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THE MICROBIOLOGICAL AND BIOCHEMICAL PARAMETERS OF MANURE FROM ANTIBIOTIC-TREATED TURKEYS*

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Abstract

The microbiological and biochemical parameters of manure should be considered in order to develop comprehensive and effective strategies for the management of manure from farmed antibiotic-treated turkeys in accordance with environmentally sustainable practices, taking into account social and economic aspects. Hence, this study was conducted on manure samples from Hybrid Converter turkeys raised to 16 weeks of age. The birds were housed in four pens (with an area of 4 m^2 each) on litter (straw pellets), at 16 turkeys per pen. Turkeys were divided into four groups: control (C) group; group M – administered monensin (M) with feed; group ME – administered M with feed and enrofloxacin (E) with drinking water; group MED – administered M and E as in group ME and doxycycline (D) with drinking water. Turkeys were raised for 112 days, and then the content of nitrogen, phosphorus, potassium, calcium, and antibiotics was determined in manure samples. The responses of culturable and non-culturable microorganisms, and seven enzymes to the antibiotics contained in manure were tested. One kilogram of manure (fresh weight) contained 4.49–5.27 mg of M, 5.11–7.83 mg of E, and 3.39 mg of D. Antibiotics exerted a highly negative effect on culturable microorganisms. In group MED, the counts of organotrophic bacteria decreased by 40%, Actinobacteria counts decreased by 89%, and fungal counts decreased by 85%. Antibiotics also modified the structure of microbial communities in turkey manure. Bacteria of the phylum Firmicutes and fungi of the phylum Mucoromycota were most susceptible to the analyzed antimicrobials. Antibiotics modified the number of amplicon sequence variants (ASVs) of bacterial families and genera and the number of operational taxonomic units (OTUs) of fungi. The examined antimicrobials decreased the diversity of non-culturable bacteria, but did not induce major changes in fungal diversity. Antibiotics clearly inhibited the activity of enzymes, in particular dehydrogenases and urease. Dehydrogenases activity was reduced by 47%, and urease activity by 72%.

Key words: organic fertilizers, monensin, enrofloxacin, doxycycline, microbial community, enzymes

Antimicrobial agents are widely applied in human and veterinary medicine for therapeutic, preventive, metaphylactic, and growth-promoting purposes (Kumar and Pal, 2018). The use of antibiotic growth promoters in animal feed has been prohibited in the European Union (EU) in 2006 and in the USA in 2017, but these compounds are still permitted in Brazil and China (Roth et al., 2019).

Enrofloxacin and doxycycline are broad-spectrum antimicrobials that are commonly used in the treatment of bacterial infections (Grabowski et al., 2022). In turkeys, early short-term administration of enrofloxacin and doxycycline exerts strong modulatory effects (both desirable and undesirable) on the composition of the gut microbiome and the activity of bacterial enzymes in the cecum (Mikulski et al., 2022, 2024). Ionophore coccidiostats such as monensin are classified as antibiotics and added to feed to prevent coccidiosis, a prevalent parasitic disease of poultry (Abdelhady et al., 2021).

The use of antibiotics in veterinary medicine has increasingly stirred controversy due to their potentially adverse effects on the immunity and antioxidant status of birds, as well as their contribution to the emergence of drug-resistant pathogens (Guillouzo and Guguen-Guillouzo, 2020). Increasing bacterial resistance to antimicrobials used to treat and prevent disease in animals has been recognized as one of the world's greatest public health concerns (WHO, 2016). Antibiotic residues are

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strictly controlled in foods of animal origin, but their presence in excreta and their potential impact on the soil environment remain insufficiently investigated.

Manure is a rich source of nutrients for microorganisms. In addition to bacteria of the genera *Enterococcus*, *Bacillus*, and *Clostridium*, manure is also colonized by *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter* spp., and *Yersinia enterocolitica* (Pachepsky et al., 2006). Their survival in manure is estimated to be up to several months. Therefore, the use of antibiotics during livestock production should be considered (Manyi-Loh et al., 2016). The transformation of antibiotics, mainly their quantification and degradation, is significantly affected by the variability of the manure matrix (Deng et al., 2024). Active drug metabolites such as epimers and isomers can also be excreted with urine and feces. These metabolites are strongly adsorbed in manure by forming complexes with humic acids, proteins, and organic matter. Stable metabolites of tetracyclines include 4-epi-oxytetracycline (EOTC), a product of oxytetracycline (OTC) degradation, and 4-epi-chlortetracycline (4-ETCT), an epimer of chlortetracycline (CTC) (Li et al., 2019).

The use of antibiotics in the prevention and treatment of animal diseases is closely linked to the presence of antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARG), and mobile genetic elements (MGE), including plasmids, integrative and conjugative elements, transposons and closely related integrons, in manure (Cabezón et al., 2014; Gillings et al., 2014). Genetic recombination, mainly mutations and transfer of ARGs between bacterial cells, promotes the acquisition of resistance in microorganisms. Genes encoding resistance to specific antibiotics can be quantified by real-time and high-throughput quantitative PCR (RT-qPCR and HT-qPCR, respectively) (Ouyang et al., 2024). Bacterial cross-resistance, collateral sensitivity, and multidrug resistance conditioned by de novo mutations have also been determined in manure (Perron et al., 2012; Santos-Lopez et al., 2019). Plasmids are thought to have the highest potential for genetic recombination in cells. In turn, ARGs located on chromosomes are mainly responsible for horizontal gene transfer (HGT) (Męcik et al., 2023).

Manure contains numerous and diverse ARGs (Qian et al., 2018), and *tet* genes responsible for ribosomal protection and enzymatic modification of tetracyclines, including doxycycline, are most commonly identified (Guo et al., 2018; Zhu et al., 2013). This group of genes, mainly *tetG*, *tetA*, *tetC*, and *tetX*, is located on plasmids and is transferred to other bacteria in manure by transposons, which is why their presence is closely linked to the transfer of genetic material (Leclercq et al., 2016; Męcik et al., 2023). Enrofloxacin, classified as a quinolone, induces an increase in the abundance of genes such as *qnrS*, *qnrA*, *aac(6')-Ib-cr*, *gyrA*, and *gnrB* (Seo and Lee, 2021). Resistance to fluoroquinolones is encoded by mutations in genes encoding topoisomerases, nuclear enzymes that play an important role in DNA replication (Jacoby et al., 2014). Monensin binds to bacterial cell membranes, disrupts the flow of sodium and potassium ions across the membrane, and modifies the electrochemical potential of sodium, thus depriving bacterial cells of energy (Łowicki and Huczyński, 2013; Simjee et al., 2012). It should be noted that the residual concentrations of monensin in poultry manure also increase the conjugation frequency of the InCFII-FIB plasmid containing genes conferring tetracycline resistance (Saraiva et al., 2022).

The group of tetracycline-resistant bacteria includes *Enterococcus faecalis* and *Clostridium perfringens* (Simjee et al., 2012), whereas the phyla Firmicutes, Bacteroidota, and Proteobacteria predominated in post-treatment samples following enrofloxacin injection (Beyi et al., 2021). The microbiome of animal manure has been researched more extensively than the enzyme activity in manure. Most of the studies conducted to date have focused on determining the sensitivity of enzymes involved in the conversion of carbon (dehydrogenases, catalase, and β-glucosidase), nitrogen (urease), and phosphorus (acid phosphatase and alkaline phosphatase) in soils fertilized with manure (Wyszkowska et al., 2022; Santás-Miguel et al., 2021). However, despite the fact that these enzymes are reliable indicators of disruptions in ecosystem homeostasis and microbial distribution and diversity (Wyszkowska et al., 2024), the biochemical parameters of manure have not yet been thoroughly analyzed. An important source of soil enzymes, in addition to microorganisms and, more specifically, the lysis products of their cells, are complexes formed with tannins or clay particles. Adsorption and copolymerization processes are responsible for the stability of these structures (Wallenstein and Burns, 2011; Menezes-Blackburn et al., 2018).

Manure containing antibiotic residues and ARB provides a pathway for their transfer to soil and water, increasing the risk of re-transmission of these xenobiotics to both animals and humans (Manyi-Loh et al., 2018). Statistical data suggest that by 2050, approximately 10 million people will die each year as a consequence of antibiotic resistance (Church and McKillip, 2021). This estimate is based on the identification of a wide range of sources of these contaminants in the environment, the most important of which are wastewater treatment plants, which collect antibiotics from the pharmaceutical industry, households, and hospitals. Antibiotics also enter soils with sewage sludge and organic fertilizers, including animal manure (Aladekoyi et al., 2024, Zhang et al., 2021). Exposure to these xenobiotics reduces the diversity of the microbial community (Iwu et al., 2020). The activity of soil enzymes is also inhibited, which slows down the decomposition of organic matter and ultimately leads to reduced crop productivity due to nutrient deficiencies (Wei et al., 2009). A better understanding of enzyme activity in manure (which is the novelty of the present research) and the structural diversity of the manure microbiome may deepen our knowledge of the key role played by this ecosystem in shaping soil biodiversity and maintaining its condition. The combined effect of two or more antibi-

otics on the microbiome of poultry manure and the agroecosystem has not been described in the literature. The combined impact of several antibiotics can differ from the effects exerted by each individual antibiotic. This is an important consideration because prolonged administration of enrofloxacin or doxycycline to birds can lead to the accumulation of these antibiotics in bodily tissues (Gbylik-Sikorska et al., 2016).

The aim of this study was to verify the research hypothesis postulating that the use of monensin, enrofloxacin, and doxycycline during turkey fattening can affect the microbiome and enzymatic activity of manure.

Material and methods

Turkey rearing and manure sampling

Manure was obtained from Hybrid Converter turkeys raised until 16 weeks of age in the laboratory of the Department of Poultry Science and Apiculture of the University of Warmia and Mazury in Olsztyn. A total of 64 one-day-old female poults were randomly divided into four equal groups (C, M, ME, and MED). Turkeys were housed in four pens (with an area of 4 m^2 each) on litter (straw pellets), at 16 birds per pen (group). Turkeys were reared according to the guidelines published by Hybrid Turkeys (2020) and the ethical and welfare standards set forth by Directive 2010/63/EU. The microclimate in the poultry house was controlled automatically, and the birds had *ad libitum* access to feed and water.

Group M birds were administered the coccidiostat monensin (M) (Coxydin 200, Huvepharma Polska, Warsaw, Poland) at 90 mg kg^{-1} feed until 84 days of age. Group ME birds received monensin as in group M and enrofloxacin (E) (Enrofloxacin 10%, Biowet, Drwalew, Poland) with drinking water at 10 mg kg^{-1} BW for 7 days (from 80 days of age). Group MED turkeys were administered M and E as in group ME and doxycycline (D) (Doxylin CT WSP 433 mg g^{-1}, Dopharma Research BV, Raamsdonksveer, Netherlands) with drinking water at 25 mg kg^{-1} BW for 5 days (from 89 days of age). The doses and duration of antibiotic administration were in accordance with the manufacturers' recommendations for prophylactic (M) or therapeutic (E and D) use. Control (C) group turkeys were not administered any antibiotics.

Turkeys were raised for 112 days to ensure compliance with the withdrawal periods for enrofloxacin (13 days) and doxycycline (12 days). At the end of rearing, feathers were removed from the surface of manure, the manure removed from each pen was weighed and pooled, and samples were collected for chemical, microbiological, and biochemical analyses.

Chemical analyses

The total content of phosphorus, potassium, and calcium in manure samples was determined by inductively coupled plasma atomic emission spectroscopy (ICP-OES) using an Avio 200 spectrometer (Perkin Elmer, USA). Manure samples were dried in a Titan MPS microwave preparation system (Perkin Elmer, USA) before analysis.

The presence of doxycycline and enrofloxacin in manure samples was determined by ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS). Liquid chromatography was performed using the Shimadzu Nexera X2 UHPLC system (Shimadzu, Japan). The mass spectrometry analysis was performed using the SCIEX QTRAP 4500 triple quadrupole mass spectrometer (SCIEX, USA) with Analyst 1.6.3 software (SCIEX, USA). Briefly, antimicrobial substances were extracted using acetonitrile with McIlvaine buffer and versenic acid. The extracts were centrifuged, and the supernatant was filtered using OASIS HLB columns. The eluate was evaporated to dryness in a stream of nitrogen. After centrifugation and filtering with PVDF syringe filters, the dissolved residue was used in chromatographic analysis. Detailed information on the analytical method for the quantitative determination of doxycycline and enrofloxacin in manure is presented in the Supplementary Materials. The UHPLC-MS/MS method was validated for linearity, precision (repeatability and within-laboratory reproducibility), and recovery. The validation parameters were calculated according to Commission Regulation (EU) 2021/808 (European Commission, 2021). The limit of quantification (LOQ) was established in accordance with the European Medicine Agency Guideline Q2 (EMA ICH Guideline Q2, 2022). The matrix-matched calibration curve for linearity was determined within a range of 5–500 μg kg^{-1} for doxycycline and 50–500 μg kg^{-1} for enrofloxacin. In the linear correlation analysis, the correlation coefficient (R^2) reached 0.997 for doxycycline and 0.995 for enrofloxacin and showed a sufficient linear response. The relative standard deviation for repeatability was 9.2% for doxycycline and 8.6% for enrofloxacin. Reproducibility was established at 12.8% for doxycycline and 11.6% for enrofloxacin. The obtained values met the recovery requirements at 102% for doxycycline and 99.8% for enrofloxacin. The LOQ was the lowest point on the matrix-matched calibration curve at 5 μg kg^{-1} for doxycycline and 50 μg kg^{-1} for enrofloxacin.

The presence of monensin in manure samples was determined by UHPLC-MS/MS. Liquid chromatography was performed using the Shimadzu Nexera X2 UHPLC system (Shimadzu, Japan). The mass spectrometry analysis was performed using a Shimadzu LCMS-8050 triple quadrupole mass spectrometer (Shimadzu, Japan) with LabSolution software 5.60 SP2 (Shimadzu, Japan). Manure samples for the monensin analysis were extracted with a solution of ammonium in methanol, followed by acetic acid in methanol. Detailed information on the analytical method for the quantitative determination of monensin is presented in the Supplementary Materials. The UHPLC-MS/MS method was validated for linearity, precision (repeatability and within-laboratory reproducibility), and recovery. The validation parameters were

calculated according to Commission Regulation (EU) 2021/808 (European Commission, 2021). The matrix-matched calibration curve for linearity was determined in the range of 0.25–12.5 mg kg^{-1} with a correlation coefficient (R^2) of 0.995. Repeatability standard deviation was 10.2%, and reproducibility standard deviation was 14.6%. The calculated recovery rate was 96.7%. The LOQ was the lowest point on the calibration curve at 0.25 mg kg^{-1}.

Microbiological analyses

Culturable microorganisms were enumerated by serial dilutions, and the counts of non-culturable microorganisms were determined by next-generation sequencing (NGS). Genomic DNA was isolated with the MagnifiQ™ 1 Genomic DNA instant kit (A&A Biotechnology, Gdańsk, Poland). Bacteria were identified by sequencing the V3–V4 hypervariable region of the rRNA gene, and fungi were identified by sequencing the ITS1 region using an Illumina MiSeq system and a MiSeq Reagent Kit v3 (Genomed S.A., Warsaw, Poland). The microbial sequences were submitted to the National Center for Biotechnology Information under GenBank accession numbers: prokaryotic 16S rRNA sequences (https://www.ncbi.nlm.nih.gov/nuccore/?term=PQ777480:PQ778041[accn]; accessed on 23 December 2024) and eukaryotic nuclear rRNA/ITS sequences (https://www.ncbi.nlm.nih.gov/nuccore/?term=PQ838995:PQ839107[accn]; accessed on 11 January 2025). Detailed information about the sequencing process and microbiological analyses is provided in the Supplementary Materials.

Biochemical analyses

The activity of seven enzymes, including oxidoreductases (dehydrogenases, catalase) and hydrolases (β-glucosidase, acid phosphatase, alkaline phosphatase, urease, arylsulfatase), was determined in biochemical analyses using a Perkin-Elmer Lambda 25 spectrophotometer (Waltham, MA, USA). The enzyme identification procedure is described in detail in the Supplementary Materials.

Statistical analysis

The results were processed statistically in Statistica 13.3 software (TIBCO, 2017), STAMP 2.1.3 (Parks et al., 2014), RStudio v1.2.5033 (RStudio Team, 2019) with a gplots library (Warnes et al., 2020), and R core (R Core Team, 2019). Homogeneous groups were identified based on the results of Tukey's HSD test. Amplicon sequence variant (ASV) and ITS reads comprising less than 1% of the total observed reads were removed from the dataset. The obtained data were subjected to principal component analysis (PCA). The results were presented as arithmetic means ± standard deviation (SD).

Results

Content of macronutrients and antibiotics in manure

The results of the proximate analysis and the content of antibiotic residues in manure are presented in Tables 1 and 2, respectively. The chemical composition of manure was highly similar in all treatments. The concentration of M in manure samples ranged from 4.49 mg kg^{-1} to 5.27 mg kg^{-1} and accounted for 7.38% of the ingested dose on average. The concentration of E was 7.83 mg kg^{-1} in ME and 5.11 mg kg^{-1} in group MED and accounted for 25.2% and 15.8% of the ingested dose, respectively. The concentration of D in group MED was 3.39 mg kg^{-1} and accounted for 6.5% of the ingested dose.

Table 1. Proximate composition of turkey manure in treatments (% FW)

Treatment	Dry matter	Nitrogen	Phosphorus	Potassium	Calcium
C	44.52±0.32 b	2.47±0.08 b	0.52±0.05 b	1.56±0.19	0.83±0.11 b
M	46.32±0.21 a	2.61±0.08 b	0.63±0.03 a	1.68±0.23	0.99±0.06 a
ME	41.45±0.20 c	2.55±0.03 b	0.60±0.04 a	1.81±0.03	1.00±0.06 a
MED	44.63±0.29 b	2.93±0.03 a	0.62±0.01 a	1.75±0.01	0.96±0.01 ab

C – control; M – monensin; E – enrofloxacin; D – doxycycline; values marked with the same letters (a–c) within each component form homogeneous groups; $P<0.050$; N = 3 for each standard deviation; N = 12 for each analyzed parameter.

Table 2. Antibiotic residues in turkey manure

Treatment	Antibiotic concentration, mg kg $^{-1}$ FW			Excretion rate, % [1]		
	M*	E*	D*	M*	E*	D*
M	4.67	nd [2]	nd	6.72	nd	nd
ME	4.49	7.83	nd	7.23	25.24	nd
MED	5.27	5.11	3.39	8.17	15.81	6.53

M – manure from turkeys administered monensin; ME – manure from turkeys administered monensin and enrofloxacin; MED – manure from turkeys administered monensin, enrofloxacin, and doxycycline; M* – monensin; E* – enrofloxacin; D* – doxycycline;

[1] ratio of excreted antibiotic to ingested dose × 100; [2] nd – not detected < LOQ .

Table 3. Counts (CFU kg^{-1}), colony development index (CD), and ecophysiological diversity index (EP) of microorganisms in manure

Microorganisms*	Unit	Treatment			
		C	M	ME	MED
Org	10^{12} CFU kg^{-1}	11.44±0.69 a	9.52±0.25 b	9.65±0.36 b	6.82±0.48 c
	CD	34.25±1.18 c	35.99±1.38 c	39.82±0.24 b	42.83±1.68 a
	EP	0.46±0.07 bc	0.52±0.03 b	0.41±0.02 c	0.70±0.04 a
Act	10^{12} CFU kg^{-1}	19.71±1.28 a	3.24±0.11 b	2.85±0.18 b	2.14±0.12 b
	CD	17.70±1.24 b	26.49±2.25 a	24.75±1.35 a	20.35±1.06 b
	EP	0.88±0.06 a	0.75±0.03 b	0.65±0.03 c	0.69±0.06 bc
Fun	10^{9} CFU kg^{-1}	37.67±1.64 a	18.74±0.73 b	16.56±0.77 c	5.50±0.37 d
	CD	35.76±2.03 a	29.77±1.42 b	30.62±1.57 b	30.80±2.50 b
	EP	0.75±0.03 ab	0.72±0.04 b	0.84±0.03 a	0.71±0.07 b

*Org – organotrophic bacteria; Act – Actinobacteria; Fun – fungi; C – control; M – monensin; E – enrofloxacin; D – doxycycline; values marked with the same letters (a–d) form homogeneous groups; $P<0.050$; N = 4 for each standard deviation; N = 16 for each analyzed parameter.

Responses of culturable microorganisms in manure to the antibiotics administered to turkeys

The counts of culturable microorganisms were significantly lower in the manure of turkeys receiving antibiotics (Table 3). Monensin decreased the counts of organotrophic bacteria by 17%, decreased Actinobacteria counts by 84%, and decreased fungal counts by 50%.

The combination of M and E induced a 26% decrease in the counts of organotrophic bacteria, an 86% decrease in Actinobacteria counts, and a 56% decrease in fungal counts. These results and a comparison of the effects of M and E indicate that M was mainly responsible for the reduction in microbial abundance. The greatest decrease in microbial abundance was noted in response to the additional application of D. In samples containing MED, the counts of organotrophic bacteria decreased by 40%, Actinobacteria counts decreased by 89%, and fungal counts decreased by 85%. Therefore, Actinobacteria and fungi were more susceptible to the tested antimicrobials than organotrophic bacteria.

Microbial responses to antibiotic residues were measured based on the rate of microbial proliferation with the use of the modified colony development (CD) index and the ecophysiological diversity (EP) index. In organotrophic bacteria, the number of fast-growing cells increased under the influence of ME and MED. The application of MED also contributed to an increase in ecological diversity. Antibiotics modified the structure of Actinobacteria in manure by promoting the development of fast-growing cells in place of slow-growing cells, and decreased the ecophysiological diversity of Actinobacteria. In contrast, antibiotics contributed to the proliferation of slow-growing fungi, and the ecophysiological diversity of fungi remained relatively stable.

Responses of non-culturable bacteria in manure to the antibiotics administered to turkeys

Turkey manure was colonized mainly by bacteria of the phylum Firmicutes (Figure 1) whose relative abundance ranged from 42.30% in treatment ME to 57.49% in the control (C) treatment. Actinobacteriota was the second most prevalent phylum with abundance ranging from 27.69% (C) to 39.70% (MED), followed by the phylum Proteobacteria with abundance ranging from 6.83% (MED) to 13.29% (M) and the phylum Bacteroidota with abundance ranging from 1.52% (MED) to 11.72% (M). Monensin decreased the population size of Firmicutes by 3.72%, decreased the population size of Actinobacteriota by 6.54%, increased the population size of Proteobacteria by 5.41%, and increased the population size of Bacteroidota by 4.85%. In contrast, in manure obtained from turkeys administered ME, a 15.19% reduction was observed in the abundance of the phylum Firmicutes and an increase in the abundance of the other three phyla, ranging from 3.05% (Proteobacteria) to 8.13% (Actinobacteriota). The additive effect of all three antibiotics (MED) was least beneficial for manure bacteria: the counts of Actinobacteriota increased by 12.01%, but the relative abundance of the other phyla decreased by 1.06% (Proteobacteria) to 5.62% (Firmicutes).

Antibiotics modified the number of ASVs associated with bacterial families (Figure 2). M, ME and MED decreased the number of ASVs affiliated with the families *Peptostreptococcales-Tissierellales*, *Lactobacillaceae*, *Dermabacteriaceae*, and *Micrococcaceae*, and increased the number of ASVs affiliated with the families *Bacillaceae*, *Staphylococcaceae*, *Aerococcaceae*, and *Aeromonadaceae*. All of the tested antibiotics completely eradicated bacteria of the families *Xandomonadaceae* and *Streptococcaceae*. ME and MED had a positive effect on bacteria of the family *Corynebacteriaceae*, and a negative effect on bacteria of the family *Sphingobacteriaceae*. *Corynebacteriaceae* was the dominant bacterial family in turkey manure. Bacteria of the families *Carnobacteriaceae*, *Peptostreptococcales-Tissierellales*, and *Bacillaceae* were also abundant.

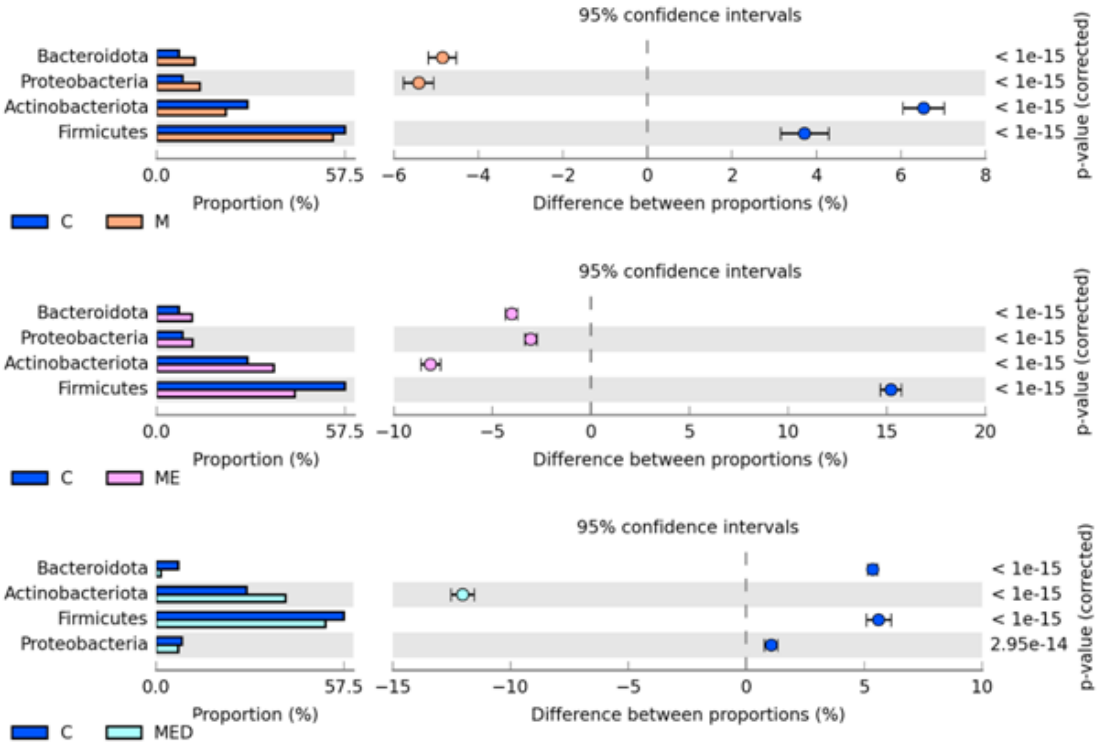


Figure 1. Relative abundance of bacterial phyla (ASV≥1%) in turkey manure.
C – manure from turkeys that were not administered antibiotics; M – manure from turkeys administered monensin; ME – manure from turkeys administered monensin and enrofloxacin; MED – manure from turkeys administered monensin, enrofloxacin, and doxycycline

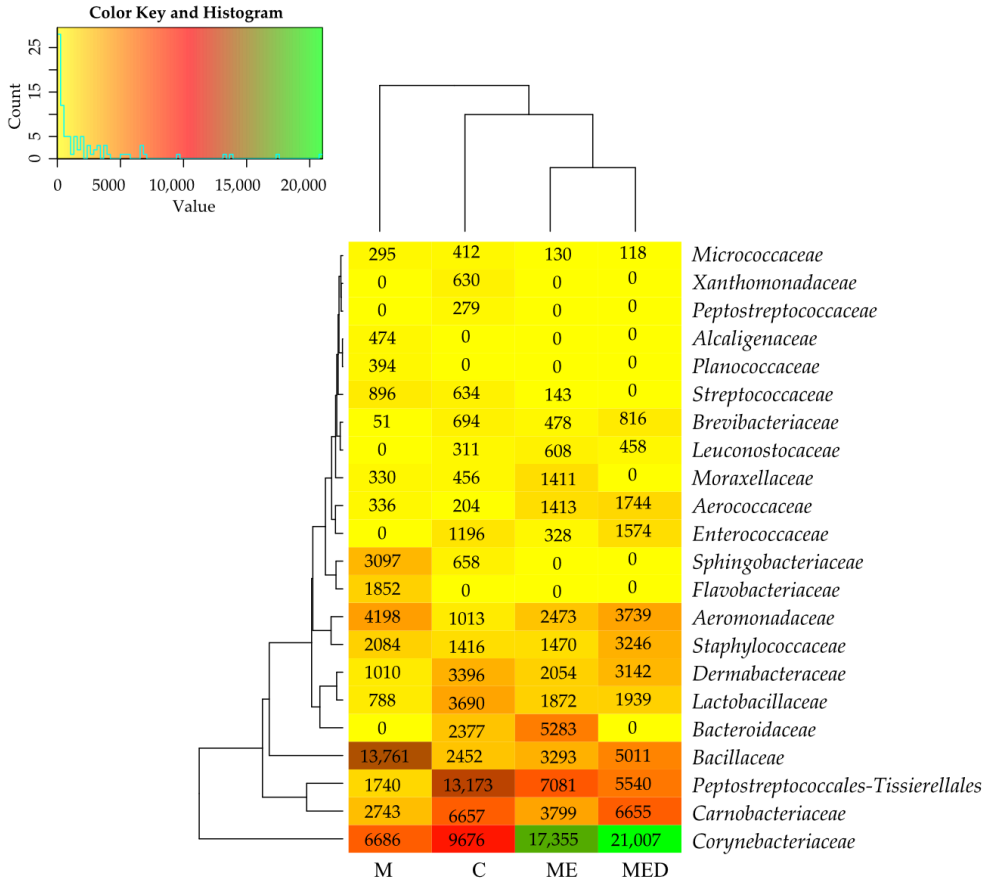


Figure 2. Number of amplicon sequence variants (ASVs) associated with bacterial families in turkey manure (ASV≥1%). C – manure from turkeys that were not administered antibiotics; M – manure from turkeys administered monensin; ME – manure from turkeys administered monensin and enrofloxacin; MED – manure from turkeys administered monensin, enrofloxacin, and doxycycline

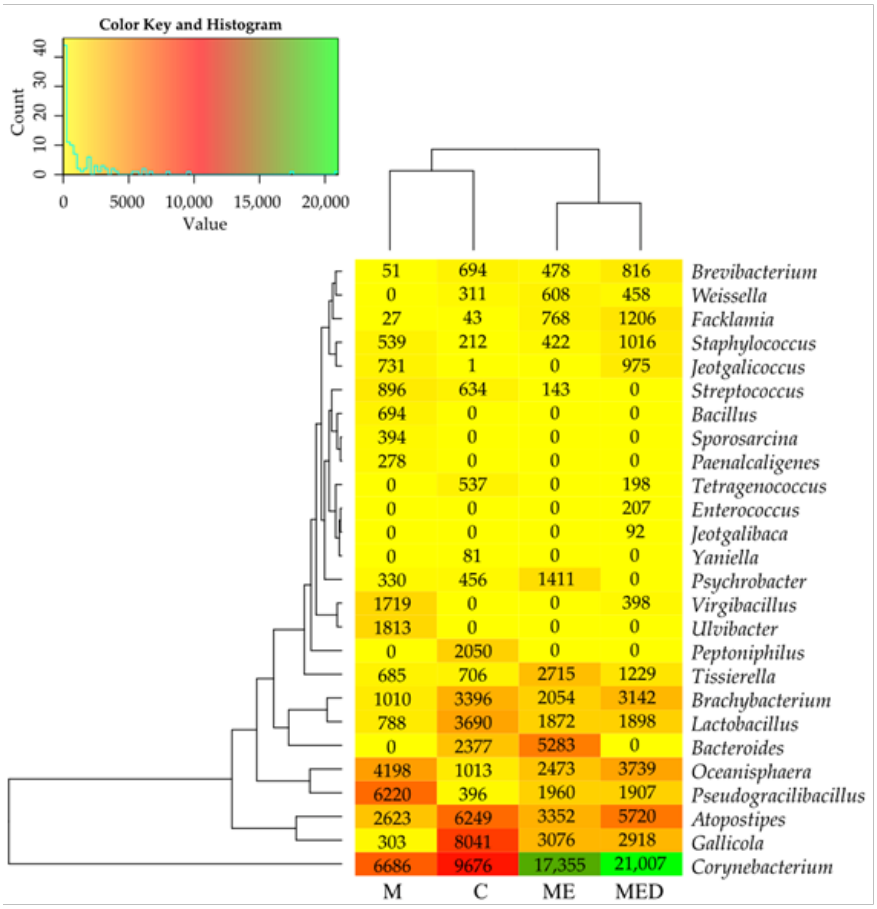


Figure 3. Number of amplicon sequence variants (ASVs) associated with bacterial genera in turkey manure (ASV≥1%). C – manure from turkeys that were not administered antibiotics; M – manure from turkeys administered monensin; ME – manure from turkeys administered monensin and enrofloxacin; MED – manure from turkeys administered monensin, enrofloxacin, and doxycycline

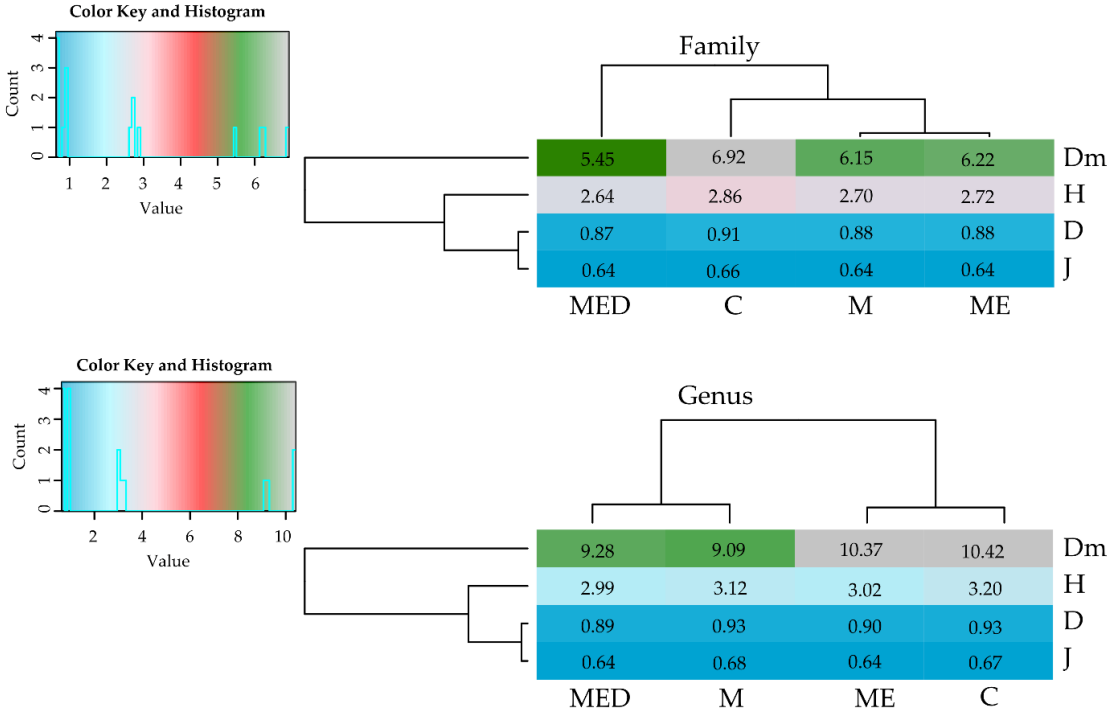


Figure 4. Diversity of bacterial families and genera in turkey manure (ASV≥1%). C – manure from turkeys that were not administered antibiotics; M – manure from turkeys administered monensin; ME – manure from turkeys administered monensin and enrofloxacin; MED – manure from turkeys administered monensin, enrofloxacin, and doxycycline; Dm – Margalef's diversity index; H – Shannon diversity index; D – Simpson's diversity index; J – Pielou's evenness index

The use of antibiotics in turkey farming has contributed to changes in the composition of bacterial genera in manure (Figure 3). The application of M resulted in the emergence of unique bacteria of the genera *Ulvibacter*, *Paenalcaligenes*, *Bacillus*, and *Sporosarcina*, while the application of MED led to the emergence of two unique bacterial genera (*Jeotgalibaca* and *Enterococcus*). None of these bacterial genera were detected after the combined application of M and E. In the control sample (C), the unique genera were *Peptoniphilus* and *Yaniella*, whereas the following genera were present in all treatments: *Corynebacterium*, *Gallicola*, *Atopostipes*, *Lactobacillus*, *Brachybacterium*, *Oceanisphaera*, *Tissierella*, *Brevibacterium*, *Pseudogracilibacillus*, *Staphylococcus*, and *Facklamia*. The highest number of sequences was assigned to the first seven genera, and *Corynobacterium* was the dominant bacterial genus. Bacteria of the genera *Peptoniphilus* and *Yaniella* were eliminated by all antibiotics in all tested combinations.

The examined antibiotics exerted a negative influence on the bacterial community in turkey manure, which was reflected in the values of diversity indices. The values of the Shannon-Wiener's diversity index (H), Simpson's diversity index (D) and Margalef's richness index (Dm) were lower in manure samples containing M, ME, and MED than in the control samples (C) at both family and genus levels (Figure 4). In turn, Pielou's evenness index (J) remained fairly stable, ranging from 0.66 to 0.64 at the family level and from 0.64 to 0.68 at the genus level.

Responses of non-culturable fungi in manure to the antibiotics administered to turkeys

Turkey manure was colonized mainly by fungi of the phylum Ascomycota, which accounted for 97.8% (ME) to 99.6% (C) of the total fungal population (Figure 5). The percentage of the remaining fungal phyla was very low. The proportion of Basidiomycota ranged from 0.07% (MED) to 1.87% (ME), and the proportion of Mucoromycota – from 0.5% (ME) to 0.52% (MED). Fungi of the phylum Glomeromycota were identified only in samples with ME and represented only 0.25% of the total fungal counts. The relative abundance of Ascomycota tended to decrease in response to all three antibiotics, while exposure to M and ME induced an increase in the abundance of Basidiomycota, and exposure to MED induced an increase in the abundance of Mucoromycota.

Eight fungal families belonging to the phylum Ascomycota were identified in turkey manure (Figure 6). The largest number of ITS sequences was assigned to *Incertae sedis*, *Microascaceae*, and *Aspergillaceae*. These families were detected in all three groups. All of the tested antibiotics completely inhibited the growth of *Saccharomycetaceae*, but stimulated the proliferation of *Erysiphaceae* and *Debartyomycetaceae*. Antibiotics partly inhibited the growth of *Incertae sedis* and *Microascaceae*. Fungi of the family *Mycosphaerellaceae* were identified only in manure samples containing M, whereas *Pleosporaceae* were detected only in samples containing MED.

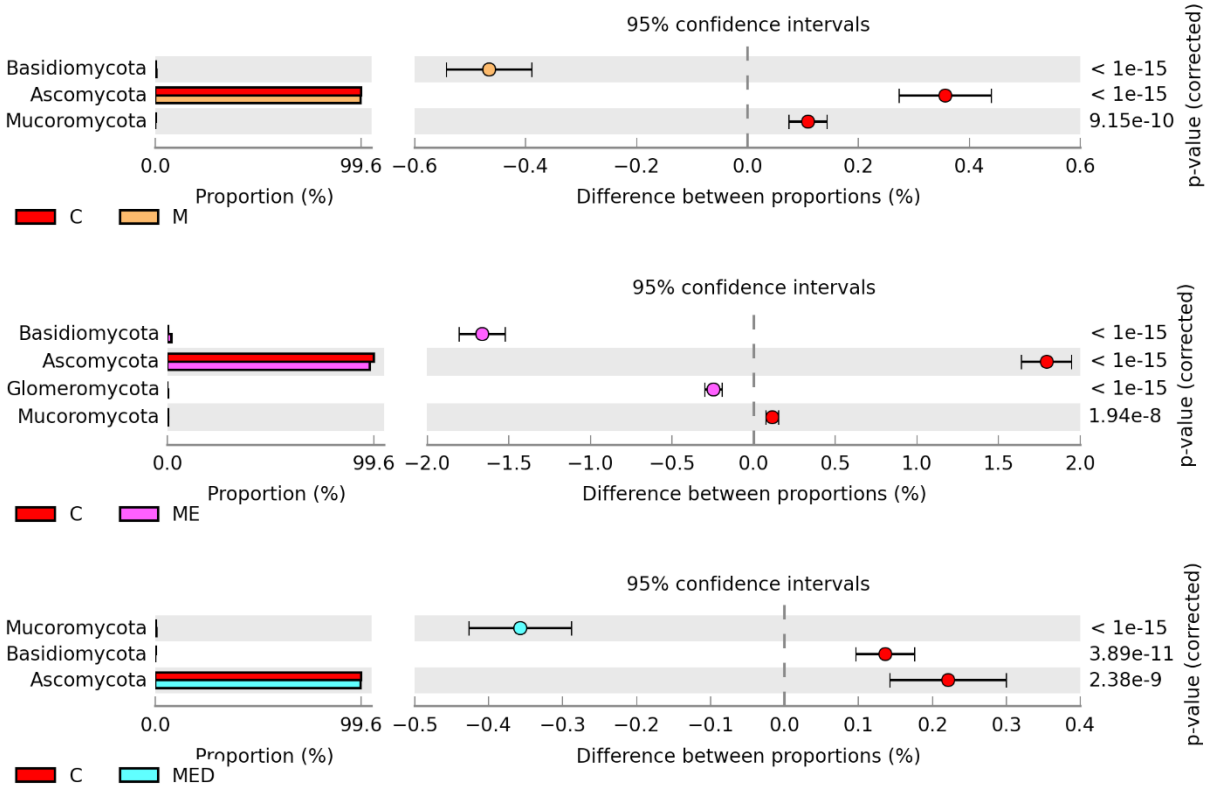


Figure 5. Relative abundance of fungal phyla (ITS≥1%) in turkey manure.
C – manure from turkeys that were not administered antibiotics; M – manure from turkeys administered monensin; ME – manure from turkeys administered monensin and enrofloxacin; MED – manure from turkeys administered monensin, enrofloxacin, and doxycycline

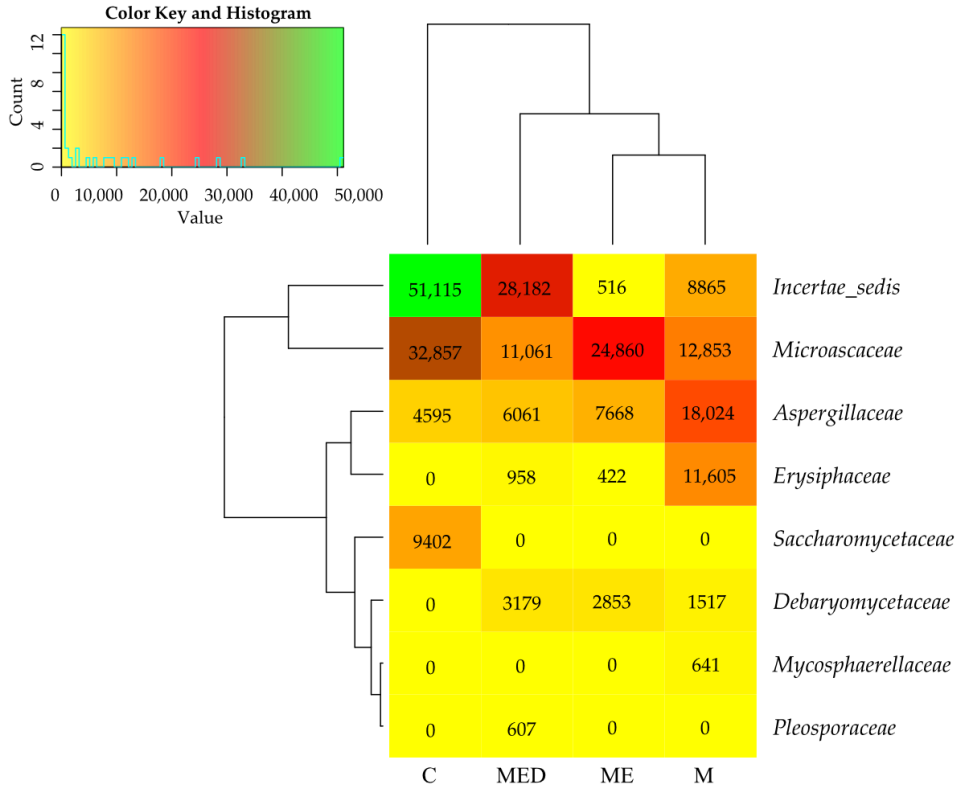


Figure 6. Number of internal transcribed space (ITS) sequences associated with fungal families in turkey manure (ITS≥1.0%).
C – manure from turkeys that were not administered antibiotics; M – manure from turkeys administered monensin; ME – manure from turkeys administered monensin and enrofloxacin; MED – manure from turkeys administered monensin, enrofloxacin, and doxycycline

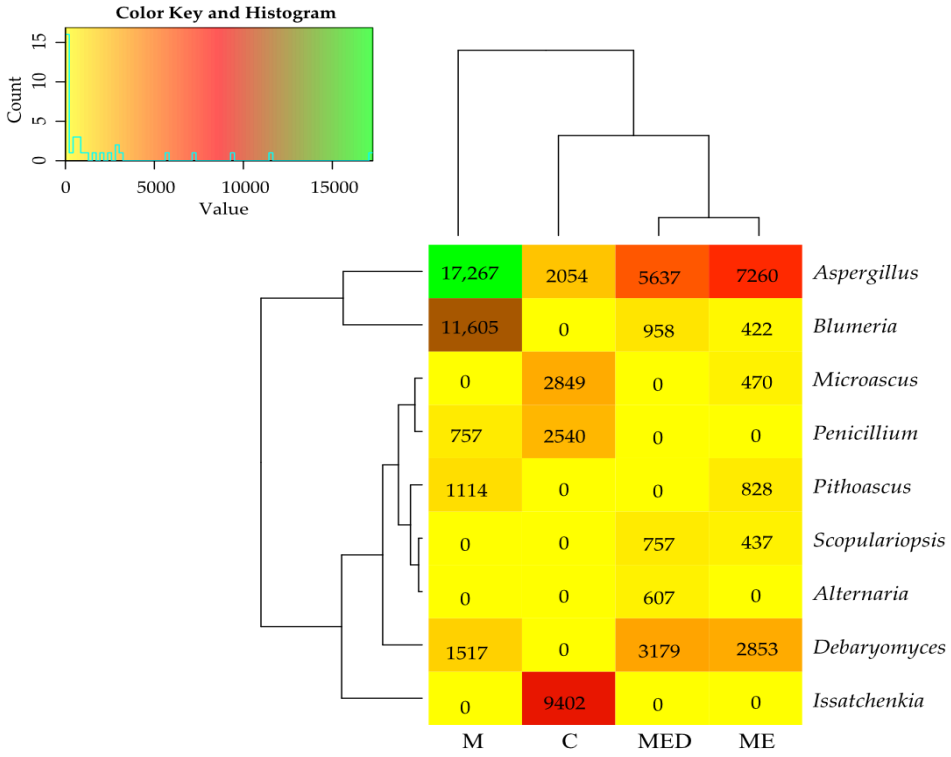


Figure 7. Number of internal transcribed space (ITS) sequences associated with fungal genera in turkey manure (ITS≥1.0%).
C – manure from turkeys that were not administered antibiotics; M – manure from turkeys administered monensin; ME – manure from turkeys administered monensin and enrofloxacin; MED – manure from turkeys administered monensin, enrofloxacin, and doxycycline

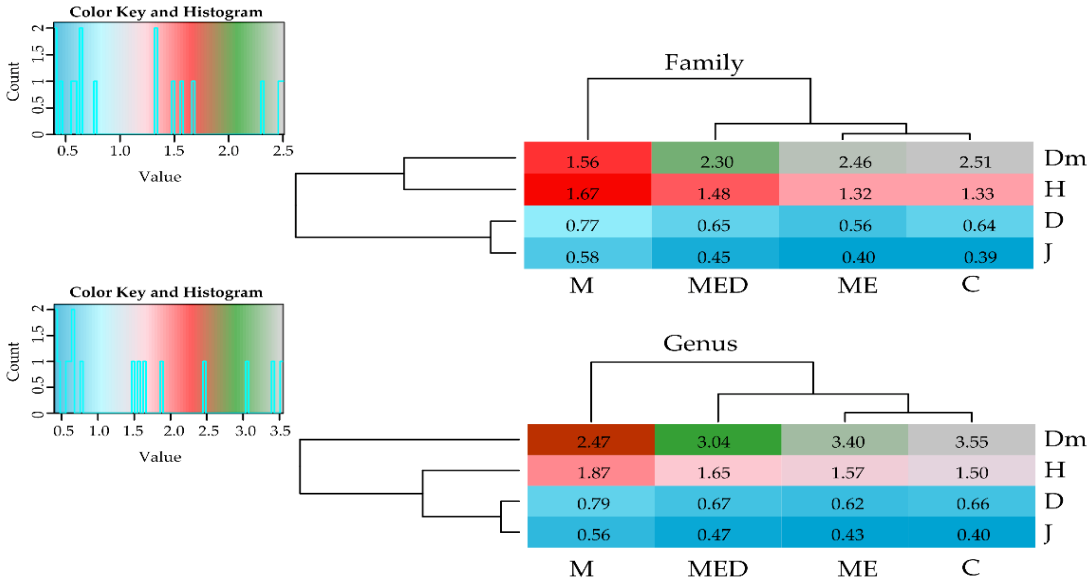


Figure 8. Diversity of fungal families and genera in turkey manure (ITS≥1%). C – manure from turkeys that were not administered antibiotics; M – manure from turkeys administered monensin; ME – manure from turkeys administered monensin and enrofloxacin; MED – manure from turkeys administered monensin, enrofloxacin, and doxycycline; Dm – Margalef's diversity index; H – Shannon diversity index; D – Simpson's diversity index; J – Pielou's evenness index

Table 4. Enzyme activity in 1 kg of manure DM h^{-1}

Enzyme*	Unit	Treatment			
		C	M	ME	MED
Deh	µmol TFF	88.78±2.07 a	52.41±1.00 b	54.49±2.22 b	46.81±1.03 c
Cat	mol O_2	12.80±0.14 b	11.90±0.07 c	13.57±0.01 a	12.86±0.07 b
Ure	mmol N-NH_4	157.06±2.35 b	176.58±2.83 a	72.74±3.79	44.56±3.52 d
AcP	mmol PN	48.89±0.56 a	46.77±0.36 b	47.33±0.28 b	45.60±0.55 c
AlP	mmol PN	153.77±3.0 b	144.40±9.94 c	150.89±4.55 bc	168.77±1.58 a
Aryl	mmol PN	15.01±0.12 a	14.66±0.30 a	13.92±0.17 b	13.97±0.23 b
Glu	mmol PN	37.66±0.38 c	35.50±0.04 d	45.66±0.52 a	42.38±0.57 b

*Deh – dehydrogenases; Cat – catalase; Ure – urease; AcP – acid phosphatase; AlP – alkaline phosphatase; Glu – *β*-glucosidase; Aryl – arylsulfatase; C – control; M – monensin; E – enrofloxacin; D – doxycycline; values marked with the same letters (a–d) within each enzyme form homogeneous groups; $P<0.050$; N = 4 for each standard deviation; and N = 16 for each analyzed parameter.

Nine fungal genera were identified: *Aspergillus* and *Penicillium* of the family *Aspergillaceae*; *Blumeria* of the family *Erysiphaceae*; *Microascus*, *Pithoascus*, and *Scopulariopsis* of the family *Microascacea*; *Alternaria* of the family *Pleosporaceae*; *Debaryomyces* of the family *Debaryomycetaceae*; and *Issatchenkia* of the family *Saccharomycetaceae* (Figure 7). Fungi of the genus *Aspergillus* were dominant in all treatments. The number of ITS sequences associated with the genus *Aspergillus* was highest in manure samples containing M (17,267) and lowest in samples C (2054). Antibiotics decreased the abundance of fungal genera *Microascus*, *Penicillium*, and *Issatchenkia*, and exerted the most inhibitory effect on the genus *Issatchenkia*. All antibiotics stimulated the growth of *Debaryomyces* spp. and *Blumeria* spp. The abundance of *Scopulariopsis* spp. increased in response to ME and MED; *Alternaria* counts increased after the application of MED and *Pithoascus* counts increased after the application of M and ME.

Despite the antibiotic-induced changes in the number of ITS sequences associated with fungal families and genera, the diversity of these fungal taxa remained stable, and a decrease was observed only in the value of Dm. The administration of M and MED resulted in an increase in the values of three indicators (H, D, and J) at the family level and two indicators (H and J) at the genus level (Figure 8). All of the tested antibiotics improved the evenness of fungal development, as indicated by the values of J at both family and genus levels.

Changes in enzyme activity in manure induced by antibiotics administered to turkeys

The analysis of enzyme activity in turkey manure indicates all of the tested antibiotics exerted significant effects (Table 4). However, not all enzymes were affected equally by the examined antimicrobials. Antibiotics exerted a clearly negative effect on dehydrogenases, enzymes that are most closely associated with the presence of live mi-

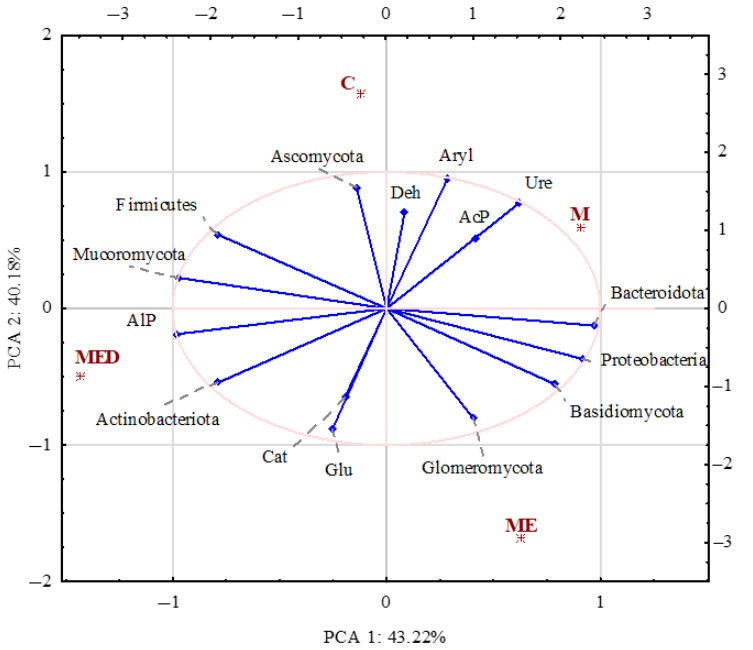
croorganisms. Dehydrogenases activity was inhibited in 41% by M, in 39% by ME, and in 47% by MED. Urease also responded strongly to antibiotics. Urease activity increased by 12% in response to M, but it was inhibited by E and D. The administration of ME and MED inhibited urease activity by 54% and by 72%, respectively. The tested antimicrobials also suppressed the activity of arylsulfatase and acid phosphatase, but to a lesser extent than the activity of dehydrogenases and urease. In turn, *β*-glucosidase activity was positively affected by ME and MED, and alkaline phosphatase activity by MED.

Interactions between the microbiome and enzyme activity in manure

The changes in the manure microbiome at phylum, family, and genus levels (subsection 3.2) were correlated with the biochemical activity of manure (subsection 3.3), as shown in Figures 9, 10, and 11. The data in Figure 9 indicate that the abundance of Ascomycota was positively correlated with dehydrogenases, arylsulfatase, acid phosphatase, and urease activity. The abundance of Glomeromycota was positively correlated with *β*-glucosidase and catalase activity, and the abundance of Actinobacteriota was positively correlated with alkaline phosphatase, *β*-glucosidase, and catalase activity. In turn, Firmicutes and Mucoromycota were bound by a positive correlation with the activity of alkaline phosphatase. In addition, a significant positive correlation was noted between Ascomycota, the dominant fungal phylum, and Firmicutes, the dominant bacterial phylum. The PCA scatterplots in Figures 9–11 indicate that antibiotics significantly influenced the microbiome and enzymatic activity of manure. ME and MED exerted similar effects on microbial populations at the family level (Figure 10) and at the genus level (Figure 11).

An analysis of the dominant sequences of bacteria and fungi at the family level (Figure 11) revealed that *Microascaceae* counts were positively correlated with Deh, Ure, AcP, and Aryl activity and negatively correlated with AlP and Cat activity; *Aspergillaceae* counts were positively correlated with Ure activity and negatively correlated with Cat, Deh, AlP, and Glu activity; *Corynebacteriaceae* counts were positively correlated with Cat, AlP, and Glu activity and negatively correlated with Aryl, Ure, Deh, and AcP activity, whereas *Carnobacteriaceae* counts were positively correlated with Del, AlP, and Cat activity and negatively correlated with Ure activity. The abundance of *Aspergillaceae* fungi was negatively correlated with the counts of *Corynebacteriaceae* and *Carnobacteriaceae* bacteria.

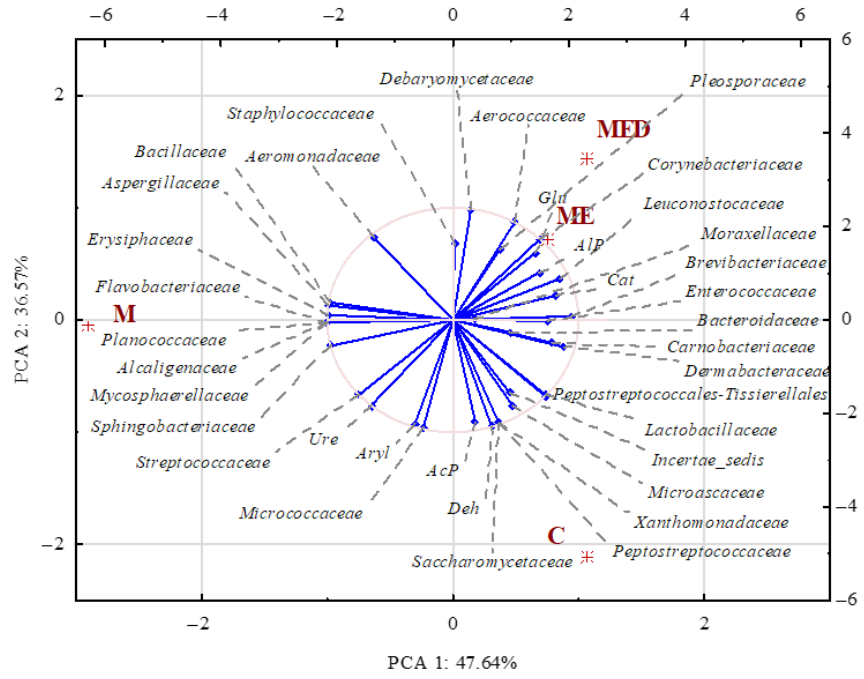
Differences were also observed in the relationships between the dominant bacterial and fungal genera and enzyme activity (Figure 11). *Aspergillus* counts were positively correlated with Ure activity and negatively correlated with Deh, Cat, AcP, AlP, and Glu activity; *Issatchenkia* counts were positively correlated with Deh, AcP, and Aryl activity and negatively correlated with Glu activity; *Blumeria* counts were positively correlated with Ure activity and negatively correlated with Cat, AlP, Glu, and Deh activity; *Corynebacterium* counts were positively correlated with Cat, AlP, and Glu activity and negatively correlated with Deh, Ure, AcP, and Aryl activity; *Pseudogracilibacillus* counts were positively correlated with Ure activity and negatively correlated with Deh, Cat, AcP, AlP, and Glu activity. The abundance of *Gallicola* and *Atopostipes* was positively correlated with Deh activity. Positive correlations were also observed between *Gallicola* counts and AcP and Aryl activity and between *Atopostipes* counts and AlP activity. In turn, the abundance of *Oceanisphaera* was negatively correlated with Deh, Cat, AlP, and Aryl activity.



Variables	PCA 1	PCA 2
Firmicutes	−0.79	0.54
Actinobacteriota	−0.79	−0.54
Proteobacteria	0.91	−0.37
Bacteroidota	0.97	−0.12
Ascomycota	−0.14	0.89
Basidiomycota	0.78	−0.55
Glomeromycota	0.40	−0.80
Mucoromycota	−0.97	0.23
Deh	0.08	0.70
Cat	−0.20	−0.65
Ure	0.62	0.78
AcP	0.41	0.52
AlP	−0.98	−0.19
Aryl	0.28	0.95
Glu	−0.25	−0.88

Treatment	PCA 1	PCA 2
C	−0.28	2.75
M	2.22	1.05
ME	1.54	−2.94
MED	−3.47	−0.86

Figure 9. PCA scatterplot presenting the relationships between the abundance of bacterial and fungal phyla and enzyme activity in manure samples. C – manure from turkeys that were not administered antibiotics; M – manure from turkeys administered monensin; ME – manure from turkeys administered monensin and enrofloxacin; MED – manure from turkeys administered monensin, enrofloxacin, and doxycycline



Treatment	PCA 1	PCA 2
C	2.30	−5.06
M	−6.28	−0.12
ME	1.65	1.72
MED	2.33	3.46

Variables	PCA 1	PCA 2
Mycosphaerellaceae	−1.00	−0.02
Pleosporaceae	0.37	0.63
Aspergillaceae	−0.99	0.13
Erysiphaceae	−0.99	0.04
Debaryomycetaceae	0.14	0.98
Saccharomycetaceae	0.37	−0.92
Incertae_sedis	0.45	−0.65
Microascaceae	0.47	−0.76
Peptostreptococcales-Tissierellales	0.73	−0.68
Corynebacteriaceae	0.69	0.72
Carnobacteriaceae	0.79	−0.20
Lactobacillaceae	0.74	−0.67

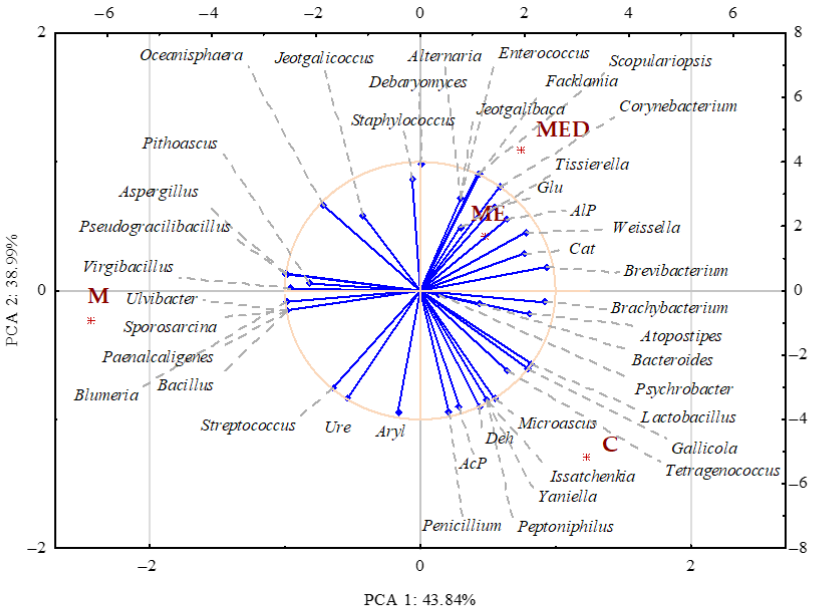
Variables	PCA 1	PCA 2
Dermabacteraceae	0.88	−0.24
Bacillaceae	−0.97	0.16
Bacteroidaceae	0.45	−0.11
Staphylococcaceae	0.01	0.68
Enterococcaceae	0.75	−0.01
Aeromonadaceae	−0.62	0.73
Brevibacteriaceae	0.94	0.03
Sphingobacteriaceae	−0.97	−0.23
Streptococcaceae	−0.75	−0.66
Xanthomonadaceae	0.37	−0.92
Moraxellaceae	0.17	0.01
Micrococcaceae	−0.23	−0.96
Leuconostocaceae	0.85	0.37

Variables	PCA 1	PCA 2
Peptostreptococcaceae	0.37	−0.92
Aerococcaceae	0.49	0.87
Alcaligenaceae	−1.00	−0.02
Flavobacteriaceae	−1.00	-0.02
Planococcaceae	−1.00	−0.02
Deh	0.31	−0.95
Cat	0.82	0.22
Ure	−0.65	−0.76
AcP	0.18	−0.92
AlP	0.69	0.42
Aryl	−0.30	−0.92
Glu	0.65	0.60

Figure 10. PCA scatterplot presenting the relationships between the abundance of bacterial and fungal families and enzyme activity in manure samples. C – manure from turkeys that were not administered antibiotics; M – manure from turkeys administered monensin; ME – manure from turkeys administered monensin and enrofloxacin; MED – manure from turkeys administered monensin, enrofloxacin, and doxycycline; Deh – dehydrogenases; Cat – catalase; Ure – urease; Pac – acid phosphatase; Pal – alkaline phosphatase; Glu – β-glucosidase; Aryl – arylsulfatase

Discussion

The chemical composition of manure is determined by many factors, including animal species, diet, type of litter, and rearing stage (Dróżdż et al., 2020). Due to its diverse composition and high nutrient content, poultry manure is used in the production of organic fertilizers and soil amendments (Regulation (EC) 1069/2009 of the European Parliament and of the Council). The present study confirmed that turkey manure is relatively abundant in nitrogen and contains large amounts of phosphorus and potassium (Nicholson et al., 1996; Huang et al., 2017; Dróżdż et al., 2020). Regardless of their pharmacokinetics and the ingested dose, the tested antibiotics were not fully degraded, which indicates that their residues can be introduced to the agroecosystem with animal excreta and can affect the environmental microbiome (Bartlett et al., 2013). Manure samples were characterized by lower concentrations of monensin and doxycycline residues (approx. 7%) than enrofloxacin residues (16–25%). High levels of tetracycline and sulfonamide residues (91 to 136 mg kg^{-1} DM) were reported in other studies (Martínez-Carballo et al., 2007; An et al., 2015). Some researchers found that up to 75% of the ingested dose of antimicrobials was eliminated with manure even after short-term treatment (several days) (Hobson-Frohock and Johnson, 1983; Winckler and Grafe, 2001; Laloučková and Skřivanová, 2019).



Variables	PCA 1	PCA 2
Alternaria	0.30	0.72
Aspergillus	−0.99	0.13
Penicillium	0.21	−0.95
Blumeria	−0.99	−0.09
Debaryomyces	0.00	0.98
Issatchenkia	0.49	−0.85
Microascus	0.55	−0.84
Pithoascus	−0.82	0.06
Scopulariopsis	0.42	0.90
Corynebacterium	0.59	0.81
Gallicola	0.79	−0.60
Atopostipes	0.81	−0.18
Lactobacillus	0.82	−0.56
Brachybacterium	0.92	−0.09
Bacteroides	0.43	−0.11
Peptoniphilus	0.49	−0.85
Oceanisphaera	−0.71	0.66
Tissierella	0.29	0.49

Variables	PCA 1	PCA 2
Brevibacterium	0.93	0.18
Streptococcus	−0.65	−0.76
Tetragenococcus	0.64	−0.62
Psychrobacter	0.14	−0.03
Pseudogracilibacillus	−0.99	0.13
Weissella	0.78	0.45
Staphylococcus	−0.06	0.86
Yaniella	0.49	−0.85
Facklamia	0.43	0.90
Jeotgalicoccus	−0.42	0.59
Virgibacillus	−0.96	0.02
Jeotgalibaca	0.30	0.72
Enterococcus	0.30	0.72
Ulvibacter	−0.98	−0.15
Paenalcaligenes	−0.98	−0.15
Bacillus	−0.98	−0.15
Sporosarcina	−0.98	−0.15

Variables	PCA 1	PCA 2
Deh	0.44	−0.90
Cat	0.77	0.29
Ure	−0.54	−0.84
AcP	0.29	−0.91
AlP	0.64	0.55
Aryl	−0.16	−0.94
Glu	0.55	0.65

Treatment	PCA 1	PCA 2
C	3.17	−5.14
M	−6.32	−0.92
ME	1.22	1.70
MED	1.93	4.35

Figure 11. PCA scatterplot presenting the relationships between the abundance of bacterial and fungal genera and enzyme activity in manure samples. C – manure from turkeys that were not administered antibiotics; M – manure from turkeys administered monensin; ME – manure from turkeys administered monensin and enrofloxacin; MED – manure from turkeys administered monensin, enrofloxacin, and doxycycline; Deh – dehydrogenases; Cat – catalase; Ure – urease; Pac – acid phosphatase; Pal – alkaline phosphatase; Glu – β-glucosidase; Aryl – arylsulfatase

In the current study, culturable microorganisms were arranged in the following order based on their susceptibility to the tested antimicrobials: Actinobacteria > fungi > organotrophic bacteria, and this order is well established. Regardless of the type and combination of antibiotics, the mechanisms responsible for the action of this group of xenobiotics have been thoroughly analyzed (Baran et al., 2023). The toxic effects of these compounds on bacteria include the inhibition of the biosynthesis of cell walls, nucleic acids and proteins, disruption of cell membrane integrity and interference with many metabolic processes (Uluseke et al., 2021). Furthermore, the metabolites of antibiotics identified in the gastrointestinal tract of animals exert a more inhibitory effect on microorganisms than the parent compound (Massé et al., 2014). A comparison of the results obtained in groups ME and M indicates that the decrease in microbial counts is induced mainly by M. In response, the antibiotic interferes with ATP synthesis and the proton motive force (PMF). In addition, interference with the transport of Na^+ ions disrupts nutrient absorption by bacterial cells, ultimately leading to cell death (Carresi et al., 2024; Łowicki and Huczyński, 2013). The negative impact of M is probably exacerbated by its prolonged retention in the manure, which can extend to 10 weeks (Carlson and Mabury, 2006). The maximum percentage inhibition of the growth of all microbial groups exposed to M, E, and D residues in manure is debatable because the conjugation rate of the plasmid, which according to Saraiva et al. (2022) confers resistance to a wide range of antibiotics (including tetracyclines such as D), increases in poultry litter under the pressure of M. However, a more

important argument supporting the observed trends is the fact that tetracyclines strongly inhibit protein synthesis by preventing the attachment of aminoacyl-tRNA to the acceptor (A) site of the bacterial 70S ribosome (Janser, 2016).

The response of Actinobacteria to the antibiotics present in manure is a complex phenomenon. Microorganisms of the phylum Actinobacteria and Actinomycetes are the most extensively studied sources of antibiotics in ecosystems. However, it should be noted that the most common classes of antibiotics, including tetracyclines and quinolones, target mainly active and proliferating cells (De Simeis and Serra, 2021). This is partly in line with the trend observed in this study, suggesting that the ecophysiological diversity of Actinobacteria (EP) decreased under the pressure of antibiotics administered in combination. It should also be emphasized that Actinobacteria are highly metabolically active, especially in environments rich in organic compounds, such as turkey manure, which may explain why this group of microorganisms was more susceptible to antibiotics.

The reasons for the high susceptibility of fungi to M, E, and D can be attributed to the disruption of fungal sterol biosynthesis by the inhibition of cytochrome P450 enzymes or cell wall synthesis, including its integrity, leading to cell lysis (Barabote et al., 2011). The subtle relationships between bacteria and selected fungal species also played a minor role. According to Liu et al. (2024), bacteria can invade and damage the cell walls of fungal chlamydospores as part of their defense strategies. In turn, fungal nucleases degrade bacterial tetracycline resistance genes.

Based on the literature data, antibiotics, especially when applied in combination, could be expected to exert negative and modulating effects on the diversity of bacterial communities (Męcik et al., 2023; Cycoń et al., 2019). The high diversity of bacteria and antibiotic residues in manure not only contributes to selective pressure but also triggers the mobilization of genes that modulate the interactions between microorganisms (Lima et al., 2020). However, it should be noted that the gut microbiota itself is a reservoir of antibiotic-resistant microorganisms in poultry (Udikovic-Kolic et al., 2014). In the present study, four bacterial phyla were dominant in turkey manure. The highest OTU abundance was found in Firmicutes, followed by Actinobacteria, Proteobacteria, and Bacteroidota (Figure 1). This result confirms the fact that the poultry gut microbiome is abundant in microorganisms representing the four bacterial phyla identified in this study (Mancabelli et al., 2016). Similar results were reported by Zhou and Yao (2020) who found that Firmicutes accounted for 39.4% and Proteobacteria for 36.7% of the total bacteria in manure. The cited authors also examined the mechanisms responsible for the structure of the microbiome and concluded that the main resistance mechanisms were deactivation of D (41.7%) and the use of efflux pumps (29.36%), whereas cellular protection accounted for 27.06% of the identified resistance mechanisms. The predominance of Firmicutes in manure could be expected because these bacteria are mostly responsible for the transfer of ARGs in different types of manure (Feng et al., 2023).

Monensin residues decreased the abundance of Firmicutes and Actinobacteria and increased the abundance of Proteobacteria and Bacteroidota. However, the combination of all three antibiotics exerted the strongest inhibitory effect, and it promoted only the proliferation of bacteria of the phylum Actinobacteria (Figure 1). The observed changes in the microbiome of turkey manure indicate that bacteria have considerable potential to degrade this group of xenobiotics. Antibiotics, including tetracyclines, can be degraded through hydrolysis or epimerization (Gong et al., 2020). However, D is eliminated mainly through bacterial transformation in the four-ring tetracyclic nucleus. The *tetX* gene encodes NADP-dependent flavoprotein monooxygenase that catalyzes the degradation of D (Yang et al., 2004). According to Gauthier et al. (2010), D can also be degraded by bacteria through cometabolism.

As demonstrated in the present study, bacteria of the genera *Bacillus* and *Enterococcu*s could be expected to proliferate most abundantly in all treatments because they belong to the phylum Firmicutes (Figure 3). *Bacillus* spp. are positively correlated with a wide range of ARGs such as *tetC*, *tetO*, *tetM*, and *tetX* (Gong et al., 2020). Agersø et al. (2002) not only identified a conjugative transposon associated with tetracycline resistance in *Bacillus cereus* isolates, but also found that this transposon was transferred via conjugation into *Enterococcus faecalis* JH2-2. Moreover, the lateral transfer of the ermB gene assigned to *Enterococcus faecium* isolated from poultry manure indicates that HGT plays the most important role in the spread of antimicrobial resistance determinants (De Leener et al., 2005). According to Simjee et al. (2012), resistance to M is a phenotypic trait in *Enterococcus faecium* and *Enterocooccus faecalis*, and is associated with the thickening of the cell wall or glycocalyx in the presence of this antibiotic. In turn, resistance to E in the genus *Enterococcus* is conditioned by two mechanisms: modification of quinolones with a piperazinyl substituent by acetyltransferase Aac(6')-Ib-cr, and active efflux by QepA and OqxAB pumps associated with the major facilitator superfamily (MFS) transporters. These mechanisms are encoded by *qnrB, qnrS*, and *qnrD* genes (Rodríguez-Martínez et al., 2011). *Oceanisphaera* and *Corynebacterium* were also identified in all treatments analyzed in the current study (Figure 3). *Corynebacterium stationis* was also detected by Męcik et al. (2023) in samples of poultry manure. In turn, Feng et al. (2023) emphasized that *Oceanisphaera* is a potential bacterial host of aac(3)-II and aac(6')-II genes that confer antibiotic resistance and, most importantly, the activity of this genus is correlated with the nitrogen content of manure. In fungi, the highest number of ITS sequences was identified mainly in the phylum Ascomycota, which was abundantly represented by the genus *Aspergillus* in

all treatments (Figure 5). It appears that resistance to M, E, and D residues in manure is due to the fact that fungi are able to degrade these antibiotics, particularly D, by cleaving their A and B rings, and increasing the accumulation of hemi-cyclins (OXY-1) (Shang et al., 2016). Gauthier et al. (2010) also demonstrated that *Aspergillus niger* degrades antibiotics via cometabolism. In *Aspergillus* spp., antibiotic resistance is conditioned by two types of transporters: the ATP-binding cassette (ABC) and MFS pumps, accounting for 85% of all active efflux pumps in fungi (Barabote et al., 2011). The increase in the abundance of Basidiomycota under exposure to ME can be attributed to the potential of fungi of this phylum to degrade antibiotics with lignolytic enzymes such as lignin and manganese peroxidases (Sun et al., 2021).

The biochemical parameters of turkey manure have not been analyzed to date, even though enzymes play a key role in maintaining the balance between the synthesis and degradation of organic matter (Wyszkowska et al., 2023). Therefore, an important biological parameter for the reliable determination of manure quality was developed in this study. However, strong inhibition of dehydrogenases activity could be expected since the abundance of all microbial groups in the manure ecosystem decreased under exposure to M, E, and D. This is closely related to the fact that dehydrogenases belong to a pool of intracellular enzymes that are found in numerous bacterial and fungal structures, including polysomes, bacterial spores and fungal spores, and the cytoplasm, and are dependent on their activity (Wallenstein and Burns, 2011). Urease is an enzyme that is involved in the hydrolysis of urea to ammonia and carbon dioxide (Miśkowiec and Olech, 2020). In the present study, urease activity was suppressed by D, and similar observations were made by Santás-Miguel et al. (2021) who found a significant negative correlation between increasing tetracycline doses and urease activity. Urease activity was stimulated by M, probably because the proportion of M residues in manure was much lower than the proportions of E and D residues (Table 1). The fact that poultry manure is a nitrogen-rich organic fertilizer due to its high protein and amino acid content also contributed to the stimulation of urease activity. The less pronounced response of phosphatase was also due to the presence of phosphorus in turkey manure, which is one of the nutrients found in this organic fertilizer, along with potassium, magnesium, calcium, and sulfur (Frąc et al., 2021). It should be noted that phosphatases participate in the mineralization of phosphate compounds by catalyzing the hydrolysis of phosphate monoesters (Janes-Bassett et al., 2022).

Conclusion

The excretion rates of the antibiotics administered to farmed turkeys with feed or drinking water were determined at 6.72–8.17% for monensin (M), 15.81–25.24% for enrofloxacin (E), and 6.53% for doxycycline (D). These antimicrobials induced significant changes in the abundance of culturable and non-culturable bacteria and fungi in the turkey manure microbiome. The analyzed antibiotics completely eliminated bacteria of the genera *Peptoniphilus* and *Yaniella* and contributed to the occurrence of unique bacteria of the genera *Ulvibacter*, *Paenalcaligenes*, *Bacillus*, and *Sporosarcina* (M) as well as *Jeotgalibaca* and *Enterococcus* (MED). The tested antimicrobials also inhibited the growth of fungi belonging to the genera *Microascus*, *Penicillium*, and *Issatchenkia*, and stimulated the growth of members of the genera *Debaryomyces* and *Blumeria* (all antibiotics), *Scopulariopsis* (ME and MED), *Alternaria (*MED), and *Pithoascus* (M and ME). Antibiotics have a negative effect on the biochemical properties of manure, which is particularly evident in their inhibitory effect on the activity of dehydrogenases, urease, arylsulfatase and acid phosphatase. This effect can be attributed to the impact of antibiotics on the manure microbiome. Consequently, additional stabilization is recommended prior to the introduction of manure into the soil to minimize the concentrations of antibiotic residues. Further research could therefore focus on evaluating the effectiveness of different manure stabilization methods, with a particular emphasis on their effects on the biodiversity of soil microorganisms. Given the importance of environmental concerns, greenhouse experiments involving this type of manure and investigating its effects on soil properties and plant growth and development may be of interest.

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