Gastrointestinal tract and neuroendocrine system responses of young turkeys to the early administration of antibiotics or feeding a diet containing a coccidiostat

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ABSTRACT This study investigated the effects of early and short-term administration of an antibiotic or feeding a diet containing a coccidiostat on gastrointestinal function and the blood levels of selected hormones in young turkeys. A total of 1540 Hybrid Converter turkeys were allocated to 4 groups on the day of hatch. Each group consisted of 7 pens with 55 birds per pen. Group ENR was treated with enrofloxacin for the first 5 d of life, group DOX received doxycycline for 5 d and group MON was administered monensin for 84 d. CON birds served as a control group without any antibiotic treatment or MON administration. An analysis of the activity of bacterial enzymes revealed that the cecal microbiota of turkeys were less sensitive to MON than to the other 2 antibiotics. Turkeys subjected to ENR and DOX treatments were characterized by lower (P < 0.05) extracellular activity of cecal bacterial β-glucosidase, compared with groups CON and MON. The extracellular activity of cecal bacterial α-galactosidase and β-galactosidase decreased significantly in response to the experimental treatment with DOX (P < 0.05 vs. CON). Turkeys treated with ENR had higher total activity of bacterial β-galactosidase than those administered DOX or MON. Despite the differences in the enzymatic activity of microbiota, the use of antibiotics did not affect the concentrations of total short-chain fatty acids or ammonia in the cecal digesta of turkeys. A diet containing MON and the early administration of ENR or DOX induced an increase in blood noradrenaline levels (P = 0.004) in 56-day-old turkeys. Early DOX use increased plasma cortisol concentrations (P < 0.001) and decreased plasma serotonin levels (P = 0.006) in 56-day-old turkeys. Over the entire experiment (up to 12 wk of age), the use of MON improved the BW gain of turkeys (P = 0.055) and feed conversion (P = 0.016), compared with the DOX treatment.

Key words: antibiotic, gastrointestinal tract, neuroendocrine system, performance, turkey

INTRODUCTION

In recent decades, antibiotics have considerably contributed to improving animal production efficiency as growth-promoting agents enhancing performance or as therapeutic and metaphylactic drugs applied to treat or prevent animal diseases (Brown et al., 2017). Due to the emergence of pathogens resistant to antimicrobials, the current challenge facing poultry production is the need to reduce the amount of antibiotics administered to birds (Bortolaia et al., 2016; Bartkiene et al., 2020). Antibiotic growth promoters were banned in the EU in 2006, in the US in 2017 and are still allowed in Brazil and China (Roth et al., 2019). In the recently adopted “Farm to fork” UE strategy, all Member States have committed to reduce the use of antimicrobials in animal production by 50% (More, 2020; Baudoin et al., 2021). United States Food and Drug Administration (FDA) issued similar guidelines to phase out the use of medically important antibiotics in livestock for production purposes (Wallenga et al., 2022).

Modern fast-growing domestic birds are characterized by increased susceptibility to adverse environmental conditions and bacterial and viral infections, which lead to gastrointestinal disorders and induce oxidative stress. Antibiotics are often used in the metaphylaxis of avian infectious diseases (Dorrestein et al., 1990; Cunha et al., 2000; Khalifeh et al., 2009;
Gutiérrez et al., 2017). Antibiotics are also added to feed to prevent coccidiosis in poultry and minimize production losses associated with infections caused by *Eimeria* spp. (Chapman et al., 2010; Kadykalo et al., 2018; Noack et al., 2019).

Phylactic antibiotics stir controversy due to their potential negative impact on the immune function and antioxidant status of birds (Silvo et al., 2013; Elamara et al., 2015; Mishra and Jha, 2019; Guillouzo and Guguen-Guillouzo, 2020). Antibiotics reduce the abundance of both harmful and saprophytic microorganisms colonizing the gut (Mehdi et al., 2018; Shang et al., 2018; Elokil et al., 2020). Research has shown that enteric colonization by microbes affects the oxidation of proteins and DNA as well as epigenetic DNA modifications not only in the gut but also in other tissues (Dunislawska et al., 2020). Negative oxidative and epigenetic changes may induce enteritis (Borrman et al., 2007; Brisbin et al., 2008; Van Deun et al., 2008; Teirlynck et al., 2009), thus disrupting intestinal barrier integrity (Lu et al., 2014).

Enrofloxacin (ENR) and doxycycline (DOX) are broad-spectrum antibiotics, commonly used in farm animals, including poultry (Gabl et al., 1992; Fife and Sledge, 1995; Khalifeh et al., 2009). A few experiments performed to date have focused on the efficacy of ENR against *Salmonella enteritidis* (Kang et al., 2019), *Escherichia coli* and *Pasteurella multocida* (Rawiet et al., 2010), as well as avian pneumovirus and *Ornithobacterium rhinotracheale* (Garmyn et al., 2009). The antibiotic was not always administered in the first week post hatch, and only bird performance was evaluated, disregarding other biological responses. Potential threats resulting from decreased extracellular activity of selected microbial enzymes in the cecal digesta and the effects of these changes on the blood levels of selected hormones in turkeys administered antibiotics in early life stages remain insufficiently investigated. A growing body of evidence indicates that the gut microbiome plays an important role in the pathogenesis of neurological diseases, as part of the gut-brain axis. Metabolites, including endotoxins released by gut bacteria, may potentially affect the expression levels of neurotransmitters as well as their precursors and receptors in the central nervous system through blood flow and vagus-dependent pathways, thus influencing brain function and cognitive performance (Farzi et al., 2018).

The research hypothesis postulates that antibiotics may lead to adverse changes in the composition of gut microbiota and, consequently, affect the neuroendocrine system. Therefore, the aim of this study was to determine whether early and short-term administration of an antibiotic (ENR or DOX) or feeding a diet containing the coccidiostat monensin (MON) affects the activity of glycolytic bacterial enzymes as well as the concentrations and profile of short-chain fatty acids (SCFAs) and selected hormones in young growing turkeys.

**MATERIALS AND METHODS**

**Ethics Statement**

The experiment was conducted in the Animal Research Laboratory of the Department of Poultry Science, University of Warmia and Mazury in Olsztyn (Poland). The protocol for this study was approved by the Local Ethics Committee for Animal Experiments in Olsztyn, Poland (decision No. 47/2021), and the animals were cared for under guidelines comparable to those laid down by EU Directive 2010/63/EU.

**Birds, Management, and Experimental Design**

A total of 1,540 Hybrid Converter female turkeys were placed in pens on litter (wood shavings) on the day of hatch, and were randomly allocated to 4 experimental groups, with 7 replicate pens (10 m² each) per treatment and 55 birds per pen. The stocking density at the initial stage of rearing was 5.5 birds/m². The experiment had a completely randomized design. The replicates (pens) were allocated to groups so as to ensure their uniform distribution in the house. The pouls were not vaccinated posthatching. The initial BW of one-day-old pouls was 69.9 to 70.5 g (P = 0.224). Environmental conditions were controlled automatically, adjusted to the birds’ age, consistent with the recommendations of Hybrid Turkeys (2020), and identical for all turkeys in the housing facility. The feed and drink lines were adjusted to the growth stage of turkeys.

The pens in the building were evenly distributed among 4 groups: ENR, DOX, MON, and negative control (CON). Group ENR received enrofloxacin (Enrofloxacin 10%, Biowet, Drwalew, Poland), group DOX received doxycycline (Doxylin CT WSP 433 mg/g, Dopharma Research B.V., Raimsdonksveer, Netherlands), and group MON was administered the coccidiostat monensin (Coxidin 200, Huvepharma Polska, Warsaw, Poland). The antibiotics ENR and DOX were added to drinking water, according to the currently advised five-day treatment schedule (10 mg ENR/kg BW and 50 mg DOX/kg BW, daily). In group MON, the coccidiostat was added to feed (90 mg/kg feed, for 84 d.). CON birds served as a control group without any antibiotic treatment or MON administration.

During each of the three feeding phases (weeks 1–4, 5–8, and 9–12), birds were fed isocaloric diets (Table 1) containing 271, 241, and 213 g/kg of crude protein, respectively, as per nutrient requirements of commercial turkeys in a given stage of rearing (Hybrid Turkeys, 2020). The trial lasted 12 wk, from 1 to 84 d of age. Starter diets were offered as crumbles, and grower diets (29–84 d) were prepared as 3 mm pellets. Throughout the experiment, all birds had unlimited access to feed and water.

**Sampling Collection and Investigations**

Samples of experimental diets were analyzed in duplicate for the content of dry matter (DM, method 934.01),
Table 1. Ingredient composition and nutrient content of turkey diets (g/100 g, as-fed basis).

| Item                        | Feeding period, wk |
|-----------------------------|-------------------|
|                             | 1–4              | 5–8              | 9–12             |
| **Ingredients**             |                  |                  |                  |
| Wheat                       | 26.280           | 41.666           | 49.103           |
| Maize                       | 20.000           | 10.000           | 10.000           |
| Soybean meal (48% CP)       | 42.690           | 34.736           | 25.199           |
| Rapseed meal                | 3.000            | 4.000            | 6.000            |
| Soybean oil                 | 3.073            | 5.083            | 5.774            |
| Sodium bicarbonate          | 0.200            | 0.200            | 0.150            |
| Sodium chloride             | 0.152            | 0.160            | 0.202            |
| Limestone                   | 1.399            | 1.413            | 1.325            |
| Monocalcium phosphate       | 2.096            | 1.696            | 1.237            |
| L Lysine HCL                | 0.397            | 0.416            | 0.394            |
| DL Methionine               | 0.291            | 0.227            | 0.190            |
| L-Threonine                 | 0.072            | 0.053            | 0.076            |
| Choline chloride            | 0.100            | 0.100            | 0.100            |
| Vitamin-mineral premix       | 0.250            | 0.250            | 0.250            |
| **Calculated nutrient content** |                |                  |                  |
| Metabolizable energy, kcal/kg | 2890            | 2950             | 3050             |
| Crude protein               | 27.00            | 24.50            | 21.50            |
| Lysine total                | 1.75             | 1.58             | 1.35             |
| Methionine total            | 0.67             | 0.58             | 0.51             |
| Methionine + Cys total      | 1.12             | 1.00             | 0.90             |
| Threonine total             | 1.08             | 0.95             | 0.85             |
| Calcium                     | 1.20             | 1.10             | 0.95             |
| Available phosphorus        | 0.58             | 0.50             | 0.40             |
| Na                          | 0.14             | 0.14             | 0.14             |
| **Analyzed chemical composition** |            |                  |                  |
| Crude protein               | 27.14            | 24.09            | 21.27            |
| Crude fat                   | 3.47             | 7.07             | 7.17             |

1Provided per kg diet (feeding periods: weeks 1–4, 5–8, 9–12): mg: retinol 3.78, 3.38, and 2.88, cholecalciferol 0.13, 0.12, and 0.10, α-tocopherol acetate 100, 90, and 80, vit. K 5.8, 5.6, and 4.8, thiamine 5.4, 4.7, and 4.0, riboflavin 9.4, 7.5, and 6.4, pyridoxine 6.4, 5.6, and 4.8, cobalamin 0.632, 0.028, and 0.024, biotin 0.32, 0.28, and 0.24, pantothentic acid 28, 24, and 20, nicotinic acid 84, 75, and 64, folic acid 3.2, 2.8, and 2.4, Fe 64, 60, and 56, Mn 120, 112, and 96, Zn 110, 103, and 88, Cu 23, 19, and 16, I 3.2, 2.8, and 2.4, Se 0.30, 0.28, and 0.24, respectively, crude protein (CP, N × 6.25; method 976.05) and crude fat (CF, method 920.39), as described by the Association of Official Analytical Chemists (AOAC, 2005). The content of monensin in MON diets was analyzed by liquid chromatography with a diode array detector (LC-DAD), according to the ISO 14183 (2005) procedure. The intended monensin concentrations in MON diets were analytically confirmed and reached 88.8, 99.9, and 90.0 mg/kg in the first, second, and third phase of feeding, respectively.

During the trial, the BW of turkeys and feed consumption were recorded on a pen basis at 4, 8, and 12 wk of age. Daily feed intake (DFI) per bird was calculated on a pen total feed consumption basis for the entire experimental period and for the number of days in the period. The feed conversion ratio (FCR; kilogram of feed/kg of BWG) was calculated based on BW gain and feed consumption. Mortality rates were recorded daily, and the weights of dead birds were used to adjust average BW gain, DFI, and FCR. Performance parameters were also determined for the entire 12-wk experiment.

At 1, 5, and 7 d of age, one bird per replicate pen (7 birds per treatment) was randomly selected and sacrificed by cervical dislocation following the recommendations for euthanasia of experimental animals (Close et al., 1997). The levels of cortisol, serotonin (5-HT), thyroxine (T4), histamine (HIS), dopamine and noradrenaline were determined in the blood plasma of 1, 7, and 56-day-old turkeys, using OxiSelect diagnostic kits (Cell Biolabs, Inc., San Diego, CA). Cortisol concentration in the yolk sac was determined in one- and 5-day-old birds. Yolk sacs were collected post mortem from 7 birds of each group (1 bird per pen), and the entire pouch (yolk sac membrane and contents) was homogenized according to the procedure described in diagnostic kits (Cell Biolabs, Inc.).

At 6 and 9 wk of age, bulk samples of fresh feces were collected (n = 7) in each group. Fecal samples were analyzed to determine oocyst counts per gram (OPG) with the modified McMaster counting chamber flotation method using a saturated salt solution, 100× magnification microscope, and standard formula calculation (Raynaud, 1970). The calculated limit of detection for standard dilution and McMaster counting chambers was ~7 OPG.

At 56 d of age, 7 birds from each treatment (1 bird per pen) were randomly selected, weighed, and sacrificed by electrocution at the Department’s slaughterhouse. Fresh samples of ileal (middle section of the ileum) and cecal contents were used for immediate analysis (ileal and cecal DM, ileal viscosity, cecal ammonia). The DM content of digesta was determined at 105°C, and digesta viscosity was measured in the supernatant fraction using the cone/plate viscometer (model LVDV II+, Brookfield Engineering Laboratories, Stoughton, MA). In the fresh cecal digesta, ammonia was extracted, trapped in a solution of boric acid in Conway’s dishes, and determined by direct titration with sulfuric acid (Hofrek and Haas, 2001). The remaining portions of the cecal contents were used immediately for the determination of enzyme activity in the gut microbiota and SCFA concentrations.

**Activity of Intestinal Microbiota**

The activity of gut microbiota was measured based on the activity of bacterial enzymes and the concentrations of SCFAs. Bacterial enzyme activity in the cecal digesta was determined spectrophotometrically based on the rate of p- or o-nitrophenol (PNP and ONP, respectively) release from their respective nitrophenylglucosides, according to the protocol described by Zary-Sikorska et al. (2021). The activity of the following microbial enzymes was assessed: α- and β-glucosidase, α- and β-galactosidase, β-glucuronidase, β-xylosidase, and α-arabinopyranosidase. The remaining samples were stored in test tubes at −70°C until analysis. In brief, to measure the activities of enzymes secreted by bacterial cells into the cecal environment (extracellular activity), a reaction mixture was prepared containing a substrate solution (5 mM) and a 1:10 (v/v) dilution of the cecal digesta, ammonia was extracted, trapped in a solution of boric acid in Conway’s dishes, and determined by direct titration with sulfuric acid (Hofrek and Haas, 2001). The remaining portions of the cecal contents were used immediately for the determination of enzyme activity in the gut microbiota and SCFA concentrations.
of selected cecal bacterial enzymes, including extracellular and intracellular activities, a cecal digesta sample diluted in phosphate buffer was mechanically disrupted by vortexing with glass beads (212–300 μm in diameter) using the FastPrep-24 homogenizer (MP Biomedicals, Santa Ana, CA). The resulting mixture was centrifuged and the supernatant was used for the enzyme assay described above. Intracellular enzyme activity was calculated by subtracting the extracellular from total activity. In order to prepare the calculation formulas, the model curves for PNP and ONP were used and the relevant equations were obtained. Extracellular enzyme activity was also calculated as the release ratio (RR), expressed as a percentage of total enzyme activity. Cecal SCFA concentrations were analyzed by gas chromatography (Shimadzu GC-2010, Shimadzu, Kyoto, Japan) on a capillary column (SGE BP21, 30 m × 0.53 mm, SGE Europe Ltd., Kiln Farm Milton Keynes, UK), as described previously (Juskiewicz et al., 2006). All analyses were performed in duplicate.

**Statistical Analysis**

For performance parameters, a pen (n = 7) was considered as a replicate experimental unit for the statistical analysis. The values of the remaining traits were determined individually in 7 birds randomly selected from each treatment. In order to improve the normality of distribution and homogeneity of variance, the data on the activity of the analyzed enzymes as well as the concentrations of iso-butyric and iso-valeric acids and histamine were subjected to the Box-Cox transformation before statistical analysis. The percentage data of mortality were transformed to arcsine of the square root before statistical analysis. All data were subjected to one-way ANOVA, according to the GLM procedure for STATISTICA software, ver. 12 (StatSoft Inc., 2014). The post-hoc Tukey’s HSD test was used to determine differences between treatment groups. The significance level was set at P < 0.05. The results were presented as the mean and the pooled standard error of the mean (SEM).

**RESULTS**

**Functional Status of the Gut**

As indicated in Table 2, ileal digesta DM concentration tended to decrease in the ENR and DOX treatments (P = 0.070). The applied treatments with ENR, DOX, and MON did not affect ileal digesta viscosity or cecal digesta DM and ammonia concentrations, compared with CON birds. Turkeys subjected to ENR and DOX treatments were characterized by lower (P < 0.05) extracellular activity of cecal bacterial β-glucosidase, relative to groups CON and MON (Table 3). The total activity of β-glucosidase, comprised of extracellular and intracellular activities, tended to be enhanced (P = 0.057) following the dietary addition of MON (21.65 μmol/h/g vs. 12.72–14.72 in the remaining groups). ENR birds had the lowest (P = 0.042) value of the calculated cecal bacterial β-glucosidase RR, compared with CON and MON turkeys. The extracellular activity of cecal bacterial α-galactosidase and β-galactosidase decreased significantly in response to the experimental treatment with DOX (P < 0.05 vs. CON). The RR of α-galactosidase was significantly lower in groups

### Table 2. Parameters of ileal and cecal digesta in turkeys at 56 d of age.

| Item                  | Small intestine (ileum) | Cecal |                      |
|-----------------------|-------------------------|-------|----------------------|
|                       | DM, %                   | Viscosity, mPa·s | DM, % | Ammonia, mg/g |
| Group (n = 7)¹        |                         |                   |       |               |
| CON                   | 18.65                   | 2.004             | 14.50 | 0.301         |
| ENR                   | 16.99                   | 2.047             | 15.45 | 0.313         |
| DOX                   | 16.99                   | 2.140             | 14.27 | 0.301         |
| MON                   | 18.76                   | 2.186             | 15.86 | 0.339         |
| SEM                   | 0.329                   | 0.058             | 0.341 | 0.009         |
| ANOVA P-value         | 0.670                   | 0.700             | 0.301 | 0.378         |

¹Group: ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 d of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 d of life; MON, treated with monensin added to feed at a dose rate of 90 mg/kg feed for 84 d; CON, untreated control. Composition of the basal diet is given in Table 1. Data represent mean values of 7 turkeys per group. RR (%) = release ratio of an enzyme (extracellular enzyme activity expressed as a percentage of total activity); SEM = standard error of the mean (SD divided by the square root of replication number, n = 28).

### Table 3. Extracellular, intracellular, and total activity of bacterial α- and β-glucosidase, α- and β-galactosidase (μmol/h/g) in the cecal digesta of turkeys at 56 d of age.

| Item                  | α-glucosidase | β-glucosidase | α-galactosidase | β-galactosidase |
|-----------------------|---------------|---------------|-----------------|-----------------|
|                       | Extra         | Intra         | Total RR        | Extra           | Intra         | Total RR        | Extra           | Intra         | Total RR        |
| Group (n = 7)¹        |               |               |                 |                 |               |                 |                 |               |                 |
| CON                   | 16.82         | 23.44         | 40.26           | 42.49           | 2.80         | 11.03          | 13.83           | 22.39          | 24.39         | 99.10          | 123.49         | 19.50          | 27.37         | 71.83          | 99.20         | 29.56         |
| ENR                   | 18.60         | 21.68         | 40.29           | 45.99           | 1.66         | 13.06          | 14.72           | 11.54          | 14.86         | 127.83         | 142.68         | 10.80          | 20.42         | 101.50         | 121.93        | 17.11         |
| DOX                   | 14.97         | 19.30         | 34.27           | 49.60           | 1.71         | 11.01          | 12.72           | 15.67          | 8.51          | 98.30          | 106.81         | 8.46           | 14.18         | 54.89         | 69.07         | 21.20         |
| MON                   | 21.08         | 19.92         | 41.00           | 55.00           | 4.25         | 17.40          | 21.65           | 21.11          | 13.02         | 102.17         | 115.18         | 11.31          | 23.32         | 53.59         | 76.92         | 29.35         |
| SEM                   | 0.942         | 2.168         | 2.630           | 2.453           | 0.280        | 1.195          | 1.306           | 1.568          | 1.089         | 5.470          | 5.947          | 1.183          | 1.651         | 5.732         | 6.369         | 1.581         |
| ANOVA P-value         | 0.120         | 0.917         | 0.319           | 0.325           | 0.001        | 0.191          | 0.057           | 0.042          | 0.018         | 0.173          | 0.171          | 0.002          | 0.018         | 0.006          | 0.008         | 0.005         |

¹Group: ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 d of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 d of life; MON, treated with monensin added to feed at a dose rate of 90 mg/kg feed for 84 d; CON, untreated control. Composition of the basal diet is given in Table 1. Data represent mean values of 7 turkeys per group. RR (%) = release ratio of an enzyme (extracellular enzyme activity expressed as a percentage of total activity); SEM = standard error of the mean (SD divided by the square root of replication number, n = 28).
ENR, DOX, and MON than in group CON ($P < 0.05$). The highest intracellular and total activity of bacterial β-galactosidase was noted in the ENR cecal digesta ($P < 0.05$ vs. groups DOX and MON). The ENR treatment was also associated with the lowest β-galactosidase RR ($P < 0.05$ vs. groups CON and MON). The dietary treatment with MON resulted in increased extracellular activity and RR of bacterial cecal α-arabinopyranosidase ($P < 0.05$ vs. the remaining groups; Table 4). The results presented in Table 5 show that the administration of ENR, DOX, and MON did not affect the concentrations of total SCFAs in the cecal digesta of turkeys ($P > 0.05$). The cecal concentrations of respective acids, i.e. acetic, propionic, iso-butyric, butyric, and iso-valeric, did not differ significantly across groups, either. A significant increase in cecal valeric acid concentration was noted in response to dietary MON inclusion, compared with CON and DOX turkeys. The percentage of acetic acid in the cecal SCFA profile tended to decrease in MON birds ($P = 0.068$).

**Effect on the Hormone Secretion in Turkeys**

In the current study, the cortisol content of the yolk sacs collected from 1- and 5-day-old turkeys was similar in all groups. No inter-group differences were found in plasma cortisol levels in 1- and 7-day-old birds, either. However, early DOX administration contributed to increasing plasma cortisol concentrations ($P < 0.001$) in turkeys aged 56 d (Table 6). Neither a diet containing MON nor the early administration of ENR or DOX affected the plasma levels of serotonin, dopamine, noradrenaline, thyroxine, or histamine in 7-day-old birds, but an increase in noradrenaline levels ($P = 0.004$) was noted in turkeys aged 56 d. At 56 d of age, blood serotonin levels were lower in group DOX than in group CON ($P = 0.006$; Table 7).

### Growth Performance of Turkeys

An analysis of the growth performance of turkeys (Table 8) indicated that the early administration of antibiotics or continuous use of the coccidiostat MON had no effect on DFI, BW gain, or FCR in the first month of rearing. In the second feeding phase (wk 5–8), turkeys receiving MON had the highest BW gain ($P = 0.030$ vs. group DOX), accompanied by a favorable reduction in the FCR ($P = 0.001$) compared with the other groups. Over the entire experiment (up to 12 wk of age), the use of a coccidiostat (group MON) resulted in better BW gain of turkeys ($P = 0.055$) and feed conversion ($P = 0.016$), compared with the DOX treatment. In

### Table 4. Extracellular, intracellular, and total activity of bacterial β-glucuronidase, α-arabinopyranosidase, and β-xylosidase (μmol/h/g) in the cecal digesta of turkeys at 56 d of age.

| Item | β-glucuronidase | α-arabinopyranosidase | β-xylosidase |
|------|----------------|-----------------------|-------------|
|      | Extra | Intra | Total | RR | Extra | Intra | Total | RR | Extra | Intra | Total | RR |
| CON  | 12.88 | 26.74 | 39.62 | 33.94 | 1.92<sup>b</sup> | 6.28 | 8.21 | 24.41<sup>b</sup> | 2.93 | 17.69 | 20.62 | 16.16 |
| ENR  | 15.94 | 47.46 | 63.41 | 25.55 | 1.34<sup>b</sup> | 5.75 | 7.09 | 19.34<sup>c</sup> | 2.01 | 14.52 | 16.54 | 12.05 |
| DOX  | 14.10 | 48.27 | 62.37 | 24.66 | 1.31<sup>b</sup> | 5.00 | 6.31 | 21.71<sup>b</sup> | 2.00 | 14.79 | 16.79 | 16.43 |
| MON  | 14.43 | 41.72 | 56.15 | 25.39 | 3.37<sup>a</sup> | 6.40 | 9.77 | 36.66<sup>c</sup> | 2.42 | 21.76 | 24.18 | 12.20 |
| SEM  | 1.119 | 3.673 | 4.393 | 1.816 | 0.200 | 0.438 | 0.552 | 1.787 | 0.197 | 1.869 | 1.900 | 1.703 |
| ANOVA P-value | 0.831 | 0.132 | 0.195 | 0.228 | <0.001 | 0.767 | 0.181 | 0.001 | 0.303 | 0.510 | 0.457 | 0.705 |

<sup>a,b</sup>Means within the same column with different superscripts differ significantly ($P < 0.05$).

<sup>1</sup>Group: ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 d of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 d of life; MON, treated with monensin added to feed at a dose rate of 90 mg/kg feed for 84 d; CON, untreated control. Composition of the basal diet is given in Table 1. Data represent mean values of 7 turkeys per group; PSCFA, putrefactive short-chain fatty acids (C4i + C5i + C5); C2, acetic acid; C3, propionic acid; C4i, iso-butyric acid; C4, butyric acid; C5i, iso-valeric acid; C5, valeric acid; SEM, standard error of the mean (SD divided by the square root of replication number, n = 28).

### Table 5. Concentrations and profile of short-chain fatty acids (SCFAs) in the cecal digesta of turkeys at 56 d of age.

| Item | SCFAs (μmol/g) | SCFA profile (% of total SCFAs) |
|------|---------------|----------------------------------|
|      | C2 | C3 | C4 | C5 | C5i | C5s | Total PSCFAs | Total SCFAs | C2 | C3 | C4 |
| CON  | 96.63 | 7.817 | 0.476 | 28.80 | 0.362 | 1.107<sup>b</sup> | 1.946 | 135.19 | 71.61 | 5.75 | 21.21 |
| ENR  | 93.22 | 7.912 | 0.569 | 28.93 | 0.435 | 1.469<sup>b</sup> | 2.472 | 133.75 | 69.89 | 6.82 | 21.39 |
| DOX  | 90.60 | 7.138 | 0.410 | 26.25 | 0.403 | 0.979<sup>b</sup> | 1.791 | 125.58 | 72.16 | 5.69 | 20.72 |
| MON  | 85.25 | 5.906 | 0.476 | 31.13 | 0.347 | 2.005<sup>b</sup> | 2.826 | 129.11 | 66.04 | 7.59 | 24.15 |
| SEM  | 2.156 | 0.488 | 0.057 | 1.337 | 0.060 | 0.129 | 0.175 | 2.851 | 0.917 | 0.328 | 0.853 |
| ANOVA P-value | 0.304 | 0.177 | 0.688 | 0.636 | 0.854 | 0.006 | 0.128 | 0.643 | 0.068 | 0.115 | 0.500 |

<sup>a,b</sup>Means within the same column with different superscripts differ significantly ($P < 0.05$).

<sup>1</sup>Group: ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 d of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 d of life; MON, treated with monensin added to feed at a dose rate of 90 mg/kg feed for 84 d; CON, untreated control. Composition of the basal diet is given in Table 1. Data represent mean values of 7 turkeys per group; PSCFA, putrefactive short-chain fatty acids (C4i + C5i + C5); C2, acetic acid; C3, propionic acid; C4i, iso-butyric acid; C4, butyric acid; C5i, iso-valeric acid; C5, valeric acid; SEM, standard error of the mean (SD divided by the square root of replication number, n = 28).
Table 6. Cortisol content of the yolk sac (ng/sac) and blood (ng/mL).

| Item          | Yolk sac 1 day of age | Yolk sac 5 days of age | Blood 1 day of age | Blood 7 days of age | Blood 56 days of age |
|---------------|-----------------------|------------------------|-------------------|--------------------|----------------------|
| Group (n = 7) |                       |                        |                   |                    |                      |
| CON           | 1641.6                | 93.62                  | 145.8             | 126.3              | 107.8                |
| ENR           | 1684.7                | 69.65                  | 136.8             | 123.0              | 130.3               |
| DOX           | 1685.9                | 72.13                  | 128.9             | 142.6              | 167.7               |
| MON           | 1607.5                | 109.91                 | 144.6             | 114.9              | 119.8               |
| SEM           | 66.52                 | 7.446                  | 3.134             | 3.855              | 5.157               |
| ANOVA P-value | 0.993                 | 0.178                  | 0.199             | 0.066              | 0.000                |

a,bMeans within the same column with different superscripts differ significantly (P < 0.05).

1Group: ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW; MON, treated with monensin added to feed at a dose rate of 90 mg/kg feed for 84 d; CON, untreated control.

**DISCUSSION**

It seems that the dietary application of the coccidiostat MON during the 8-wk feeding trial had a lower impact on the activity of cecal microbiota than the antibiotics ENR and DOX administered to birds via drinking water from 1 to 5 d of age. It can be assumed that the cecal microbial community in turkeys was less sensitive to MON than to the other two antibiotics. It should also be stressed that a short 5-d antibiotic treatment in the first days of birds’ life considerably affected cecal bacterial enzyme activity measured more than seven weeks after the antibiotic treatments had been completed. In an experiment performed by Chen et al. (2017) on 7-wk-old Balb/c nude mice, 4-d antibiotic treatment caused a significant decrease in fecal β-glucuronidase activity on day 4 and then the activity gradually increased. Morales-Barrera et al. (2016) demonstrated that ENR administration to chickens (via drinking water for 1–5 d) that were additionally challenged with Salmonella Enteritidis or Salmonella Heidelberg contributed to Salmonella colonization in the gut, enhanced intestinal permeability and caused a shift in the microbial community from Firmicutes and Bacteroidetes towards a higher proportion of Proteobacteria. According to some authors, both α- and γ-Proteobacteria are capable of producing the enzyme β-galactosidase (Cheng et al., 2017). In the present study, the total activity of bacterial β-galactosidase was significantly higher in group ENR, compared with groups DOX and MON. β-galactosidase is able to hydrolyze the β-glicosidic bond formed between galactose and its organic moiety. It might also cleave fucosides and arabinosides but with much lower efficiency. It is well known that *Escherichia coli* can easily produce large amounts of β-galactosidase (Matthews, 2005), and other authors reported that DOX, alone or in combination, might efficiently reduce the incidence of *E. coli* infections (Lai et al., 2016). An in vitro study (Chatterjee, 1993) revealed that human proximal tubular cells incubated with MON had 2.5- to 3-fold higher activity of α-galactosidase, β-galactosidase, and β-glucosidase than control cells. Interestingly, the dietary treatment with MON resulted in higher extracellular activity of bacterial β-glucosidase in the cecal digesta, in comparison with groups ENR and DOX, and the activity of this enzyme in group MON was comparable with that in control untreated turkeys. β-glucosidase is a microbial multienzyme that acts as a key factor in the hydrolysis of plant polysaccharides, including cellulose, oligosaccharides, and polyphenolic derivatives which are widely present in fodder (Juśkiewicz et al., 2002; Zhang et al., 2018). Some authors have classified bacterial β-glucosidase along with β-glucuronidase as biomarkers of undesirable changes in intestinal microbial enzyme activity, pointing to a possible release of toxic substances from glucoside or glucuronide conjugates in the digesta with high activity of such bacterial enzymes (Michlmayr and Kneifel, 2014). For instance, glycosylation provides the chemical stability of aglycone and effectively detoxifies metabolites/xenobiotics (e.g., mycotoxins), whereas high activity of bacterial β-glucosidase may interfere with such a mechanism of action. β-glucosidase may also enhance the uptake of dietary phenols, especially flavonoids, which is important for maintaining the redox balance in the body (Modrackova et al., 2020). Since the activity of bacterial β-glucuronidase was unchanged by ENR, DOX, and MON treatments in the current study, the effect of MON on other bacterial enzymes in the cecal digesta of turkeys should be regarded as neutral or even beneficial. It is also noteworthy that the extracellular activity of bacterial α-arabinopyranosidase was highest in the MON treatment, even higher than in CON birds. Additionally, group MON was characterized by the highest RR of α-arabinopyranosidase from bacterial cells into the cecal environment. Such a mechanism provides additional energy by the microbial fermentation of polysaccharides and oligosaccharides that escape digestion in the upper gastrointestinal tract (Gugołek et al., 2014).
L-arabinose is a common component of several polysaccharides and glycosides whose digestion is dependent on the activity of arabinofuranosidases and arabinopyranosidases (Shin et al., 2003). In the present study, despite the differences in microbial enzyme activity, the experimental groups did not differ from one another with regard to cecal SCFA concentrations. It is well known that SCFAs are bacterial metabolites that generally exert beneficial effects in the gut, serve as a source of energy and maintain intestinal homeostasis, for instance through anti-inflammatory effects and cell proliferation/differentiation. Research has shown that Bacteroidetes (Gram-negative) and Firmicutes (Gram-positive) are the most abundant intestinal phyla; the former mainly produce acetic and propionic acids, and the latter mostly act as butyric acid producers (Parada Venegas et al., 2019). Since SCFA and ammonia concentrations remained unchanged in turkeys subjected to ENR, DOX and MON treatments, it can be concluded that the antibiotics and coccidiostat applied in this study did not disrupt the large gut microbial homeostasis but only slightly affected the enzymatic pattern of cecal microbes.

The gut-brain axis is an information exchange network that connects the gut and brain. The 2-way communication between the gut microbiome and brain includes central nervous and endocrine systems (Chen et al., 2021). In the present study, a diet containing MON and ENR or DOX administered to turkeys during the first five days of their life increased the plasma levels of the neurotransmitter noradrenaline. Moreover, early DOX use also increased plasma cortisol concentrations and decreased plasma serotonin levels. According to previous research, antibiotics lead to the acquired deprivation of gut bacteria and can also alter the levels of neurotransmitters and their precursors in the gut and blood (Fujisaka et al., 2018; Gao et al., 2018). Changes in the abundance of gut microbiota are accompanied by changes in the expression of neurotransmitter receptors in the brain (Sudo et al., 2004; Bravo et al., 2011; Neufeld et al., 2011). Gao et al. (2018) demonstrated that the plasma levels of serotonin (5-HT) and dopamine decreased in piglets receiving ileal antibiotic infusions (a mixture of ampicillin, gentamicin and metronidazole). Some bacterial taxa may signal, through their metabolites, the synthesis and release of neurotransmitters by enteroendocrine cells (e.g., metabolites produced by spore-forming bacteria serve as signaling molecules to regulate the biosynthesis of serotonin by increasing the expression of its rate-limiting gene TPH1 in enterochromaffin cells) (Chen et al., 2021). The present findings indicate that the biosynthesis of 5-HT in the hypothalamus may be hindered by antibiotic administration. It was found that 5-HT and dopamine in neurons of the hypothalamus play an important role in regulating feeding behaviors and BW (Meister, 2007), which indicates that a decrease in 5-HT levels in the hypothalamus may contribute to dysregulating feed intake and BW gain in turkeys. It cannot be excluded that the noted decrease in plasma 5-HT levels in turkeys that received DOX
during the first few days post hatch could be associated with lower BW gain at 5 to 8 wk of age. There is evidence to suggest that metabolites produced by intestinal bacteria (e.g., SCFAs, neurotransmitters and their precursors), particularly in early life stages, can affect the levels of related metabolites in the brain via the bloodstream, thus regulating brain and cognitive functions as well as neuroendocrine responses to stress (Dinan and Cryan, 2012; Cox and Weiner, 2018; Caspani et al., 2019; Cryan et al., 2020). The increased plasma cortisol levels, observed in this experiment in turkeys administered DOX during early life, could result from the above relationships. According to Farzi et al. (2018), antibiotic-induced dysbiosis of the gut microbiota increases serum corticosterone levels.

This study confirms previous observations that the early short-term administration of antibiotics or MON in the recommended doses does not compromise the growth performance of birds (Watkins et al., 1993; Watkins and Novilla, 1994; Chapman and Saleh, 1999; Sureshkumar et al., 2013). However, Madubuike et al. (2020) reported that DOX administered in early life to vaccinated chickens decreased their BW gain. Several anticoccidials decreased the BW of 8-wk-old turkeys that were not exposed to a coccidial challenge (Cabel and Waldroup, 1991). However, after removal of the anticoccidials, compensatory gains were observed in almost every instance at market age. The results of other studies revealed a beneficial effect of MON on BW gain and feed conversion compared with infected untreated broilers and turkeys (Cabel et al., 1991; Logan et al., 1993; Varga et al., 1994; McDougald et al., 1996; Sims and Hooge, 2002; Chapman et al., 2004).

Part of the differential effects of MON versus DOX on the growth performance of birds may be related to the different modes of action of these 2 disease management programs. Monensin is a carboxylic polyether ionophore used to control coccidiosis in poultry, which interferes with ion transport across the cell membrane of sporozoites (Mollenhauer et al., 1990). Doxycycline (a tetracycline antibiotic) and ENR (a fluoroquinolone antibiotic) are agents with antibacterial activity against both Gram-positive and Gram-negative bacteria (Gbylik-Sikorska et al., 2016; Tronchon and Lefebvre, 2016; Gutiérrez et al., 2017). Their mechanism of action is based on the inhibition of bacterial protein synthesis by binding DNA and RNA.

### CONCLUSIONS

This study demonstrated that the early administration (from 1 to 5 d of age) of antibiotics ENR (10 mg/kg BW) or DOX (50 mg/kg BW) to turkeys resulted in a decrease in the extracellular activity of selected microbial enzymes in the cecal digesta of birds aged 56 d. The opposite results, comparable with those observed in untreated CON birds, were noted in MON-treated birds (90 mg/kg BW) despite the fact that the coccidiostat was administered during the entire feeding period (1–56 d). Interestingly, the above changes in the enzyme activity of microbiota did not affect cecal SCFA concentrations. It appears that the dietary antibiotic treatment, even when provided over a short period of time during the first days of turkeys’ life, considerably altered cecal microbial enzyme activity in subsequent weeks after the completion of the treatment. A diet containing MON and the early administration of ENR or DOX induced an increase in blood noradrenaline (catecholamine neurotransmitter) levels in turkeys. Early DOX use increased plasma cortisol concentrations and decreased plasma serotonin levels, pointing to stress induction in young birds, most likely due to changes in the functions of the large intestine.

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### DISCLOSURES

D. Mikulski, J. Juśkiewicz, K. Ognik, P. Zdunićzyk, R. Smagiel, and J. Jankowski declare no financial and personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.
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