doi- Kurokawa, H. and Arakawa, Y. 2005. Global spread of multiple aminoglycoside resistant genes. *Emerging Infectious Diseases* 11 (6): 951-953.

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