

# Drug Enrichment of Commercial Poultry Feeds and Human Health in the Tropical Developing Countries

By Adé A. Ojeniyi

Gastro-Enterology Division, Department of Medicine, Rigshospitalet (National University Hospital), Copenhagen, Denmark.

**Ojeniyi Ade, A: Drug enrichment of commercial poultry feeds and human health in the tropical developing countries. Acta vet. scand. 1989, 30, 133–139.** – Eighty per cent of *Enterobacteriaceae* isolates from battery poultry exhibited drug resistance in a survey among university and commercial poultry flocks. These birds, being a source of human food, may serve as an important reservoir for human pathogenic drug-resistant enteric organisms. Feeds used on University and Commercial poultry farms were found to be inhibitory to standard test organisms – Oxford strain of *Staphylococcus aureus* and *Escherichia coli* K 12 J5 NA+ Lac-. Feed additives, which purportedly were mineral and vitamin supplements, were found to be highly laden with antibacterials, a quantitative estimation of which revealed that one of them, termed A & D Crumbles contained as much as 3000 (three thousand) µg of antibiotic per g, while another feed additive known as ADVIT contained 130 µg of antibiotic per g of the feed supplement. These are routinely added to poultry feeds, a practice which may easily lead to development of drug resistance among enteric pathogens that may, in turn, reach humans and complicate therapy of human bacterial infections. This may be of a considerable public health significance.

feed additives; drug resistance.

## Introduction

During an epidemiological survey of *Enterobacteriaceae* among poultry reared under various conditions in some parts of Nigeria, 80% of the isolates were found to exhibit drug resistance (Ojeniyi 1980). As these birds serve as sources of food for humans, drug resistance among enteric pathogens could have adverse public health effects. In countries with intensive poultry husbandry practice, like the Scandinavian countries, Britain and the United States of America, incorporation of antibiotics and chemotherapeutics in the birds' feeds has been practised for quite some time. However, it has been shown in these countries that a large reservoir of antibiotic resistant *Escherichia*

*coli* exists in poultry (Siegel *et al.* 1975), with these organisms being regularly excreted in large numbers in their faeces (Howe *et al.* 1976), while drug resistant organisms can reach man through poultry products (Harbour *et al.* 1978). These drugs which have been given to animals extensively either as growth promoters or for prophylaxis, must account for the large number of resistant *Escherichia coli* present in the gastro-intestinal tract of poultry (Siegel *et al.* 1974, Smith 1974, Linton 1977, Harbour *et al.* 1978, Edit 1979, Ojeniyi 1980, Holmberg *et al.* 1984a). Poultry husbandry, like other livestock husbandry in developing countries, is still in its infancy. However, the poultry industry in these countries is already aiming

at the trends of large flock rearing systems of Europe and America. As may be expected, the same problems of drug resistance would arise, especially as there is no effective control of drug use in the tropical developing countries. Such drug resistance problems may reach alarming proportions as plasmid-mediated transmissible resistance factors are involved (Ojeniyi 1980). It was considered desirable therefore, to examine the feeds available in Nigeria, for example, for the contents of antimicrobial agents, in the wake of the appearance among battery poultry of multiply resistant *Escherichia coli* – resistant to Streptomycin, Sulphonamide and Tetracycline.

#### Materials and methods

Feed lots at the University of Ibadan Teaching and Research Poultry Farm, Olaogun Commercial Poultry Farm and University of Ife Institute of Agricultural Research and Training's Bora Poultry Farm were sampled over a period of 2 1/2 years. All 3 poultry establishments have their own feed mills. The following feeding stuffs were sampled and examined for antibacterial activity.

##### *University of Ibadan:*

Feeding stuffs:	Chicks' mash	Feed additives:	A & D Crumbles
	Growers' mash		ADVIT
	Layers' mash		Agricare Mix
	Breeders' mash		Chick Formula

##### *Commercial Farm:*

Feeding stuffs:	Growers' mash	Feed additives:	A & D Crumbles
	Breeders' mash		ADVIT
	Broilers' mash		
	Vitality Chicks' mash		
	Goldlay hybrid layers' mash		

##### *University of Ife:*

Feeding stuffs:	Chicks' mash	Feed additives:	A & D Crumbles
	Broilers' mash		Chick Formula
	Breeders' mash		

##### *Antibacterial activity in feeding stuffs and feed additives*

Twenty-five g portions of each feeding stuffs and 10 g portions of feed additives were weighed out into 250 ml conical flasks. Quarter strength Ringer's solution was added to each flask and the mixture was allowed to stand for several minutes. This extraction procedure was repeated using Todd Hewitt Broth to ensure that inhibition of the test organisms was not due to the acidity of the medium. The mixture was filtered, first through a filter paper to remove particles and obtain a clear extract, and later through a Seitz filter with a grade EK Asbestos Pad for sterilisation. Each sterilised filtrate was pipetted into sterile round bottomed test tubes and double dilutions up to 1 in 32 were prepared. Young broth cultures of each of two test organisms – Oxford strain of *Staphylococcus aureus* and *Escherichia coli* K 12 J5 NA+ Lac- were prepared and the concentrations of viable bacterial cells in each case was adjusted by use of a nephelometer to match McFarland's Standards Numbers 0.5 and 0.25. Each of the two test organisms was subsequently drop inoculated into the

various filtrate dilutions. Test tubes containing broth only and organisms only were also prepared. All the test tubes were hereafter incubated for 24 h at 37°C.

*Qualitative and Quantitative analysis of Feeding Stuffs for Antibiotics*

This was carried out according to the procedures enumerated in the *European Common Market Gazette* (1972). A spectrum of bacteria at various hydrogen ion concentrations and at different temperatures was employed in the detection and identification of antibiotics present in animal feeds. The following organisms were used:

*Staphylococcus aureus*  
*Micrococcus flavus* BR  
*Escherichia coli*  
*Micrococcus flavus*  
*Bacillus subtilis*  
*Micrococcus luteus* DHSR  
*Staphylococcus epidermidis*  
*Bacillus cereus* TR + Penicillinase  
*Bacillus cereus* TR  
*Streptococcus faecalis*  
*Bacillus cereus*

**Extraction:** Three-gram samples of various feeding stuffs were agitated for 15 min with 15 ml solvent consisting of methanol and phosphate buffer. This was centrifuged and 0.1 ml of each extract pipetted into a well of 10 mm in diameter which was bored in sensitivity agar in plates of 200 mm in diameter.

**Plate preparation:** Assay culture medium, consisting of the following, was prepared and boiled:

glucose 1 g  
 peptone 10 g  
 dried meat extract 1.5 g  
 yeast extract 3 g  
 agar 15 g  
 water to 100 ml.

Half of this volume was adjusted to pH 6 and the other half to pH 8. Both were sterilised at 120°C for 15 min and cooled to 50°C hereafter. 100 ml of the pH 6 medium were taken and to this volume was added 1 ml penicillinase solution capable of inactivating 1000 i.u. penicillin per ml within 1 h at 37°C. Meanwhile bacterial suspensions were prepared and added to each antibiotic solution. These were inoculated into bore holes of 10 mm in diameter made in petri dish agar. The test was set up as an agar diffusion test on plates with 4 concentration levels of standard solutions ( $S_8, S_4, S_2, S_1$ ) and four concentration levels of each extract ( $U_8, U_4, U_2, U_1$ ). Each plate contained all 4 concentrations of standard and 4 concentrations of extract. Four inoculations of each of standard and each of extract were made such that 32 inhibition zones were recorded for each test. Thus, extracts of A & D Crumbles and ADVIT, supposedly mineral and vitamin supplements, were inoculated into the bore holes. The plates were incubated overnight at 30°C and at 37°C.

*Calculation of Antibiotic contents*

The diameter of inhibition zones were measured by projection. Inhibition zones, in mm were plotted against the logarithms of concentrations. Straight lines were drawn through the plotted points of the standards and of the extracts. The logarithms of antibiotic activity were calculated according to the formula:

$$(U_1 + U_2 + U_4 + U_8 - S_1 - S_2 - S_4 - S_8) \times 0.602$$

$$U_4 + U_8 + S_4 - S_8 - U_1 - U_2 - S_1 - S_2$$

U = Unknowns (A & D Crumbles and ADVIT)

S = Standards (EEC Values)

### Results

Filtrates of all the feeding stuffs used on all 3 farms, including Chicks' mash were inhibitory to the 2 test organisms. Table 1 shows the results of the tube dilution tests. Of the Feed Additives, A & D Crumbles and Chick Formula were most inhibitory to the test organisms with growth inhibition taking place even at the highest dilution prepared: 1 in 32. Agricare Mix was found to be inhibitory at all dilutions except at 1 in 32; at this dilution, inhibition was rather inconclusive. ADVIT was the least inhibitory of all the Feed additives tested; inhibition being inconclusive even with the undiluted filtrate. Table 2 shows the performance of a spectrum of test organisms against a range of antibiotics, and the effect of 2 of the Feed Additives on these organisms. It will be seen from this table that A & D Crumbles were inhibitory to most of the test organisms, while ADVIT was active against only 4 of them. The results of the quantitative estimation of Tetracycline content in A & D Crumbles and in ADVIT are reflected in Tables 3A, 3B and 3C. Trials at various assumed concentration levels and calculations showed that the content of Tetracy-

line is about 3000 µg per g of A & D Crumbles while that of ADVIT was about 130 µg per g of this feed supplement.

### Discussion

Despite the denial of feed enrichment with antibiotics by the management of the Universities' and Commercial Poultry Farms, antibacterial agents were detectable in appreciable quantities in the Feed Additives. It was claimed that the supplements added to feedlots were made up exclusively of vitamins and minerals: thus A & D Crumbles and ADVIT were said to be no more than vitamins A and D. This practice was thought to prevent disease, promote growth and offset stresses due to vaccinations against Newcastle Disease, Fowl Pox and Fowl Typhoid. In industrialised countries with intensive poultry husbandry, many problems have arisen from feed enrichment with antibacterial agents (Quarles *et al.* 1977, Linton 1977, Smuth 1978, Holmberg *et al.* 1984a, b), the most important of which are development of resistance against such drugs and the phenomenon of resistance factor transfer to otherwise drug-susceptible organisms. The public health aspects center around the possibility

Table 1. Inhibition dilutions of feeding stuffs and feed additives.

	Chicks' Mash	Broilers' Mash	Growers' Mash	Layers' Mash	Breeders' Mash	A & D Crumbles	ADVIT	Agircare Mix	Chick Formula	Broth Control	Organism Control
B: <i>St. au.</i>	16	—	16	16	16	32	1	16	32	—	—
B: <i>E. coli</i>	16	—	16	16	16	16	1	8	32	—	—
C: <i>St. au.</i>	16	2	8	2	4	32	1	—	—	—	—
C: <i>E. coli</i>	8	2	4	4	2	32	—	—	—	—	—
F: <i>St. au.</i>	8	8	—	—	4	32	—	—	32	—	—
F: <i>E. coli</i>	8	4	—	—	4	16	—	—	32	—	—

B = University of Ibadan Teaching and Research Poultry Farm

C = Olaogun Commercial Poultry Farm

F = University of Ife, Institute of Agricultural Research & Training Bora Poultry Farm.

*St. au.* = *Staphylococcus aureus*

Table 2. Detection and identification of antibiotics by means of a bacterial spectrum.

	µg/ml	Bacillus cereus pH 6 30° C	Streptococcus faecalis pH 6 37° C	Bacillus cereus TR pH 6 37° C	Bacillus cereus TR pH 6 30° C + penicillinase	Staphylococcus epidermidis pH 8 37° C	Micrococcus luteus DHSR pH 8 37° C	Bacillus subtilis pH 8 30° C	Micrococcus flavus pH 6 37° C	Escherichia coli pH 6 37° C	Micrococcus flavus BR pH 6 37° C	Staphylococcus aureus pH 6 37° C
Chlortetracycline	0.2	+	+	-	-	-	*	*	*	-	+	*
Penicillin	0.4 i.u./ml	-	-	+	-	-	+	-	+	-	+	*
Streptomycin	0.8	+	-	+	+	-	-	+	-	+	-	+
Bacitracin	10	-	+	-	-	-	+	-	+	-	-	-
Flavomycin	0.2	+	-	+	+	-	-	-	-	-	-	-
Virginiamycin	1	+	+	+	+	+	+	-	+	-	-	-
Spiramycin	1	+	+	+	+	+	+	+	+	-	-	-
Oleandomycin	4	+	+	+	+	*	+	+	+	-	-	*
Neomycin	10	+	+	+	+	+	+	+	+	+	+	+
Furazolidone	10	+	-	+	+	+	-	+	*	*	+	+
Nitrovin	10	+	+	+	+	+	-	+	-	-	+	+
Monensin Na	8	+	-	+	+	*	-	*	-	-	+	*
Chloramphenicol	40	+	+	+	+	*	*	*	*	+	-	*
ADVIT		17.3	-	15.5	-	17.8	-	-	21.8	-	-	-
		+	-	-	-	+	-	-	-	-	-	-
A & D Crumbles		41.0	-	16.6	-	17.5	42.8	27.3	43.3	32.5	44.7	39.2
		+	-	-	-	+	+	+	+	+	+	+
		Inhibition zones in millimeters										

+ = Inhibition    - = No inhibition    \* = Delayed inhibition

of transmission to humans of such drug resistant organisms that arise as a direct consequence of antibacterial agents' use. More serious still is the possibility of spread of resistance factors among enteric bacteria that may be pathogenic for humans. The tropical developing countries are characterised by poor environmental sanitation, while at the same time, antibiotics are readily available without a prescription and are freely used in the belief that these drugs confer advantages enumerated above. It is obvious therefore,

that public health may be jeopardised as a widespread drug resistance can complicate the therapy of many human bacterial infections. Since veterinary and pharmacy laws and regulations prohibit the use of drugs except on prescription, a stricter enforcement of drug legislation is necessary to avoid the problems of drug resistance which, in the tropical developing countries, may very readily assume epidemic proportions and pose hazard for public health.

Table 3 A. Quantitative analysis of antibacterial agent content in A & D Crumbles at an assumed content level of 100 µg/ml.

	Inhibition zones in millimeters				Average
U 1	34.9	35.3	34.5	35.6	35.1
U 2	36.2	36.6	35.9	35.1	36.0
U 4	38.7	36.2	37.5	37.7	37.5
U 8	40.3	39.4	39.1	39.8	39.6
S 1	24.4	24.6	25.1	24.8	24.7
S 2	26.8	27.0	26.0	27.5	26.8
S 4	29.8	29.1	29.2	29.4	29.4
S 8	31.5	31.2	32.2	29.8	31.2

U = Unknown . . . (A & D Crumbles)

S = Standards for Tetracycline

Result = 3000 µg of Tetracycline per g of  
A & D Crumbles

Table 3 B. Quantitative analysis of antibacterial agent content in A & D Crumbles at an assumed content level of 500 µg/ml.

	Inhibition zones in millimeters				Average
U 1	30.5	30.4	30.8	30.6	30.6
U 2	32.4	32.5	32.5	32.6	32.5
U 4	34.4	34.4	34.7	34.3	34.4
U 8	36.7	36.9	36.4	36.9	36.7
S 1	24.1	25.1	24.8	24.6	24.6
S 2	27.0	27.6	28.1	27.4	27.5
S 4	29.1	29.8	29.1	28.8	29.2
S 8	31.5	31.3	31.2	31.3	31.3

U = Unknown . . . (A & D Crumbles)

S = Standards for Tetracycline

Result = 3000 µg of Tetracycline per g of  
A & D Crumbles

Table 3 C. Quantitative analysis of antibacterial agent content in ADVIT at an assumed content level of 1000 µg/ml.

	Inhibition zones in millimeters				Average
U 1	Contaminated	17.8	Contaminated	Contaminated	-
U 2	Contaminated	21.7	21.4	Contaminated	-
U 4		24.3	23.5	25.7	25.3
U 8	Contaminated	24.0	Contaminated	23.9	24.0
S 1		24.9	25.1	24.9	25.1
S 2		28.2	28.0	27.7	27.8
S 4		31.1	30.9	30.8	30.7
S 8		33.3	33.3	33.3	33.6

U = Unknown . . . (ADVIT)

S = Standards for Tetracycline

Result = 130 µg of Tetracycline per g of ADVIT

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### Sammendrag

*Antibiotikatilskud i hønsefoder. betydning for menneskets sundhed i de tropiske udviklingslande*

En epidemiologisk undersøgelse blandt universitets- og kommercielle fjerkræflokke viste antibiotikaresistens hos 80 % af *Enterobacteriaceae* isolater. Da fjerkræprodukter indgår i levnedsmidler, kan hønsene udgøre et betydeligt reservoir for humanpatogene antibiotikaresistente enterobakterier. Foderet til såvel universitets- som kommercielle fjerkræflokke hæmmede testorganismerne *Staphylococcus aureus* og *Escherichia coli* K12 J5 NA+ Lac-.

Det påvistes, at fodertilskuddene A & D Crumbles og ADVIT, der angiveligt bestod af mineraler og vitaminer indeholdt 3000 µg henholdsvis 130 µg antibiotika(tetracyklin) per gram af tilskuddet. Da disse tilskud ofte anvendes på de større fjerkræhold, kan denne praksis føre til, at der let opstår antibiotikaresistens hos patogene tarmbakterier, der kan overføres og besværliggøre behandling af bakterielle infektioner hos mennesker.

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Reprints may be requested from: Ade A. Ojeniyi, Telemarksgade 9, DK-2300 Sundby, Copenhagen, Denmark.

