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Survey of Enterobacteria and Variation in Blood Parameters of Birds (Broilers) on Prolonged Treatment with Antimicrobial Agents

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Abstract
The study evaluated the effects of prolonged prophylactic use of common antimicrobial agents on enterobacteria and haematology of broiler chickens. Fifty chicks were randomly assigned to 3 different experimental groups comprising a control (T1) group given only vitastress as chemotherapeutic agent while T2 and T3 had neo-terramycin (neomycin and oxytetracycline) and terramycin antigen 77 and vitastress (oxytetracycline and antistress) as antimicrobial agents respectively over a period of 8 weeks. Faecal and blood samples were collected before and after treatments from each experimental unit. Blood and MacConkey agar were used for isolation of bacteria from the faecal samples. All isolates were identified by standard biochemical procedures. Blood samples were subjected to Hb concentration, PCV and ESR assays. The results showed that *Escherichia coli*, *Klebsiella* spp., *Proteus* spp. and *Enterococcus faecalis* were enterobacteria present before treatment. *Escherichia coli* persisted in all the treatment groups. *Klebsiella* and *Enterococcus* species persisted in T2, while *Proteus* species persisted in T3. The study also showed that prolonged use of these antimicrobial agents did not affect the haematology of birds. However, due to the resistance to these antimicrobial agents exhibited by these bacteria, the use of these antimicrobials in poultry production is not recommended
Keywords: enterobacteria, antimicrobial agents, haematology, antistress, treatment

1. Introduction

The development and profitability of any commercial poultry farm is totally dependent on the health of the entire birds. Health, when ignored can bring about high mortality on the farm which reduces the economic value. Antimicrobials are used in food animals to treat or prevent disease and also to promote growth. Data on such uses of antimicrobials in animals, including dosing schedules, contraindications, and withdrawal times have been provided by many researchers (McEwen & Fedorka-Cray 2002).

Therapeutic treatment is commonly administered to animals that are sick. But in food animal production, treatment of entire groups by medicating feed or water is more efficient especially in poultry. Prophylaxis is used during high-risk periods for infectious diseases. Shortly after the introduction of the therapeutic use of antibiotics, the growth-promoting effect of these products in chickens was discovered by feeding fermentation offal from the chlortetracycline production of *Streptomyces aureofaciencs* (Jukes & Williams 1953; Butaye *et al*. 2003). Butaye *et al*. (2003) reported that these antibiotics improved feed conversion and animal growth and reduced morbidity and mortality due to clinical and subclinical diseases. Ewing & Cole (1994) and Butaye *et al*. (2003) estimated the average growth improvement to be between 4 and 8%, and feed utilization was improved by 2 to 5%.

However, the mechanisms of growth promotion are not exactly known. Butaye *et al*. (2003) cited the following 4 hypotheses by Feighner & Dashkevicz (1987) to explain their action: (i) nutrients may be protected against bacterial destruction; (ii) absorption of nutrients may improve because of a thinning of the small intestinal barrier; (iii) the antibiotics may decrease the production of toxins by intestinal bacteria; and (iv) there may be a reduction in the incidence of subclinical intestinal infections.

The effects of antimicrobials on enteropathogens have been documented by many researchers. Treatment of animals with antimicrobials that are active against enteropathogens such as *Salmonella* can reduce faecal shedding (McEwen & Fedorka-Cray 2002).

Conversely, there are reports that treatment with these antimicrobials may increase pathogen loads in the food chain by selecting for resistant nontarget pathogens with increased fitness, thus increasing the likelihood that animals will be infected with resistant pathogens having altered duration of infection (Holcomb 1997; McEwen & Fedorka-Cray 2002; van den Bogaard *et al*. 2001).

The effects of antimicrobials on intestinal microbiota have been reported by many researchers. Bambermycin reduces the number of *Clostridium perfringens* in the intestines (Butaye *et al*. 2003), while no influence was noted on the counts of enterococci, coliforms, and lactobacilli in the faeces of broilers (Brenes *et al*. 1989). The additions of virginiamycin and avilamycin to feed reduce the number of *C. perfringens* in the intestines of chickens (van den Bogaard *et al*. 1997; Elwinger *et al*. 1998).

Khan & Zafar (2005) reported that most workers who studied the avian blood found a great degree of variation and considered it to be normal. According to Elagib & Ahmed (2011), haematological parameters in birds have been shown to be influenced by various factors such as age, sex, season and nutrition.

Despite the obvious consequences of the use of antimicrobials, they are indiscriminately used in poultry in Benue State, Nigeria. Also previous studies on the haematology of chicken did not consider the effects of antimicrobial agents on haematological parameters. This study was therefore carried out to evaluate the effect of prolonged prophylactic use of common antimicrobial agents on enterobacteria and haematology of broiler chickens.

2. Materials and Methods

2.1 Experimental animal

Fifty day-old broiler chicks obtained from a local hatchery were used for the study. On arrival, they were distributed at random to three equal groups, which were randomly assigned into three different experimental units which had been cleaned and disinfected previously. The first week was used to acclimatize the chicks in their new environment and thus no treatment was given and no parameter was taken. The birds were brooded in the poultry house of the Animal Production farm, Akperan Orshi College of Agriculture, Yandev. The brooding lasted for 5 weeks after which the brooding pens were converted to broiler finisher units.

2.2 Experimental units and collection of sample

After acclimatization at the second week, faecal samples were taken from each experimental unit. Blood samples were collected from the wing vein of birds using 3 ml disposable syringe (23 gauge) and immediately transferred into sterile containers containing anticoagulant (EDTA). The various treatments were then administered as follows:

T1: Treatment 1 = Vitastress – (control group)
T2: Treatment 2 = Neo-terramycin (neomycin + oxytetracycline)
T3: Treatment 3 = Terramycin antigen 77 and vitastress (oxytetracycline and antistress)

These were all Pfizer products. The treatment lasted for 8 weeks, at the end of which blood and faecal samples were collected again for laboratory analyses.

2.3 Sample analysis

Faecal samples from individual birds in each treatment group were pulled together and directly plated out in duplicate on blood and MacConkey agar. The inoculated plates were aerobically incubated at 37°C for 24 hours. After incubation, the resultant colonies were subsequently streak-purified on nutrient agar and stock cultured on nutrient agar slants for further study. All isolates were identified by standard biochemical procedures as described by Barrow & Feithan (1993).

Blood samples collected before and after treatments were subjected to the following assays as described by Schalm et al. (1975):

Haemoglobin (Hb) concentration
Packed cell volume (PCV)
Erythrocyte sedimentation rate (ESR)

3. Results

Enterobacteria isolated from the different experimental units before treatments were administered were *Escherichia coli*, *Klebsiella* spp., *Proteus* spp. and *Enterococcus faecalis*. Bacteria isolated from the different groups after prolonged antimicrobial treatment are shown on Table 1. Three out of the 4 bacterial genera were re-isolated post-treatment in T2 while in T3 two were re-isolated.

Table 1: Enterobacteria isolated following prolonged antimicrobial treatments

Treatment	***E. coli***	***Klebsiella* spp.**	***Proteus* spp.**	***Enterococcus faecalis***
T1	+	+	+	+
T2	+	+	-	+
T3	+	-	+	-

The average blood parameters recorded for each experimental unit before treatments were given were Hb concentration 5.2g/100ml, PCV 30% and ESR 3 mm/hr. after prolonged antimicrobial treatments, the following parameters were obtained as shown in Table 2.

Table 2: Blood parameters after prolonged antimicrobial treatments

Treatment	Hb (g/100ml)	PCV (%)	ESR (mm/hr)
T1	5.2	30	3
T2	5.5	31	1
T3	5.1	29	2

4. Discussion and Conclusion

The results of this study highlight the effect of commonly used antimicrobial agents that are routinely used in brooding in poultry farms in Benue State. The combination of neomycin and terramycin eliminated only *Proteus* spp. from T2, while the combination terramycin (antimicrobial) and vitastress (anti-stress) eliminated both *Klebsiell* spp. and *Enterococcus faecalis* from T3. Oxytetracycline and neomycin act by inhibition of protein synthesis via blocking of 30S ribosomal activity (Quinn & Markey 2003). Similar results on enterococci were reported by Barnes *et al.* (1978) and Torres *et al.* (1985) where bactracin was included in the animal feed. Kaukas *et al.* (1988) reported that the effect was mainly on *Enterococcus faecalis* organisms. *Escherichia coli* was resistant to both antimicrobials in T2 and T3. This finding was not surprising because many researchers have reported resistance patterns of faecal *E. coli* isolates from poultry. Chah & Nweze (2001) reported high resistance rates of *E. coli* in poultry in Nsukka, Nigeria. Karyuki *et al.* (1997) recorded different resistance patterns and multidrug resistance with *E. coli* from poultry in Kenya.

Mean haematological values observed in different treatment groups in the present study were similar. Values recorded for the two antimicrobial treatment groups were not significantly different from the control group. It was shown in this study that prolonged antimicrobial treatment of birds does not cause any significant haematological changes.

Though working on different factors (treatments), the haematological values reported by Durotoye *et al.* (2000), Khan & Zafar (2005), and Elagib & Ahmed (2011) in their studies were far much higher than the values observed in this present study. Durotoye *et al.* (2000) documented haematological values ranging from 32.25 – 34.22% (PCV) and 11.1 – 11.33g/dl (Hb). Values ranging from 8.45 – 10.23g/dl (Hb) and 5.01 – 27.25 mm/hr (ESR) were reported by Khan & Zafar (2005) in broiler chicken. Elagib & Ahmed (2011) documented haematological values ranging 39.95 – 44.40% (PCV) and 17.35 – 18.70g/dl (Hb).

Many reports have shown a great degree of variation for haematological parameters of birds, and it was concluded that these parameters vary among species, breed, sex and the nutrition supplied to the birds (Sturkie 1965; Khan & Zafar 2005).

In conclusion, the resistance exhibited by the bacterial organisms to the antimicrobial agents used in this study is worrisome. Thus the use of these antimicrobials in poultry production is not recommended. However, prolonged use of these agents does not affect the haematology of birds.

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