Comparative Antibiotic and Probiotic Effects on Antimicrobial Sensitivity of *Escherichia coli* Isolates and Performance of Broiler Chickens

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Abstract. The study evaluated the growth performance, haematology, serum biochemistry, intestinal microbial count, and antimicrobial resistance profile of *Escherichia coli* (*E. coli*) from broiler chicks fed diets supplemented with antibiotics (neomycin, and oxytetracycline), and probiotic (*Saccharomyces cerevisiae*). One hundred and twenty Abor acre broiler chicks randomly alloted to four treatment groups (30 birds/group; 10 birds/replicate) were used in the 49 days study. Group one (G 1, control) were fed basal diet while G 2, 3, and 4 received basal diet containing *S. cerevisiae* (0.80g/kg; 10⁸ cfu/g), neomycin (0.50g/kg) and oxytetracycline (0.30g/kg), respectively. Results showed significant treatment effects on body weight, feed intake, linear body values, some haematological indices, intestinal, caecal and combined caecal and intestinal bacteria counts, diameter of *E. coli* inhibition zone, and mortality. Body weight and feed intake were significantly higher in the supplemented groups. Intestinal bacterial count was highest in neomycin and control groups (5.29 ± 0.01 and 5.22 ± 0.02 Log₁₀ cfu/ml, respectively) while *S. cerevisiae* and neomycin groups yielded the highest caecal, and combined caecal and intestinal bacterial counts. *Eimeria* Oocyst count did not differ significantly between groups. *Escherichia coli* from antibiotic fed groups had reduced sensitivity or were resistant to the antibiotics. It was concluded that subtherapeutic use of antibiotics as growth promoters in broiler chickens caused the development of antibiotic resistance, and therefore, should be avoided.

Keywords: antimicrobial resistance, growth performance, neomycin, oxytetracycline, *S. cerevisiae*

Introduction

Animal productivity is hampered by pathogenic organisms and unfavourable environmental conditions. Intestinal pathogens limit nutrient digestion, absorption, and utilization, as well as depressing immune status and performance and exacerbating other disease condition. Antibiotic growth promoters have been used to boost the performance of broiler chickens by limiting the growth of pathogenic microflora (He et al., 2019). The practice, however, eliminates susceptible bacteria allowing resistant phenotypes to multiply and spread (Neveling et al., 2017).

To stem this tide, the elimination of subtherapeutic in-feed antibiotic growth promoters and the use of alternative feed additives, such as probiotics have been advocated. *Saccharomyces cerevisiae* (a probiotic) has been extensively evaluated and found effective to enhance the performance of chickens by enhancing intestinal microbial balance and intestinal epithelial development, integrity and function (He et al., 2019). *Saccharomyces cerevisiae* fed in the diet of broiler chickens improved growth rate, feed efficiency, carcass yield and quality, and immune status (Jadhav et al., 2015; He et al., 2019).

The campaign to eliminate antibiotic growth promoters has not been successful due to ignorance of the development of antimicrobial resistance, fear of depressed performance, and high mortality among farmers’ livestock who doubt the possibility of efficient production without antibiotic supplementation. There is, hence, a need to demonstrate the effect of antibiotic growth promoters on antimicrobial resistance development and the feasibility of profitable broiler chicken production without in-feed antibiotic growth promoters. The study,
therefore, aims to evaluate the performance and antimicrobial sensitivity of \textit{E. coli} from broiler chickens fed basal diet, and diets supplemented with antibiotics and a probiotic.

**MATERIALS AND METHODS**

**Experimental animals and design**

A total of 120 Abor-acre broiler chicks were used for the study. The birds were purchased at day old, brooded until 21 days of age and then randomly allocated (30 birds) to each of four treatment groups (G) with three replicates of 10 birds per group namely control (basal diet, G1), basal diet supplemented with \textit{S. cerevisiae} at $8.0 \times 10^8$ cfug/kg (G2), neomycin at 0.5g/kg (G3), and oxytetracycline at 0.3 g/kg (G4). They were fed starter mash (21% CP; 3100kcal/kg ME) during brooding, finisher mash (18 % CP; 2800kcal/kg ME) until 49 d, and water \textit{ad libitum}. Management practices and study protocols followed the guideline of the College of Veterinary Medicine Animal Care Ethics Committee (CVM-ACEC).

**Measurement of performance variables**

Body weight was obtained weekly by weighing individual birds while feed intake was measured daily. Head circumference, breast girth, neck, keel, body, and thigh lengths were obtained with a measuring tape.

**Haematological and serum biochemical indices**

Blood samples were collected from 6 birds per treatment at two weekly intervals for haematological indices (erythrocyte (RBC) count, packed cell volume (PCV), haemoglobin concentration (Hb), total and differential leukocyte (WBC) counts), and at the end of the study (day 49) for serum biochemical indices (albumin, globulin, cholesterol, creatinine, aspartate amino transferase, AST; and alanine amino transferase, ALT).

**Aerobic bacteria and \textit{Eimeria} oocyst counts**

On 49 day old, three birds were randomly selected from each treatment and sacrificed by cervical dislocation for enumeration of intestinal and caecal aerobic bacteria colonies, and \textit{Eimeria} oocysts. Thoroughly mixed samples of intestinal or caecal or combined caecal and intestinal digester were diluted 1:9 (wt vol$^{-1}$) in peptone water followed by tenfold serial dilution from $10^{-1}$ to $10^9$ for the determination of total aerobic bacteria concentration. Enumeration was done on nutrient agar after incubation at $37^\circ$C for 48 hours and results expressed as log colony forming units per ml (log cfu/ml) (Cruickshank, 1975). \textit{Eimeria} oocysts count was done using the floatation technique (Cheesbrough, 1998) and results expressed as number of oocysts per gram digester (oocyst/g).

**Isolation, identification, and antibiotic sensitivity test for \textit{Escherichia coli}**

Thoroughly mixed intestinal digester from each treatment was enriched in thioglycolate broth and incubated at $37^\circ$C for 24 h followed by inoculation onto Mackonkey agar, and incubation at $37^\circ$C for 24 h. Colonies resembling \textit{E. coli} (round, opaque, rose pink colour, and ~ 1mm diameter) were carefully collected, subcultured in Eosin Methylene Blue agar and incubated at $37^\circ$C for 24 h. Colonies with greenish metallic sheen suggestive of \textit{E. coli} were then subjected to indole, methyl-red, Voges-Proskauer and citrate tests for confirmation of \textit{E. coli}. Representative \textit{E. coli} colonies were subcultured in peptone water and incubated at $37^\circ$C for 24 h. A suspension of the broth culture was gently swabbed over nutrient agar (NA), allowed standing for 4 min and then incubated at $37^\circ$C for 3 h. Thereafter, oxytetracycline (30µg) and neomycin (30µg) discs were carefully pressed onto the NA surface and incubated at $37^\circ$C for 24 h. The zones of inhibition were measured to the nearest millimeter using a caliper and zone.
Data analysis

Data were subjected to Analysis of Variance in SPSS version 9.0 and significantly different means were separated using the Duncan Multiple Range Test.

Results and discussion

Growth performance

Birds in the control had the lowest body weight followed by those fed oxytetracycline compared to the groups fed S. cerevisiae and neomycin (Table 1).

Body weight gain and feed conversion ratio were not significantly different across dietary treatments. Feed intake did not differ significantly between groups except for day 49 when feed intake was significantly higher in the treated groups compared to the control. The enhanced body weight values observed in the supplemented groups demonstrate the growth enhancing effects of probiotics and antibiotic growth promoters as reported by other studies (Bonsu et al., 2012; El-Hammady et al., 2014; He et al., 2019). The similar body weight of neomycin and probiotic fed birds indicates that probiotics can replace in-feed antibiotics as growth promoters in broiler diets. In the present study, birds fed oxytetracycline had consistently lower body weight than those fed probiotic. This shows that the continued use of some antibiotics could depress growth performance in broiler chickens. He et al. (2019) observed lower body weight gains in broilers fed clotetracycline compared to those fed probiotics. Probiotics enhance growth performance by inhibiting pathogenic intestinal microflora, enhancing beneficial intestinal microflora, intestinal integrity and function, nutrient retention and utilization, immune and health status (Mohamed et al., 2014; Neveling et al., 2017). Saccharomyces cerevisiae is a type of anaerobic bacteria rich in protein, nucleic acid, vitamins, polysaccharides, and other nutrients (He et al., 2019). Its cell wall is believed to be effective in reducing the toxicity of mycotoxins in animals (Jadhav et al., 2015).

Table 1. Effect of antibiotics and probiotic on growth parameters of Abor acre broiler chicks

| Variable     | Basal diet | S. cerevisiae | Neomycin | oxytetracycline |
|--------------|------------|---------------|----------|-----------------|
| BWT<sub>31</sub> | 586.84 ± 28.32<sup>a</sup> | 696.43 ± 17.24<sup>a</sup> | 673.08 ± 14.74<sup>ab</sup> | 632.76 ± 19.42<sup>bc</sup> |
| BWT<sub>35</sub> | 831.58 ± 61.08 | 946.43 ± 26.84 | 976.00 ± 39.59 | 882.76 ± 31.90 |
| BWT<sub>35</sub> | 1129.41 ± 53.22<sup>b</sup> | 1356.00 ± 45.85<sup>a</sup> | 1314.00 ± 25.25<sup>ab</sup> | 1210.71 ± 32.26<sup>b</sup> |
| BWT<sub>42</sub> | 1600.00 ± 56.07<sup>c</sup> | 1833.33 ± 58.72<sup>ab</sup> | 1900.00 ± 35.00<sup>ab</sup> | 1698.00 ± 46.95<sup>bc</sup> |
| BWT<sub>49</sub> | 2113.33 ± 59.25<sup>b</sup> | 2352.17 ± 85.70<sup>a</sup> | 2468.18 ± 36.86<sup>ab</sup> | 2170.83 ± 45.64<sup>b</sup> |
| BWG<sub>21-28</sub> | 36.09 ± 8.98 | 35.71 ± 4.35 | 43.43 ± 5.36 | 35.71 ± 3.99 |
| BWG<sub>28-35</sub> | 38.24 ± 8.49 | 56.12 ± 6.76 | 42.57 ± 7.99 | 50.74 ± 7.56 |
| BWG<sub>35-42</sub> | 67.23 ± 9.49 | 70.15 ± 9.00 | 75.14 ± 6.68 | 72.41 ± 8.30 |
| BWG<sub>42-49</sub> | 68.57 ± 4.72 | 70.15 ± 13.79 | 71.43 ± 7.43 | 75.86 ± 8.47 |
| FL<sub>21-28</sub> | 84.47 ± 6.03 | 94.39 ± 6.03 | 87.30 ± 6.03 | 86.82 ± 6.03 |
| FL<sub>28-35</sub> | 111.18 ± 6.03 | 127.12 ± 6.03 | 136.95 ± 6.03 | 132.65 ± 6.03 |
| FL<sub>35-42</sub> | 145.82 ± 6.03 | 146.29 ± 6.03 | 161.57 ± 6.03 | 148.35 ± 6.03 |
| FL<sub>42-49</sub> | 142.86 ± 6.03 | 161.71 ± 6.03 | 160.56 ± 6.03 | 156.01 ± 6.03 |
| FCR<sub>21-28</sub> | 1.71 ± 1.31 | 4.04 ± 0.69 | 2.10 ± 0.35 | 3.76 ± 0.54 |
| FCR<sub>28-35</sub> | 2.00 ± 1.54 | 2.57 ± 0.36 | 1.63 ± 1.23 | 2.74 ± 0.68 |
| FCR<sub>35-42</sub> | 2.49 ± 0.62 | 2.13 ± 0.37 | 2.57 ± 0.43 | 2.22 ± 0.24 |
| FCR<sub>42-49</sub> | 2.05 ± 0.20 | 1.68 ± 0.71 | 2.53 ± 0.42 | 2.05 ± 0.42 |

<sup>a,b,c</sup>Significantly different means on the same row (p < 0.05), BWT: live body weight, BWG: body weight gain, FL: daily feed intake, FCR: feed conversion ratio.
The higher feed intake in the supplemented groups at finisher phase (day 49) was necessary to support the enhanced growth rate in these groups while the predominant similarity in feed intakes agrees with He et al. (2019) that in-feed antibiotics and probiotics has no effect on daily feed intake. Some earlier studies (Ashayerizadeh et al., 2009; Toghyan et al., 2011) however, reported significantly higher cumulative feed intake in broiler chickens fed probiotics, prebiotics and antibiotics compared to control while Odefemi (2016) reported higher feed intake in broilers fed probiotics compared to those fed antibiotics. Variations in response to feed additives relate to differences in probiotic species and antibiotic agents, species combination and dose, production standards, environmental factors and management, intestinal ecosystem, as well as inter-microbial and microbiota – host interactions. The similar feed conversion ratio indicates equivalent feed efficiency across treatments which suggests that optimal environmental control eliminates the need for antibiotic supplementation to enhance feed utilization (Gunal et al., 2006).

Comparison of linear body values revealed inconsistent trends over the age periods (Table 2). On day 49, the head circumference was similar across treatment groups (P > 0.05). Neck length was lower in the neomycin group compared to others which had higher and similar values. The control group and the group fed oxytetracycline had similar and higher breast girth than the groups fed neomycin and S. cerevisiae which did not differ significantly. Keel length was lowest in the group fed oxytetracycline and highest in control and neomycin groups. Birds fed S. cerevisiae had a similar final body length as those of the control and oxytetracycline groups, but this was lower compared to that of neomycin group. Birds fed neomycin had the highest final value for thigh length compared to other groups. Similar linear body values between experimental groups indicated similarity in the development of the different body parts of the broiler chickens. Islam et al. (2004) reported no effect of different levels of probiotics on linear body traits of broiler chickens.

| Variable | Basal diet | S. cerevisiae | Neomycin | Oxytetracycline |
|----------|------------|---------------|----------|-----------------|
| HC | 9.53 ± 0.17 * | 9.96 ± 0.14 * | 9.76 ± 0.14 ** | 10.03 ± 0.14 * |
| HC | 13.15 ± 0.18 * | 13.04 ± 0.15 * | 12.56 ± 0.14 * | 12.41 ± 0.14 * |
| HC | 13.34 ± 0.18 * | 13.37 ± 0.15 * | 13.57 ± 0.15 * | 13.39 ± 0.15 * |
| NL | 4.42 ± 0.18 | 4.59 ± 0.15 | 4.60 ± 0.15 | 4.59 ± 0.14 |
| NL | 6.74 ± 0.19 * | 7.24 ± 0.15 * | 6.29 ± 0.16 * | 6.38 ± 0.15 * |
| NL | 8.02 ± 0.19 * | 7.84 ± 0.16 * | 7.00 ± 0.16 * | 7.80 ± 0.16 * |
| BG | 7.97 ± 0.24 * | 8.86 ± 0.20 * | 8.29 ± 0.21 * | 7.97 ± 0.24 * |
| BG | 12.88 ± 0.26 | 12.78 ± 0.21 | 12.29 ± 0.22 | 12.88 ± 0.26 |
| BG | 13.33 ± 0.26 * | 12.56 ± 0.22 * | 12.70 ± 0.22 * | 13.33 ± 0.26 * |
| KL | 8.79 ± 0.28 * | 9.41 ± 0.18 * | 9.25 ± 0.19 ** | 9.16 ± 0.18 * |
| KL | 12.88 ± 0.23 ** | 12.94 ± 0.19 * | 12.73 ± 0.19 ** | 12.43 ± 0.18 * |
| KL | 13.48 ± 0.23 * | 12.76 ± 0.20 * | 13.14 ± 0.20 ** | 12.20 ± 0.19 * |
| BL | 16.10 ± 0.44 * | 17.64 ± 0.37 * | 16.42 ± 0.38 ** | 17.31 ± 0.36 ** |
| BL | 27.06 ± 0.47 * | 27.40 ± 0.39 * | 26.21 ± 0.39 * | 25.95 ± 0.37 * |
| BL | 28.77 ± 0.47 * | 27.63 ± 0.40 * | 29.18 ± 0.40 * | 28.46 ± 0.39 * |
| TL | 5.11 ± 0.21 * | 5.75 ± 0.17 * | 5.54 ± 0.18 ** | 6.00 ± 0.17 ** |
| TL | 7.77 ± 0.22 * | 8.28 ± 0.18 * | 7.38 ± 0.18 * | 7.96 ± 0.17 ** |
| TL | 7.98 ± 0.22 * | 7.83 ± 0.19 * | 8.92 ± 0.19 * | 8.20 ± 0.18 * |

* indicates significantly different means on same row (p<0.05); HC: head circumference, BG: breast girth, NL, KL, BL and TL: neck, keel, body and thigh lengths, respectively.
Haematological and serum biochemical indices

Probiotic and antibiotic supplementation did not affect erythrocyte (RBC), and white blood cell (WBC) counts, and haemoglobin (Hb) concentration (Table 3). At day 21 and 49, PCV was higher in the antibiotic groups compared to probiotic group but similar to control group which in turn did not differ significantly from the probiotic group. Birds fed neomycin had significantly (p < 0.05) lower percent lymphocytes compared to S. cerevisiae group at day 21 while those fed oxytetracycline had lower lymphocytes than probiotic group at day 49. The probiotic and control groups had the highest percent lymphocytes across the age periods. Birds fed antibiotics also had higher heterophils than the probiotic group at these age periods. Ratio of Heterophil to lymphocyte (H:L) was highest (p < 0.05) in the antibiotic group at day 21 and 35, and in the oxytetracycline and control groups at day 49 compared to the group fed S. cerevisiae. The non-significant effect of treatments on RBC and WBC counts, and Hb concentration agrees with Tang et al. (2017) who reported non-significant effect of prebiotics, probiotics, and synbiotics on blood indices in laying hens, and Neveling et al. (2017) who reported non-significant differences in RBC, Hb, PCV, and WBC in broiler chickens fed chlotetracycline, and probiotics.

The lower ratio of heterophils to lymphocytes (H:L) in the control group (day 21 to 35) and the group fed S. cerevisiae indicated lower stress profile in birds of these groups (Campos et al., 2006). while the higher percent lymphocytes in these groups indicated stronger immunological status compared to the group fed antibiotics (Lee et al., 2004).

Table 3. Effect of antibiotics and probiotic on haematological indices of Abor acre broiler chicks

| Variable        | Basal diet | S. cerevisiae | Neomycin | oxytetracycline | SEM  |
|-----------------|------------|---------------|----------|----------------|------|
| RBC<sub>21</sub> (x 10<sup>9</sup>) | 2.67       | 2.56          | 3.01     | 3.24           | 0.24 |
| RBC<sub>35</sub> | 2.80       | 2.89          | 3.02     | 2.78           | 0.24 |
| RBC<sub>49</sub>| 2.82       | 2.50          | 3.07     | 3.24           | 0.24 |
| PCV<sub>21</sub> (%) | 32.25<sup>ab</sup> | 28.50<sup>b</sup> | 35.00<sup>a</sup> | 37.00<sup>a</sup> | 2.53 |
| PCV<sub>35</sub> | 35.25     | 33.00         | 34.50    | 31.75          | 2.53 |
| PCV<sub>49</sub>| 32.25<sup>ab</sup> | 28.50<sup>b</sup> | 35.00<sup>a</sup> | 37.00<sup>a</sup> | 2.53 |
| Hb<sub>21</sub> (g/dl) | 7.73       | 8.15          | 9.70     | 7.68           | 1.08 |
| Hb<sub>35</sub> | 7.43       | 8.63          | 8.05     | 7.70           | 1.08 |
| Hb<sub>49</sub>| 7.73       | 8.15          | 9.20     | 7.68           | 1.08 |
| WBC<sub>21</sub> (x 10<sup>3</sup>) | 5.77       | 6.09          | 6.94     | 6.76           | 7.48 |
| WBC<sub>35</sub> | 12.03      | 10.20         | 13.39    | 11.48          | 7.48 |
| WBC<sub>49</sub>| 12.79      | 38.58         | 13.58    | 12.56          | 7.48 |
| Lym<sub>21</sub> (%) | 68.00<sup>a</sup> | 63.00<sup>ab</sup> | 51.00<sup>c</sup> | 54.00<sup>bc</sup> | 3.92 |
| Lym<sub>35</sub> | 69.25<sup>a</sup> | 61.75<sup>ab</sup> | 52.75<sup>b</sup> | 57.50<sup>b</sup> | 3.92 |
| Lym<sub>49</sub>| 57.50<sup>ab</sup> | 66.25<sup>a</sup> | 65.00<sup>a</sup> | 52.00<sup>b</sup> | 3.92 |
| Het<sub>21</sub> (%) | 27.00<sup>c</sup> | 34.25<sup>bc</sup> | 46.25<sup>a</sup> | 42.25<sup>ab</sup> | 4.14 |
| Het<sub>35</sub> | 26.50<sup>c</sup> | 34.25<sup>bc</sup> | 44.50<sup>b</sup> | 38.50<sup>ab</sup> | 4.14 |
| Het<sub>49</sub>| 40.75<sup>ab</sup> | 29.75<sup>c</sup> | 32.00<sup>bc</sup> | 45.50<sup>a</sup> | 4.14 |
| Het:Lym<sub>21</sub> | 0.40<sup>b</sup> | 0.53<sup>b</sup> | 0.93<sup>a</sup> | 0.84<sup>a</sup> | 0.13 |
| Het:Lym<sub>35</sub> | 0.39<sup>c</sup> | 0.57<sup>bc</sup> | 0.91<sup>a</sup> | 0.72<sup>ab</sup> | 0.13 |
| Het:Lym<sub>49</sub>| 0.73<sup>bc</sup> | 0.46<sup>c</sup> | 0.53<sup>bc</sup> | 0.88<sup>a</sup> | 0.13 |

*<sup>a</sup>* significantly different means on same row (p<0.05); RBC: red blood cell; PCV: packed cell volume; Hb: haemoglobin concentration; WBC: white blood cell; Lym: lymphocyte; Het: heterophil.
Table 4. Effect of antibiotics and probiotic supplementation on serum biochemical indices of Abor acre broiler chicks

| Variable               | Basal diet       | S. cerevisiae | Neomycin    | oxytetracycline |
|------------------------|------------------|---------------|-------------|----------------|
| Albumin (g/dl)         | 1.12 ± 0.21      | 1.18 ± 0.24   | 0.70 ± 0.17 | 1.10 ± 0.24    |
| Globulin (g/dl)        | 1.83 ± 0.38      | 1.78 ± 0.65   | 1.97 ± 0.28 | 1.80 ± 0.08    |
| Total protein (g/dl)   | 2.95 ± 0.25      | 2.95 ± 0.54   | 2.67 ± 0.17 | 2.90 ± 0.23    |
| Cholesterol (mg/dl)    | 97.09 ± 5.89     | 100.5 ± 13.19 | 103.04 ± 6.90 | 95.32 ± 9.41  |
| Creatinine (mg/dl)     | 0.35 ± 0.47      | 0.28 ± 0.12   | 0.25 ± 0.06 | 0.54 ± 0.20    |
| AST (iu/l)             | 69.61 ± 2.83     | 66.80 ± 2.29  | 78.07 ± 6.53 | 69.21 ± 5.50   |
| ALT (iu/l)             | 2.58 ± 0.34      | 4.53 ± 1.11   | 3.78 ± 0.70 | 3.50 ± 1.10    |

AST: aspartate amino transferase; ALT: alanine amino transferase

The observed low values of H:L in the probiotic group agrees with Beski and Al-Sardar (2015) who reported that probiotics ameliorate environmental stress, resulting in lower heterophil to lymphocyte ratio. He et al. (2019) reported higher IgA levels in broiler chickens fed probiotics compared to control and those fed antibiotics. IgA are antibodies generated by the activation of B lymphocytes. In the same study, probiotic fed broiler chickens also had lower values of malondialdehyde (MDA) which indicate lower oxidative stress profile, and higher glutathione peroxidase and super oxide dismutase which indicate higher cellular antioxidant status (He et al., 2019). Islam et al. (2004) however, reported significant effects of probiotic supplementation in broiler diet on packed cell volume, haemoglobin concentration, and red blood cell count. Neveling et al. (2017) reported significant effects of probiotic and antibiotic treatments on percent lymphocyte of 19-day broiler chickens and non-significant effects in 29-day broiler chickens. The higher stress profile observed in the groups fed antibiotics could result from the continuous intake of antibiotics (Kalghatgi et al., 2013). It has been suggested that continuous administration of bactericidal antibiotics induce the formation of toxic reactive oxygen species (ROS). Bactericidal antibiotics cause cell death by oxidation of the guanine nucleotide pool. Kalghatgi et al. (2013) showed that bactericidal antibiotics cause mitochondrial dysfunction and ROS overproduction in mammalian cells leading to oxidative damage to DNA, proteins, and membrane lipids.

Serum biochemical indices did not differ significantly between treatment groups (Table 4) however, albumin tended to be lower in birds fed neomycin compared to other groups while total protein tended to be higher in the control group and the group fed probiotic compared to those fed neomycin. Birds fed oxytetracycline tended to have higher creatinine while AST tended to be higher in birds fed neomycin. These results were similar to those of Elsayed et al. (2014) who reported non-significant effects of antibiotic and probiotic feed additives on serum biochemical indices of broiler chickens, and Tang et al. (2017) who reported non-significant differences in AST and total serum protein in laying hens fed prebiotics, probiotics and synbiotics. Generally, the observed values were within the ranges for chickens as reported by previous studies (Albokhadaim et al., 2012; Rezende et al. 2017) and this suggests no special advantages from the antibiotic additives with regards to the metabolic functions of the experimental birds.

Intestinal bacteria and Eimeria oocyst counts

Intestinal, caecal, and combined caecal and intestinal bacteria counts differed significantly between treatment groups (Table 5). Colony count in the small intestine was highest in neomycin and control groups resulting in the highest bacteria population (1.96.25 x 10^5 and 1.65 x 10^5 cfu/ml, respectively) compared to
oxytetracycline and probiotic groups which had the least colony count and hence the least bacteria population (9.85 x 10^4, and 1.12 x 10^5 cfu/ml, respectively). Birds fed *S. cerevisiae*, and neomycin had highest caecal bacteria colony counts (1.66 x 10^5, and 1.55 x 10^5 cfu/ml, respectively) compared to control and oxytetracycline groups (1.11 x 10^5, and 9.60 x 10^4 cfu/ml, respectively). Colony count from combined caecal and intestinal digestor was highest in neomycin and probiotic groups (1.92 x 10^5, and 1.65 x 10^5 cfu/ml, respectively) and least in oxytetracycline group (1.06 x 10^5 cfu/ml). The lower bacteria counts from birds fed oxytetracycline suggest stronger antimicrobial effect compared to neomycin. Early in vitro studies (Gunnison et al., 1955; Williams, 1971) showed that some antibiotics (e.g., neomycin, polymyxin, and streptomycin) are more effective in the absence of nutrients while others (e.g., oxytetracycline and bacitracin) are not influenced by presence or absence of nutrients.

We hence speculate that the presence of nutrients may have reduced the bactericidal effects of neomycin in the present study. Other factors that may have reduced the effectiveness of neomycin include (1) the presence of sodium and potassium salts (Gunnison et al., 1955) which are components of poultry ration, and (2) the anaerobic and acidic pH of the lower intestinal environment which favour the efficacy of oxytetracycline but limit that of neomycin (Williams, 1971). The high bacteria counts observed in birds fed *S. cerevisiae* may have resulted from increased population of beneficial bacteria due to competitive exclusion and inhibition of pathogenic microflora (Ologhobo et al., 2015; Neveling et al., 2017). Koc et al. (2010) reported consistent increases in lactic acid bacteria and decreases in *E. coli* counts in ileum and caecum of broiler chickens fed yeast probiotic, and a prebiotic (mannan oligosaccharide). Neveling et al. (2017) reported lower levels of bioiluminescent *L. monocytogenes* in the gastrointestinal tract of broiler chickens fed a multistrain probiotic compared to control, and the group fed combined antibiotic agents. The authors suggested that the multistrain probiotic inhibited colonization and growth of bacterium in vivo due to production of organic acids, diacetyl, acetoin, hydrogen peroxide, and bacteriocins, or through competitive exclusion from the gastrointestinal tract. The low intestinal bacteria population (order, 10^4 to 10^5 cfu/ml) reported in the present study can be attributed to the culture-based technique employed which enumerates only culturable bacteria species and species that could grow on nutrient agar under aerobic condition (Shang et al., 2018; Yadav and Jha, 2019).

| Variable       | Basal diet       | *S. cerevisiae* | Neomycin       | Oxytetracycline |
|---------------|------------------|----------------|----------------|----------------|
| **Intestine** |                  |                |                |                |
| Mean colony count | 164.75 ± 8.34^b | 111.75 ± 7.27^c | 196.25 ± 7.92^a | 98.50 ± 5.51^c |
| Log (cfu/ml) | 5.22^a           | 5.05^c          | 5.29^c          | 4.99^c          |
| *Emeria* (oocyst/g) | 0.67 ± 0.33      | 0.25 ± 0.25     | 1.25 ± 0.48     | 0.50 ± 0.29     |
| **Caecae**  |                  |                |                |                |
| Mean colony count | 111.25 ± 5.02^b | 149.75 ± 5.02^a | 165.75 ± 7.65^a | 96.00 ± 6.25^b |
| Log (cfu/ml) | 5.05^b           | 5.18^a          | 5.22^a          | 4.98^c          |
| *Emeria* (oocyst/g) | 1.33 ± 0.67      | 0.70 ± 0.48     | 1.25 ± 0.48     | 1.00 ± 0.41     |
| **Intestine + caecae** |                |                |                |                |
| Mean colony count | 132.50 ± 6.06^c | 163.25 ± 8.63^b | 192.25 ± 4.31^a | 105.50 ± 5.91^d |
| Log (cfu/ml) | 5.12^c           | 5.21^b          | 5.28^a          | 5.02^d          |
| *Emeria* (oocyst/g) | 1.50 ± 0.65      | 0.50 ± 0.29     | 0.75 ± 0.25     | 1.50 ± 0.50     |

^a,b,c significantly different means on the same row (p<0.05).
Corduk et al. (2008) reported coliform and total aerobic bacteria counts in the order $10^5$, and $10^6$ cfu/g, respectively using enumeration in nutrient agar after incubation at 37°C for 48 hours.

**Sensitivity of *Escherichia coli* isolates**

The between group comparative sensitivity of *E. coli* isolates to neomycin and oxytetracycline revealed significantly different diameter of inhibition zones by the antibiotic agents (Table 6). *Escherichia coli* from control group and birds fed probiotic had the highest diameter of inhibition zone by neomycin (22.30 ± 2.90 and 18.00 ± 2.90 mm, respectively) and oxytetracycline (24.30 ± 2.90 and 19.30 ± 2.90mm, respectively). Organisms from birds fed neomycin had diameter of inhibition zone of 11.70 ± 2.90 mm by oxytetracycline while those from birds fed oxytetracycline had inhibition zone diameter of 14.30 ± 2.90 mm by neomycin.

*Escherichia coli* from the groups fed neomycin, and oxytetracycline, were least inhibited by neomycin (zone diameter: 5.70 ± 2.90mm), and oxytetracycline (zone diameter: 3.39 ± 2.90mm), respectively. *Escherichia coli* from control and probiotic groups were therefore sensitive to neomycin and intermediately sensitive to oxytetracycline while organisms from neomycin group were resistant to neomycin and intermediately sensitive to oxytetracycline (CLSI, 2013). On the other hand, *E. coli* from oxytetracycline group were resistant to both neomycin and oxytetracycline. These results indicate that oxytetracycline had a stronger selection pressure for resistant bacteria phenotypes, and that the use of antibiotics for growth enhancement in the present study resulted in the development of resistant bacteria phenotypes and expression of antimicrobial resistant genes in agreement with other studies (Diarra and Malouin 2014; Cosby et al 2015). It has been reported that the use of antibiotics for growth enhancement in animals creates a selection pressure that favours the survival and spread of antimicrobial resistant bacteria phenotypes (Neveling et al., 2017; He et al., 2019). The continuous intake of antibiotics through feed in the present study may have constituted a stress factor for bacteria leading to increased frequency of mutations, and recombinations, and the emergence of resistant bacteria phenotypes (Diarra et al. 2007; Wistrand-Yuen et al., 2018). Fairchild et al. (2005) had reported the presence and increased expression of tetracycline resistance determinant genes in *E. coli* and *Campylobacter jejuni* isolated from caecal digester of chickens fed oral tetracycline antibiotic. The observed partial antimicrobial resistance of *E. coli* from control and probiotic groups to oxytetracycline could result from acquisition of resistant phenotypes or resistance genes from the environment (litter material, feed, water, feeders, drinkers, and farm personnel). These findings by the present study have grave consequences for animal production, public health and the therapeutic use of antibiotics in animals and man.

The environmental limitations to the antimicrobial effects of neomycin presented earlier could also enhance the development of resistance to this antibiotic and this may be an additional explanation for the higher colony counts (higher bacteria populations) observed in the group fed neomycin. Kumar et al. (2018) reported that birds fed bacitracin dimethyl salicylate had higher prevalence of food borne pathogens like Campylobacter and Salmonella species and did not differ in number of bacterial phyla present in the intestinal tract compared to untreated control group while Neveling et al. (2017) observed higher levels of bioiluminescent *L. monocytogenes* in the intestinal tracts of broilers fed a combination of antibiotics (sulphadiazine, colistin and trimethoprim) compared to control group and those fed probiotics.
Mortality

Percent mortality did not differ significantly (p > 0.05) between treatments across the age periods. Mortality tended to be higher at 42 d of age when birds in the control group had mortality of 5.50 % as against 3.70 % for neomycin, and 0.00 % other treatments (Table 7).

Apart from this, mortality was generally low (range: 0.00 to 3.70 %) across treatment groups at the other age periods. The lowest mortality observed in birds fed S. cerevisiae reflects the beneficial effects of probiotics on health and survival of broiler chickens. Bonsu et al (2012) reported lower percent mortality in broiler chickens fed probiotics (direct fed microbials) compared to the control diet. Giggs and Jacobs (2005) however reported non-significant effect of treatment on percent mortality in chickens fed antibiotic growth promoters, probiotics and basal diet.

Table 7. Mortality in Abor acre broiler chicks fed basal diet or diet supplemented with probiotic and antibiotics

| Age (days) | Basal diet | S. cerevisiae | Neomycin | Oxytetracycline |
|-----------|------------|---------------|----------|-----------------|
| 28        | 0.00 ± 0.00 | 0.00 ± 0.00   | 0.00 ± 0.00 | 3.33 ± 3.33     |
| 35        | 0.00 ± 0.00   | 3.33 ± 3.33   | 3.70 ± 3.70 | 0.00 ± 0.00     |
| 42        | 5.50 ± 3.22a | 0.00 ± 0.00c  | 3.70 ± 3.70b | 0.00 ± 0.00c   |
| 49        | 0.00 ± 0.00 | 0.00 ± 0.00   | 0.00 ± 0.00 | 3.33 ± 3.33     |

a,b,c: significantly different means on the same row (p<0.05)

Conclusions

Probiotics should be used in place of antibiotics as feed additives for broiler chicken production to enhance growth performance. The use of antibiotics in feed to enhance growth performance should be discouraged to minimize the development and spread of antibiotic resistant bacteria phenotypes.

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