UNIWERSYTET PRZYRODNICZY w LUBLINIE

Wydział Nauk o Zwierzętach i Biogospodarki
Dyscyplina naukowa Zootechnika i Rybactwo

mgr inż. Radosław Smagieł

Rozprawa doktorska

Wpływ wczesnej antybiotykoterapii i stosowania kokcydiostatyków na rozwój układu immunologicznego i antyoksydacyjnego u młodych indyków

Effects of early-life antibiotic therapy and coccidiostat administration on the development of the immune and antioxidant systems in young turkey

Rozprawa doktorska wykonana w Katedrze Biochemii i Toksykologii

Promotor: prof. dr hab. Katarzyna Ognik

Praca doktorska została zrealizowana w ramach grantu badawczego Narodowego Centrum Nauki nr. 2020/39/B/NZ9/00765 pt.: „Odpowiedź układu immunologicznego i systemu oksydoredukcyjnego indyków żywionych dietami z lub bez dodatku kokcydiostatyku na wczesne podanie antybiotyków”.

Lublin, 2026

Serdeczne podziękowania składam wspaniałej

Pani Promotor prof. dr hab. Katarzynie Ognik

Dziękuję też całemu zespołowi.

Oświadczenie promotora rozprawy doktorskiej

Oświadczam, że niniejsza rozprawa doktorska została przygotowana pod moim kierunkiem i stwierdzam, że spełnia ona warunki do przedstawienia jej w postępowaniu o nadanie stopnia naukowego.

Data 21.05.2026 r. Podpis promotora Katarzyna Ozmik

Oświadczenie autora rozprawy doktorskiej

Świadom/a odpowiedzialności prawnej oświadczam, że:

- niniejsza rozprawa doktorska została przygotowana przez mnie samodzielnie pod kierunkiem Promotora oraz Promotora pomocniczego i nie zawiera treści uzyskanych w sposób niezgodny z obowiązującymi przepisami.
- przedstawiona rozprawa doktorska nie była wcześniej przedmiotem procedur związanych z uzyskaniem stopnia naukowego.
- niniejsza wersja rozprawy doktorskiej jest tożsama z załączoną na pendrive wersją elektroniczną.

Data 21.05.2026 r. Podpis autora Radosław Smagieł

Wykaz prac naukowych wchodzących w skład cyklu

1. Mikulski D., Juśkiewicz J., Ognik K., Zduńczyk P., Smagieł R., Jankowski J. **2022**. Gastrointestinal tract and neuroendocrine system responses of young turkeys to the early administration of antibiotics or feeding a diet containing a coccidiostat. *Poultry Science* 101: 102098.https://doi.org/10.1016/j.psj.2022.102098
   MNiSW = 140; IF = 4,400

2. Smagieł R., Ognik K., Cholewińska E., Stępniowska A., Listos P., Tykałowski B., Mikulski D., Koncicki A., Jankowski J. **2023**. The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys. *Poultry Science* 102: 102876. https://doi.org/10.1016/j.psj.2023.102876
   MNiSW = 140; IF = 3,800

3. Smagieł R., Tutaj K., Cholewińska E., Sołek P., Mikulski D., Stępniowska A., Jankowski J., Ognik K. **2024**. The effect of early administration of antibiotics or feeding a diet containing coccidiostats on the level of their accumulation in liver and the redox status of turkeys. *Animal* 18: 101321. https://doi.org/10.1016/j.animal.2024.101321
   MNiSW = 200; IF = 4,200

4. Ognik K., Tykałowski B., Mikulski D., Smagieł R., Cholewińska E., Koncicki A., Stępniowska A., Jankowski J. **2025**. Early administration of antibiotics to turkey poults impairs maternal immunity and post-vaccination antibody synthesis. *Annals of Animal Science* 25, 1: 239-257.https://doi.org/10.2478/aoas-2024-0063
   MNiSW = 140; IF = 2,200

5. Smagieł R.,Ognik K., Cholewińska E.,Sołek P., Mikulski D., Tykałowski B., Jankowski J., Tutaj K. **2026**. Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency? *Annals of Animal Science.* https://doi.org/10.2478/aoas-2025-0125

MNiSW = 140; IF=2,200

Suma MNiSW = 760; IF = 16,800

Spis treści

Wykaz skrótów i akronimów

ACTB – beta-aktyna

aMPV – ptasi metapneumowirus

AOPP – zaawansowane produkty utleniania białek

APV – ptasi pneumowirus

CAT – katalaza

cDNA – komplementarny DNA

Cp – ceruloplazmina

CT – próg cyklu

DOX – doksycyklina

ELISA – test immunoenzymatyczny

ENR – enrofloksacyna

GAPDH – dehydrogenaza aldehydu 3-fosfoglicerynowego

GCH1 – cyklohydrolaza GTP 1

GPx – peroksydaza glutationowa

GSH – zredukowany glutation

H+E – hematoksylina i eozyna

IFN-γ – interferon gamma

IgA – immunoglobulina A

IgM – immunoglobulina M

IgY – immunoglobulina Y

IL-1β – interleukina 1 beta

IL-2 – interleukina 2

IL-6 – interleukina 6

IL-8 – interleukina 8

IL-12 – interleukina 12

iNOS – indukowalna syntaza tlenku azotu

LPS – lipopolisacharyd

MON – monenzyna

MPO – mieloperoksydaza

MRL – maksymalny poziom pozostałości

MRM – monitorowanie wybranych reakcji fragmentacji

NADPH – fosforan dinukleotydu nikotynoamidoadeninowego

ND – rzekomy pomór drobiu

NDV – wirus rzekomego pomoru drobiu

NO – tlenek azotu

NOX – oksydaza NADPH

ORT – Ornithobacterium rhinotracheale

PCR – reakcja łańcuchowa polimerazy

PM – Mycoplasma gallisepticum / mykoplazmoza ptaków

QPCR – ilościowa reakcja łańcuchowa polimerazy

RNA – kwas rybonukleinowy

ROS – reaktywne formy tlenu

RT – odwrotna transkrypcja

SCFA – krótkołańcuchowe kwasy tłuszczowe

sIgM – powierzchniowa immunoglobulina M

SOD – dysmutaza ponadtlenkowa

TAS – całkowity status antyoksydacyjny

TCRγδ – receptor limfocytów T gamma-delta

TLR-4 – receptor Toll-podobny 4

TNF-α – czynnik martwicy nowotworu alfa

TRT – zakaźne zapalenie tchawicy indyków

Streszczenie

Celem pracy była ocena wpływu wczesnej antybiotykoterapii oraz stosowania monenzyny na rozwój układu immunologicznego i antyoksydacyjnego u młodych indyków, ze szczególnym uwzględnieniem transferu przeciwciał matczynych oraz kształtowania odporności. Założono, że wczesne podawanie antybiotyków może zaburzać wykorzystanie odporności biernej oraz równowagę oksydoredukcyjną organizmu.

Badania przeprowadzono na indyczkach Hybrid Converter w dwóch doświadczeniach o układzie dwuczynnikowym. Antybiotyki (enrofloksacyna, doksycyklina) podawano w pierwszych 5 dniach życia, natomiast monenzynę stosowano w paszy w okresie odchowu. Ocenie poddano poziom przeciwciał matczynych i poszczepiennych, subpopulacje limfocytów, ekspresję genów oraz parametry stresu oksydacyjnego i aktywność enzymów antyoksydacyjnych. Przeprowadzono również analizę histologiczną narządów limfatycznych oraz oznaczenie pozostałości badanych substancji w wątrobie.

Uzyskane wyniki wykazały, że badane czynniki nie wpływały na resorpcję woreczka żółtkowego, jednak obniżały poziom przeciwciał matczynych oraz ograniczały odpowiedź humoralną, co wskazuje na bezpośredni wpływ antybiotyków na funkcjonowanie układu immunologicznego. Stwierdzono także zmiany w subpopulacjach limfocytów oraz ekspresji genów związanych z odpowiedzią zapalną.

Wykazano, że antybiotyki, szczególnie enrofloksacyna, mogą indukować stres oksydacyjny i zaburzać równowagę oksydoredukcyjną organizmu. Doksycyklina wykazywała częściowo efekt adaptacyjny, natomiast stosowanie monenzyny nie nasilało reakcji oksydacyjnych.

Uzyskane wyniki wskazują, że wczesna antybiotykoterapia oraz stosowanie kokcydiostatyków mogą prowadzić do niekorzystnych zmian w funkcjonowaniu układu immunologicznego młodych indyków, co uzasadnia konieczność racjonalizacji ich stosowania w praktyce produkcyjnej.

Słowa kluczowe: indyki, antybiotyki, monenzyna, immunomodulacja, przeciwciała matczyne

Summary

The aim of the study was to evaluate the effects of early-life antibiotic therapy and the administration of monensin on the development of the immune and antioxidant systems in young turkeys, with particular emphasis on maternal antibody transfer and the development of immunity. It was hypothesized that early antibiotic administration may impair the utilization of passive immunity and disturb the redox balance of the organism.

The study was conducted on Hybrid Converter turkey hens in two experiments with a two-factorial design. Antibiotics (enrofloxacin and doxycycline) were administered during the first 5 days of life, while monensin was included in the diet throughout the rearing period. The evaluation included the levels of maternal and post-vaccination antibodies, lymphocyte subpopulations, gene expression, oxidative stress parameters, and the activity of antioxidant enzymes. Histological analysis of lymphoid organs and determination of residues of the tested substances in the liver were also performed.

The results showed that the applied factors did not affect yolk sac resorption; however, they reduced the levels of maternal antibodies and impaired the humoral immune response, indicating a direct effect of antibiotics on immune system function. Changes in lymphocyte subpopulations and in the expression of genes related to inflammatory responses were also observed.

It was demonstrated that antibiotics, particularly enrofloxacin, may induce oxidative stress and disrupt redox balance. Doxycycline showed a partial adaptive effect, whereas monensin administration did not intensify oxidative reactions.

The obtained results indicate that early antibiotic therapy and the use of coccidiostats may lead to adverse changes in immune system function in young turkeys, highlighting the need for rational use of these substances in poultry production.

Keywords: turkeys, antibiotics, monensin, immunomodulation, maternal antibodies

1. Wstęp

Polska zajmuje 1. miejsce w Europie i 3. na świecie w produkcji mięsa indyczego (około 600 tys. ton rocznie). Współczesna, wysoka wydajność indyków jest efektem postępu genetycznego i racjonalnego żywienia ptaków, a także bieżącego wykorzystania wyników badań ukierunkowanych na niedostatecznie jeszcze rozpoznane czynniki, warunkujące zdrowie i szybki wzrost ptaków oraz jakość uzyskiwanego mięsa. Aktualnie użytkowane, szybkorosnące indyki charakteryzuje zwiększona podatność na niekorzystne czynniki środowiska i infekcje skutkujące często koniecznością stosowania antybiotykoterapii. Z drugiej strony istnieje olbrzymi nacisk społeczny, aby ograniczyć ilości antybiotyków zużywanych w produkcji zwierzęcej, w tym drobiarskiej. W krajach UE już w 2006 roku wprowadzono zakaz stosowania antybiotyków jako stymulatorów wzrostu. Pozostawiono jednak możliwość ciągłego stosowania w paszy kokcydiostatyków, w tym jonoforowych, będących także antybiotykami. Doszło do paradoksalnych sytuacji, kiedy zabronione jest stosowanie konkretnego antybiotyku jak stymulatora wzrostu a dopuszczone jako kokcydiostatyku (np. salinomycyny u kurcząt). Aktualnym wyzwaniem stojącym przed produkcją drobiarską, w tym indyków, jest uzasadniona wieloma względami konieczność ograniczenia ilości zużywanych antybiotyków. UE zobowiązała kraje członkowskie do ograniczenia o 50% zużycia antybiotyków w produkcji zwierzęcej. Zdobycie nowej wiedzy dotyczącej biologicznych skutków wczesnego stosowania antybiotyków i ciągłego stosowania kokcydiostatyków u młodych indyków (do 8-12 tygodnia życia) jest jedną z najważniejszych dróg prowadzących do osiągnięcia tego celu. Brakuje bowiem empirycznych dowodów, że takie postępowanie, w tym „profilaktyczne" podawanie antybiotyków w dawkach leczniczych już w pierwszym tygodniu życia indyków (zazwyczaj od 2. dnia życia), nie koliduje z fizjologicznymi mechanizmami przekazywania matczynej odporności pisklętom i nabywania przez nie osobniczej obrony przed patogenami.

System odpornościowy wyklutego pisklęcia jest tylko częściowo rozwinięty i nie jest w stanie zapewnić całkowitej ochrony organizmu przed patogenami. W pierwszych dniach życia w dużej mierze odporność piskląt na czynniki zakaźne zapewniają przeciwciała matczyne IgY, IgA i IgM, które są przenoszone za pośrednictwem jaja do zarodków a następnie do piskląt (Grindstaff i in., 2003; Chrząstek i in., 2011, Murai, 2013, Murai i in., 2020). U większości ptaków przekazanie przeciwciała IgY z matki na pisklęta następuje głównie za pośrednictwem żółtka jaja, natomiast przeciwciała IgM i IgA w odróżnieniu do

IgY nie są przekazywane do żółtka jaja, a niewielkie ich koncentracje jakie stwierdza się w żółtku pochodzą z wydzieliny jajowodu (Kaspers i in., 1991). Przeciwciała IgY są absorbowane z żółtka przez rozwijający się zarodek do krążenia embrionalnego, natomiast przeciwciała IgM i IgA nie są transportowane do krążenia embrionalnego, ale do przewodu pokarmowego rozwijającego się pisklęcia (Rose and Orlans, 1981), gdzie pełnią istotną rolę w odporności miejscowej w pierwszych dniach po wykluciu (Kowalczyk i in., 2019). Wykazano, że transfer przeciwciał matczynych do żółtka jest proporcjonalny do poziomu przeciwciał w surowicy matki, jednak tylko około 30% tych przeciwciał przenoszonych jest z żółtka do układu krążenia zarodka (Al.-Natour i in., 2004). Stwierdzono też, że poziom IgY u 3-dniowych kurcząt mieścił się w zakresie 0,99 - 1,52 mg/ml, co stanowiło 30% tych przeciwciał oznaczonych w surowicy niosek (Hamal i in., 2006). Zgodnie z najlepszą wiedzą w dostępnej literaturze ostatnio opublikowano jedyne badania przeprowadzone na nioskach indyczych (Kowalczyk i in., 2019), u których odnotowano tę samą tendencję, bowiem stwierdzono, że średni transfer przeciwciał IgY z niosek indyczych do piskląt osiągnął 31,4% w całym cyklu produkcyjnym jaj. Ustalono także, że zależność między stężeniami przeciwciał matczynych różnych klas (w tym IgM) w różnych strukturach jaj jest podobna u indyków i kur (Kowalczyk i in., 2019).

W nielicznych publikacjach wykazywano wpływ niektórych czynników (wiek i rasa nioski, warunki inkubacji, stopień uwodnienia pisklęcia w czasie klucia, warunki transportu, termin pierwszego pojenia, rodzaj wody i paszy, stres) na resorpcję woreczka żółtkowego (Jamroz i in., 2004, van der Wagt i in., 2020). Wiadomo, że u piskląt tuż po wylęgu istotnym problemem zdrowotnym są zaburzenia resorpcji woreczka żółtkowego. U indyków, fizjologicznie w 4-5 dobie po wylęgu zanik treści i inwolucja ściany woreczka żółtkowego powinna zostać zakończona, jednak w praktyce często ten proces jest znacząco wydłużony lub całkowicie zahamowany. Z ostatnio opublikowanego przeglądu Van der Wagt i in., (2020), uwzględniającego wyniki badań z ostatnich 88 lat wynika, że opóźnienie resorpcji woreczka żółtkowego i zaburzenie fizjologicznego procesu wchłaniania jego treści może negatywnie wpływać na zdrowie i wzrost ptaków. Jest to ważna przesłanka uzasadniająca podjęcie projektowanych badań w tym rozstrzygnięcie czy i w jaki sposób wczesne podanie antybiotyku pisklętom wpływa na resorpcję woreczka żółtkowego, a w konsekwencji na transfer przeciwciał matczynych z tego woreczka do pisklęcia (odporność humoralna).

Prawidłowe funkcjonowanie układu odpornościowego obejmujące różnorodne reakcje obronne, z wykorzystaniem wrodzonej, ale także adaptacyjnej odpowiedzi immunologicznej, zapewnia homeostazę organizmu. Stada rodzicielskie kur oraz indyczek

są wielokrotnie szczepione (żywymi i/lub inaktywowanymi szczepionkami) w celu wytworzenia swoistych przeciwciał przeciwko patogenom, które chronią przed infekcjami, a dodatkowo przeciwciała te są przekazywane potomstwu. W pierwszych dniach życia piskląt matczyne przeciwciała swoiste mogą zapobiegać lub łagodzić konsekwencje zakażeń bakteryjnych i wirusowych. W opublikowanym raporcie naukowym określono całkowite stężenie przeciwciał IgY i IgM oraz miana swoistych przeciwciał anty-APV, -NDV, -ORT i -PM w surowicach niosek i piskląt indyczych oraz w żółtku i białku jaj indyczych (Kowalczyk i in., 2019), a dane uzyskane przez tych autorów korespondują z wynikami wcześniejszych badań na kurach nieśnych (Gharaibeh i in, 2008). W przypadku kur stwierdzono, że poziom matczynych przeciwciał swoistych IgY przeciwko różnym patogenom jest bardzo zróżnicowany w organizmie nioski, a zróżnicowanie to jest również widoczne w organizmie piskląt, po tym jak nastąpi transfer przeciwciał swoistych z organizmu nioski poprzez żółtko do piskląt. W badaniu tym transfer swoistych przeciwciał anty-AEV z nioski do piskląt wynosił średnio 4,3%, natomiast transfer specyficznych przeciwciał anty-IBDV wynosił średnio 73,6%. Uzyskano też dowody, że transfer przeciwciał swoistych z matki do potomstwa zależy również od gatunku ptaków. Transfer przeciwciał swoistych anty-NDV z kury nieśnej do organizmu piskląt wynosił 29,2 - 40,7% (Hamal i in, 2006; Gharaibeh i in, 2008), natomiast transport przeciwciał swoistych anty-NDV z indyczki nieśnej do organizmu piskląt wynosił 51,9% (Kowalczyk i in., 2019).

Wiedza na temat transferu przeciwciał swoistych do potomstwa i ich okresów półtrwania, umożliwia opracowanie odpowiednich programów immunoprofilaktyki przeciwko wybranym chorobom (Al-Natour i in., 2004; Ahmed, 2015). Z niektórych badań wiadomo jednak, że szczepienie piskląt przeciwko ND było mało skuteczne w okresie 5 dniowej antybiotykoterapii, bowiem podawanie antybiotyku pisklętom obniżyło poziom swoistych przeciwciał anty-NDV, a zatem wykazało działanie immunosupresyjne (Khalifeh i in., 2009). Obecnie nie jest jeszcze wiadomo, czy podawanie pisklętom antybiotyku wpływa na zahamowanie resorpcji woreczka żółtkowego, co niewątpliwie mogłoby zaburzyć transfer przeciwciał swoistych do organizmu piskląt. Należy zakładać, że poprzez wczesną antybiotykoterapię zostanie zahamowany transfer swoistych przeciwciał matczynych do organizmu pisklęcia, a dodatkowo zahamowane zostanie endogenne (poszczepienne) wytwarzanie swoistych przeciwciał. Na taką możliwość wskazują doniesienia, że system immunologiczny piskląt może nie poradzić sobie z czynnikiem zakaźnym (Khalifeh i in., 2009). Stałe podawanie w mieszankach paszowych kokcydiostatyków (będących antybiotykami) jest metodą profilaktyki kokcydiozy u ptaków.

Jednoczesne podanie dodatkowego antybiotyku ptakom (nawet przez krótki-kilkudniowy okres) może skutkować wystąpieniem w organizmie reakcji biologicznych będących wynikiem sumującego działania tych antybiotyków. Zdaniem Madadi i in. (2009), efekt sumującego działania dwóch lub większej liczby antybiotyków na organizm może być zupełnie inny niż każdego z osobna. Ważny jest również fakt, że kokcydiostatyki są podawane w całym okresie odchowu. Dane dostępne w literaturze dowodzą, że badania dotyczące stosowania kokcydiostatyków u drobiu ukierunkowane są głównie na ocenę skuteczności ich stosowania w kontekście nabywania odporności swoistej ptaków przeciwko kokcydiozie (Chapman, 2008, Kadykalo i in., 2018). Brakuje natomiast informacji, w jaki sposób długotrwałe podawanie kokcydiostatyku wpływa na reakcje biologiczne organizmu, przede wszystkim system immunologiczny i oksydoredukcyjny. Jest to ważne bowiem długotrwałe podawanie kurczętom enrofloksacyny lub doksycykliny może skutkować kumulacją tych antybiotyków w tkankach kurcząt (Gbylik-Sikorska i in., 2016). Z innych badań wynika, że długotrwałe (30-dniowe) podawanie enrofloksacyny kurczętom niekorzystnie wpłynęło na morfologię krwi (spowodowało anemię i leukopenię), a szczególnie kiedy podawano dawki 10 i 20-krotnnie większe od rekomendowanych (Ibrahim i in., 2009).

Enrofloksacyna i doksycyklina to antybiotyki o szerokim spektrum działania, jedne z najczęściej stosowanych u zwierząt gospodarskich, w tym drobiu (Khalifeh i in., 2009). W ograniczonej liczbie eksperymentów, przeprowadzonych jedynie na jednym gatunku drobiu, badano dotychczas efekty podawania enrofloksacyny u kurcząt zakażonych *Salmonella Enteritidis* (Kangi in., 2019), zakażonych *E. coli* lub *Pasteurella multiciola*, a także zakażonych wirusem APV *i Ornithobacterium rhinotracheale* (Garmyn i in., 2009), jednak badania te koncentrowały się na ocenie skuteczności działania antybiotyku. Ponadto nie zawsze antybiotyk zastosowano w pierwszym tygodniu życia, a poza efektami produkcyjnymi nie oceniano innych reakcji biologicznych u ptaków. Zgodnie z dostępną wiedzą dane literaturowe dotyczące reakcji systemu immunologicznego w efekcie stosowania antybiotykoterapii i jednoczesnego oddziaływania czynnika zakaźnego na organizm są nieliczne. Nie jest też wiadomo jak w odpowiedzi na ww. czynniki kształtują się reakcje oksydoredukcyjne w organizmie ptaków. W jednym z doświadczeń (Chrzastek i Wieliczko) stwierdzono, że w efekcie stosowania antybiotyków u piskląt zmniejszył się odsetek subpopulacji limfocytów T CD3(+), TCRγδ(-), CD4(+), CD8(-) i B (Bu-1(+)),a wzrósł odsetek subpopulacji limfocytów T CD8(+) i CD4(+), a ponadto jednoczesne podanie kurczętom LPS z *E. coli* w okresie stosowania antybiotyków nasiliło ten efekt. Autorzy

cytowanych badań stwierdzili, że wczesna antybiotykoterapia może działać immunosupresyjnie, a obrona przed endotoksynami u kurcząt może być nieskuteczna. Zatem należy przypuszczać, że ewentualną konsekwencją wczesnego podawania antybiotyku pisklętom może być zahamowanie resorpcji woreczka żółtkowego, co niewątpliwie w przypadku oddziaływania na organizm czynnika zakaźnego może skutkować nasileniem immunosupresji oraz pogorszeniem statusu antyoksydacyjnego.

Dostępna literatura, w dużej mierze prezentująca wyniki badań przeprowadzonych na zwierzętach laboratoryjnych oraz trzodzie chlewnej, dostarcza wielu dowodów na to, że antybiotyki mają istotny wpływ na funkcjonowanie układu immunologicznego w zakresie odporności nieswoistej (Pomorska-Mól i Pejsak, 2012). W obecności niektórych antybiotyków dochodzi do modyfikacji funkcji komórek żernych, zmian w wytwarzaniu markerów i cytokin zapalnych oraz zmian w intensywności procesów oksydacyjnych (fagocytoza), (Bodei in., 2014). Z kolei wpływ antybiotyków na odpowiedz swoistą jest wciąż relatywnie mało poznany, aczkolwiek większość dostępnych wyników badań, choć także wykonanych na zwierzętach laboratoryjnych lub trzodzie chlewnej, pozwala na stwierdzenie istnienia zależności pomiędzy antybiotykoterapią a produkcją swoistych przeciwciał (Khalifehi in., 2009, Pomorska-Mól i in., 2015, Pomorska-Mól i in., 2016).

W nielicznych badaniach podejmowano próbę ustalenia wpływu antybiotyków na niektóre reakcje biologiczne (głównie immunologiczne oraz rzadziej antyoksydacyjne) także u kurcząt, jednakże termin i długość okresu ich podawania był bardzo zróżnicowany. Dotychczas stwierdzono, że enrofloksacyna może pogarszać morfologię krwi, powodować anemię i leukopenię (Ibrahim i in., 2009), może działać chondrotoksycznie (Maślanka i in., 2009), kardiotoksycznie (Hruba i in., 2019), modyfikować skład mikroflory saprofitycznej jelit (Elokil i in., 2020), a także może kumulować się w tkankach kurcząt (Marchetti i in., 2019). W przeciwieństwie do enrofloksacyny istotnie mniej badań poświęcono dotychczas ocenie oddziaływania doksycykliny na reakcje biologiczne ptaków. W jednym z badań stwierdzono, że enrofloksacyna podawana w późniejszym okresie odchowu kurcząt (43-47 dzień życia) miała działanie immunosupresyjne i ograniczała humoralną odpowiedz immunologiczną u kurcząt sczepionych przeciwko NDV (Sureshkumar i in., 2013). Autorzy cytowanych badań ustalili, że podawanie enrofloksacyny spowodowało obniżenie swoistych przeciwciał anty-NDV oraz zmiany histopatologiczne w bursie Fabrycjusza i śledzionie (dyspersja i zubożenie limfocytarne z kilkoma obszarami zwyrodnienia limfoblastycznego). W innym doświadczeniu u kurcząt, którym podawano przez 5 dni (15-20 dzień życia) enrofloksacynę lub doksycyklinę stwierdzono pogorszenie humoralnej odpowiedzi

immunologicznej (Pavlova i Milanova, 2017). Z badań przeprowadzonych przez Elamarani i in., (2015) wynika, że podawanie enrofloksacyny kurczętom przez 5 dni (38-42 dzień życia) indukowało stres oksydacyjny w organizmie kurcząt, co manifestowało się zwiększeniem aktywności SOD, CAT i GPx oraz zawartości GSH i MDA w wątrobie (Elamaran i in., 2015). Opublikowano także nieliczne doniesienia, w których podjęto próbę ustalenia wpływu wczesnego podania antybiotyku kurczętom na reakcje układu immunologicznego, jednak według naszej najlepszej wiedzy nie podejmowano dotychczas próby ustalenia takiego wpływu na reakcje statusu antyoksydacyjnego. Khalifeh i in., (2009) ustalili, że wczesna antybiotykoterapia (1-5 dzień życia) ograniczała wytwarzanie swoistych przeciwciał anty-NDV u szczepionych kurcząt. Z kolei Madubuike i in., (2020) podają, że wczesna antybiotykoterapia nie miała wpływu na poziom swoistych przeciwciał anty-NDV u szczepionych kurcząt. Chociaż w niektórych badaniach na kurczętach wykazano, że enrofloksacyna pomimo obniżania humoralnej odpowiedzi immunologicznej może mieć korzystny wpływ na komórkową odpowiedź immunologiczną u kurcząt (Khalifeh i in., 2009; Hassanin i in., 2014; Chrząstek i Wieliczko, 2015). Jest to dodatkowy argument do celowości zbadania, czy wczesne podanie antybiotyku może hamować resorpcję woreczka żółtkowego i wpływać w ten sposób na transfer przeciwciał matczynych a w konsekwencji na odporność nieswoistą oraz swoistą i obronę antyoksydacyjną u indyków rzeźnych.

2. Hipoteza badawcza i cel badań

Założono, że wczesne podawanie antybiotyku poprzez hamowanie resorpcji woreczka żółtkowego, a tym samym zmniejszenie transferu przeciwciał matczynych do krwiobiegu może obniżyć odporność humoralną w pierwszych dniach życia ptaków, a w konsekwencji pogorszyć funkcjonowania systemu immunologicznego (immunosupresja) i statusu antyoksydacyjnego ptaków. Podobne skutki może powodować żywienie ptaków mieszankami zawierającymi kokcydiostatyk, będący także antybiotykiem.

Celem badań było pogłębienie wiedzy o skutkach wczesnego podawania antybiotyków pisklętom indyczym, najczęściej w ramach tzw. metafilaktyki, jak też stałego stosowania w mieszankach paszowych dla młodych indyków kokcydiostatyków jonoforowych, będących także antybiotykami. Jest to ważne w kontekście współczesnych wyzwań i problemów intensywnej produkcji drobiarskiej.

Celem doświadczenia 1 było ustalenie czy wczesne podawanie antybiotyku lub żywienie dietą zawierającą kokcydiostatyk wpływa na tempo resorpcji woreczka żółtkowego i transfer przeciwciał matczynych oraz czy może mieć wpływ na sprawność systemu immunologicznego (szczególnie odpowiedzi swoistej, w tym poszczepiennej) i antyoksydacyjnego rosnących indyków.
Celem doświadczenia 2 było ustalenie czy na tempo resorpcji woreczka żółtkowego i transfer przeciwciał matczynych, a także na sprawność systemu immunologicznego rosnących indyków może mieć wpływ wczesne podawanie antybiotyku i jednoczesne żywienie dietą zawierającą kokcydiostatyk.

3. Materiał i metody

Badania wykonano w ramach finansowania Narodowego Centrum Nauki nr 2020/39/B/NZ9/00765.

Dwa doświadczenia wykonywano w Fermie Doświadczalnej Katedry Drobiarstwa Uniwersytetu Warmińsko-Mazurskiego w Olsztynie, która spełnia odpowiednie standardy w zakresie warunków środowiskowych, dobrostanu ptaków, liczby powtórzeń i dokładności pomiarów. Eksperymenty przeprowadzono na młodych indyczkach Hybrid Converter zakupionych jako pisklęta jednodniowe z komercyjnej wylęgarni Grelavi w Kętrzynie (Polska), która również dostarczała pisklęta indycze do wielu wcześniejszych eksperymentów prowadzonych przez nasz zespół. Protokół badawczy dla niniejszego doświadczenia został zatwierdzony przez Lokalną Komisję Etyczną do spraw Doświadczeń na Zwierzętach w Olsztynie, Polska (nr zgody 47/2021; Olsztyn, Polska), a zwierzęta utrzymywano zgodnie z wytycznymi porównywalnymi do określonych w Dyrektywie 2010/63/UE (2010). Ptaki utrzymywano na ściółce w kojcach po 8 powtórzeń w każdej grupie i żywiono mieszankami pełnoporcjowymi o wartości pokarmowej zgodnej z zalecaniami firmy Hybrid (2020). Każdy kojec stanowił powtórzenie w analizie statystycznej wyników odchowu. Wartości pozostałych cech określono indywidualnie, u 1 lub 3 ptaków z każdego powtórzenia (24 lub 8 ptaków na grupę) o masie ciała zbliżonej do średniej w grupie.

3.1 Doświadczenie 1

Szczegółowy opis metodologiczny doświadczenia przedstawiono w publikacjach Smagieł i in, 2023 (Poultry Science), Mikulski i in, 2022 (Poultry Science), Smagieł i in, 2024 (Animal), Ognik i in, 2025 (Annals of Animal Science)

Układ doświadczenia zaprojektowano jako dwuczynnikowy (antybiotyk × szczepienie), co umożliwiło ocenę zarówno bezpośredniego wpływu antybiotyków i kokcydiostatyku, jak i ich interakcji z odpowiedzią immunologiczną indukowaną szczepieniem.

Układ doświadczenia

Antybiotyk / Szczepienia	Control C	Monenzyna M	Enrofloksacyna E	Doksycyklina D
$V_{(-)}$	$CV_{(-)}$	$MV_{(-)}$	$EV_{(-)}$	$DV_{(-)}$
$V_{(+)}$	$CV_{(+)}$	$MV_{(+)}$	$EV_{(+)}$	$DV_{(+)}$

$V_{(-)}$ brak szczepień, $V_{(+)}$ szczepienie

W doświadczeniu wykorzystano 3080 jednodniowych indyczek Hybrid Converter rozmieszczonych losowo do 8 grup doświadczalnych. Każda grupa składała się z 7 powtórzeń po 55 ptaków utrzymywanych na ściółce z trocin drzewnych. Ptaki odchowywano do 56 lub 84. dnia życia, zależnie od zakresu analiz. Szczepione grupy ptaków (cztery grupy z ośmiu) zaszczepiono przeciwko TRT/aMPV (Poulvac TRT; Zoetis) oraz NDV (Nobilis ND Clone 30; MSD Animal Health) w 1. dniu życia metodą „grubej kropli", w oprysku. W 28. dniu życia podano inaktywowaną szczepionkę przeciwko ORT (Ornitin; Phibro, Polska) drogą iniekcji podskórnej. Ptaki nieszczepione utrzymywano w oddzielnych częściach budynku i obsługiwano przez różne osoby w celu ograniczenia ryzyka krzyżowego przenoszenia czynników biologicznych.

Ptaki z grup kontrolnych nie otrzymywały antybiotyków ani kokcydiostatyku. W grupach MON do mieszanek paszowych dodawano monenzynę (Coxidin 200, Huvepharma Polska, Warszawa, Polska) w ilości 90 mg/kg mieszanki paszowej przez 56 lub 84 dni odchowu, zależnie od zakresu analiz. W grupach ENR enrofloksacynę (Enrofloxacin 10%, Biowet, Drwalew, Polska) podawano w wodzie do picia przez pierwszych 5 dni życia w dawce 10 mg/kg masy ciała dziennie. W grupach DOX doksycyklinę (Doxylin CT WSP 433 mg/g, Dopharma Research B.V., Holandia) podawano w wodzie do picia przez pierwszych 5 dni życia w dawce 50 mg/kg masy ciała dziennie.

Kompleksowa ocena reakcji biologicznych indyków w doświadczeniu 1:

1) Ocena resorpcji woreczka żółtkowego oraz transferu przeciwciał matczynych

W 1, 3, 5 dniu życia, od 21 sztuk z grupy (3 ptaki z każdego powtórzenia) pobrane zostały woreczki żółtkowe oraz krew. Krew pobierano do probówek bez antykoagulantu w celu uzyskania surowicy. Po wykrzepieniu próbki wirowano przez 10 minut przy 3000 × g w temperaturze 4°C, a uzyskaną surowicę przechowywano w temperaturze −80°C. Woreczki żółtkowe pobierano aseptycznie po otwarciu jamy brzusznej i również przechowywano w temperaturze −80°C. Zgodnie z procedurą opisaną przez Chamblee i in. (1992) oceniono resorpcję woreczka żółtkowego u piskląt indyczych na podstawie stosunku masy woreczka żółtkowego do masy ciała ptaków. Dla oceny transferu przeciwciał matczynych z żółtka do piskląt w woreczku żółtkowym oraz we krwi zbadano ogólny poziom przeciwciał klasy IgY i IgM przy użyciu zestawów diagnostycznych ELISA (Shanghai Qayee Biotechnology Co., Ltd.) oraz poziom swoistych przeciwciał matczynych klasy IgY w stosunku do metapneumowirusa ptasiego (anty-aMPV), wirusa rzekomego

pomoru drobiu (anty-NDV) i Ornithobacterium rhinotracheale (anty-ORT) przy użyciu komercyjnych zestawów ELISA firmy IDEXX (aMPV Ab, NDV Ab, ORT Ab).

Wszystkie etapy oznaczeń wykonano automatycznie z wykorzystaniem stacji pipetującej Eppendorf EpMotion 5075LH oraz płuczki BioTek ELx405. Odczyt absorbancji przeprowadzono przy użyciu czytnika mikropłytek BioTek ELx800, a analizę wyników wykonano w środowisku oprogramowania xCheck (IDEXX).

2) Ocena reakcji systemu immunologicznego, w tym nieswoistej odporności komórkowej oraz swoistej odporności humoralnej i adaptacyjnej

W 7 i 56 dniu życia, od 7 ptaków z każdej grupy (po jednym ptaku z każdego powtórzenia) pobrana została krew. W osoczu krwi indyków metodami immunoenzymatycznymi (ELISA, Qayee Biotechnology Co., Ltd.) oznaczono poziom wybranych markerów odpowiedzi immunologicznej, w tym białka C-reaktywnego (CRP), ceruloplazminy (Cp), jądrowego czynnika transkrypcyjnego NF-κB, immunoglobuliny A (IgA), receptora Toll-like 4 (TLR-4) oraz amyloidu A.

W krwi indyków określono również poziom miana przeciwciał poszczepiennych przeciwko ORT, aMPV i NDV przy użyciu komercyjnych zestawów ELISA firmy IDEXX (ORT Ab, aMPV Ab, NDV Ab).

3) Ocena funkcjonowania przewodu pokarmowego i aktywności mikrobioty jelitowej

W doświadczeniu oceniano aktywność enzymatyczną mikrobioty jelita ślepego, profil krótkołańcuchowych kwasów tłuszczowych (SCFA) metodą chromatografii gazowej (GC) przy użyciu chromatografu Shimadzu GC-2010), oraz poziom amoniaku w treści jelitowej. Analizy aktywności enzymatycznej obejmowały oznaczenie aktywności: α-galaktozydazy, β-galaktozydazy, β-glukozydazy, β-glukuronidazy, α-glukozydazy, β-ksylozydazy metodą kolorymetryczną z wykorzystaniem syntetycznych substratów p-nitrofenylowych (PNP) oraz o-nitrofenylowych (ONP). Aktywność enzymatyczną wyrażano jako ilość uwolnionego p-nitrofenolu lub o-nitrofenolu w jednostce czasu i masy próbki. Oceniano zarówno aktywność całkowitą, jak i pozakomórkową.

4) Ocena reakcji oksydoredukcyjnych w organizmie

W 7 i 56 dniu życia, od 7 ptaków z każdej grupy (po jednym ptaku z każdego powtórzenia) pobrana została krew. W osoczu krwi indyków metodami immunoenzymatycznymi, przy użyciu komercyjnych zestawów ELISA, zgodnie z intrukcją producenta oznaczono aktywność wybranych enzymów oksydacyjno-antyoksydacyjnych, w tym oksydazy NADPH (NOX), mieloperoksydazy (MPO), dysmutazy ponadtlenkowej (SOD), katalazy (CAT) oraz cyklohydrolazy GTP (GCH1).

Ponadto oznaczono poziom wybranych markerów stresu oksydacyjnego również metodami immunoenzymatycznymi, przy użyciu komercyjnych zestawów ELISA, zgodnie z intrukcją producenta, w tym zaawansowanych produktów utleniania białek (AOPP), 8-izoprostanów oraz 8-hydroksydeoksyguanozyny (8-OHdG). Dodatkowo określono poziom tlenku azotu (NO) oraz całkowity status antyoksydacyjny (TAS) oraz kaspazy 3 i kaspazy 8.

5) Ocena ekspresji genów związanych z systemem immunologicznym

W 7 i 56 dniu życia, od 7 ptaków z każdej grupy (po jednym ptaku z każdego powtórzenia) pobrana została krew. W celu analizy ekspresji genów z krwi indyków izolowano całkowite RNA przy użyciu zestawu RNeasy Protect Animal Blood Kit (Qiagen, Polska), zgodnie z instrukcją producenta. Ilość RNA określano spektrofotometrycznie przy użyciu spektrofotometru UV–VIS Nabi (MicroDigital Co. Ltd., Korea), natomiast integralność RNA oceniano metodą elektroforezy w 0,8% żelu agarozowym. Do syntezy cDNA wykorzystano zestaw NG dART RT kit (EURx, Gdańsk, Polska). Analizę ekspresji genów przeprowadzono metodą Real-Time PCR z użyciem barwnika SYBR Green (SG qPCR Master Mix, EURx). Analizowano ekspresję genu iNOS. Jako geny referencyjne zastosowano ACTB oraz GAPDH.

6) Ocena histologiczna narządów limfatycznych

W 7 i 56 dniu życia po uprzedniej eutanazji od 7 sztuk z grupy, po jednej sztuce z każdego powtórzenia pobrane zostały wycinki śledziony, natomiast w 56 dniu życia dodatkowo pobrane zostały grasica i bursa Fabrycjusza. Próbki śledziony, grasicy oraz bursy Fabrycjusza przecięto wzdłużnie i utrwalono w 5% formalinie (pH = 7,2) przez 24 godziny. Następnie materiał poddano standardowej procedurze odwodnienia w rosnących stężeniach alkoholu, przeprowadzono przez aceton i ksylen, a następnie zatopiono w parafinie. Z przygotowanych bloczków parafinowych wykonano skrawki o grubości 5 μm, które poddano barwieniu hematoksyliną i eozyną (H+E). Ocenę histologiczną przeprowadzono przy użyciu mikroskopu świetlnego Nikon Eclipse E600 wyposażonego w kamerę cyfrową Nikon DS-Fi1 oraz system analizy obrazu NIS-Elements BR-2.20.

7) Wyniki odchowu indyków

W trakcie doświadczenia oceniano parametry produkcyjne, w tym masę ciała ptaków, przyrosty masy ciała, spożycie paszy oraz współczynnik jej wykorzystania i śmiertelność. Pomiary wykonywano w ustalonych terminach, zgodnie z przyjętym schematem doświadczenia.

8) Analiza poziomu antybiotyków i kokcydiostatyku - monenzyny

W 7. i 56. dniu życia od 7 ptaków z każdej grupy, po jednym ptaku z każdego powtórzenia, pobrano próbki krwi oraz wątroby. W próbkach wątroby indyków oznaczono poziom pozostałości antybiotyków (enrofloksacyny i doksycykliny) oraz kokcydiostatyku (monenzyny). Analizę przeprowadzono metodą wysokosprawnej chromatografii cieczowej sprzężonej z tandemową spektrometrią mas (LC-MS/MS) z wykorzystaniem systemu ExionLC AD (Sciex, USA) połączonego ze spektrometrem mas QTRAP 6500+ (Sciex, USA).

Próbki tkanek poddano odpowiedniemu przygotowaniu obejmującemu homogenizację oraz ekstrakcję analitów, a następnie oczyszczanie uzyskanych ekstraktów. Rozdział chromatograficzny przeprowadzono przy użyciu chromatografu cieczowego sprzężonego ze spektrometrem mas pracującym w trybie monitorowania wybranych reakcji fragmentacji (MRM). Identyfikację oraz ilościowe oznaczenie badanych związków przeprowadzono na podstawie porównania czasów retencji oraz charakterystycznych przejść jonowych z odpowiednimi standardami analitycznymi. Uzyskane wyniki odniesiono do obowiązujących maksymalnych poziomów pozostałości (MRL), co umożliwiło ocenę bezpieczeństwa stosowania badanych substancji w produkcji drobiarskiej.

3.2 Doświadczenie 2

Szczegółowy opis metodologiczny doświadczenia przedstawiono w publikacji Smagieł i in, 2026 (Annals of Animal Science)

Układ doświadczenia miał charakter dwuczynnikowy (antybiotyk × monenzyna), co umożliwiło ocenę efektu addytywnego oraz interakcji między tymi czynnikami.

Układ doświadczenia

Antybiotyk / Monenzyna	Control C	Enrofloksacyna E	Doksycyklina D
$M_{(-)}$	$CM_{(-)}$	$EM_{(-)}$	$DM_{(-)}$
$M_{(+)}$	$CM_{(+)}$	$EM_{(+)}$	$DM_{(+)}$

$M_{(-)}$ brak monenzyny, $M_{(+)}$ monenzyna

W doświadczeniu wykorzystano 1152 jednodniowe indyczki Hybrid Converter Novo rozmieszczone losowo do 6 grup doświadczalnych utworzonych w układzie czynnikowym 3 × 2 (antybiotyk × monenzyna). Każda grupa składała się z 8 powtórzeń po 24 ptaki.

Wszystkie ptaki szczepiono przeciwko aMPV i NDV w 1. dniu życia metodą „grubej kropli" w oprysku oraz przeciwko ORT w 28. dniu życia drogą iniekcji podskórnej.
W grupach ENR podawano enrofloksacynę (10 mg/kg m.c.) przez pierwszych 5 dni życia, natomiast w grupach DOX doksycyklinę (50 mg/kg m.c.) przez pierwszych 5 dni życia. Antybiotyki podawano w wodzie do picia. W grupach „+" stosowano monenzynę w ilości 90 mg/kg mieszanki paszowej.

Ocena wybranych reakcji biologicznych indyków w doświadczeniu 2 obejmowała:

1) Ocena resorpcji woreczka żółtkowego i transferu odporności biernej

W 1., 3. i 5. dniu życia oceniano tempo resorpcji woreczka żółtkowego na podstawie stosunku jego masy do masy ciała ptaków zgodnie z metodą Chamblee i in. (1992).
Jednocześnie oceniano efektywność transferu odporności matczynej z woreczka żółtkowego do organizmu piskląt poprzez oznaczenie: całkowitego poziomu immunoglobulin IgY i IgM, poziomu swoistych przeciwciał przeciwko: avian metapneumovirus (aMPV), Newcastle disease virus (NDV), Ornithobacterium rhinotracheale (ORT). Analizy prowadzono zarówno w zawartości woreczka żółtkowego, jak i w surowicy krwi indyków.

2) Ocena odpowiedzi immunologicznej organizmu

W 7. i 56. dniu życia analizowano funkcjonowanie układu immunologicznego ptaków, obejmujące ocenę odporności humoralnej i komórkowej oraz wybranych markerów odpowiedzi immunologicznej metodą immunoenzymatyczną (ELISA).

W osoczu krwi oznaczano poziom: immunoglobulin IgY, IgM i IgA, wybranych cytokin pro- i przeciwzapalnych, markerów aktywacji układu immunologicznego. Analizie poddano między innymi: IL-1β, IL-2, IL-4, IL-6, IL-8, IL-12, IL-13, TNF-α, IFN-γ, TLR-4, NF-κB, CRP, z wykorzystaniem komercyjnych zestawów firm MyBioSource i Qayee-Bio zgodnie z instrukcją producenta. Dodatkowo oznaczano poziom przeciwciał poszczepiennych przeciwko aMPV, NDV i ORT, przy użyciu zestawów firmy IDEXX.

3) Immunofenotypowanie komórek immunokompetentnych

W celu oceny wpływu zastosowanych czynników na dojrzewanie i funkcjonowanie komórek immunokompetentnych wykonywano analizę immunofenotypową komórek jednojądrzastych izolowanych z krwi oraz śledziony. Przy użyciu cytometrii przepływowej oznaczano udział procentowy: limfocytów CD4+, limfocytów CD8+, limfocytów CD4+CD8+, komórek IgM+.

Analizy te umożliwiły ocenę zmian w odpowiedzi komórkowej indukowanych przez antybiotyki, monenzynę oraz ich wzajemne oddziaływanie.

4) Ocena ekspresji genów związanych z odpowiedzią immunologiczną

W wybranych punktach czasowych oceniano również ekspresję genów związanych z regulacją odpowiedzi immunologicznej i procesów zapalnych. Ocenie poddano ekspresję genów IL-6, IFN-γ oraz IgY, związanych odpowiednio z regulacją procesów zapalnych, aktywacją odpowiedzi komórkowej oraz odpowiedzią humoralną. Analizy prowadzono metodą Real-Time PCR po wcześniejszej izolacji całkowitego RNA z krwi i syntezie cDNA.

5) Wyniki odchowu indyków

W trakcie doświadczenia oceniano parametry produkcyjne, w tym masę ciała ptaków, przyrosty masy ciała, spożycie paszy oraz współczynnik jej wykorzystania i śmiertelność. Pomiary wykonywano w ustalonych terminach, zgodnie z przyjętym schematem doświadczenia.

3.3 Analiza statystyczna

Analizę statystyczną wyników przeprowadzono przy użyciu programu STATISTICA 13 (StatSoft Inc., Tulsa, OK, USA). Dane poddano analizie wariancji (ANOVA), stosując modele statystyczne odpowiednie dla schematu doświadczenia wykorzystanego w poszczególnych publikacjach. W większości analiz zastosowano model dwuczynnikowy uwzględniający wpływ zastosowanego antybiotyku (brak antybiotyku, enrofloksacyna, doksycyklina), obecności monenzyny w paszy oraz interakcji pomiędzy tymi czynnikami. W analizach dotyczących transferu odporności biernej i odpowiedzi poszczepiennej uwzględniano natomiast wpływ zastosowanego antybiotyku oraz wieku ptaków lub terminu pobrania próbek. Istotność różnic pomiędzy średnimi oceniano testem Tukeya przy poziomie istotności $P \leq 0,05$. Wyniki przedstawiono jako średnie ± SEM. W analizie parametrów produkcyjnych pojedynczy kojec stanowił jednostkę doświadczalną, natomiast w przypadku analiz biochemicznych, immunologicznych, molekularnych oraz histologicznych jednostkę doświadczalną stanowił pojedynczy ptak.

4. Omówienie wyników i dyskusja

Uzyskane wyniki badań z doświadczenia 1 wykazały, że badane substancje nie hamowały resorpcji woreczka żółtkowego, jednak obniżały miano przeciwciał matczynych u kilkudniowych indyków. ENR i DOX dodatkowo hamowały endogenną (poszczepienną) syntezę swoistych przeciwciał (Ognik i in, 2025). Wyniki opublikowane w Ognik i in, (2025) wskazują, że wczesna antybiotykoterapia nie zaburza fizjologicznego procesu resorpcji woreczka żółtkowego, jednak istotnie ogranicza efektywność transferu i wykorzystania odporności biernej. Sugeruje to, że kluczowym mechanizmem odpowiedzialnym za obniżenie poziomu przeciwciał nie jest upośledzenie procesu absorpcji składników z woreczka żółtkowego, lecz bezpośredni wpływ antybiotyków na funkcjonowanie układu immunologicznego. Antybiotyki mogą modulować aktywność limfocytów B oraz zaburzać proces różnicowania komórek plazmatycznych, prowadząc do ograniczenia syntezy immunoglobulin i osłabienia odpowiedzi humoralnej. Mechanizm ten może częściowo tłumaczyć obserwowane obniżenie poziomu IgY i IgM oraz zmniejszoną skuteczność odpowiedzi poszczepiennej. Dodatkowo, zgodnie z doniesieniami literaturowymi (Khalifehi in, 2009; Pomorska-Mól i Pejsak, 2012), antybiotyki mogą hamować odpowiedź poszczepienną poprzez ograniczenie proliferacji komórek immunokompetentnych oraz zaburzenie komunikacji cytokinowej, co znajduje odzwierciedlenie w uzyskanych wynikach.

Warto podkreślić, że obniżenie poziomu przeciwciał matczynych może mieć szczególnie istotne znaczenie w pierwszych dniach życia indyków, kiedy własny układ odpornościowy ptaków nie jest jeszcze w pełni rozwinięty (Noy i Sklan, 1999; Rodrigues i in., 2021)

W tym okresie odporność bierna stanowi podstawowy mechanizm ochrony przed infekcjami środowiskowymi. Ograniczenie transferu IgY i IgM może zatem zwiększać podatność młodych ptaków na zakażenia oraz osłabiać skuteczność pierwszych szczepień ochronnych. Uzyskane wyniki sugerują również, że nawet krótkotrwała ekspozycja na antybiotyki w krytycznym okresie rozwoju immunologicznego może wywoływać długofalowe konsekwencje dla dojrzewania odpowiedzi humoralnej. W badaniach Ognik i in. (2025) wykazano ponadto, że obniżenie poziomu przeciwciał dotyczyło zarówno odporności swoistej wobec aMPV, NDV i ORT, jak i całkowitej puli immunoglobulin, co wskazuje na szeroki zakres oddziaływania zastosowanych antybiotyków na układ odpornościowy.

Dodatkowe badania wskazują, że prawidłowy transfer przeciwciał matczynych zależy nie tylko od efektywnej resorpcji woreczka żółtkowego, ale również od integralności rozwijającego się układu immunologicznego piskląt (Noy i Sklan, 1999; Wang i in., 2020). Zaburzenia równowagi mikrobiologicznej wywołane antybiotykoterapią mogą dodatkowo ograniczać dojrzewanie odporności humoralnej i osłabiać odpowiedź poszczepienną (Schokker i in., 2017; Rodrigues i in., 2021). Podobne zależności pomiędzy wczesną antybiotykoterapią a osłabieniem odpowiedzi humoralnej obserwowali również Jankowski i in. (2022) oraz Khalifeh i in. (2009).

W doświadczeniu 1 stwierdzono, że w wątrobie indyków ubijanych 56. dnia życia, stężenie ENR i DOX było niższe od MRL (Maximum Residue Level), natomiast MON ponad trzykrotnie wyższe od MRL. Jednak kokcydiostatyk ten nie nasilał reakcji oksydacyjnych. Stwierdzono, że DOX i ENR nasilały reakcje oksydacyjne szczególnie widoczne w krótkim okresie po zakończeniu ich podawania. Niekorzystny efekt indukowania reakcji oksydacyjnych przez ENR może utrzymywać się znacznie dłużej, nawet do 8. tygodnia życia. W przypadku DOX, w okresie spadku jej poziomu w organizmie, odnotowano korzystną stymulację układu antyoksydacyjnego, niwelująco wcześniejsze reakcje oksydacyjne indukowane przez ten antybiotyk. Szczepienie indyków może zmniejszyć reakcje oksydacyjne i apoptozę w organizmie (Smagieł i in., 2024). Uzyskane wyniki potwierdzają, że wczesna ekspozycja na antybiotyki może prowadzić do zaburzeń równowagi oksydoredukcyjnej (Smagieł i in., 2024). Mechanizm tego zjawiska może być związany z nasileniem produkcji reaktywnych form tlenu (ROS) w wyniku oddziaływania antybiotyków na procesy mitochondrialne oraz metabolizm komórkowy. Szczególnie istotne jest długotrwałe działanie enrofloksacyny, które wskazuje na możliwość trwałego przeprogramowania odpowiedzi oksydacyjnej organizmu. Z kolei obserwowany w późniejszym okresie efekt adaptacyjny w przypadku doksycykliny może wynikać z aktywacji endogennych mechanizmów obronnych, takich jak zwiększona aktywność enzymów antyoksydacyjnych. Jedną z obserwacji był brak zależności pomiędzy wysokim poziomem pozostałości monenzyny w wątrobie a nasileniem procesów oksydacyjnych. Może to wskazywać, że potencjalna toksyczność tej substancji nie jest bezpośrednio związana z indukcją stresu oksydacyjnego, lecz wynika z innych mechanizmów oddziaływania na komórki gospodarza. Ponadto, zmniejszenie reakcji oksydacyjnych po szczepieniu może być związane z aktywacją mechanizmów immunoregulacyjnych ograniczających nadmierną odpowiedź zapalną.

Uzyskane wyniki wskazują również, że organizm młodych indyków wykazuje zróżnicowaną zdolność adaptacji do działania poszczególnych substancji przeciwdrobnoustrojowych. W przypadku enrofloksacyny obserwowano bardziej nasilone i długotrwałe zaburzenia parametrów redoks, co może świadczyć o większym obciążeniu mechanizmów detoksykacyjnych wątroby. Natomiast doksycyklina, mimo początkowego nasilenia reakcji oksydacyjnych, wydaje się uruchamiać procesy kompensacyjne związane ze wzrostem aktywności układu antyoksydacyjnego. Może to sugerować, że poszczególne antybiotyki różnią się nie tylko siłą działania przeciwdrobnoustrojowego, ale również wpływem na homeostazę komórkową i zdolność organizmu do adaptacji metabolicznej. Istotne znaczenie może mieć także fakt, że stres oksydacyjny i aktywacja procesów apoptycznych są ściśle związane z funkcjonowaniem układu odpornościowego, dlatego obserwowane zaburzenia redoks mogą częściowo tłumaczyć równoczesne zmiany immunologiczne opisane w pozostałych publikacjach cyklu.

W literaturze podkreśla się, że fluorochinolony mogą indukować stres oksydacyjny poprzez zaburzenie funkcjonowania mitochondriów oraz nasilenie produkcji reaktywnych form tlenu (ROS), co może prowadzić do uszkodzeń lipidów, białek i DNA (Simon i in., 2016; Roh i Sohn, 2018). Z kolei aktywacja mechanizmów antyoksydacyjnych po ekspozycji na doksycyklinę może stanowić element odpowiedzi adaptacyjnej organizmu na wcześniejsze zaburzenia homeostazy komórkowej. Ścisły związek pomiędzy stresem oksydacyjnym a funkcjonowaniem układu odpornościowego opisują również Jarosz i in. (2018) oraz Broom i Kogut (2018).

W doświadczeniu 1 stwierdzono, że wczesne podanie ENR lub DOX indykom spowodowało spadek aktywności zewnątrzkomórkowej wybranych enzymów bakteryjnych w treści jelita ślepego ptaków w wieku 56 dni. Efektu tego nie odnotowano u ptaków otrzymujących MON, pomimo faktu, że kokcydiostatyk podawano przez cały okres doświadczenia (1–56 dni). Co ciekawe, powyższe zmiany aktywności enzymatycznej mikrobioty nie wpłynęły na stężenie SCFA w jelicie ślepym. Dieta zawierająca MON i wczesne podanie ENR lub DOX indukowały wzrost poziomu noradrenaliny we krwi u indyków. DOX powodowała wzrost stężenia kortyzolu w osoczu i spadek poziomu serotoniny w osoczu, co wskazuje na indukcję stresu u młodych ptaków, najprawdopodobniej z powodu zmian w funkcjonowaniu jelita grubego (Mikulski i in., 2022). Wyniki te wskazują, że wczesna antybiotykoterapia prowadzi do długotrwałych zmian w funkcjonowaniu mikrobioty jelitowej, co potwierdzają obserwacje ujęte w publikacji Mikulski i in., (2022). Spadek aktywności enzymatycznej bakterii przy braku

zmian w poziomie SCFA sugeruje, że dochodzi do zmiany struktury funkcjonalnej mikrobioty, a nie całkowitego zahamowania jej aktywności metabolicznej. Antybiotyki mogą selektywnie eliminować określone grupy mikroorganizmów, co prowadzi do zaburzenia równowagi mikrobiologicznej (dysbiozy). Zmiany te mogą mieć bezpośredni wpływ na funkcjonowanie osi jelito–mózg, czego potwierdzeniem są obserwowane zmiany w poziomie hormonów stresu. Wzrost poziomu noradrenaliny i kortyzolu oraz spadek serotoniny wskazują na aktywację odpowiedzi stresowej, która może być wynikiem zaburzeń mikrobioty jelitowej i zmienionej produkcji metabolitów bakteryjnych oddziałujących na układ nerwowy.

Pomimo zmian w aktywności enzymatycznej mikrobioty nie odnotowano istotnych zmian w poziomie krótkołańcuchowych kwasów tłuszczowych. Może to świadczyć o uruchomieniu mechanizmów kompensacyjnych przez pozostałe populacje bakterii jelitowych lub o utrzymaniu podstawowej aktywności fermentacyjnej mimo zmian jakościowych mikrobioty. Jednocześnie obserwowane zaburzenia hormonalne potwierdzają, że nawet subtelne zmiany funkcjonalne w obrębie jelit mogą wpływać na regulację neuroendokrynną organizmu. Wyniki te wpisują się w koncepcję osi mikrobiota–jelito–mózg, zgodnie z którą mikroorganizmy jelitowe mogą modulować aktywność układu nerwowego i hormonalnego poprzez produkcję metabolitów, wpływ na integralność bariery jelitowej oraz aktywację szlaków immunologicznych. Długotrwałe konsekwencje takich zmian mogą obejmować nie tylko zaburzenia odporności, ale również pogorszenie zdolności adaptacyjnych organizmu do czynników stresowych.

Wyniki te są zgodne z doniesieniami wskazującymi, że nawet krótkotrwała antybiotykoterapia we wczesnym okresie życia może prowadzić do długotrwałych zmian w składzie i aktywności mikrobioty jelitowej (Schokker i in., 2017; Simon i in., 2016). Zaburzenia mikrobioty mogą wpływać na funkcjonowanie osi jelito–mózg poprzez modulację produkcji metabolitów bakteryjnych oraz aktywację szlaków immunologicznych i neuroendokrynnych (Broom i Kogut, 2018; Rodrigues i in., 2021). Znaczenie mikrobioty jelitowej w regulacji dojrzewania układu odpornościowego ptaków podkreślają również Zenner i in. (2021)

Z przeprowadzonych badań w doświadczeniu 1 wynika, że antybiotyki podawane ptakom przez pierwsze 5 dni życia w wodzie hamowały odpowiedź immunologiczną u indyków zaszczepionych przeciwko ND i TRT w pierwszym dniu życia. Stosowanie MON w paszy było najmniej skuteczne w hamowaniu reakcji zapalnych po szczepieniu. Zmiany histopatologiczne w narządach układu odpornościowego (zwyrodnienie tłuszczowe) były

również największe u ptaków otrzymujących MON, a następnie u traktowanych DOX i ENR (Smagieł i in, 2023). Uzyskane wyniki wskazują, że wczesna antybiotykoterapia może prowadzić do zaburzeń dojrzewania układu odpornościowego oraz osłabienia odpowiedzi poszczepiennej, co wykazano w publikacji Smagieł i in, (2023). Mechanizm tego zjawiska może być związany zarówno z bezpośrednim działaniem antybiotyków na komórki układu odpornościowego, jak i pośrednio poprzez wpływ na mikrobiotę jelitową, która odgrywa kluczową rolę w kształtowaniu odpowiedzi immunologicznej. Szczególnie interesujący jest fakt, że monenzyna, mimo słabszego wpływu na odpowiedź zapalną, powodowała najpoważniejsze zmiany histopatologiczne w narządach limfatycznych. Może to wskazywać na jej długotrwały wpływ na strukturę i funkcję tych narządów, co w konsekwencji może prowadzić do trwałego osłabienia odporności.

Zmiany histopatologiczne obserwowane w śledzionie, grasicy i kaletce Fabrycjusza mogą świadczyć o przewlekłym obciążeniu układu odpornościowego i zaburzeniu procesów dojrzewania komórek immunokompetentnych. Szczególnie niekorzystne wydaje się występowanie zwyrodnienia tłuszczowego w narządach limfatycznych, które może ograniczać ich prawidłowe funkcjonowanie oraz zdolność do efektywnej odpowiedzi na antygeny. Warto również podkreślić, że nasilenie zmian po szczepieniu wskazuje na dodatkowe obciążenie organizmu związane z aktywacją odpowiedzi immunologicznej w warunkach wcześniejszej ekspozycji na substancje przeciwdrobnoustrojowe. Uzyskane wyniki sugerują więc, że nawet krótkotrwała antybiotykoterapia zastosowana w krytycznym okresie rozwoju może wywoływać trwałe konsekwencje strukturalne i funkcjonalne dla układu odpornościowego ptaków.

Według Lee i in. (2012) oraz Wisselink i in. (2017), zarówno antybiotyki, jak i jonoforowe kokcydiostatyki mogą modulować populacje limfocytów oraz ekspresję cytokin, wpływając na dojrzewanie układu odpornościowego ptaków. Dodatkowo zaburzenia mikrobioty jelitowej indukowane przez substancje przeciwdrobnoustrojowe mogą prowadzić do przewlekłej aktywacji odpowiedzi zapalnej oraz zmian strukturalnych w narządach limfatycznych (Broom i Kogut, 2018; Rodrigues i in., 2021). Podobne zależności pomiędzy dysbiozą jelitową a zaburzeniami dojrzewania odporności opisywali również Rubio (2019) oraz Zenner i in. (2021).

Wyniki przeprowadzonych badań w ramach doświadczenia 2 wskazują, że podanie ENR indykom w pierwszych 5 dniach życia i żywienie ich dietą zawierającą MON skutkowało silnymi reakcjami układu odpornościowego, wskazującymi na immunosupresję. Efektu immunosupresyjnego nie obserwowano u indyków otrzymujących wyłącznie MON

lub MON i DOX. Wczesne podanie antybiotyków, niezależnie od stosowania MON w paszy nie miało wpływu na wyniki odchowu indyków (Smagieł i in, 2026). Uzyskane wyniki wskazują na istotną rolę interakcji pomiędzy antybiotykami a kokcydiostatykami w modulacji odpowiedzi immunologicznej. Szczególnie silny efekt immunosupresyjny obserwowany w przypadku jednoczesnego stosowania enrofloksacyny i monenzyny sugeruje działanie addytywne lub synergistyczne tych substancji. Może to wynikać z nakładania się ich wpływu na mikrobiotę jelitową, metabolizm komórkowy oraz mechanizmy regulujące odpowiedź immunologiczną. Brak takiego efektu w przypadku doksycykliny może wskazywać na jej odmienny mechanizm działania oraz mniejszy wpływ na kluczowe szlaki regulujące odporność. Jednocześnie brak wpływu na wyniki odchowu sugeruje, że zmiany immunologiczne mogą nie przekładać się bezpośrednio na parametry produkcyjne w krótkim okresie, jednak mogą mieć znaczenie długofalowe.

W piśmiennictwie podkreśla się, że równoczesna ekspozycja na kilka substancji przeciwdrobnoustrojowych może prowadzić do silniejszego zaburzenia dojrzewania odpowiedzi immunologicznej niż działanie pojedynczych preparatów (Lee i in., 2012; Tykałowski i in., 2025). Szczególnie istotną rolę może odgrywać tutaj wpływ na populacje limfocytów T oraz cytokiny regulujące odpowiedź komórkową i humoralną. Zgodnie z obserwacjami Lee i in. (2018), zmiany udziału populacji CD4+CD8+ mogą wskazywać na zaburzenia dojrzewania limfocytów oraz przewlekłą aktywację układu odpornościowego.

Uzyskane rezultaty podkreślają również znaczenie właściwej oceny zasadności stosowania antybiotykoterapii w pierwszych dniach życia ptaków, szczególnie w sytuacji jednoczesnego podawania kokcydiostatyków. Mimo braku wyraźnych zmian w parametrach produkcyjnych, obserwowane zaburzenia immunologiczne mogą zwiększać podatność ptaków na infekcje w późniejszym okresie życia oraz wpływać na skuteczność profilaktyki szczepiennej. Wyniki doświadczenia 2 wskazują ponadto, że skutki działania substancji przeciwdrobnoustrojowych mogą być zależne od rodzaju zastosowanego antybiotyku oraz obecności dodatkowych czynników modulujących, takich jak monenzyna. Potwierdza to konieczność ostrożnego stosowania preparatów przeciwdrobnoustrojowych w intensywnym chowie drobiu oraz potrzebę dalszych badań nad ich długofalowym wpływem na rozwój i funkcjonowanie układu odpornościowego indyków. Łącznie wyniki wszystkich doświadczeń wskazują, że konsekwencje wczesnej ekspozycji na antybiotyki i kokcydiostatyki wykraczają poza okres ich podawania i obejmują długotrwałe zmiany w funkcjonowaniu mikrobioty jelitowej, statusie oksydacyjno-redukcyjnym oraz dojrzewaniu układu odpornościowego indyków.

5. Wnioski

1. Badane środki przeciwdrobnoustrojowe nie hamowały resorpcji woreczka żółtkowego, jednak obniżały transfer przeciwciał matczynych z woreczka żółtkowego do organizmu piskląt.
2. Enrofloksacyna (ENR) i doksycyklina (DOX) dodatkowo hamowały endogenną (w tym poszczepienną) syntezę swoistych przeciwciał.
3. Uzyskane wyniki wskazują, że zarówno wczesne podanie antybiotyków, jak i żywienie dietą z kokcydiostatykiem mogą niekorzystnie wpływać na rozwój i funkcjonowanie układu immunologicznego oraz antyoksydacyjnego młodych indyków, a ich stosowanie w pierwszych dniach życia powinno być rozważane wyłącznie w sytuacjach klinicznego uzasadnienia.
4. Wczesne podanie enrofloksacyny i doksycykliny indukowało przejściowe, a w przypadku enrofloksacyny także długotrwałe nasilenie stresu oksydacyjnego, co wskazuje, że krótkotrwała ekspozycja na antybiotyki w okresie postnatalnym może prowadzić do trwałej modulacji równowagi oksydacyjno-antyoksydacyjnej organizmu.
5. Interakcja pomiędzy wczesną antybiotykoterapią a żywieniem dietą zawierającą monenzynę może nasilać efekt immunomodulacyjny, prowadząc do subklinicznej immunosupresji, która nie znajduje odzwierciedlenia w standardowych parametrach produkcyjnych, lecz może zwiększać podatność ptaków na czynniki infekcyjne w późniejszym okresie odchowu.

Bibliografia

1. Ahmed A.S. 2015. Assessment of maternal immunity against Newcastle Disease in offspring chicks as affected by parents genetic crossing. *Asian Journal of Animal and Veterinary Advances* 10: 35–42. https://doi.org/10.3923/ajava.2015.35.42
2. Al-Natour M.Q., Ward L.A., Saif Y.M., Stewart-Brown B., Keck L.D. 2004. Effect of different levels of maternally derived antibodies on protection against infectious bursal disease virus. *Avian Diseases* 48: 177–182. https://doi.org/10.1637/5319
3. Bode C., Diedrich B., Muenster S., Hentschel V., Weisheit C., Rommelsheim K., Hoeft A., Meyer R., Boehm O., Knuefermann P., Baumgarten G. 2014. Antibiotics regulate the immune response in both presence and absence of lipopolysaccharide through modulation of Toll-like receptors, cytokine production and phagocytosis in vitro. *International Immunopharmacology* 18: 27–34. https://doi.org/10.1016/j.intimp.2013.10.025
4. Broom L.J., Kogut M.H. 2018. The role of the gut microbiome in shaping the immune system of chickens. *Veterinary Immunology and Immunopathology* 204: 44–51. https://doi.org/10.1016/j.vetimm.2018.10.002
5. Chapman H.D. 2008. Coccidiosis in the turkey. *Avian Pathology* 37: 205–210. https://doi.org/10.1080/03079450802050689
6. Chrząstek K., Woźniak A., Wieliczko A. 2011. Maternal antibodies in young birds. *Medycyna Weterynaryjna* 67: 250–252.
7. Chrząstek K., Wieliczko A. 2015. The influence of enrofloxacin, florfenicol, ceftiofur and E. coli LPS interaction on T and B cells subset in chicks. *Veterinary Research Communications* 39: 53–60. https://doi.org/10.1007/s11259-015-9632-7
8. Chamblee T.N., Brake J.D., Schultz C.D., Thaxton J.P. 1992. Yolk sac absorption and initiation of growth in broilers. *Poultry Science* 71: 1811–1816. https://doi.org/10.3382/ps.0711811
9. Elamaran A., Hariharan P., Ramesh S., Vairamuthu S. 2015. Enrofloxacin induced oxidative stress and its amelioration with antioxidants in liver homogenates of broilers. *Indian Journal of Veterinary and Animal Sciences Research* 44: 124–128. https://doi.org/10.56093/ijvasr.v44i2.173623
10. Elokil A.A., Abouelezz K.F.M., Ahmad H.I., Pan Y., Li S. 2020. Investigation of the impacts of antibiotic exposure on the diversity of the gut microbiota in chicks. *Animals* 10: 896. https://doi.org/10.3390/ani10050896

11. Garmyn A., Martel A., Froyman R., Nauwynck H., Duchateau L., Haesebrouck F., Pasmans F. 2009. Efficacy of four enrofloxacin treatment regimens against experimental infection in turkey poults with avian pneumovirus and Ornithobacterium rhinotracheale. *Avian Pathology* 38: 287–292. https://doi.org/10.1080/03079450903055413
12. Gbylik-Sikorska M., Posyniak A., Śniegocki T., Sell B., Gajda A., Tomczyk G., Żmudzki J. 2016. Effect of doxycycline concentrations in chicken tissues as a consequence of permanent exposure to enrofloxacin traces in drinking water. *Journal of Veterinary Research* 60: 233–238. https://doi.org/10.1515/jvetres-2016-0045
13. Gharaibeh S., Mahmoud K., Al-Natour M. 2008. Field evaluation of maternal antibody transfer to a group of pathogens in meat-type chickens. *Poultry Science* 87: 1550–1555. https://doi.org/10.3382/ps.2008-00119
14. Grindstaff J.L., Brodie E.D. 3rd, Ketterson E.D. 2003. Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. *Proceedings of the Royal Society B: Biological Sciences* 270: 2309–2319. https://doi.org/10.1098/rspb.2003.2485
15. Hamal K.R., Burgess S.C., Pevzner I.Y., Erf G.F. 2006. Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. *Poultry Science* 85: 1364–1372. https://doi.org/10.1093/ps/85.8.1364
16. Hassanin O., Abdallah F., Awad A. 2014. Effects of florfenicol on the immune responses and the interferon-inducible genes in broiler chickens under the impact of E. coli infection. *Veterinary Research Communications* 38: 51–58. https://doi.org/10.1007/s11259-013-9585-7
17. Hruba H., Abdelsalam E.E.E., Anisimov N., Bandouchova H., Havelkova B., Heger T., Kanova M., Kovacova V., Nemcova M., Piacek V., Sedlackova J., Vitula F., Pikula J. 2019. Reproductive toxicity of fluoroquinolones in birds. *BMC Veterinary Research* 15: 209. https://doi.org/10.1186/s12917-019-1957-y
18. Ibrahim I.G., Atmaca N., Kanici A., Yarsan E. 2011. Evaluation of effects of enrofloxacin on some haematological parameters in broilers. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi* 6: 97–102. https://izlik.org/JA59HD83BC
19. Jamroz D., Wertelecki T., Orda J., Skorupińska J. 2004. Dynamics of yolk sac resorption and post-hatching development of the gastrointestinal tract in chickens, ducks and geese. *Journal of Animal Physiology and Animal Nutrition* 88: 239–250. https://doi.org/10.1111/j.1439-0396.2004.00481.x

20. Jarosz Ł.S., Marek A., Grądzki Z., Kwiecień M., Kaczmarek B. 2018. The effect of feed supplementation with a copper-glycine chelate and copper sulphate on selected humoral and cell-mediated immune parameters, plasma superoxide dismutase activity, ceruloplasmin and cytokine concentration in broiler chickens. *Journal of Animal Physiology and Animal Nutrition* 102: e326–e336. https://doi.org/10.1111/jpn.12750
21. Jankowski J., Tykałowski B., Stępniowska A., Konieczka P., Koncicki A., Matusevičius P., Ognik K. 2022. Immune parameters in chickens treated with antibiotics and probiotics during early life. *Animals* 12: 1133. https://doi.org/10.3390/ani12091133
22. Kadykalo S., Roberts T., Thompson M., Wilson J., Lang M., Espeisse O. 2018. The value of anticoccidials for sustainable global poultry production. *International Journal of Antimicrobial Agents* 51: 304–310. https://doi.org/10.1016/j.ijantimicag.2017.09.004
23. Kang J., Hossain M.A., Park H.C., Kim Y., Lee K.J., Park S.W. 2019. Pharmacokinetic and pharmacodynamic integration of enrofloxacin against Salmonella Enteritidis after administering to broiler chicken by per-oral and intravenous routes. *Journal of Veterinary Science* 20: e15. https://doi.org/10.4142/jvs.2019.20.e15
24. Kaspers B., Schranner I., Lösch U. 1991. Distribution of immunoglobulins during embryogenesis in the chicken. *Zentralblatt für Veterinärmedizin A* 38: 73–79. https://doi.org/10.1111/j.1439-0442.1991.tb00986.x
25. Khalifeh M.S., Amawi M.M., Abu-Basha E.A., Yonis I.B. 2009. Assessment of humoral and cellular-mediated immune response in chickens treated with tilmicosin, florfenicol, or enrofloxacin at the time of Newcastle disease vaccination. *Poultry Science* 88: 2118–2124. https://doi.org/10.3382/ps.2009-00215
26. Kowalczyk J., Smiałek M., Tykałowski B., Dziewulska D., Stenzel T., Koncicki A. 2019. Field evaluation of maternal antibody transfer from breeder turkey hens to egg yolks, egg whites, and poults. *Poultry Science* 98: 3150–3157. https://doi.org/10.3382/ps/pez126
27. Lee I.K., Gu M.J., Ko K.H., Bae S., Kim G., Jin G.D., Kim E.B., Kong Y.Y., Park T.S., Park B.C., Jung H.J., Han S.H., Yun C.H. 2018. Regulation of CD4+CD8−CD25+ and CD4+CD8+CD25+ T cells by gut microbiota in chicken. *Scientific Reports* 8: 8627. https://doi.org/10.1038/s41598-018-26763-0
28. Lee K.W., Lillehoj H.S., Lee S.H., Jang S.I., Park M.S., Bautista D.A., Ritter G.D., Hong Y.H., Siragusa G.R., Lillehoj E.P. 2012. Effect of dietary antimicrobials on immune status in broiler chickens. *Asian-Australasian Journal of Animal Sciences* 25: 382–392. https://doi.org/10.5713/ajas.2011.11259

29. Madadi M.S., Bojmehrani H., Azari M. 2014. Evaluation of drug interactions and prescription errors of poultry veterinarians in North of Iran. *Poultry Science Journal* 2: 25–35.
30. Madubuike K.G., Okoroafor O.N., Asuzu I.U. 2020. Effects of early-life antibiotics administration on the immune response to Newcastle disease Lasota vaccination and weight indices of broiler chicken. *Folia Veterinaria* 64: 74–79. https://doi.org/10.2478/fv-2020-0020
31. Marchetti M.L., Buchamer A., Buldain D., Mestorino N. 2019. Enrofloxacin and ciprofloxacin residues in broiler chicken feathers after enrofloxacin oral administration. *EC Veterinary Science* 4: 180–186.
32. Maślanka T., Jaroszewski J.J., Mikołajczyk A., Rotkiewicz T. 2009. Effect of increasing doses of enrofloxacin on chicken articular cartilage. *Polish Journal of Veterinary Sciences* 12: 21–33.
33. Mikulski D., Juśkiewicz J., Ognik K., Zduńczyk P., Smagieł R., Jankowski J. 2022. Gastrointestinal tract and neuroendocrine system responses of young turkeys to the early administration of antibiotics or feeding a diet containing a coccidiostat. *Poultry Science* 101: 102098.https://doi.org/10.1016/j.psj.2022.102098
34. Murai A. 2013. Maternal transfer of immunoglobulins into egg yolks of birds. *Journal of Poultry Science* 50: 185–193. https://doi.org/10.2141/jpsa.0120194
35. Murai A., Hamano T., Kakiuchi M., Kobayashi M., Horio F. 2020. Evaluation of a receptor gene responsible for maternal blood IgY transfer into egg yolks using bursectomized IgY-depleted chickens. *Poultry Science* 99: 1914–1920. https://doi.org/10.1016/j.psj.2019.11.045
36. Noy Y., Sklan D. 1999. Different types of early feeding and performance in chicks and poults. *Journal of Applied Poultry Research* 8: 16–24. https://doi.org/10.1093/japr/8.1.16
37. Ognik K., Tykałowski B., Mikulski D., Smagieł R., Cholewińska E., Koncicki A., Stępniowska A., Jankowski J. 2025. Early administration of antibiotics to turkey poults impairs maternal immunity and post-vaccination antibody synthesis. *Annals of Animal Science* 25, 1: 239-257.https://doi.org/10.2478/aoas-2024-0063
38. Pavlova A., Milanova A. 2017. Different effect of doxycycline and enrofloxacin on cathelicidin-3 mRNA expression in chickens with or without probiotics supplementation. *Bulgarian Journal of Veterinary Medicine* 20: 357–366. https://doi.org/10.15547/bjvm.1026

39. Pomorska-Mól M., Pejsak Z. 2012. Effects of antibiotics on acquired immunity in vivo – current state of knowledge. *Polish Journal of Veterinary Sciences* 15: 583–588. https://doi.org/10.2478/v10181-012-0089-0
40. Pomorska-Mól M., Czyżewska E., Kwit K., Rachubik J., Lipowski A., Pejsak Z. 2015. Immune response in pigs treated with therapeutic doses of enrofloxacin at the time of vaccination against Aujeszky's disease. *Research in Veterinary Science* 100: 68–74. https://doi.org/10.1016/j.rvsc.2015.04.003
41. Pomorska-Mól M., Kwit K., Wierzchosławski K., Dors A., Pejsak Z. 2016. The effects of amoxicillin, ceftiofur, doxycycline, tiamulin and tulathromycin on pig humoral immune response induced by erysipelas vaccination. *Veterinary Record* 178: 559. https://doi.org/10.1136/vr.103533
42. Rodrigues D.R., Wilson K.M., Bielke L.R. 2021. Proper immune response depends on early exposure to gut microbiota in broiler chicks. *Frontiers in Physiology* 12: 758183. https://doi.org/10.3389/fphys.2021.758183
43. Roh J.S., Sohn D.H. 2018. Damage-associated molecular patterns in inflammatory diseases. *Immune Network* 18: e27. https://doi.org/10.4110/in.2018.18.e27
44. Rose M.E., Orlans E. 1981. Immunoglobulins in the egg, embryo and young chick. *Developmental and Comparative Immunology* 5: 15–20. https://doi.org/10.1016/S0145-305X(81)80003-1
45. Rubio L.A. 2019. Possibilities of early life programming in broiler chickens via intestinal microbiota modulation. *Poultry Science* 98: 695–706. https://doi.org/10.3382/ps/pey416
46. Schokker D., Jansman A.J.M., Veninga G., De Bruin N., De Bree F.M., Bossers A., Rebel J.M.J., Smits M.A. 2017. Perturbation of microbiota in one-day old broiler chickens with antibiotic for 24 hours negatively affects intestinal immune development. *BMC Genomics* 18: 241. https://doi.org/10.1186/s12864-017-3625-6
47. Simon K., Verwoolde M.B., Zhang J., Smidt H., de Vries Reilingh G., Kemp B., Lammers A. 2016. Long-term effects of early life microbiota disturbance on adaptive immunity in laying hens. *Poultry Science* 95: 1543–1554. https://doi.org/10.3382/ps/pew088
48. Smagieł R., Ognik K., Cholewińska E., Stępniowska A., Listos P., Tykałowski B., Mikulski D., Koncicki A., Jankowski J. 2023. The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys. *Poultry Science* 102: 102876. https://doi.org/10.1016/j.psj.2023.102876

49. Smagieł R., Tutaj K., Cholewińska E., Sołek P., Mikulski D., Stępniowska A., Jankowski J., Ognik K. 2024. The effect of early administration of antibiotics or feeding a diet containing coccidiostats on the level of their accumulation in liver and the redox status of turkeys. *Animal* 18: 101321. https://doi.org/10.1016/j.animal.2024.101321
50. Smagieł R.,Ognik K., Cholewińska E.,Sołek P., Mikulski D., Tykałowski B., Jankowski J., Tutaj K. 2026. Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency? *Annals of Animal Science*. https://doi.org/10.2478/aoas-2025-0125
51. Sureshkumar V., Sarathchandra G., Ramesh J. 2013. Effect of enrofloxacin on zootechnical performance, behaviour and immunohistopathological response in broiler chicken. *Veterinary World* 6: 337–342. https://doi.org/10.5455/vetworld.2013.337-342
52. Tykałowski B., Kowalczyk J., Ognik K., Mikulski D., Koncicki A., Kozłowski K., Jankowski J. 2025. Early post-hatching effects of antibiotics and ionophore coccidiostat supplementation on immune parameters in turkeys. *Poultry Science* 104: 105782. https://doi.org/10.1016/j.psj.2025.105782
53. van der Wagt I., de Jong I.C., Mitchell M.A., Molenaar R., van den Brand H. 2020. A review on yolk sac utilization in poultry. *Poultry Science* 99: 2162–2175. https://doi.org/10.1016/j.psj.2019.11.041
54. Wang J.S., Hu H.J., Xu Y.B., Wang D.C., Jiang L., Li K.X., Wang Y.Y., Zhan X.A. 2020. Effects of posthatch feed deprivation on residual yolk absorption, macronutrients synthesis, and organ development in broiler chicks. *Poultry Science* 99: 5587–5597. https://doi.org/10.1016/j.psj.2020.08.032
55. Wisselink H.J., Cornelissen J.B.W.J., Mevius D.J., Smits M.A., Smidt H., Rebel J.M. 2017. Antibiotics in 16-day-old broilers temporarily affect microbial and immune parameters in the gut. *Poultry Science* 96: 3068–3078. https://doi.org/10.3382/ps/pex133
56. Zenner C., Hitch T.C.A., Riedel T., Wortmann E., Tiede S., Buhl E.M., Abt B., Neuhaus K., Velge P., Overmann J., Kaspers B., Clavel T. 2021. Early-life immune system maturation in chickens using a synthetic community of cultured gut bacteria. *mSystems* 6: e01300-20. https://doi.org/10.1128/mSystems.01300-20

Kopie opublikowanych prac wchodzących w skład cyklu publikacji (dane bibliograficzne)

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

Dariusz Mikulski [*,1] Jerzy Juśkiewicz [†] Katarzyna Ognik,[‡] Przemysław Zduńczyk [†] Radosław Smagieł,[‡] and Jan Jankowski [*]

[*]*Department of Poultry Science, University of Warmia and Mazury in Olsztyn, 10-719, Olsztyn, Poland;* [†]*Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, 10-748, Olsztyn, Poland; and* [‡]*Department of Biochemistry and Toxicology, University of Life Sciences, 20-950, Lublin, Poland*

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

2022 Poultry Science 101:102098
https://doi.org/10.1016/j.psj.2022.102098

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 (Wallinga 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;

© 2022 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Received May 10, 2022.
Accepted July 25, 2022.

[1]Corresponding author: dariusz.mikulski@uwm.edu.pl

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).

Prophylactic antibiotics stir controversy due to their potential negative impact on the immune function and antioxidant status of birds (Sihvo et al., 2013; Elamaran 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 than 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 (Borrmann 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 (Gabler 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 multiciola* (Rawiwet 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 m^2 each) per treatment and 55 birds per pen. The stocking density at the initial stage of rearing was 5.5 birds/m^2. 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 poults were not vaccinated posthatching. The initial BW of one-day-old poults 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., Raamsdonksveer, 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
Rapeseed 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[1]	0.250	0.250	0.250
Calculated nutrient content			
Metabolizable energy, kcal/kg	2800	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

[1]Provided 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-tocopheryl acetate 100, 90, and 80, vit. K_3 5.8, 5.6, and 4.8, thiamine 5.4, 4.7, and 4.0, riboflavin 8.4, 7.5, and 6.4, pyridoxine 6.4, 5.6, and 4.8, cobalamin 0.032, 0.028, and 0.024, biotin 0.32, 0.28, and 0.24, pantothenic 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 (Hofirek 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 Żary-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 sample in 100mM phosphate buffer (pH 7.0) after centrifugation at 7,211 *g* for 15 min. Incubation was carried out at 39°C, and p- or o-nitrophenol was quantified at 400 and 320 nm, respectively after the addition 0.25 M-cold sodium carbonate to stop the reaction. Enzyme activity was expressed as μmol product formed per hour per g of digesta. In order to determine the total activity

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 (Juśkiewicz 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 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).

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

Item	Small intestine (ileum)		Ceca	
	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	0.070	0.700	0.301	0.378

[1]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. DM, dry matter.SEM = standard error of the mean (SD divided by the square root of replication number, n = 28).

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 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	Extra	Intra	Total	RR
Group (n = 7)[1]																
CON	16.82	23.44	40.26	42.49	2.80^{a}	11.03	13.83	22.39^{a}	24.39^{a}	99.10	123.49	19.50^{a}	27.37^{a}	71.83^{ab}	99.20^{ab}	29.56^{a}
ENR	18.60	21.68	40.29	45.99	1.66^{b}	13.06	14.72	11.54^{b}	14.86^{ab}	127.83	142.68	10.80^{b}	20.42^{ab}	101.50^{a}	121.93^{a}	17.11^{b}
DOX	14.97	19.30	34.27	49.60	1.71^{b}	11.01	12.72	15.67^{ab}	8.51^{b}	98.30	106.81	8.46^{b}	14.18^{b}	54.89^{b}	69.07^{b}	21.20^{ab}
MON	21.08	19.92	41.00	55.00	4.25^{a}	17.40	21.65	21.11^{a}	13.02^{ab}	102.17	115.18	11.31^{b}	23.32^{ab}	53.59^{b}	76.92^{b}	29.35^{a}
SEM	0.942	2.168	2.630	2.453	0.280	1.195	1.306	1.568	1.689	5.470	5.947	1.183	1.661	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

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

[1]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. 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 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
Group ($n = 7$) [1]												
CON	12.88	26.74	39.62	33.94	1.92[b]	6.28	8.21	24.44[b]	2.93	17.69	20.62	16.16
ENR	15.94	47.46	63.41	25.55	1.34[b]	5.75	7.09	19.34[b]	2.01	14.52	16.54	12.05
DOX	14.10	48.27	62.37	24.66	1.31[b]	5.00	6.31	21.71[b]	2.00	14.79	16.79	16.43
MON	14.43	41.72	56.15	25.39	3.37[a]	6.40	9.77	36.66[a]	2.42	21.76	24.18	12.20
SEM	1.119	3.673	4.383	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

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

[1]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.

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 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	C4i	C4	C5i	C5	Total PSCFAs	Total SCFAs	C2	C3	C4
Group (n = 7) [1]											
CON	96.63	7.817	0.476	28.80	0.362	1.107[b]	1.946	135.19	71.61	5.75	21.21
ENR	93.22	9.124	0.569	28.93	0.435	1.469[ab]	2.472	133.75	69.89	6.82	21.39
DOX	90.60	7.138	0.410	26.05	0.403	0.979[b]	1.791	125.58	72.16	5.69	20.72
MON	85.25	9.906	0.476	31.13	0.347	2.003[a]	2.826	129.11	66.04	7.59	24.15
SEM	2.156	0.488	0.057	1.337	0.060	0.120	0.175	2.851	0.917	0.328	0.851
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

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

[1]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 in turkeys (ng/mL).

Item	Yolk sac		Blood		
	1 day of age	5 days of age	1 day of age	7 days of age	56 days of age
Group (n = 7) [1]					
CON	1641.6	93.62	145.8	126.3	107.8[b]
ENR	1684.7	69.65	136.8	123.0	130.3[b]
DOX	1685.9	72.13	128.9	142.6	167.7[a]
MON	1697.5	109.91	144.6	114.9	119.8[b]
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,b]Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]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.Data represent mean values of 7 turkeys per group; SEM, standard error of the mean (SD divided by the square root of replication number, n = 28).

general, no significant differences in the average mortality rates of turkeys were noted during the 12-wk experimental period, which ranged from approximately 3% in groups CON and ENR to 7% in the DOX treatment. Overall deaths were normally distributed among replicate pens. Coccidial lesions were not found in any of the dead birds. The counts of coccidial oocysts did not exceed the limit of detection in any of the fecal samples collected from turkeys aged 6 and 9 wk.

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-glycosidic 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.,

Table 7. Blood hormone levels in turkeys.

	7 days of age					56 days of age				
Item	Serotonin (ng/mL)	Thyroxine (ng/mL)	Histamine (ng/mL)	Dopamine (pg/mL)	Noradrenaline (pg/mL)	Serotonin (ng/mL)	Thyroxine (ng/mL)	Histamine (ng/mL)	Dopamine (pg/mL)	Noradrenaline (pg/mL)
Group ($n = 7$) [1]										
CON	139.0	98.93	1.326	135.1	157.9	157.8[a]	118.9	4.224	328.4	902.3[b]
ENR	137.2	98.54	1.296	127.0	149.3	148.2[a,b]	121.0	4.682	394.6	1497.6[a]
DOX	141.1	95.62	1.503	136.0	142.2	131.2[b]	129.2	4.158	389.3	1273.0[a]
MON	137.8	71.68	1.296	139.0	145.7	156.9[a]	104.4	4.921	338.9	1497.8[a]
SEM	1.674	6.176	0.067	3.518	4.609	3.256	4.806	0.296	15.216	64.50
ANOVA *P*-value	0.864	0.353	0.675	0.684	0.685	0.006	0.337	0.860	0.301	0.004

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

[1]Group: ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life; MON, treated with monensin added to feed at a dose rate of 90 mg/kg feed for 84 days; CON, untreated control.Data represent mean values of 7 turkeys per group; SEM = standard error of the mean (SD divided by the square root of replication number, n = 28).

2015). 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

Table 8. The effect of different antibiotics on the growth performance of turkeys at 1 to 12 wk of age.

Item	DFI (g/bird)				BWG (kg/bird)				FCR (kg feed/kg BWG)				Mortality (%)
	wk 1−4	wk 5−8	wk 9−12	wk 1−12	wk 1−4	wk 5−8	wk 9−12	wk 1−12	wk 1−4	wk 5−8	wk 9−12	wk 1−12	wk 1−12
Group ($n = 7$) [1]													
CON	67.3	228.8	379.3	220.3	1.30	3.45^{ab}	4.10	8.85^{ab}	1.48	1.86^{a}	2.60	2.13^{ab}	3.12
ENR	68.1	232.4	384.2	223.9	1.31	3.49^{ab}	4.11	8.91^{ab}	1.49	1.87^{a}	2.61	2.14^{ab}	3.64
DOX	68.4	233.4	377.2	221.8	1.30	3.43^{b}	4.04	8.77^{b}	1.51	1.90^{a}	2.62	2.16^{a}	7.27
MON	67.1	226.0	383.3	221.0	1.31	3.56^{a}	4.18	9.05^{a}	1.47	1.78^{b}	2.57	2.09^{b}	4.42
SEM	0.425	1.512	2.443	1.204	0.005	0.017	0.025	0.038	0.010	0.012	0.011	0.008	0.774
ANOVA *P*-value	0.649	0.291	0.731	0.759	0.803	0.030	0.253	0.055	0.488	0.001	0.495	0.016	0.384

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

[1]Group: ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days 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.Data represent mean values of 7 replications per treatment; DFI, daily feed intake; BWG, body weight gain; FCR, feed conversion ratio; SEM, standard error of the mean (SD divided by the square root of replication number, n = 28).

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. Monenesin is a carboxylic polyeter 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; Trouchon 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.

ACKNOWLEDGMENTS

This work was supported by the National Science Centre, grant no. 2020/39/B/NZ9/00765.

DISCLOSURES

D. Mikulski, J. Juśkiewicz, K. Ognik, P. Zduńczyk, R. Smagieł, 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.

REFERENCES

Association of Official Analytical Chemists (AOAC). 2005. Official Methods of Analysis. 18th ed. AOAC, Arlington, VA.

Bartkiene, E., M. Ruzauskas, V. Bartkevics, I. Pugajeva, P. Zavistanaviciute, V. Starkute, E. Zokaityte, V. Lele, A. Dauksiene, M. Grashorn, L. E. Hoelzle, A. Mendybayeva, R. Ryshyanova, and R. Gruzauskas. 2020. Study of the antibiotic residues in poultry meat in some of the EU countries and selection of the best compositions of lactic acid bacteria and essential oils against *Salmonella enterica*. Poult. Sci. 99:4065–4076.

Baudoin, F., H. Hogeveen, and E. Wauters. 2021. Reducing antimicrobial use and dependence in livestock production systems: a social and economic sciences perspective on an interdisciplinary approach. Front. Vet. Sci. 8:584–593.

Borrmann, E., A. Berndt, L. Hanel, and H. Kohler. 2007. Campylobacter-induced interleukin-8 responses in human intestinal epithelial cells and primary intestinal chick cells. Vet. Microbiol. 124:115–124.

Bortolaia, V., C. Espinosa-Gongora, and L. Guardabassi. 2016. Human health risks associated with antimicrobial-resistant enterococci and *Staphylococcus aureus* on poultry meat. Clin. Microbiol. Infect. 22:130–140.

Bravo, J. A., P. Forsythe, M. V. Chew, E. Escaravage, H. M. Savignac, T. G. Dinan, J. Bienenstock, and J. F. Cryan. 2011. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. USA 108:16050–16055.

Brisbin, J. T., J. Gong, and S. Sharif. 2008. Interactions between commensal bacteria and the gut-associated immune system of the chicken. Anim. Health Res. Rev. 9:101–110.

Brown, K., R. R. E. Uwiera, M. L. Kalmokoff, S. P. J. Brooks, and G. D. Inglis. 2017. Antimicrobial growth promoter use in livestock: a requirement to understand their modes of action to develop effective alternatives. Int. J. Antimicrob. Agents 49:12–24.

Cabel, M. C., and P. W. Waldroup. 1991. Effects of different coccidiostats on performance of large white turkeys. Poult. Sci. 70:241–249.

Cabel, M. C., R. Norton, T. Yazwinski, and P. W. Waldroup. 1991. Efficacy of different anticoccidials against experimental coccidiosis in large white turkeys. Poult. Sci. 70:289–292.

Caspani, G., and J. Swann. 2019. Small talk: microbial metabolites involved in the signaling from microbiota to brain. Curr. Opin. Pharmacol. 48:99–106.

Chapman, H. D., T. K. Jefferss, and R. B. Williams. 2010. Forty years of monensin for the control of coccidiosis in poultry. Poult. Sci. 89:1788–1801.

Chapman, H. D., P. L. Matsler, and M. E. Chapman. 2004. Control of coccidiosis in turkeys with diclazuril and monensin: effects upon performance and development of immunity to *Eimeria* species. Avian Dis. 48:631–634.

Chapman, H. D., and E. Saleh. 1999. Effect of different concentrations of monensin and monensin withdrawal upon control of coccidiosis in the turkey. Poult. Sci. 78:50–56.

Chatterjee, S. 1993. Effects of monensin on glycosphingolipid metabolism in cultured human proximal tubular cells. Indian J. Biochem. Biophys. 30:346–352.

Chen, M., K. W. Cheng, Y. J. Chen, C. H.Wang, T. C. Cheng, K. C. Chang, A. P. Kao, and K. H. Chuang. 2017. Real-time imaging of intestinal bacterial β-glucuronidase activity by hydrolysis of a fluorescent probe. Sci. Rep. 7:3142.

Chen, Y., J. Xu, and Y. Chen. 2021. Regulation of neurotransmitters by the gut microbiota and effects on cognition in neurological disorders. Nutrients 13:2099.

Cheng, J., T. Romantsov, K. Engel, A. C. Doxe, D. R. Rose, J. D. Neufeld, and T. C. Charles. 2017. Functional metagenomics reveals novel β-galactosidases not predictable from gene sequences. PLoS One 12:e0172545.

Close, B., K. Banister, V. Baumans, E. M. Bernoth, N. Bromage, J. Bunyan, W. Erhardt, P. Flecknell, N. Gregory, H. Hackbarth, D. Morton, and C. Warwick. 1997. Recommendations for euthanasia of experimental animals. Part 2. Lab. Anim. 31:1–32.

Cox, L. M., and H. L Weiner. 2018. Microbiota signaling pathways that influence neurologic disease. Neurotherapeutics 15:135–145.

Cryan, J. F., K. J. O'Riordan, K. Sandhu, V. Peterson, and T. G. Dinan. 2020. The gut microbiome in neurological disorders. Lancet Neurol. 19:179–194.

Cunha, B. A., P. Domenico, and C. B. Cunha. 2000. Pharmacodynamics of doxycycline. Clin. Microbiol. Infect. 6:270–273.

Dinan, T. G., and J. F. Cryan. 2012. Regulation of the stress response by the gut microbiota: implications for psychoneuroendocrinology. Psychoneuroendocrinology 37:1369–1378.

Dorrestein, G., M. J. Welink, and N. Haagsma. 1990. Pharmacokinetic differences for doxycyc1ine between racing pigeons (*Columba livia*) and collared doves (*Streptopelia decaveto*) and the effect of a Salmonella infection. Proc. VII Tagung über Vogelkrankheiten.

Dunislawska, A., A. Slawinska, and M. Siwek. 2020. Hepatic DNA methylation in response to early stimulation of microbiota with *Lactobacillus* synbiotics in broiler chickens. Genes 11:579.

Elamaran, A., P. Hariharan, S. Ramesh, and S. Vairamuthu. 2015. Enrofloxacin induced oxidative stress and its amelioration with antioxidants in liver homogenates of broilers. India J. Vet. Anim. Sci. Res. 44:124–128.

Elokil, A. A., K. F. M. Abouelezz, H. I. Ahmad, Y. Pan, and S. Li. 2020. Investigation of the impacts of antibiotic exposure on the diversity of the gut microbiota in chicks. Animals (Basel) 10:896.

Farzi, A., E. E. Fröhlich, and P. Holzer. 2018. Gut microbiota and the neuroendocrine system. Neurotherapeutics 15:5–22.

Fife, R. S., and G. W. Sledge. 1995. Effects of doxycycline on in vitro growth, migration, and gelatinase activity of breast carcinoma cells. J. Lab. Clin. Med. 125:407–411.

Fujisaka, S., J. Avila-Pacheco, M. Soto, A. Kostic, J. M. Dreyfuss, H. Pan, S. Ussar, E. Altindis, N. Li, L. Bry, C. B. Clish, and C. R. Kahn. 2018. Diet, genetics, and the gut microbiome drive dynamic changes in plasma metabolites. Cell Rep. 22:3072–3086.

Gabler, W. L., J. Smith, and N. Tsukuda. 1992. Comparison of doxycycline and a chemically modified tetracycline inhibition of leukocyte functions. Res. Commun. Chem. Pathol. Pharmacol. 78:151–160.

Gao, K., Y. Pi, C. L Mu, Y. Peng, Z. Huang, and W. Y. Zhu. 2018. Antibiotics-induced modulation of large intestinal microbiota altered aromatic amino acid profile and expression of neurotransmitters in the hypothalamus of piglets. J. Neurochem. 146:219–234.

Garmyn, A., A. Martel, R. Froyman, H. Nauwynck, L. Duchateau, F. Haesebrouck, and F. Pasmans. 2009. Efficacy of four enrofloxacin treatment regimens against experimental infection in turkey poults with avian pneumovirus and *Ornithobacterium rhinotracheale*. Avian Pathol. 38:287–292.

Gbylik-Sikorska, M., A. Posyniak, T. Śniegocki, B. Sell, A. Gajda, G. Tomczyk, and J. Żmudzki. 2016. Effect of doxycycline concentrations in chicken tissues as a consequence of permanent exposure to enrofloxacin traces in drinking water. J. Vet. Res. 60:293–299.

Guillouzo, A., and Ch. Guguen-Guillouzo. 2020. Antibiotics-induced oxidative stress. Curr. Opin. Toxicol. 20−21:23–28.

Gugołek, A., J. Juśkiewicz, J. Strychalski, M. Konstantynowicz, and C. Zwoliński. 2015. Nutrient digestibility and colonic fermentation processes in species of the families *Mustelidae* and *Canidae* fed the same diet. J. Exp. Zool. 323:637–644.

Gutiérrez, L., J. Zermeño, Y. Alcalá, and H. Sumano. 2017. Higher bioavailability of doxycycline in broiler chickens with a novel in-feed pharmaceutical formulation. Poult. Sci. 96:2662–2669.

Hofirek, B., and D. Haas. 2001. Comparative studies of ruminal fluid collected by oral tube or by puncture of the caudorental ruminal sac. Acta Vet. Brno 70:27–33.

Hybrid Turkeys. 2020. Commercial Management Guide and Nutrient Guidelines for Hybrid Turkeys. Accessed July 2021. http://www.hybridturkeys.com

Juśkiewicz, J., J. Jankowski, Z. Zduńczyk, and D. Mikulski. 2006. Performance and gastrointestinal tract metabolism of turkeys fed diets with different contents of fructooligosaccharides. Poult. Sci. 85:886–891.

Juśkiewicz, J., Z. Zduńczyk, J. Jankowski, and B. Król. 2002. Caecal metabolism in young turkeys fed diets supplemented with oligosaccharides. Arch. Geflugelk. 66:206–210.

Kadykalo, S., T. Roberts, M. Thompson, J. Wilson, M. Lang, and O. Espeisse. 2018. The value of anticoccidials for sustainable global poultry production. Int. J. Antimicrob. Agents 51:304–310.

Kang, J., M. A. Hossain, H. C. Park, Y. Kim, K. J. Lee, and S. W. Park. 2019. Pharmacokinetic and pharmacodynamic integration of enrofloxacin against *Salmonella* Enteritidis after administering to broiler chicken by per-oral and intravenous routes. J. Vet. Sci. 20:e15.

Khalifeh, M., M. M. Amawi, E. A. Abu-Basha, and I. Bani Yonis. 2009. Assessment of humoral and cellular-mediated immune response in chickens treated with tilmicosin, florfenicol, or enrofloxacin at the time of Newcastle disease vaccination. Poult. Sci. 88:2118–2124.

Lai, C. C., C. C. Chen, H. L. Huang, Y. C. Chuang, and H. J. Tang. 2016. The role of doxycycline in the therapy of multidrug-resistant E. coli − an in vitro study. Sci. Rep. 6:31964.

Logan, N. B., M. E. Mc Kenzie, D. P. Conway, L. R. Chappel, and N. C. Hammel. 1993. Anticoccidial efficacy of Semduramicin. 2. Evaluation against field isolates including comparisons with salinomycin, maduramicin, and monensin in battery tests. Poult. Sci. 72:2058–2063.

Lu, H., S. A. Adedokun, L. Adeola, and K. M. Ajuwon. 2014. Anti-inflammatory effects of non-antibiotic alternatives in coccidia challenged broiler chickens. J. Poult. Sci. 51:14–21.

Madubuike, K. G., O. N. Okoroafor, and I. U. Asuzu. 2020. Effects of early-life antibiotics administration on the immune response to Newcastle disease lasota vaccination and weight indices of broiler chicken. Folia Vet 64:74–79.

Matthews, B. W. 2005. The structure of E. coli β-galactosidase. Comptes. Rendus. Biologies 328:549–556.

McDougald, L. R., G. F. Mathis, and D. P. Conway. 1996. Effect of semduramicin, salinomycin, and monensin on performance, shank pigmentation, and coccidial lesions in broiler chickens in floor pens. Avian Dis. 40:68–71.

Mehdi, Y., M.-P. Letourneau-Montminy, M.-L. Gaucher, Y. Chorfi, G. Suresh, T. Rouissi, S. K. Brar, C. Cot, A. A. Ramirez, and S. Godbout. 2018. Use of antibiotics in broiler production: global impacts and alternatives. Anim. Nutr. 4:170e178.

Meister, B. 2007. Neurotransmitters in key neurons of the hypothalamus that regulate feeding behavior and body weight. Physiol. Behav. 92:263–271.

Michlmayr, H., and W. Kneifel. 2014. β-Glucosidase activities of lactic acid bacteria: mechanisms, impact on fermented food and human health. FEMS Microbiol. Lett. 352:1–10.

Mishra, B., and R. Jha. 2019. Oxidative stress in the poultry gut: potential challenges and interventions. Front. Vet. Sci. 6:60.

Modrackova, N., E. Vlkova, V. Tejnecky, C. Schwab, and V. Neuzil-Bunesova. 2020. Bifidobacterium β-glucosidase activity and fermentation of dietary plant glucosides is species and strain specific. Microorganisms 8:839.

Mollenhauer, H. H., D. J. Morré, and L. D. Rowe. 1990. Alteration of intracellular traffic by monensin; mechanism, specificity and relationship to toxicity. Biochim. Biophys. Acta 1031:225–246.

Morales-Barrera, E., N. Calhoun, J. L. Lobato-Tapia, V. Lucca, O. Prado-Rebolledo, X. Hernandez-Velasco, R. Merino-Guzman, V. M. Petrone-García, J. D. Latorre, B. D. Mahaffey, K. D. Teague, L. E. Graham, A. D. Wolfenden, M. F. A Baxter, B. M. Hargis, and G. Tellez. 2016. Risks involved in the use of enrofloxacin for *Salmonella Enteritidis* or *Salmonella Heidelberg* in commercial poultry. Front. Vet. Sci. 3:72.

More, S. J. 2020. European perspectives on efforts to reduce antimicrobial usage in food animal production. Ir. Vet. J. 73:2.

Neufeld, K. M., N. Kang, J. Bienenstock, and J. A. Foster. 2011. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol. Motil. 23:255–e119.

Noack, S., H. D. Chapman, and P. M. Selzer. 2019. Anticoccidial drugs of the livestock industry. Parasitol. Res. 118:2009–2026.

Parada Venegas, D., M. K. De la Fuente, G. Landskron, M. J. González, R. Quera, G. Dijkstra, H. J. M. Harmsen, K. N. Faber, and M. A. Hermoso. 2019. Short chain fatty acids (SCFAs)-mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. Front. Immunol. 10:277.

Rawiwet, V., C. Piyarat, and C. Niwat. 2010. Comparison of the efficacy of enrofloxacin against *Escherichia coli* or *Pasteurella multocida* infection in chickens. Thai J. Vet. Med. 40:297–301.

Raynaud, J. P. 1970. Etude de l'efficacited'une technique de coproscopie quantitative pour le diagnostic de routine et le controle des infestations parasitaires des bovins, ovins, equines et porcins. Ann. Parasitol. 45:321–342.

Roth, N., A. Käsbohrer, S. Mayrhofer, U. Zitz, Ch. Hofacre, and K. J. Domig. 2019. The application of antibiotics in broiler production and the resulting antibiotic resistance in Escherichia coli: a global overview. Poult. Sci. 98:1791–1804.

Shang, Y., S. Kumar, B. Oakley, and W. K. Kim. 2018. Chicken gut microbiota: importance and detection technology. Front. Vet. Sci. 5:254.

Shin, H. Y., S. Y. Park, J. H. Sung, and D. H. Kim. 2003. Purification and characterization of α-L-arabinopyranosidase and α-L-arabinofuranosidase from *Bifidobacterium breve* K-110, a human intestinal anaerobic bacterium metabolizing ginsenoside Rb2 and Rc. Appl. Environ. Microbiol. 69:7116–7123.

Sihvo, H.-K., K. Immonen, and E. Puolanne. 2013. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet. Pathol. 51:619–623.

Sims, M. D., and D. M. Hooge. 2002. Efficacy of nitarsone, monensin or nitarsone followed by monensin, in female turkeys in litter pens and exposed to coccidial (25 days) and cochlosomal (28 days) inoculations. Int. J. Poult. Sci. 1:164–166.

ISO 14183. 2005. Animal feeding stuffs - determination of monensin, narasin and salinomycin contents - liquid chromatographic method using post-column derivatisation. Accessed Sep. 2021. http://www.scribd.com/document/465200942/ISO-14183-2005.

StatSoft Inc. STATISTICA (Data Analysis Software System), Version 12.0. 2014. Accessed Jan. 2022. http://www.statsoft.com.

Sudo, N., Y. Chida, Y. Aiba, J. Sonoda, N. Oyama, X. N. Yu, C. Kubo, and Y. Koga. 2004. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J. Physiol. 558:263–275.

Sureshkumar, V., G. Saratchandra, and J. Ramesh. 2013. Effect of enrofloxacin on zootechnical performance, behaviour and immunohistopathological response in broiler chicken. Vet. World 6:337–342.

Teirlynck, E., L. Bjerrum, V. Eeckhaut, G. Huygebaert, F. Pasmans, F. Haesebrouck, J. Dewulf, R. Ducatelle, and F. van Immerseel. 2009. The cereal type in feed influences gut wall morphology and intestinal immune cell infiltration in broiler chickens. Br. J. Nutr. 102:1453–1461.

Trouchon, T., and S. Lefebvre. 2016. A review of enrofloxacin for veterinary use. Open J. Vet. Med. 6:40–58.

Van Deun, K., F. Pasmans, R. Ducatelle, B. Flahou, K. Vissenberg, A. Martel, W. van den Broeck, F. van Immerseel, and F. Haesebrouck. 2008. Colonization strategy of *Campylobacter jejuni* results in persistent infection of the chicken gut. Vet. Microbiol. 130:285–297.

Varga, I., P. Laczay, J. Lehel, Z. Mora, A. Romvary, and J. Fekete. 1994. Potentiation on ionophorous anticoccidials with dihydroquinolines: battery trials against *Eimeria tenella* in chickens. Int. J. Parasitol. 5:689–694.

Wallinga, D., L. A. M. Smit, M. F. Davis, J. A. Casey, and K. E. Nachman. 2022. A review of the effectiveness of current US policies on antimicrobial use in meat and poultry production. Curr. Environ. Health Rep. 9:339–354.

Watkins, K. L., M. N. Novilla, and T. Campi. 1993. Effects of feed restriction and subsequent gorging with limited access to water on male turkeys fed graded levels of monensin. Poult. Sci. 72:677–683.

Watkins, K. L., and M. N. Novilla. 1994. Feed gorging and extended water restriction do not produce knockdown in male turkeys fed monensin. Poult. Sci. 73:587–590.

Zhang, X., M. Liu, Y. Fan, J. Xu, X. Xu, and H. Li. 2018. The structural and functional contributions of β-glucosidase-producing microbial communities to cellulose degradation in composting. Biotechnol. Biofuels 11:51.

Żary-Sikorska, E., B. Fotschki, M. Kosmala, J. Milala, P. Matusevicius, A. Rawicka, and J. Juśkiewicz. 2021. Strawberry polyphenol-rich fractions can mitigate disorders in gastrointestinal tract and liver functions caused by a high-fructose diet in experimental rats. Pol. J. Food Nutr. Sci. 71:423–440.

Poultry Science

The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys

Radosław Smagieł,[*] Katarzyna Ognik,[*,1] Ewelina Cholewińska,[*] Anna Stępniowska,[*] Piotr Listos,[†] Bartłomiej Tykałowski,[‡] Dariusz Mikulski,[§] Andrzej Koncicki,[‡] and Jan Jankowski[§]

[*]*Department of Biochemistry and Toxicology, University of Life Sciences in Lublin, 20-950 Lublin, Poland;* [†]*Department of Pathomorphology and Forensic Medicine, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, 20-612 Lublin, Poland;* [‡]*Department of Poultry Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland; and* [§]*Department of Poultry Science and Apiculture, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland*

ABSTRACT It was assumed that early administration of enrofloxacin or doxycycline may impair immune function and alter the morphology of organs of the immune system in turkeys, and that diets containing the coccidiostat monensin, an ionophore antibiotic, can exert similar effects. The aim of this study was to determine whether early antibiotic administration or feeding a diet containing a coccidiostat affect immune function in young turkeys. The experiment had a completely randomized design, with 8 groups (a total of 3,080 one-day-old turkeys), 7 replicate pens per group and 55 birds per pen. The experiment had a 2-factorial design, with 4 treatments (C—control, M—monensin, E—enrofloxacin, and D—doxycycline) and 2 groups of birds (vaccinated and unvaccinated) per treatment. Control group birds did not receive the coccidiostat or antibiotics. Group M was administered monensin at 90 mg/kg feed for the first 5 d of life, group E received enrofloxacin at 10 mg/kg BW, added to drinking water, for the first 5 d of life, and group D received doxycycline at 50 mg/kg BW, added to drinking water, for the first 5 d of life. One-day old turkeys from groups C+, M+, E+, and D+ were administered live-attenuated vaccines against turkey rhinotracheitis (**TRT**) (Poulvac TRT; Zoetis, Parsippany, NJ) and Newcastle disease (**ND**) (Nobilis ND clone 30; Merck, Rahway, NJ) by coarse spray; 28-day-old birds were administered a subcutaneously injected inactivated vaccine against *Ornithobacterium rhinotracheale* (**ORT**) (Ornitin, Phibro, Poland). Turkeys from groups C−, M−, E−, and D− were not vaccinated. It was found that early administration of enrofloxacin or doxycycline, or feeding a diet containing monensin, did not weaken the immune system of turkeys. The administration of monensin, in particular when combined with vaccination, was least effective in inhibiting inflammatory responses. Histological changes in immunocompetent organs (fatty degeneration) were also most severe in birds receiving monensin, followed by those administered doxycycline and enrofloxacin. The observed changes were exacerbated by vaccination.

Key words: turkey, antibiotic, coccidiostat, blood, immunology

2023 Poultry Science 102:102876
https://doi.org/10.1016/j.psj.2023.102876

INTRODUCTION

Modern fast-growing meat-type turkeys are characterized by increased susceptibility to infections that are usually treated with antibiotics. Antibiotics are often administered to turkeys already in the first days after hatch. On the other hand, there has been a tremendous pressure to reduce antibiotic use in animal production. In the European Union, the use of antibiotics as growth promoters in animal feed was banned in 2006 to protect public health and limit the spread of antimicrobial resistance. However, the use of ionophore coccidiostats, which are also classified as antibiotics, is still permitted. The current challenge faced by poultry and livestock producers is the need to reduce the amount of antibiotics administered to animals. Irrational use of antibiotics exerts adverse effects on the immune system and gastrointestinal microbiome. Enrofloxacin and doxycycline are broad-spectrum antibiotics with immunomodulatory properties, extensively used in poultry to treat bacterial

© 2023 The Authors. Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Received May 15, 2023.
Accepted June 13, 2023.

[1]Corresponding author: kasiaognik@poczta.fm

infections. Grabowski et al. (2022) administered enrofloxacin to chickens infected with *Salmonella Typhimurium* and found that this antibiotic decreased the levels of proinflammatory cytokines and inhibited the potential of anti-inflammatory cytokines, which was reflected in a decrease in the serum levels of interleukin 10 (**IL-10**) and interleukin 4 (**IL-4**). According to Riesbeck (2002), quinolones, including enrofloxacin, deregulate the mRNA levels of genes encoding cytokines such as interleukin 1a (**IL-1a**), tumor necrosis factor a (**TNF-a**), interleukin 2 (**IL-2**), interleukin 3 (**IL-3**), and IL-4. The immunosuppressive effect of enrofloxacin was also observed as the antibiotic reduced the levels of immunoglobulin Y (**IgY**) in the blood serum of laying hens and the egg yolk after experimental infection with *Salmonella enterica* (Tokarzewski, 2002). Khalifeh et al. (2009) demonstrated that enrofloxacin had a negative effect on serum ND virus (**NDV**) antibody titers. Madubuike et al. (2020) found that the exposure of broiler chickens to doxycycline in the first week of life did not modulate their immune responses to the NDV.

Coccidiostats are administered with feed to prevent coccidiosis in poultry, but they can also affect the immune status of birds. Abdelhady et al. (2021) investigated the effect of different coccidiostats on the immune response of broiler chickens challenged with *Eimeria* spp. and found that monensin downregulated the gene expression of interleukin 6 (**IL-6**) and interferon γ (**IFN-γ**). Moreover, coccidiostats decrease the intestinal loads of protozoans and pathogenic bacteria, thus reducing microbial colonization of gut-associated lymphoid tissue (**GALT**) and inflammatory responses in the host (Abdelhady et al., 2021). According to Lee et al. (2012), coccidiostats stimulate the immune system. In turn, Kozłowski et al. (2021) reported that monensin administered to uninfected birds had no influence on their immune responses. Antibiotics also affect vaccine-induced antibody titers. In a study by Khalifeh et al. (2009), a combination of both live-attenuated vaccine and inactivated adjuvanted vaccine activated a high level of antibody production and induced an increase in the cell-mediated immune response represented by an increase in IFN-γ levels.

It was assumed that early administration of enrofloxacin or doxycycline may impair immune function and alter the morphology of organs of the immune system in turkeys, and that diets containing the coccidiostat monensin, an ionophore antibiotic, can exert similar effects. The aim of this study was to determine whether early antibiotic administration or feeding a diet containing a coccidiostat affect immune function in young turkeys.

MATERIALS AND METHODS

Ethical 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 and Housing

The experiment had a completely randomized design, with 8 groups (a total of 3,080 one-day-old Hybrid Converter female turkeys). Each group consisted of 7 replicate pens bedded with wood shavings, with 55 birds per pen. The replicates (pens) were allocated to groups so as to ensure their uniform (homogeneous) distribution in the house. The birds were reared until 12 wk of age. Stocking density in the initial stage of rearing was 5.5 birds/m^2. Vaccinated and unvaccinated birds were kept in separate sections of the building and were handled by different people to prevent cross-contamination. Environmental conditions were controlled automatically. They were adjusted to the birds' age, consistent with the recommendations of Hybrid Turkeys (2020), and identical for all turkeys in the 2 separate sections of the building.

Diets and Experimental Design

Turkeys were fed complete diets whose nutritional value met their nutriment requirements in successive stages of rearing (Hybrid Turkeys, 2020). The detailed composition of the diets was presented in Table 1. The diets, produced by a local feed mill, were offered as crumbles (d 1−28) and pellets (d 29−84). The birds had ad libitum access to feed and water. The experiment had a 2-factorial design, with 4 treatments (C—control, M—monensin, E—enrofloxacin, and D—doxycycline) and 2 groups of birds (vaccinated, unvaccinated; +, −) per treatment. Control group birds did not receive the coccidiostat or antibiotics. Group M was administered monensin at 90 mg/kg feed for the first 5 d of life, group E received enrofloxacin at 10 mg/kg BW, added to drinking water, for the first 5 d of life, and group D received doxycycline at 50 mg/kg BW, added to drinking water, for the first 5 d of life.

Challenge

One-day old turkeys from groups C+, M+, E+, and D + were administered live-attenuated vaccines against turkey rhinotracheitis (**TRT**) (Poulvac TRT; Zoetis, Parsippany, NJ) and Newcastle disease (**ND**) (Nobilis ND clone 30; Merck, Rahway, NJ) by coarse spray; 28-day-old birds were administered a subcutaneously injected inactivated vaccine against *Ornithobacterium rhinotracheale* (**ORT**) (Ornitin, Phibro, Poland). Turkeys from groups C−, M−, E−, and D− were not vaccinated.

Table 1. Ingredient composition and nutrient content of turkey diets (g/100 g, as-fed basis) (presented in Mikulski et al., 2022).

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
Rapeseed 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[1]	0.250	0.250	0.250
Calculated nutrient content			
Metabolizable energy, kcal/kg	2800	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

[1]Provided per kg diet (feeding periods: wk 1–4, 5–8, 9–12): mg: retinol 3.78, 3.38, and 2.88, cholecalciferol 0.13, 0.12, and 0.10, α-tocopheryl acetate 100, 90, and 80, vit. K_3 5.8, 5.6, and 4.8, thiamine 5.4, 4.7, and 4.0, riboflavin 8.4, 7.5, and 6.4, pyridoxine 6.4, 5.6, and 4.8, cobalamin 0.032, 0.028, and 0.024, biotin 0.32, 0.28, and 0.24, pantothenic 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.

Sample Collection and Analyses

At 7 and 56 d of age, blood samples were collected from 7 birds from each group (1 bird per replicate), and 1 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 weights of the spleen and the bursa of Fabricius were determined relative to the live body weight (**BW**) of birds. Samples of the spleen, the thymus, and the bursa of Fabricius were collected for histological examination.

The plasma levels of C-reactive protein (**CRP**), ceruloplasmin (**Cp**), nuclear factor kappa B (**NF-κB**), immunoglobulin A (**IgA**), toll-like receptor 4 (**TLR-4**), and amyloid A were determined in 7-day-old and 56-day-old turkeys, using Qayee-Bio diagnostic kits (Qayee Biotechnology Co., Ltd., Shanghai, China). The respiratory burst activity of the heterophils was quantified by nitroblue tetrazolium (**NBT**) reduction to formazan as a measurement of production of oxygen radicals (Park et al., 1968).

Samples of the spleen, the thymus, and the bursa of Fabricius were cut in 2 lengthwise and fixed for 24 h in 5% formalin, pH = 7.2. Within 24 h, the fixed tissue fragments were passed through increasing concentrations of alcohol solutions, acetone, and xylene into paraffin blocks in a tissue processor (Leica TP-20). Paraffin-embedded microscope sections, 5 μm thick, were stained with hematoxylin and eosin (**H&E** staining). Morphometric evaluation of the tissues was carried out using a computer-assisted microscopic image analysis system. The system includes a light microscope (Nikon Eclipse E600) with a digital camera (Nikon DS-Fi1) and a PC with image-analysis software (NIS-Elements BR-2.20, Laboratory Imaging).

Statistical Analysis

The values of the all traits and parameters were determined individually in 7 birds per group (1 bird per replicate), whose BW was representative of the average BW in the group. The data were analyzed by 2-way ANOVA with the general linear model (**GLM**) procedure to examine the main effects of treatment (C, M, E, D), challenge (vaccinated vs. unvaccinated; V effect), and their interaction. When the model was significant, Tukey's HSD test was used to separate treatment means. The statistical analysis was performed using STATISTICA software version 13.1 (TIBCO Software Inc, 2017) at a significance level of $P < 0.05$. The results were presented as the mean and the pooled standard error of the mean (**SEM**).

RESULTS

The experimental treatments had no effect on % NBT in the blood of 7-day-old and 56-day-old turkeys. Antibiotic × vaccine interactions were found for CRP ($P < 0.001$) and Cp ($P = 0.006$) in the blood of 7-day-old birds, and for CRP, NF-kB ($P < 0.001$, both) and amyloid A ($P = 0.032$) in the blood plasma of 56-day-old birds (Table 4). The above interactions indicate that the levels of CRP and Cp were affected by antibiotics administered via drinking water, in particular in 7-day-old turkeys vaccinated against ND and TRT. As regards blood CRP levels in 56-day-old turkeys, the noted interaction was due to the fact that early administration of enrofloxacin and vaccination of 1-day-old birds against TRT and ND, and 28-day-old birds against ORT decreased CRP levels, which was not observed in turkeys receiving monensin or doxycycline. The interaction noted for blood Cp levels in 7-day-old turkeys resulted from the fact that vaccination of 1-day-old birds against TRT and ND and the administration of enrofloxacin or doxycycline for the first 5 d of life decreased the value of this parameter, which was not observed in vaccinated birds that received monensin (Table 3). The administration of enrofloxacin and vaccination against TRT, ND, and ORT led to an in increase in blood amyloid A levels in 56-day-old birds, which was not noted in vaccinated turkeys that received monensin or doxycycline. In turn, vaccination against TRT, ND, and ORT and simultaneous administration of monensin decreased blood NF-kB levels in 56-day-old turkeys, which was not observed

Table 2. Effect of treatments on the weights of the spleen and the bursa of Fabricius at 7 and 56 d of age.

	D 7					D 56				
		Spleen		Bursa of Fabricius			Spleen		Bursa of Fabricius	
Item	BW (g)	Total weight (g)	Relative weight (% BW)	Total weight (g)	Relative weight (% BW)	BW (g)	Total weight (g)	Relative weight (% BW)	Total weight (g)	Relative weight (% BW)
Antibiotic[1]										
C	185.4	0.110	0.059	0.266	0.145	4.450	3.743	0.084	3.885	0.087
M	186.7	0.106	0.057	0.265	0.142	4.489	4.424	0.099	4.246	0.095
E	177.1	0.116	0.067	0.239	0.137	4.471	4.142	0.092	4.026	0.090
D	192.0	0.120	0.062	0.240	0.125	4.486	4.011	0.090	4.114	0.092
Vaccine[2]										
−	189.4	0.110	0.058	0.251	0.133	4.518	3.960	0.088	4.065	0.090
+	181.2	0.116	0.064	0.253	0.141	4.430	4.200	0.094	4.071	0.092
Group										
C−	191.1	0.111	0.058	0.235	0.122	4.500	3.640	0.081	3.847	0.086
C+	179.8	0.108	0.061	0.297	0.167	4.400	3.846	0.087	3.923	0.089
M−	192.8	0.097	0.051	0.276	0.144	4.521	4.299	0.095	4.167	0.092
M+	180.5	0.114	0.063	0.253	0.141	4.457	4.549	0.102	4.326	0.097
E−	175.7	0.118	0.068	0.242	0.139	4.464	4.066	0.091	4.154	0.093
E+	178.4	0.115	0.066	0.236	0.134	4.479	4.219	0.093	3.899	0.087
D−	198.0	0.114	0.057	0.253	0.129	4.586	3.834	0.083	4.090	0.089
D+	186.1	0.126	0.068	0.227	0.121	4.386	4.189	0.096	4.139	0.095
SEM	2.532	0.004	0.002	0.007	0.004	0.024	0.104	0.002	0.106	0.002
P value										
Antibiotic (A)	0.215	0.618	0.461	0.364	0.369	0.936	0.147	0.141	0.707	0.675
Vaccine (V)	0.107	0.462	0.182	0.897	0.379	0.073	0.250	0.297	0.976	0.903
A × V interaction	0.660	0.753	0.523	0.116	0.069	0.463	0.988	0.973	0.919	0.949

[1]Treatment: C, untreated control; M, treated with monensin; E, treated with enrofloxacin; D, treated with doxycycline.
[2]Unvaccinated, − or vaccinated, +.BW, body weight.

Table 3. Immunological parameters in the blood plasma of turkeys at 7 d of age.

Item	CRP (ng/mL)	Cp (μg/mL)	NF-kB (ng/mL)	IgA (ng/mL)	NBT (%)	TLR-4 (ng/mL)	Amyloid A (μg/mL)
Antibiotic[1]							
C	8.678[a]	65.27[a]	24.28	10792	41.28	4.485[a]	1.057[b]
M	8.793[a]	59.79[ab]	21.87	10513	40.55	4.211[a]	1.497[a]
E	7.380[b]	59.74[ab]	24.19	10694	39.98	3.596[b]	1.411[ab]
D	7.940[ab]	54.21[b]	21.32	10339	40.25	3.177[c]	1.343[ab]
Vaccine[2]							
−	8.562[a]	65.32[a]	26.28[a]	11167[a]	40.54	4.569[a]	1.135[b]
+	7.834[b]	54.18[b]	19.55[b]	10002[b]	40.49	3.165[b]	1.519[a]
Groups							
C−	8.518[ab]	64.31[ab]	28.94	11408	41.74	5.648	0.736
C+	8.837[a]	66.23[ab]	19.62	10176	40.83	3.322	1.378
M−	8.418[ab]	64.48[ab]	24.84	10701	40.16	4.784	1.296
M+	9.168[a]	55.10[abc]	18.90	10325	40.94	3.638	1.697
E−	8.642[ab]	67.50[a]	28.08	11849	40.22	4.280	1.255
E+	6.119[c]	51.98[bc]	20.29	9540	39.75	2.912	1.566
D−	8.670[ab]	65.00[ab]	23.28	10710	40.04	3.564	1.252
D+	7.211[bc]	43.42[c]	19.36	9969	40.46	2.790	1.434
SEM	0.170	1.530	0.759	164.1	0.296	0.166	0.064
P value							
Antibiotic (A)	<0.001	0.015	0.196	0.690	0.477	0.001	0.048
Vaccine (V)	0.004	<0.001	<0.001	<0.001	0.940	<0.001	<0.001
A × V interaction	<0.001	0.006	0.433	0.105	0.747	0.134	0.536

[a,b,c]Means within the same column with different superscripts differ significantly ($P < 0.05$).
[1]Treatment: C, untreated control; M, treated with monensin; E, treated with enrofloxacin; D, treated with doxycycline.
[2]Unvaccinated, − or vaccinated, +.CRP, C-reactive protein; Cp, ceruloplasmin; NF-κB, nuclear factor kappa B; IgA, immunoglobulin A; NBT, respiratory burst activity of the heterophils; TLR-4, toll-like receptor 4.

in vaccinated birds administered enrofloxacin or doxycycline (Table 4).

Effect of Antibiotics and/or a Coccidiostat

Table 2 data show that feeding a diet containing monensin and early administration of enrofloxacin or doxycycline had no effect on the weights of the organs of the immune system, that is, the spleen and the bursa of Fabricius in turkeys at 7 and 56 d of age.

Monensin added to feed induced an increase in blood amyloid A levels ($P = 0.048$) in 7-day-old turkeys, but not in 56-day-old birds (Tables 3 and 4). Early administration of doxycycline contributed to a decrease in blood TLR-4 levels ($P = 0.001$) in 7-day-old turkeys. In 56-day-old birds, blood TLR-4 levels ($P = 0.002$) decreased in response to early administration of both doxycycline and enrofloxacin.

A histological image analysis of the bursa of Fabricius and the thymus, collected from 7-day-old turkeys,

Table 4. Immunological parameters in the blood plasma of turkeys at 56 d of age.

Item	CRP (ng/mL)	Cp (μg/mL)	NF-kB (ng/mL)	IgA (ng/mL)	NBT (%)	TLR-4 (ng/mL)	Amyloid A (μg/mL)
Antibiotic[1]							
C	6.162[a]	53.23	19.01[a]	16454	45.67	5.579[a]	0.228
M	6.635[a]	56.32	16.20[a]	16032	44.43	4.612[ab]	0.299
E	5.338[b]	52.49	11.78[b]	15420	44.75	4.051[b]	0.341
D	4.599[b]	44.48	11.41[b]	12954	44.21	4.116[b]	0.180
Vaccine[2]							
−	6.637[a]	60.96[a]	17.70	16337	45.34	6.607[a]	0.251
+	4.730[b]	42.30[b]	11.50[b]	14093	44.20	2.572[b]	0.273
Groups							
C−	7.406[a]	65.14	26.73[a]	17986	46.54	6.854[a]	0.254[b]
C+	4.917[bc]	41.32	11.29[b]	14922	44.80	4.303[b]	0.203[b]
M−	7.326[a]	61.56	21.67[a]	14536	45.23	6.330[a]	0.376[ab]
M+	5.943[ab]	51.08	10.74[b]	17528	43.63	2.893[bc]	0.221[b]
E−	7.103[a]	66.05	11.96[b]	17466	45.26	6.545[a]	0.208[b]
E+	3.573[c]	38.93	11.60[b]	13374	44.25	1.556[c]	0.474[a]
D−	4.712[bc]	51.10	10.45[b]	15362	44.32	6.698[a]	0.165[b]
D+	4.487[bc]	37.87	12.38[b]	10547	44.10	1.534[c]	0.195[b]
SEM	0.221	2.086	0.943	810.1	0.373	0.325	0.027
P value							
Antibiotic (A)	0.000	0.069	0.000	0.419	0.549	0.002	0.119
Vaccine (V)	0.000	0.000	0.000	0.166	0.139	0.000	0.650
A × V interaction	0.000	0.200	0.000	0.304	0.892	0.008	0.032

[a,b,c]Means within the same column with different superscripts differ significantly ($P < 0.05$).
[1]Treatment: C, untreated control; M, treated with monensin; E, treated with enrofloxacin; D, treated with doxycycline.
[2]Unvaccinated, − or vaccinated, +.CRP, C-reactive protein; Cp, ceruloplasmin; NF-κB, nuclear factor kappa B; IgA, immunoglobulin A; NBT, respiratory burst activity of the heterophils; TLR-4, toll-like receptor 4.

revealed normal histology of both organs in unvaccinated birds from the control group, the group receiving monensin, and the groups administered enrofloxacin or doxycycline for the first 5 d of life. The bursa of Fabricius and the thymus of 7-day-old unvaccinated turkeys from the control group were characterized by fatty degeneration, which progressed to a more advanced form in 56-day-old birds. A histological image analysis of the bursa of Fabricius and the thymus in 7-day-old unvaccinated turkeys also revealed the presence of adipose tissue foci, which were least visible in the control group, more visible in enrofloxacin and doxycycline-treated groups, and most visible in the group receiving monensin, where fatty degeneration was most severe (Figures 1 and 2). A histological image analysis of the spleen collected from 7-day-old unvaccinated turkeys revealed normal histology of the organ. Spleens collected from control group birds were characterized by multiple

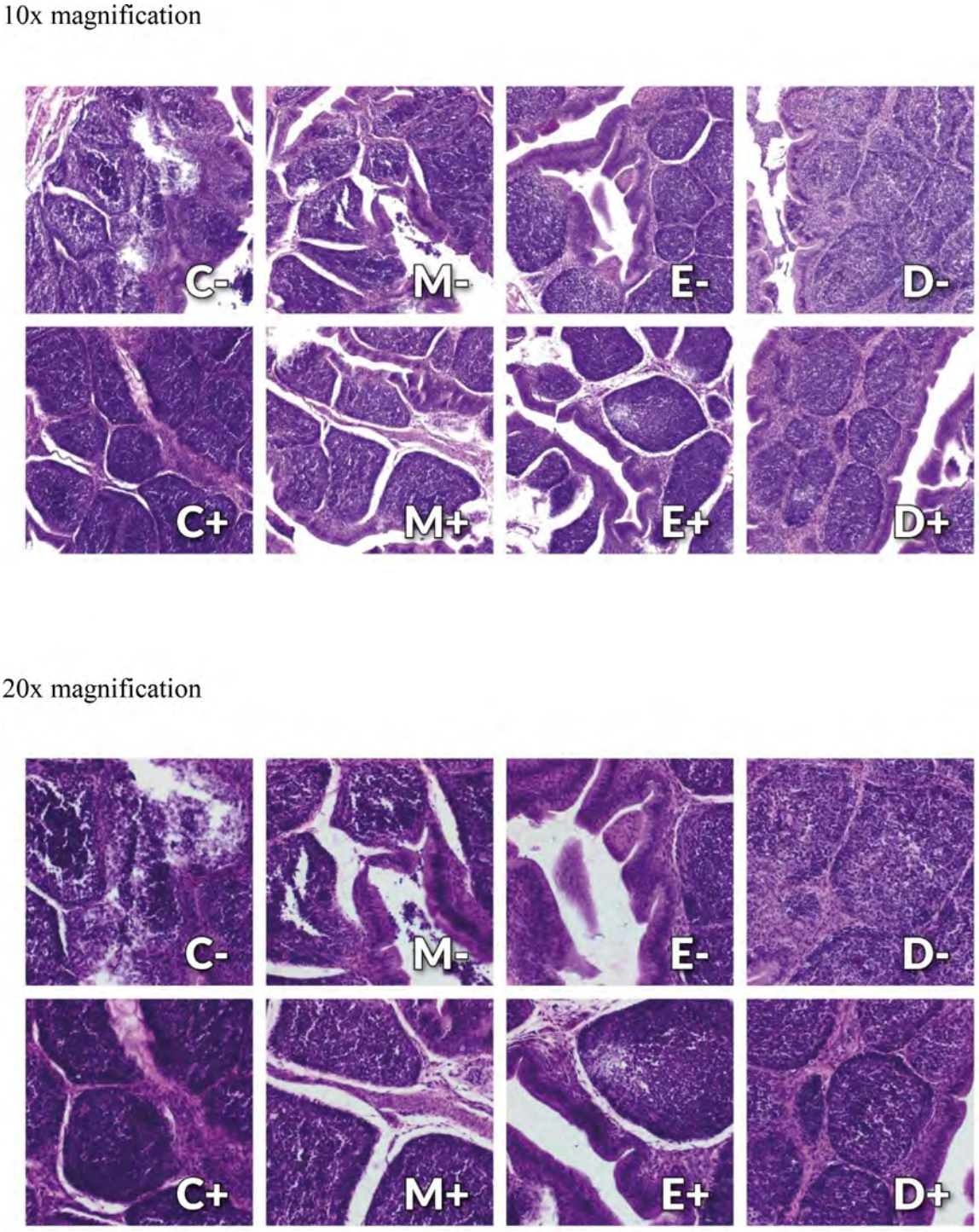

Figure 1. Morphological effects of early administration of antibiotics or feeding a diet containing a coccidiostat and vaccination on the bursa of Fabricius of 7-day-old turkeys. Treatment: (C−) unvaccinated control group; (M−) unvaccinated group treated with monensin; (E−) unvaccinated group treated with enrofloxacin; (D−) unvaccinated group treated with doxycycline; (C+) vaccinated untreated control group; (M+) vaccinated group treated with monensin; (E+) vaccinated group treated with enrofloxacin; (D+) vaccinated group treated with doxycycline.

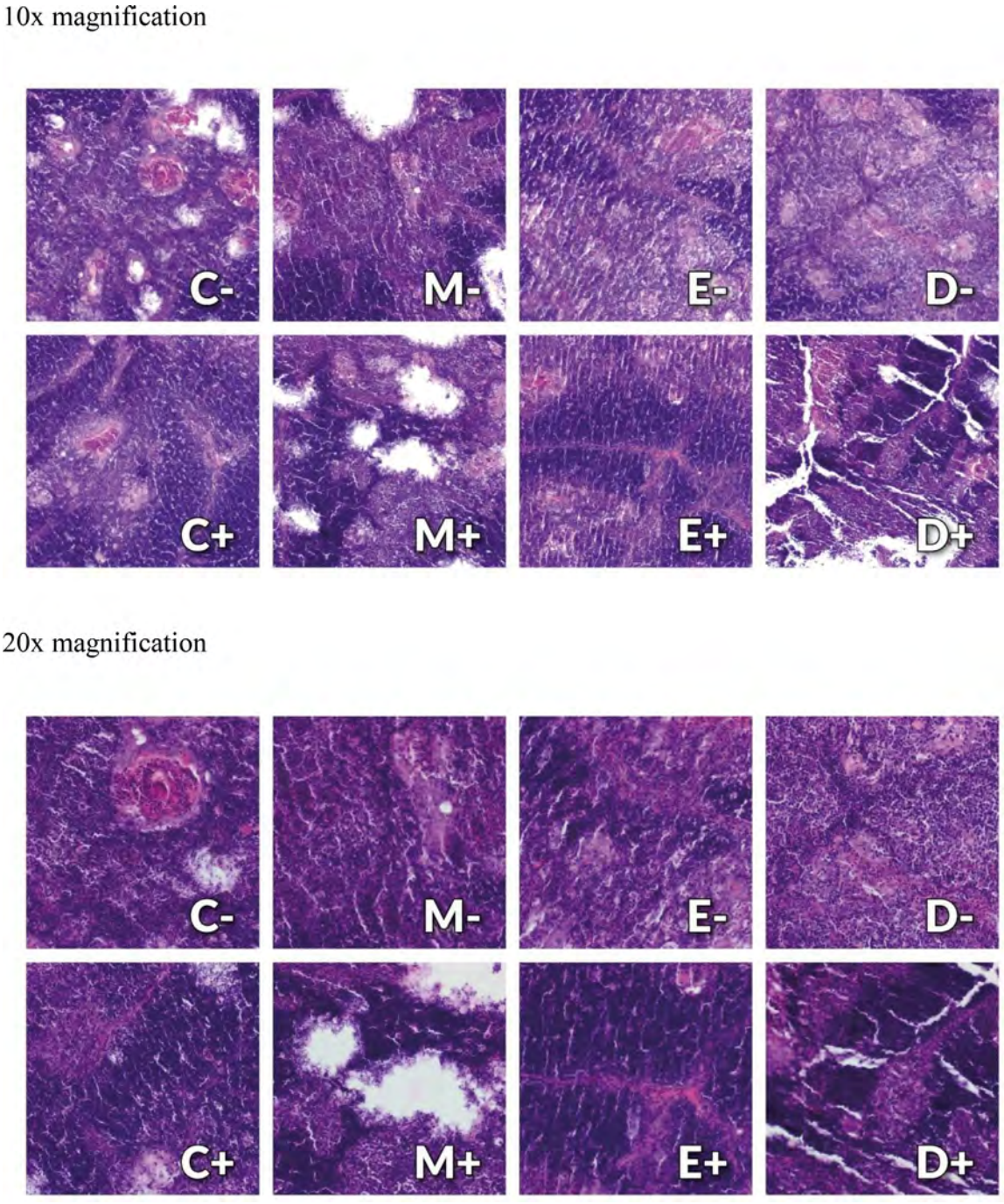

Figure 2. Morphological effects of early administration of antibiotics or feeding a diet containing a coccidiostat and vaccination on the thymus of 7-day-old turkeys. Treatment: (C−) unvaccinated control group; (M−) unvaccinated group treated with monensin; (E−) unvaccinated group treated with enrofloxacin; (D−) unvaccinated group treated with doxycycline; (C+) vaccinated untreated control group; (M+) vaccinated group treated with monensin; (E+) vaccinated group treated with enrofloxacin; (D+) vaccinated group treated with doxycycline.

foci of local congestion and the presence of single clusters of adipocytes with no pathological changes. Similarly to the bursa of Fabricius and the thymus, a histological image analysis of the spleen collected from 7-day-old unvaccinated turkeys revealed the presence of multiple fatty degeneration foci, which were more visible in the groups administered enrofloxacin and doxycycline, and most visible in the group receiving monensin, where fatty degeneration was most severe (Figure 3). Apart from foci of fatty degeneration, the analyzed organs collected from unvaccinated turkeys in all experimental groups were also characterized by the presence of local congestion foci. A histological image analysis of the bursa of Fabricius, the thymus and the spleen in 56-day-old unvaccinated birds confirmed the previously noted relationship: monensin applied throughout the feeding period resulted in the most severe fatty degeneration of these organs. In 56-day-old turkeys, the spleen was least prone to fatty degeneration of all examined organs (Figures 4, 5, and 6).

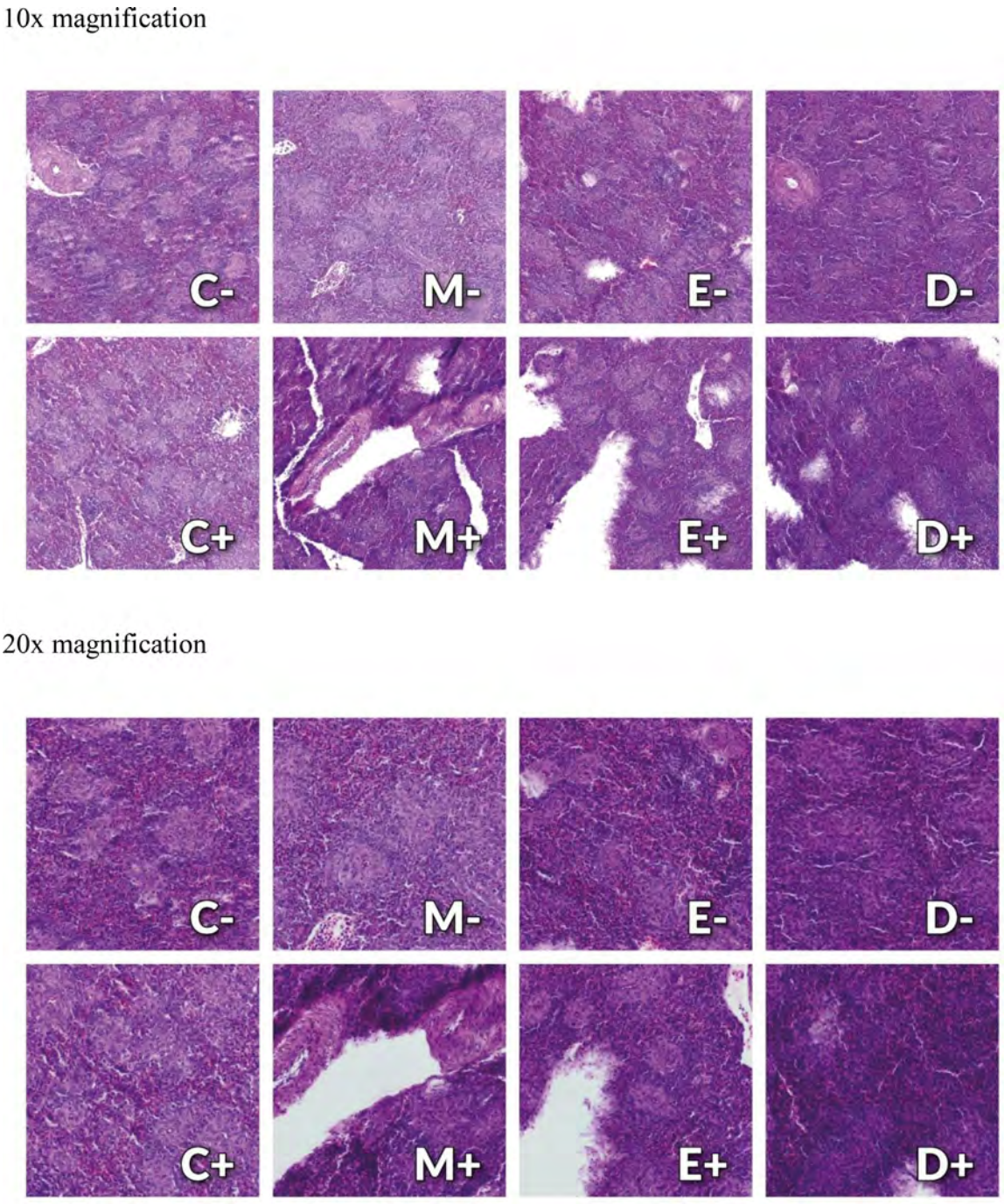

Figure 3. Morphological effects of early administration of antibiotics or feeding a diet containing a coccidiostat and vaccination on the spleen of 7-day-old turkeys. Treatment: (C−) unvaccinated control group; (M−) unvaccinated group treated with monensin; (E−) unvaccinated group treated with enrofloxacin; (D−) unvaccinated group treated with doxycycline; (C+) vaccinated untreated control group; (M+) vaccinated group treated with monensin; (E+) vaccinated group treated with enrofloxacin; (D+) vaccinated group treated with doxycycline.

Effect of Vaccination

Turkeys vaccinated against TRT and ND on the first day of their life had lower blood levels of NF-kB, IgA, and TLR-4 ($P < 0.001$, all) at 7 d of age than unvaccinated birds. Moreover, vaccination on the first day of life increased blood amyloid A levels ($P = 0.001$) in 7-day-old turkeys. Vaccination against ORT at 28 d of age had no influence on blood IgA levels in 56-day-old birds, but it decreased Cp and TLR-4 levels ($P < 0.001$, both).

A histological image analysis of the bursa of Fabricius, the thymus and the spleen, collected from 7-day-old turkeys vaccinated against TRT and ND, revealed normal histology of these organs. Foci of fatty degeneration were noted in vaccinated birds from the control group, and their image was similar to that of unvaccinated turkeys from the control group. A histological image analysis of the bursa of Fabricius, the thymus and the spleen, collected from vaccinated 7-day-old turkeys receiving enrofloxacin, doxycycline, or monensin revealed a higher number of fatty degeneration and local congestion foci than in their respective unvaccinated counterparts (Figures 1, 2, and 3). A histological analysis of the organs collected at 56 d of age from turkeys vaccinated against

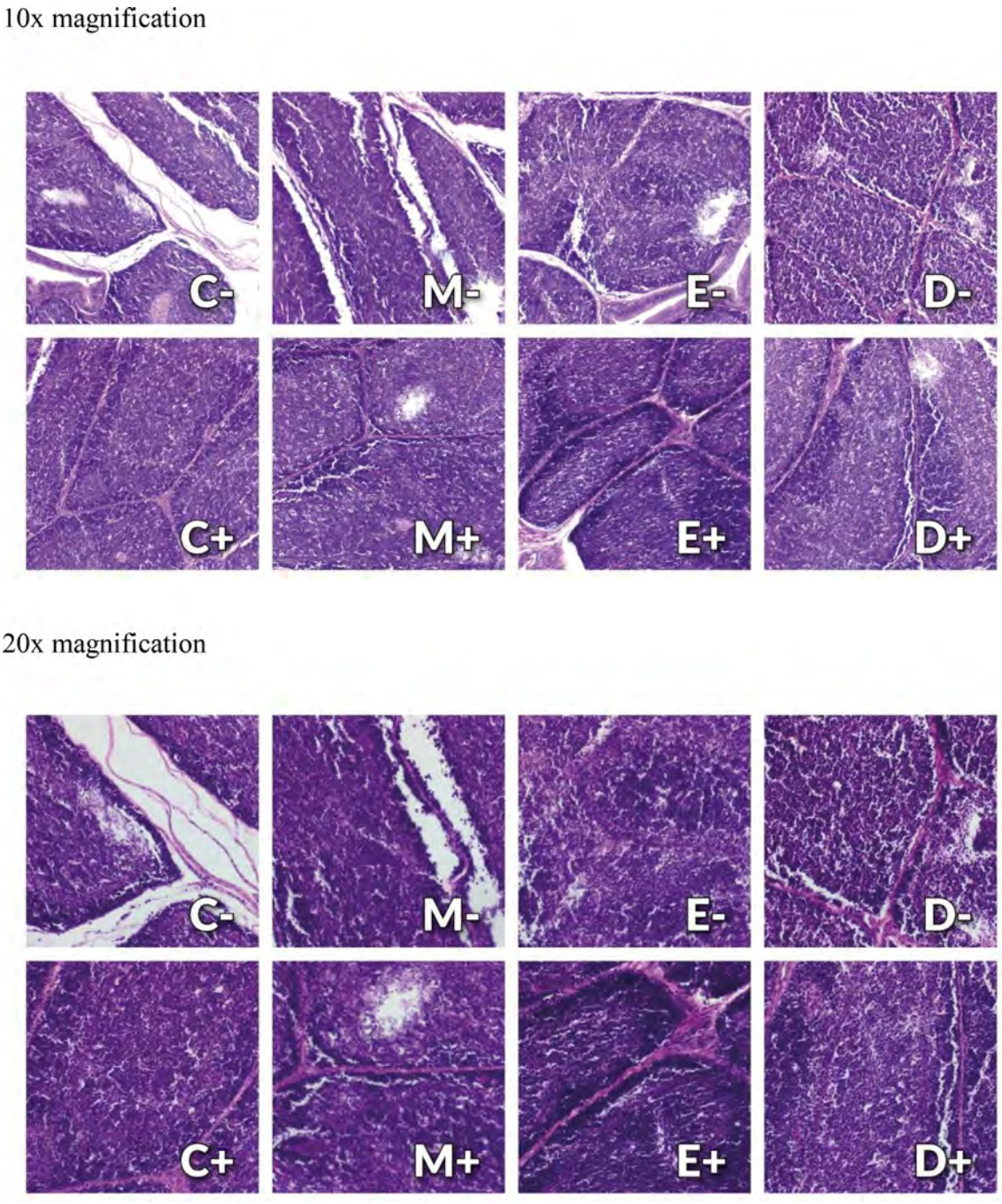

Figure 4. Morphological effects of early administration of antibiotics or feeding a diet containing a coccidiostat and vaccination on the bursa of Fabricius of 56-day-old turkeys. Treatment: (C−) unvaccinated control group; (M−) unvaccinated group treated with monensin; (E−) unvaccinated group treated with enrofloxacin; (D−) unvaccinated group treated with doxycycline; (C+) vaccinated untreated control group; (M+) vaccinated group treated with monensin; (E+) vaccinated group treated with enrofloxacin; (D+) vaccinated group treated with doxycycline.

TRT, ND, and ORT, similarly to those collected at 7 d of age, demonstrated that feeding a diet with the addition of monensin made a greater contribution to the development of fatty degeneration than early administration of enrofloxacin or doxycycline. An evaluation of the organs of the immune system collected from vaccinated turkeys revealed that the spleen is least prone to fatty degeneration resulting from antibiotic treatment. Similar observations were made in unvaccinated birds (Figures 4, 5, and 6).

DISCUSSION

In poultry farms, antibiotics are added to feed to prevent coccidiosis in broiler chickens and meat-type turkeys. Enrofloxacin and doxycycline are broad-spectrum antibiotics, commonly used in farm animals, including poultry (Gabler et al., 1992; Fife and Sledge, 1995; Khalifeh et al., 2009). Ibrahim et al. (2011) demonstrated that enrofloxacin administered to broiler chickens for 30 consecutive days had a negative effect on their

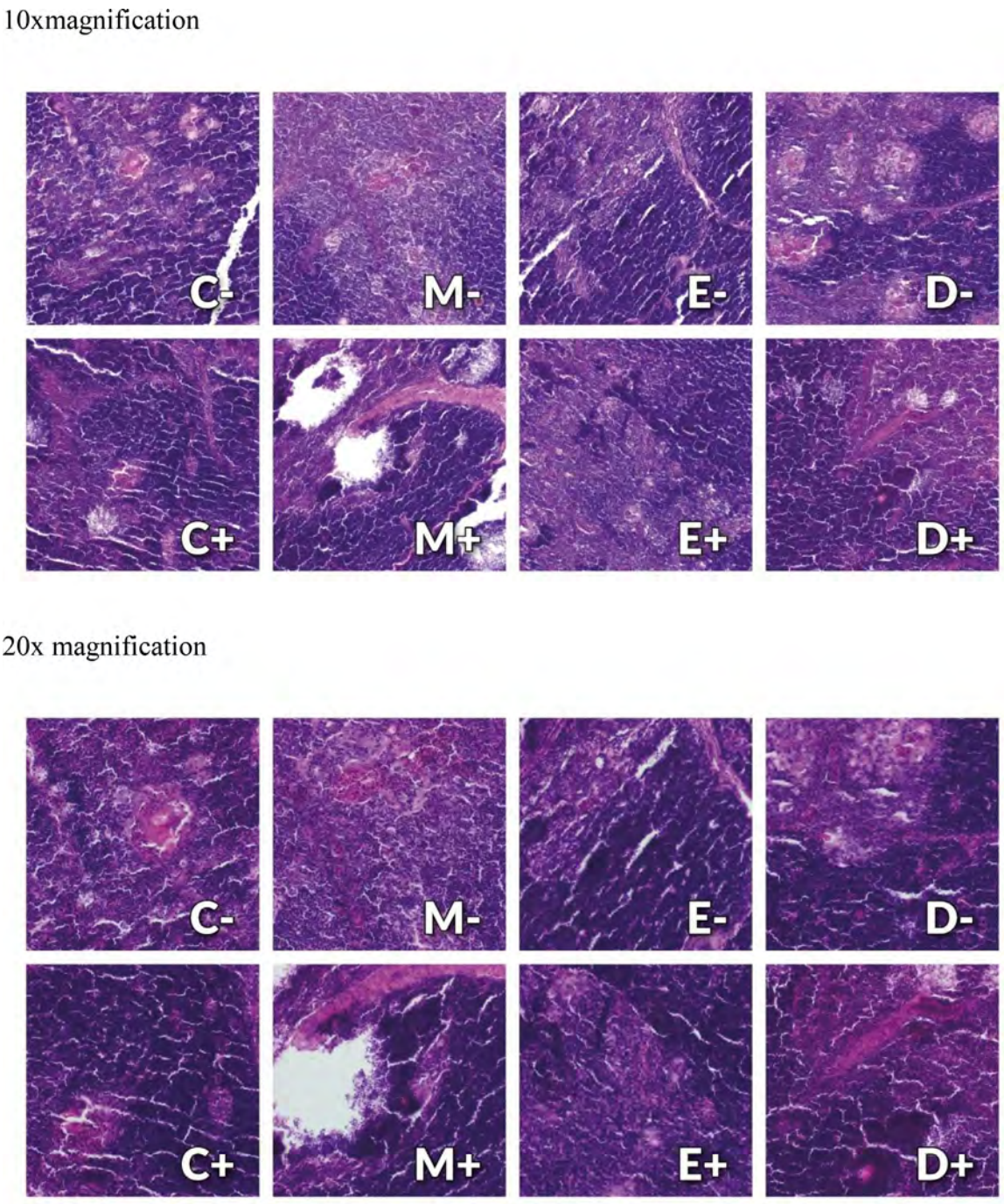

Figure 5. Morphological effects of early administration of antibiotics or feeding a diet containing a coccidiostat and vaccination on the thymus of 56-day-old turkeys. Treatment: (C−) unvaccinated control group; (M−) unvaccinated group treated with monensin; (E−) unvaccinated group treated with enrofloxacin; (D−) unvaccinated group treated with doxycycline; (C+) vaccinated untreated control group; (M+) vaccinated group treated with monensin; (E+) vaccinated group treated with enrofloxacin; (D+) vaccinated group treated with doxycycline.

hematological parameters. Enrofloxacin doses that were 10- and 20-fold higher than the recommended dose resulted in leukopenia, which indicates that the antibiotic affected the immune system of birds.

It has long been recognized that several antibiotic classes, including quinolones and tetracyclines, exhibit both immunomodulatory and antimicrobial activity (Chrząstek et al., 2011; Chrząstek and Wieliczko 2015). They diminish TLR-2 and TLR-4 activity, resulting in anti-inflammatory actions. Toll-like receptors are an evolutionarily conserved group of pattern recognition receptors that play an important role in mediating host responses to pathogens (Lagos Silva et al., 2022). They are expressed on the surface of various immune cells, including macrophages, dendritic cells, B cells, and selected T cell subpopulations. The extracellular portion of TRLs recognizes pathogen-associated molecular patterns (**PAMPs**) and the intracellular portion constitutes the Toll/interleukin-1 receptor (**TIR**) domain (Akira et al., 2001). The TIR domain initiates intracellular responses to PAMPs, leading to the activation of the transcription factor NF-kB, activator protein 1 (**AP-1**),

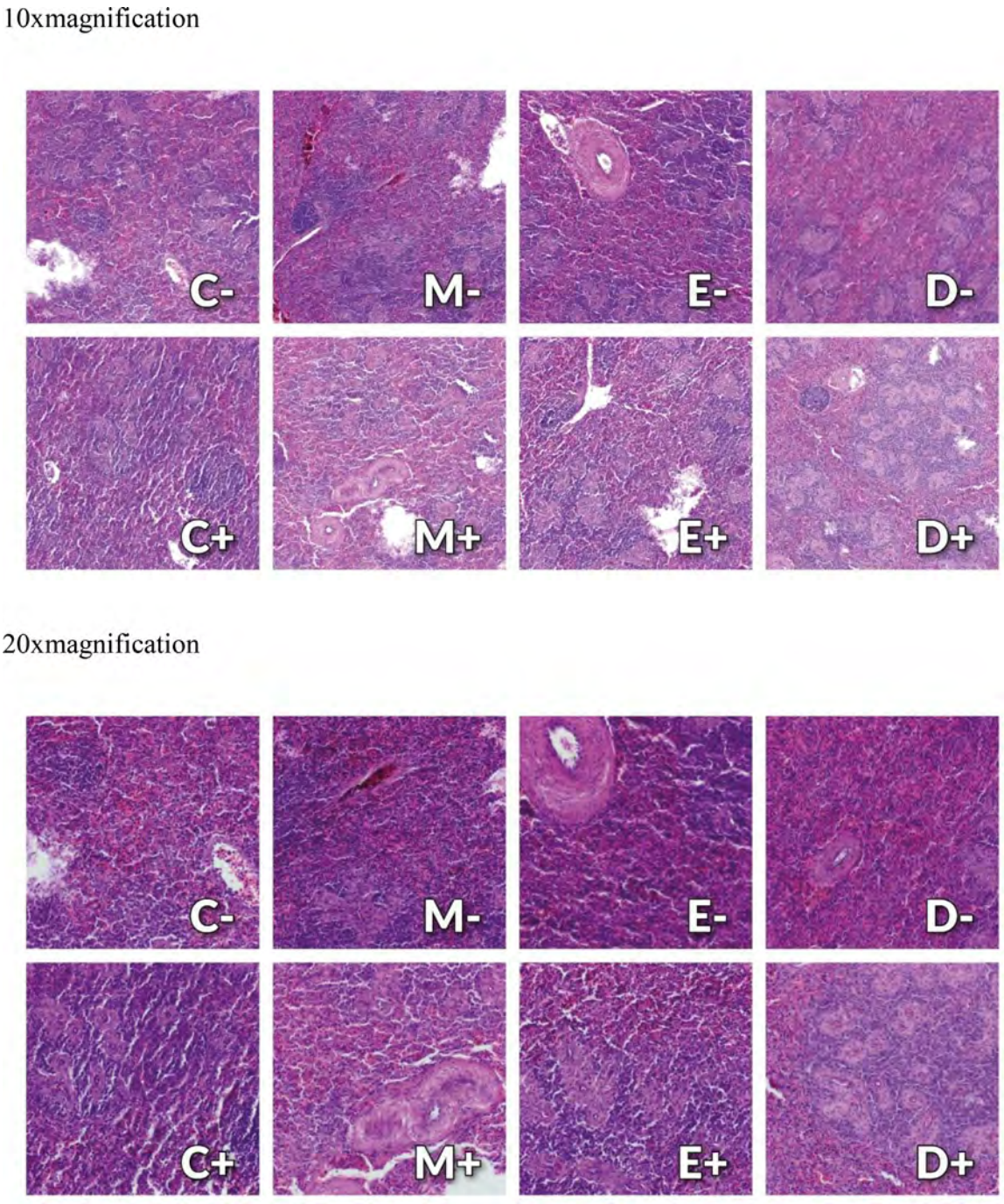

Figure 6. Morphological effects of early administration of antibiotics or feeding a diet containing a coccidiostat and vaccination on the spleen of 56-day-old turkeys. Treatment: (C−) unvaccinated control group; (M−) unvaccinated group treated with monensin; (E−) unvaccinated group treated with enrofloxacin; (D−) unvaccinated group treated with doxycycline; (C+) vaccinated untreated control group; (M+) vaccinated group treated with monensin; (E+) vaccinated group treated with enrofloxacin; (D+) vaccinated group treated with doxycycline.

and interferon regulatory factors (**IRFs**), and, in consequence, to the production of proinflammatory cytokines. This receptor is usually activated in response to a pathogenic factor and inflammation. Its activation is followed by NF-kB secretion and the production of proinflammatory cytokines (Richmond and Yang, 2016). Free-floating variants of TLRs are known as soluble TLRs (**sTLRs**). These free-floating protein complexes are considered structurally identical to their membrane-bound counterparts, but they do not participate in the TLR pathway. Instead, they reduce inflammatory responses by competing with TLRs for ligands. In the present study, the administration of enrofloxacin or doxycycline to turkeys during the first 5 d of their life induced a decrease in serum soluble TLR-4 levels. This suggests that these antibiotics reduced the microbial load in birds to a greater extent than monensin, thus decreasing the inflammatory response to the microbes.

The above is confirmed by the fact that 7-day-old turkeys receiving monensin were characterized by the highest levels of amyloid A, which is an important marker of inflammation. The levels of this receptor decreased in 7-

day-old turkeys that had been vaccinated against TRT and ND, which is surprising because vaccination induces host inflammatory responses. Toll-like receptors constitute the first line of defense against invading pathogens and they enable immune cells to differentiate between own and foreign antigens.

In the current study, antibiotics administered in the first days after hatch and pathogens contained in vaccines decreased serum NF-kB levels in turkeys, which could be related to the fact that enrofloxacin and doxycycline induced a significant decrease in sTLR-4 levels, which was not observed in birds receiving monensin. NF-kB plays a key role in the transcription of numerous genes. The first group includes genes directly related to immunity, such as immunoreceptors, proteins involved in antigen presentation, cytokines, and acute phase proteins. The second group of genes regulated by NF-κB is associated with stress adaptation and homeostasis maintenance under various stress conditions. The third group of NF-κB-regulated genes, including regulators of apoptosis, cell-surface receptors, cell adhesion molecules, growth factors, and their modulators, is responsible for the regulation of various cellular functions. There are also NF-κB-regulated genes related to viruses, enzymes, and some other important signaling molecules. Therefore, the great variety of NF-κB-regulated genes explains the pivotal role of this transcription factor in major physiological and pathophysiological processes in mammalian and avian species (Surai et al., 2021). Increased expression and activation of NF-kB usually results from progressive inflammation (Richmond and Yang, 2016; Liu et al., 2017). This study demonstrated that even short-term antibiotic treatment may inhibit inflammatory responses in turkeys, especially when combined with vaccination.

To the best of our knowledge, there is a general scarcity of published studies investigating the immune responses of birds receiving antibiotics and simultaneously coping with infectious agents contained in live and inactivated vaccines.

In the present study, antibiotics administered to vaccinated turkeys inhibited the secretion of inflammation-regulating factors (sTLR-4 and NF-kB), which was reflected in lower plasma levels of acute phase proteins (CRP and Cp). Most probably, antibiotics exerted an inhibitory effect on the gut microbiome of poults. Similar relationships were observed in newborn piglets that were administered antibiotics in the first days of life (Jiang et al., 2012). The current study demonstrated that early administration of enrofloxacin and doxycycline (first 5 d after hatch) significantly decreased plasma CRP levels in turkeys included in the vaccination program, compared with control group birds and those receiving monensin throughout the rearing period. Similarly to CRP, plasma Cp levels decreased in vaccinated 7-day-old turkeys in response to antibiotics, in particular doxycycline. The effect of enrofloxacin and doxycycline on Cp levels weakened over time, as indicated by the fact that the decrease in this parameter in 8-wk-old birds resulted solely from vaccination against ORT at 28 d of age. In our previous study, blood CRP and Cp levels also decreased in 35-day-old chickens in response to enrofloxacin administered at 0.5 mL of Scanoflox 10% Oral (Lavet Pharmaceuticals Ltd., Budapest, Hungary) per 1 l of water for the first 5 d of life (Jankowski et al., 2022). Acute phase proteins are produced in the liver under the influence of various proinflammatory factors. Their physiological levels point to the absence of inflammation (Du Clos and Mold, 2004). Many classes of antibiotics used in human and veterinary medicine exert not only antimicrobial but also immunomodulatory effects that involve the inhibition of innate immune responses, including inflammatory and acute phase responses (Tkalčević et al., 2011; Parnham and Haber, 2016), which may negatively affect the development of vaccine-induced and natural immunity.

An analysis of serum amyloid A levels in 7-day-old turkeys revealed that they increased in response to a diet containing monensin, and vaccination against TRT and ND. This indicates that the coccidiostat exerted a weaker anti-inflammatory effect than enrofloxacin and doxycycline. Moreover, monensin had a particularly negative influence on the development of histopathological changes, mostly foci of fatty degeneration, in the analyzed organs of the immune system in turkeys. Monensin contributed to undesirable changes in internal organs also in other animal species (Anderson et al., 1984; Van Vleet and Ferrans, 1984).

It was also found that serum IgA levels decreased in 7-day-old turkeys as a result of vaccination against TRT and ND. It appears that the decrease in maternal-IgA levels in the blood serum of vaccinated turkeys could be due to the fact that some of the pathogen-specific antibodies used in vaccines formed antigen-antibody complexes. Turkeys were vaccinated against TRT and ND with live-attenuated vaccines administered by coarse spray and after vaccination, IgA antibodies could be transferred from the blood serum to the mucosal surface where vaccine viruses were present. Mucosal immunity is largely dependent on IgA produced locally by B cells. After infection, IgA is synthesized and transported through epithelial cells to the mucosal surface via a secretory component that neutralizes pathogens. As a result, the proliferation of pathogens is limited, but not completely inhibited, and serum IgA levels may be lower due to their secretion at the mucosal surface (Ganapathy et al., 2005; Feng et al., 2021). Vaccination of 28-day-old turkeys with a subcutaneously injected inactivated vaccine against ORT had no influence on serum IgA levels, most likely because such vaccines usually induce systemic IgY-dependent immunity, whereas local immune responses involving IgA are limited.

A histopathological analysis of immunocompetent organs revealed fatty degeneration and congestion that aggravated over time in all experimental groups, which could be associated with the diets applied in intensive turkey farming. Nevertheless, the extent of the observed changes was greater in turkeys that received antibiotics (enrofloxacin or doxycycline) in early life or were fed a diet containing the coccidiostat monensin. Monensin

exerted the most negative effect on the morphology of immunocompetent organs. It should also be stressed that the undesirable histological changes in the examined organs were exacerbated by vaccination. Apart from fatty degeneration, no undesirable morphological changes were found in the bursa of Fabricius, the spleen and the thymus of turkeys. Chrząstek et al. (2011) also reported that early administration of antibiotics (including enrofloxacin) to chickens did not impair the microstructure of the bursa of Fabricius.

CONCLUSIONS

The results of this study indicate that early administration of enrofloxacin or doxycycline, or feeding a diet containing monensin, may affect the immune system of young turkeys. Antibiotics administered to birds for the first 5 d of life, with drinking water, inhibited innate immune responses in turkeys that were vaccinated against ND and TRT on the first day of life. The administration of monensin was least effective in inhibiting inflammatory responses after vaccination. Histological changes in the organs of the immune system (fatty degeneration) were also most severe in birds receiving monensin, followed by those administered doxycycline and enrofloxacin.

ACKNOWLEDGMENTS

This work was supported by the National Science Centre in Poland, Grant No. 2020/39/B/NZ9/00765.

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present study.

REFERENCES

Abdelhady, A. Y., S. A. El-Safty, M. Hashim, M. A. Ibrahim, F. F. Mohammed, A. M. Elbaz, and A. M. E. Abdel-Moneim. 2021. Comparative evaluation of single or combined anticoccidials on performance, antioxidant status, immune response, and intestinal architecture of broiler chickens challenged with mixed Eimeria species. Poult. Sci. 100:101162.

Akira, S., K. Takeda, and T. Kaisho. 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat. Immunol. 2:675–680.

Anderson, T. D., W. G. Van Alstine, M. D. Ficken, D. W. Miskimins, T. L. Carson, and G. D. Osweiler. 1984. Acute monensin toxicosis in sheep: light and electron microscopic changes. Am. J. Vet. Res. 45:1142–1147.

Chrząstek, K., J. P. Madej, E. Mytnik, and A. Wieliczko. 2011. The influence of antibiotics on B-cell number, percentage, and distribution in the bursa of Fabricius of newly hatched chicks. Poult. Sci. 90:2723–2729.

Chrząstek, K., and A. Wieliczko. 2015. The influence of enrofloxacin, florfenicol, ceftiofur and E. coli LPS interaction on T and B cells subset in chicks. Vet. Res. Commun. 39:53–60.

Close, B., K. Banister, V. Baumans, E. M. Bernoth, N. Bromage, J. Bunyan, W. Erhardt, P. Flecknell, N. Gregory, H. Hackbarth, D. Morton, and C. Warwick. 1997. Recommendations for euthanasia of experimental animals: part 2. Lab. Anim. 31:1–32.

Du Clos, T. W., and C. Mold. 2004. C-reactive protein. Immunol. Res. 30:261–277.

Feng, H., X. Wang, J. Zhang, K. Zhang, W. Zou, K. Zhang, L. Wang, Z. Guo, Z. Qiu, G. Wang, R. Xin, and J. Li. 2021. Combined effect of shegandilong granule and doxycycline on immune responses and protection against avian infectious bronchitis virus in broilers. Front. Vet. Sci. 8:756629.

Fife, R. S., and G. W. Sledge. 1995. Effects of doxycycline on in vitro growth, migration, and gelatinase activity of breast carcinoma cells. J. Lab. Clin. Med. 125:407–411.

Gabler, W. L., J. Smith, and N. Tsukuda. 1992. Comparison of doxycycline and a chemically modified tetracycline inhibition of leukocyte functions. Res. Commun. Chem. Pathol. Pharmacol. 78:151–160.

Ganapathy, K., P. W. Cargill, and R. C. Jones. 2005. A comparison of methods of inducing lachrymation and tear collection in chickens for detection of virus-specific immuoglobulins after infection with infectious bronchitis virus. Avian Pathol. 34:248–251.

Grabowski, L., G. Węgrzyn, A. Węgrzyn, and M. Podlacha. 2022. Highly different effects of phage therapy and antibiotic therapy on immunological responses of chickens infected with Salmonella enterica serovar Typhimurium. Front. Immunol. 13:956833.

Hybrid Turkeys. 2020. Commercial Management Guide and Nutrient Guidelines for Hybrid Turkeys. Accessed July 2021. http://www.hybridturkeys.com.

Ibrahim, I. G., N. Atmaca, A. Kanici, and E. Yarsan. 2011. Evaluation of effects of enrofloxacin on some haematological parameters in broilers. Atatürk Üniv. Vet. Bil. Derg. 6:97–102.

Jankowski, J., B. Tykałowski, A. Stępniowska, P. Konieczka, A. Koncicki, P. Matusevičius, and K. Ognik. 2022. Immune parameters in chickens treated with antibiotics and probiotics during early life. Animals 12:1133.

Jiang, P., M. L. Jensen, M. S. Cilieborg, T. Thymann, J. M. Wan, W. H. Sit, G. L. Tipoe, and P. T. Sangild. 2012. Antibiotics increase gut metabolism and antioxidant proteins and decrease acute phase response and necrotizing enterocolitis in preterm neonates. PLoS One 7:e44929.

Khalifeh, M. S., M. M. Amawi, E. A. Abu-Basha, and I. B. Yonis. 2009. Assessment of humoral and cellular-mediated immune response in chickens treated with tilmicosin, florfenicol, or enrofloxacin at the time of Newcastle disease vaccination. Poult. Sci. 88:2118–2124.

Kozłowski, K., K. Ognik, A. Stępniowska, J. Juśkiewicz, Z. Zduńczyk, B. Kierończyk, A. Benzertiha, D. Józefiak, and J. Jankowski. 2021. Growth performance, immune status and intestinal fermentative processes of young turkeys fed diet with additive of full fat meals from *Tenebrio molitor* and *Hermetia illucens*. Anim. Feed Sci. Technol. 278:114994.

Lagos Silva, L., T. V. Luu, B. De Haan, M. Faas, and P. De Vos. 2022. TLR2 and TLR4 activity in monocytes and macrophages after exposure to amoxicillin, ciprofloxacin, doxycycline and erythromycin. J. Antimicrob. Chemother. 77:2972–2983.

Lee, K. W., H. S. Lillehoj, S. H. Lee, S. I. Jang, M. S. Park, D. A. Bautista, G. D. Ritter, Y. H. Hong, G. R. Siragusa, and E. P. Lillehoj. 2012. Effect of dietary antimicrobials on immune status in broiler chickens. Asian-Aust. J. Anim. Sci. 25:382–392.

Liu, T., L. Zhang, D. Joo, and S. C. Sun. 2017. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2:17023.

Madubuike, K. G., O. N. Okoroafor, and I. U. Asuzu. 2020. Effects of early-life antibiotics administration on the immune response to Newcastle disease lasota vaccination and weight indices of broiler chicken. Folia Vet. 64:74–79.

Mikulski, D., J. Juśkiewicz, K. Ognik, P. Zduńczyk, R. Smagieł, and J. Jankowski. 2022. Gastrointestinal tract and neuroendocrine system responses of young turkeys to the early administration of antibiotics or feeding a diet containing a coccidiostat. Poult. Sci. 101:102098.

Park, B. H., S. M. Fikrig, and E. M. Smithwick. 1968. Infection and nitroblue tetrazolium reduction by neutrophils. Lancet 7:532–534.

Parnham, M. J., and V. E. Haber. 2016. Antibiotics as antiinflammatory drugs. Pages 1−11 in Compendium of Inflammatory Diseases. M. J. Parnham, ed. Springer, Basel, Switzerland.

Richmond, A., and J. Yang. 2016. The role of NF-kB in modulating antitumor immunity. Oncoimmunology 5:e1005522.

Riesbeck, K. 2002. Immunomodulating activity of quinolones: review. J. Chemother. 14:3–12.

Surai, P. F., I. I. Kochish, and M. T. Kidd. 2021. Redox homeostasis in poultry: regulatory roles of NF-κB. Antioxidants 10:186.

TIBCO Software Inc. 2017. STATISTICA (data analysis software system), version 13. Accessed February 2023. http://statistica.io.

Tkalčević, V. I., B. Hrvačić, I. Pašalić, V. E. Haber, and I. Glojnarić. 2011. Immunomodulatory effects of azithromycin on serum amyloid a production in lipopolysaccharide-induced endotoxemia in mice. J. Antibiotics 64:515–517.

Tokarzewski, S. 2002. Influence of enrofloxacin and chloramphenicol on the level of IgY in serum and egg yolk after immunostimulation of hens with Salmonella Enteritidis antigens. Pol. J. Vet. Sci. 5:151–158.

Van Vleet, J. F., and V. J. Ferrans. 1984. Ultrastructural alterations in the atrial myocardium of pigs with acute monensin toxicosis. Am. J. Pathol. 114:367–379.

Animal 18 (2024) 101321

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

The effect of early administration of antibiotics or feeding a diet containing coccidiostats on the level of their accumulation in liver and the redox status of turkeys

R. Smagieł [a], K. Tutaj [a], E. Cholewińska [a], P. Sołek [a], D. Mikulski [b], A. Stępniowska [a,*], J. Jankowski [b], K. Ognik [a]

[a] Department of Biochemistry and Toxicology, University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland
[b] Department of Poultry Science and Apiculture, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-719 Olsztyn, Poland

ARTICLE INFO

Article history:
Received 10 January 2024
Revised 28 August 2024
Accepted 29 August 2024
Available online 4 September 2024

Keywords:
Antioxidant
Doxycycline
Enrofloxacin
Monensin
Tissue

ABSTRACT

Early administration of antibiotics may worsen the functioning of the turkeys' antioxidant system. It was also assumed that the longer the time of administration of an antibiotic, e.g. a coccidiostat, the greater the risk of its accumulation in the liver. The study aimed to determine whether early administration of antibiotics or feeding a diet containing coccidiostats causes accumulation in the liver and whether it affects the deterioration of the antioxidant system, and whether preventive vaccinations can intensify it. A total of 3 080 female turkeys were randomly allocated to eight groups. The experiment had a two-factorial design, with four treatments (C, M, E, D) and two groups of birds (vaccinated +, unvaccinated −). The C group did not receive the coccidiostat or antibiotics. Group M was administered monensin at 90 mg/kg feed for 56 days of life. Group E received enrofloxacin at 10 mg/kg BW, and group D received doxycycline at 50 mg/kg BW, added to drinking water, for the first 5 days of life. One-day-old turkeys from groups C+, M+, E+, and D+ were administered live-attenuated vaccines against turkey rhinotracheitis and Newcastle disease by coarse spray; 28-day-old birds were administered a subcutaneously injected inactivated vaccine against *Ornithobacterium rhinotracheale*. Turkeys from groups C-, M-, E-, and D- were not vaccinated. It was determined that as a result of administration of enrofloxacin or doxycycline until the 5th day of life, biotransformation of these antibiotics occurred in the liver until the 56th day of life of the turkeys, which was confirmed by their lower level than the Maximum Residue Level. Because the concentration of monensin in the liver of turkeys gradually increased with the extension of the time of its administration in the diet, it is probable that discontinuing its addition a day before the slaughter of birds will result in the presence of this coccidiostat in the liver of turkeys. Despite the accumulation of monensin in the liver of turkeys, this coccidiostat did not increase oxidative reactions in the organism of turkeys. Vaccination of turkeys can reduce oxidative reactions and apoptosis in the body. However, the effect of the redox system reaction is different immediately after vaccination, which is due to the mechanism of action of the immune system. If it is necessary to administer an antibiotic in the early rearing period, the effects of doxycycline on the organism's immunity including antioxidant defence will be less severe than those of enrofloxacin.
© 2024 The Authors. Published by Elsevier B.V. on behalf of The Animal Consortium. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Implications

Despite the ban on the use of antibiotics as growth promoters, the possibility of administering coccidiostats, which are antibiotics, has been left open. The need to administer antibiotics in the early rearing period can have many negative effects. In the case of antibiotic administration in the first 5 days of life, their presence is not detected in the liver of 8-week-old turkeys, and long-term administration of monensin causes its accumulation in direct proportion to the time of use. It has been established that the disruption of antioxidant defence is less severe in the case of administration of doxycycline than enrofloxacin.

Introduction

The current challenge facing poultry production, including turkey production, is the need to limit the antibiotics used, justified for many reasons (Śmialek et al., 2023). The European Union has obliged member states to reduce the use of antibiotics in animal

* Corresponding author.
E-mail address: anna.stepniowska@up.lublin.pl (A. Stępniowska).

https://doi.org/10.1016/j.animal.2024.101321
1751-7311/© 2024 The Authors. Published by Elsevier B.V. on behalf of The Animal Consortium.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

production by 50% by 2030. This is one of the goals of the European Green Deal as part of the fight against antimicrobial resistance (European Medicines Agency, 2023). Gaining new knowledge about the biological effects of early use of antibiotics and continuous use of coccidiostats in young turkeys (up to 8–12 weeks of age) is one of the most important ways to achieve this goal. There is a lack of empirical evidence that such treatment, including "prophylactic" administration of antibiotics in therapeutic doses already in the 1st week of life of turkeys (usually from the 2nd day of life), does not interfere with the physiological mechanisms of transferring maternal immunity to chicks and acquiring their defence against pathogens. We assume that by potentially limiting the transfer of maternal antibodies in turkey poults' first courses of life, the birds' immune system may be weakened. Maternal antibodies constitute humoral immunity, which acts through antibodies produced by stimulated B lymphocytes, which, by binding to the antigen, initiate the phagocytosis reaction by phagocytic cells. During phagocytosis, phagocytic cells produce free radicals, and the intensity of this process may modify systemic redox reactions (Splettstoesser and Schuff-Werner, 2002). In EU countries, a ban on the use of antibiotics as growth stimulants was introduced in 2006 (Regulation EC, 2003). However, the possibility of continuous use of coccidiostats in the feed, including ionophores, which are also antibiotics, has been left. According to Rajendran et al. (2018), apart from its antibacterial effect, monensin has antifungal, anti-parasitic, antiplasmodial, anti-viral, anti-trypanosomiasis, anti-toxoplasmosis and antitileishmaniasis properties. Monensin can be used until turkeys are 16 weeks old, and the withdrawal period is only 1 day (WHO, 2009). Paradoxical situations have arisen when the use of a specific antibiotic as a growth stimulator is prohibited and allowed as a coccidiostat. A commonly used method of preventing coccidiosis in birds is the constant administration of coccidiostats in feed mixtures, but their combined administration with another antibiotic may result in biological reactions in the body resulting from their interaction. The effect of such an interaction in the body may be completely different than the effect of each antibiotic individually (Madadi et al., 2014).

Data available in the literature prove that research on the use of coccidiostats in poultry is mainly focused on assessing the effectiveness of their use in the context of acquiring specific immunity in birds against coccidiosis. Drug treatment does not eliminate coccidia and may facilitate or interfere with the immune response against coccidia (Chapman, 2008, Kadykalo et al., 2018). However, there is no information on how long-term administration of coccidiostats affects the body's biological reactions, primarily their accumulation in tissues and, consequently, the response to the redox status. Research conducted by Gbylik-Sikorska et al. (2016) indicates that long-term administration of enrofloxacin or doxycycline to chickens may result in the accumulation of these antibiotics in chicken tissues. Research conducted by Elamaran et al. (2015) shows that the administration of enrofloxacin to chickens for 5 days (38–42 days of age) induced oxidative stress in the chickens' body, which was manifested by an increase in the activity of superoxide dismutase, catalase and glutathione peroxidase as well as the content of glutathione reduced and malondialdehyde in the liver (Elamaran et al., 2015). Stimulation of oxidative reactions in the body due to the administration of xenobiotics is unfavourable because it may result in a weakening of the antioxidant potential, induction of oxidative stress, and consequently, deterioration of the immunity and growth performance of birds. It is worth emphasising that the intensification of oxidative reactions may also result in deterioration of the quality of poultry meat.

Our previous research on the discussed research topic showed that the constant use of monensin improved turkey BW gain and feed efficiency compared to doxycycline treatment (Mikulski et al., 2022). In turn, histopathological changes in immunocompetent organs were observed in turkeys that were constantly administered monensin or briefly doxycycline or enrofloxacin, and the most visible changes were in the case of constant use of monensin (Smagieł et al., 2023). Therefore, determining the impact of antibiotic administration on the redox status and their possible accumulation in the liver, as the tissue responsible for the biotransformation of xenobiotics, is important in the context of a better understanding of the previously noted histopathological changes.

It was assumed that early administration of antibiotics may worsen the functioning of the turkeys' antioxidant system. Feeding birds with mixtures containing a coccidiostat, which is also an antibiotic, may cause similar effects. Additionally, it was assumed that the longer the antibiotic administration time, the greater the risk of its accumulation in the liver. The aim of the study was to determine whether early administration of antibiotics or feeding a diet containing coccidiostats causes accumulation in the liver and whether it affects the deterioration of the antioxidant system, and whether preventive vaccinations can intensify it.

Material and methods

Birds and housing

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 (Approval No. 47/2021; Olsztyn. Poland), and the animals were cared for under guidelines comparable to those laid down by EU Directive 2010/63/EU. The study was carried out in compliance with the ARRIVE guidelines. Every effort was made to minimise the suffering of the animals used in the experiment.

In the presented experiment, 3 080 one-day-old Hybrid Converter female turkeys were used. The birds were divided randomly into eight groups, each consisting of seven pens with 55 birds per pen. Each pen had an area of 10 m^2 and was lined with litter. Environmental conditions were under the recommendations of Hybrid Turkeys (2020), controlled automatically, adjusted to the age of the birds, and identical for all turkeys. The experiment was conducted for 8 weeks from 1 to 56 days of life. During two feeding phases (weeks 1–4 and 5–8), birds were fed a diet formulated based on Hybrid Turkeys, (2020) to meet the nutritional requirements of commercial turkeys at a given rearing stage. The detailed composition of the diets was presented in Supplementary Material (Supplementary Table S1) and Mikulski et al. (2022) and Smagieł et al. (2023). Feed and water were available for turkeys *ad libitum*.

Experimental design

The experiment used a two-factor design with four treatments (C, M, E, D) and two groups of birds (vaccinated, +, unvaccinated, −). Turkeys from group C did not receive the coccidiostat monensin added to their feed or antibiotics added to their drinking water. Birds from group M received monensin (Coxydin 200, Huvepharma Polska, Warsaw, Poland) in an amount of 90 mg/kg of feed for 56 days. Birds from group E received the addition of enrofloxacin (Enrofloxacin 10%, Biowet, Drwalew, Poland) to drinking water for first 5 days of life in an amount of 10 mg E/kg BW, while birds from group D received the addition of doxycycline (Doxylin CT WSP 433 mg/g, Dopharma Research B.V., Raamsdonksveer, Netherlands) into drinking water for first 5 days of life in an amount of 50 mg D/kg BW.

In four experimental groups (C+, M+, E+, D+), 1-day-old turkeys were administered live-attenuated vaccines against turkey rhino-

tracheitis (**TRT**) (Poulvac TRT; Zoetis) and Newcastle disease (**ND**) (Nobilis ND clone 30; MSD Animal Health) by coarse spray, and 28-day-old birds were administered a subcutaneously injected inactivated vaccine against omitobacteriosis (Ornitin, Phibro, Poland). Turkeys from groups C-, M-, E-, and D- were not vaccinated. Vaccinated birds were kept in a separate area of the same building and were handled by different people to prevent cross-contamination.

Sample collection and investigations

During the experiment, the BW of turkeys and feed consumption were recorded on a pen basis for 56 days of life. Daily feed intake and the feed conversion ratio were calculated. Mortality rates were recorded daily.

At 7 and 56 d of age, blood samples were collected from seven birds from each group (one bird per replicate). At 1, 3, 5, 7, and 56 d of life, one bird per replicate pen (seven birds per treatment) was euthanised by cervical dislocation (Close et al., 1997). Liver samples were collected from seven birds from each group.

Sample preparation and liquid chromatography- mass spectrometry analysis

Samples were prepared by simple protein precipitation with acetonitrile. For liver analysis, 200 mg of tissue was mixed up with 200 μL of water and 300 mg of ceramic beads (1.4 mm dimension) in 2 mL Bead Mill Tubes and homogenised using a soft tissue program (Bead Mill Max Homogeniser, VWR International LLC, Randor, USA). Then, 1 000 μL of acetonitrile was added. Before the homogenisation and precipitation step, an equivalent amount of internal standard as in the calibration standards was added (final concentration 50 ng of nigericin in mL/mg of the sample). The precipitated sample was centrifuged at 14 000 rpm for 5 min, and the supernatant was transferred into autosampler vials and immediately analysed.

The concentration of monensin, doxycycline, and enrofloxacin was determined using a high-performance liquid chromatograph (ExionLC AD, AB Sciex, Framingham, MA, USA) coupled with a mass spectrometer (QTRAP 6500+, AB Sciex, Framingham, MA, USA). Chromatographic separation of sample supernatants was carried out on a Kinetex Biphenyl (100 mm × 3 mm, 2.6 μm particle size) column (Phenomenex, Torrance, CA, USA). Column temperature was set at 40 °C. The mobile phase flow rate was 0.4 mL/min, and the injection volume was 5 μL. The mobile phase consisted of water containing 0.1% v/v of formic acid (component A) and ACN containing 0.1% v/v of formic acid (component B). Mobile phase gradient conditions: 0.0–0.5 min 45% B, 0.5–3.0 min 45–95% B, 3.0–4.5 min hold 95% B, 4.5–4.6 min 95–45% B, and 4.6–6.0 min 45% B. Electrospray ionisation (ESI) in the positive ion mode was used. The ion source parameters and MRM transitions (e.g., precursor (Q1), product ions (Q2), collision energy (CE), and retention times) are listed in the Supplementary Materials (Supplementary Table S2). Analyst 1.7.2 software (AB Sciex, Framingham, MA, USA) was used to control the LC/MS/MS system. SCIEX OS Version 2.1.6.59781 (AB Sciex, Framingham, MA, USA) was used for data processing. An internal standard calibration method for the quantification of enrofloxacin, doxycycline, and monensin was applied. Nigericin was used as an internal standard. Mobile phase constituent (LC-MS grade) and standards were purchased from Sigma Aldrich (St. Louis, MO, USA).

RNA extraction from the blood and quantitative real-time PCR

The total RNA extraction from the blood of turkeys was performed using the RNeasy Protect Animal Blood Kit (Qiagen, Wrocław, Poland) following the manufacturer's recommendation. The quantity of isolated RNA samples was assessed spectrophotometrically with a UV–VIS Nabi spectrophotometer (MicroDigital Co. Ltd., Gyeonggi, Republic of Korea), and its integrity was confirmed through agarose gel electrophoresis (0.8% concentration). To synthesise complementary cDNA, 1 μg of total RNA underwent reverse transcription using the NG dART RT kit (EURX Ltd., Gdańsk, Poland), following the manufacturer's protocol.

Specific primers (sequences presented in Table 1) designed for assessing inducible nitric oxide synthase (iNOS) gene expression were created utilising Primer 3 software from the Whitehead Institute (Cambridge, MA, USA), and synthesised by Genomed (Warsaw, Poland). The Real-time PCR method was performed on a Quantabio thermocycler (VWR International LLC, Radnor, PA, USA) using a universal solution for quantitative real-time PCR (SG qPCR Master Mix, EURX Ltd., Gdańsk, Poland). The amplification was conducted for 35 cycles: denaturation at 95 °C for 10 s, annealing at 58–59 °C for 15 s, and elongation at 72 °C for 20 s. The experiment was normalised to β-actin (*ACTB*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) reference genes. The target gene relative mRNA level analysis was evaluated using the $2 - \Delta Ct$ method.

Analysis of redox parameters in serum

The serum levels of 8-isoprostane (cat. no. QY-E80208), advanced oxidation protein products (cat. no. QY-E80192), 8 – hydroxydeoxyguanosine (**8-OHdG**) (cat. no. QY-E80001), NADPH oxidase (**NOX**) (cat. no. QY-E80199), cyclohydrolase 1(**GCH1/GTP**) (cat. no. QY-E80195), catalase (cat. no. QY-E80173), oxoguanine glycosylase 1 (**OGG1**) (cat. no. QY-E80159), caspase 3 (**Casp 3**) (cat. no. QY-E80111), caspase 8 (**Casp 8**) (cat. no. QY-E80112), nitric oxide (cat. no. QY-E80065) and activity of myeloperoxidase (cat. no. QY-E80209), superoxide dismutase (cat. no. QY-E80142) were determined in serum of 7-day-old and 56-day-old turkeys, using Qayee-Bio diagnostic kits (Qayee Biotechnology Co., Ltd., Shanghai, China). According to Qayee-Bio for all tested parameters repeatability: the plate CV was less than 15%, and accuracy: standard linear regression correlation coefficient R with the expected concentration value was greater than or equal to 0.9900. Total antioxidant status (**TAS**) was determined using a Randox TAS kit (cat. no. NX2332, Randox Laboratories Ltd., Warszawa, Polska).

Statistical analysis

An individual bird (n = 7) was considered as the experimental unit in analyses of antibiotic levels in the liver, and other parameters in the serum of turkeys. The data were analysed by two-way ANOVA with the GLM procedure to examine the main effects of antibiotics used (C, M, E, D), the applied challenge (vaccinated vs unvaccinated; V effect), and their interaction. When the model was significant, Tukey's HSD test was performed to separate treatment means. The results were presented as means and pooled SEs of the mean (SEM). The statistical analysis was performed using STATISTICA software version 13.1 (2017) at a significance level of $P < 0.05$ (TIBCO Software Inc., 2017).

Results

Antibiotic levels in turkey liver

The conducted research shows that the use of a diet containing monensin resulted in a time-dependent increase in the level of this coccidiostat in the livers of turkeys, amounting to 0.75 ± 0.61 μg/kg on the 1st day of life vs 32 ± 16 μg/kg on the 56th day of life, respectively (Fig. 1). In the livers of turkeys that received doxycycline for the first 5 days of life, the level of this antibiotic was

Table 1
The sequences of all using primers for turkey genes.

Gene	Primer	Sequence (5′-3′)	Melting temperature (°C)	Product size (nt)	GenBank access no.
iNOS	Forward	CAACTCTCACAAAGACGCGG	59	91	NM_001303213
	Reverse	TTTGTGTGATGTGGGAACGC			
ACTB	Forward	TACCCCATTGAACACGGCAT	58	96	NM_001303173
	Reverse	CTCCTCAGGGGCTACTCTCA			
GAPDH	Forward	AGGATACACAGAGGACCAGGTTG	58	71	NM_001303179
	Reverse	CCGCATCAAAGGTGGAGGAATG			

iNOS - inducible nitric oxide synthase; *ACTB* - β-actin; *GAPDH* - glyceraldehyde-3-phosphate dehydrogenase.

13572.2 ± 11397.4 μg/kg on the 1st day of life and increased to 49109.61 ± 612.5 μg/kg on the 5th day of life. The level of this antibiotic in turkey livers decreased in the following days of life, reaching the value of 3.92 ± 0.78 μg/kg on day 56 (Fig. 2). Similarly, in the case of enrofloxacin, the level of this antibiotic in the liver increased in the first 5 days of life, when the birds received this antibiotic (3157.48 ± 973.4 μg/kg on the 1st day of life vs 9526.5 4 ± 227.1 μg/kg on the 5th day of life), and then on the 56th day of life, the level of this antibiotic in turkey livers was 2.86 ± 0.07 μg/kg (Fig. 3).

Interaction antibiotic × vaccination

The conducted research showed an antibiotic × vaccination interaction in 7- and 56-day-old turkeys in the case of analysis of iNOS gene expression ($P < 0.001$, both) and the case of analysis of nitric oxide level ($P = 0.007$ and $P < 0.001$, respectively) in the blood (Table 2 and Table 3). An antibiotic × vaccination interaction was also found in 7-day-old turkeys when analysing the levels of advanced oxidation protein products, 8-OHdG, NOX, TAS ($P < 0.001$, respectively), Casp 3 ($P = 0.008$) and Casp 8 ($P = 0.018$) in blood serum (Table 4). Statistical analysis of the results obtained from blood tests of 56-day-old turkeys also showed an antibiotic × vaccination interaction in the case of the levels of advanced oxidation protein products ($P < 0.001$), 8-OHdG ($P = 0.001$), myeloperoxidase ($P = 0.029$), NOX ($P = 0.012$), Casp 8 ($P < 0.001$), TAS ($P = 0.001$) (Table 5). The observed interactions indicate that the tested blood parameters were influenced by both early antibiotic administration and vaccination.

Effects of antibiotics and/or a coccidiostat

Compared to the control group, higher BW and higher BWG ($P = 0.006$, both) were found in 56-day-old turkeys with lower feed

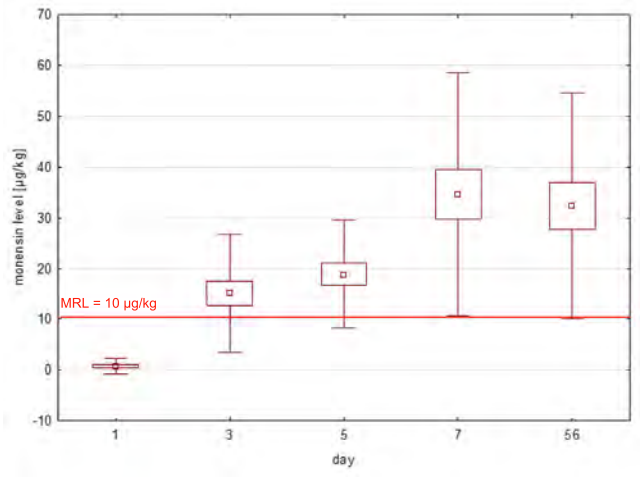

Fig. 1. Changes in monensin level in turkey liver (μg/kg of tissue) during the subsequent days of the experiment (n = 7) MRL - Maximum Residue Level.

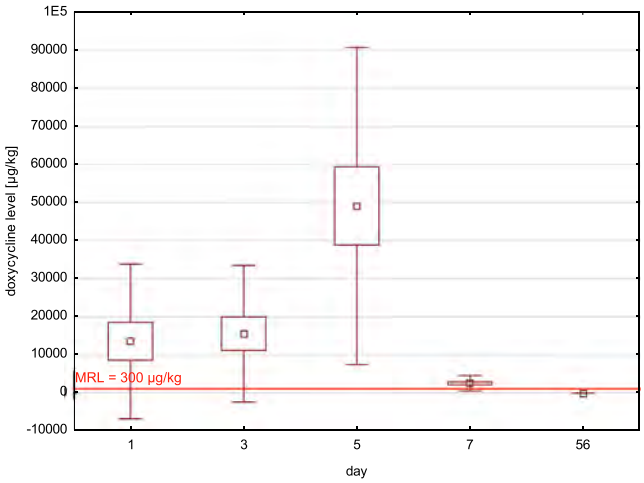

Fig. 2. Changes in doxycycline level in turkey liver (μg/kg of tissue) during the subsequent days of the experiment (n = 7) MRL - Maximum Residue Level.

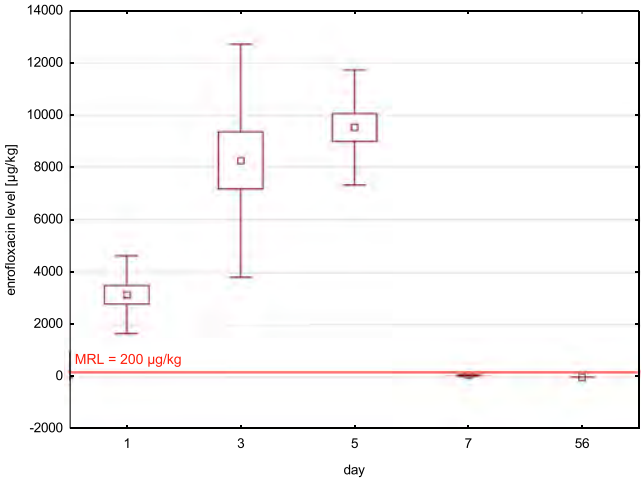

Fig. 3. Changes in enrofloxacin level in turkey liver (μg/kg of tissue) during the subsequent days of the experiment (n = 7) MRL - Maximum Residue Level.

conversion ratio ($P < 0.001$) in the group of turkeys receiving monensin supplements to feed (Table 6).

The conducted research showed increased catalase activity ($P < 0.001$) in the blood of 7-day-old turkeys receiving doxycycline. Administration of enrofloxacin and doxycycline to turkeys resulted in a reduction in OGG1 levels ($P = 0.006$) in the blood of 7-day-old turkeys (Table 4). A decrease in the level of 8-isoprostanes was found in the blood serum of 56-day-old turkeys receiving doxycycline in the first days of life ($P = 0.047$). Early administration of enrofloxacin resulted in an increase in GCH1/GTP levels ($P = 0.001$) and a decrease in catalase activity ($P = 0.027$) in the blood serum of 56-day-old turkeys (Table 5).

Table 2
iNOS expression and NO level in the blood of turkeys at 7 days of age (n = 7).

	Antibiotic[1]				Vaccine[2]		SEM	*P*-value		
Item	C	M	E	D	–	+		Antibiotic (A)	Vaccine (V)	A × V interaction
iNOS expression	0.571[a]	0.514[c]	0.554[b]	0.549[b]	0.565[a]	0.529[b]	0.005	<0.001	<0.001	<0.001
NO (μmol/L)	4.043[a]	2.887[b]	3.780[ab]	3.551[ab]	3.978[a]	3.152[b]	0.161	0.022	0.003	0.007

iNOS - inducible nitric oxide synthase; NO - nitric oxide.
[1] Treatment: C - untreated control; M - treated with monensin; E - treated with enrofloxacin; D – treated with doxycycline.
[2] Unvaccinated (–) or vaccinated (+).
[a,b,c] Means within the same row with different superscripts differ significantly ($P < 0.05$).

Table 3
iNOS expression and NO level in the blood of turkeys at 56 days of age (n = 7).

	Antibiotic[1]				Vaccine[2]		SEM	*P*-value		
Item	C	M	E	D	–	+		Antibiotic (A)	Vaccine (V)	A × V interaction
iNOS expression	0.573[c]	0.589[b]	0.603[a]	0.581[bc]	0.576[b]	0.597[a]	0.004	<0.001	<0.001	<0.001
NO (μmol/L)	8.388	8.730	9.518	8.857	8.478[b]	9.268[a]	0.192	0.084	0.014	<0.001

iNOS - inducible nitric oxide synthase; NO - nitric oxide.
[1] Treatment: C - untreated control; M - treated with monensin; E - treated with enrofloxacin; D - treated with doxycycline.
[2] Unvaccinated (–) or vaccinated (+).
[a,b,c] Means within the same row with different superscripts differ significantly ($P < 0.05$).

Table 4
Redox parameters in blood plasma of turkey at 7 days of age (n = 7).

	Antibiotic[1]				Vaccine[2]		SEM	*P*-value		
Item	C	M	E	D	–	+		Antibiotic (A)	Vaccine (V)	A × V interaction
8-isoprostanes (pg/mL)	221.9	202.1	217.8	211.8	211.3	215.5	6.550	0.748	0.759	0.436
AOPP (ng/mL)	91.33	84.76	87.67	95.51	93.87[a]	85.77[b]	2.077	0.075	0.009	<0.001
8-OHdG (ng/mL)	13.27[ab]	11.72[b]	14.43[ab]	16.23[a]	12.27[b]	15.56[a]	0.636	0.009	0.001	<0.001
MPO (U/L)	95.83	88.61	96.11	92.22	76.39[b]	110.00[a]	3.456	0.732	<0.001	0.510
NOX (pg/mL)	440.2[ab]	422.3[b]	468.8[a]	377.8[c]	450.7[a]	403.8[b]	8.622	<0.001	<0.001	<0.001
GCH1/GTP (ng/mL)	5.869	5.890	6.320	6.536	5.737[b]	6.570[a]	0.195	0.524	0.032	0.333
SOD (U/mL)	252.3	239.6	255.3	250.1	251.1	247.6	3.252	0.376	0.601	0.769
CAT (ng/mL)	15.81[b]	16.99[b]	17.18[b]	23.42[a]	16.94[b]	19.76[a]	0.773	<0.001	0.032	0.129
OGG1 (pg/mL)	601.9[a]	595.0[ab]	572.5[b]	570.3[b]	597.8[a]	572.0[b]	4.375	0.006	0.001	0.298
Casp 3 (pg/mL)	956.4	891.0	871.8	826.5	997.8[a]	775.0[b]	24.42	0.075	<0.001	0.008
Casp 8 (ng/mL)	119.1	120.3	106.7	119.5	121.2[a]	111.6[b]	17.58	0.073	0.025	0.018
TAS (mmol trolox/L)	0.989[a]	0.719[b]	0.735[b]	0.728[b]	0.862[a]	0.723[b]	0.034	0.001	0.008	<0.001

AOPP - advanced oxidation protein products; 8-OHdG - 8-hydroxy-2′ -deoxyguanosine; MPO - myeloperoxidase; NOX - NADPH oxidase; GCH1/GTP - cyclohydrolase 1; SOD - superoxide dismutase; CAT - catalase; OGG1 - oxoguanine glycosylase 1; Casp 3 - caspase 3; Casp 8 - caspase 8; TAS – total antioxidant status.
[1] Treatment: C - untreated control; M - treated with monensin; E - treated with enrofloxacin; D - treated with doxycycline.
[2] Unvaccinated (–) or vaccinated (+).
[a,b,c] Means within the same row with different superscripts differ significantly ($P < 0.05$).

Table 5
Redox parameters in blood plasma of turkey at 56 days of age (n = 7).

	Antibiotic[1]				Vaccine[2]		SEM	*P*-value		
Item	C	M	E	D	–	+		Antibiotic (A)	Vaccine (V)	A × V interaction
8-isoprostanes (pg/mL)	499.9[a]	453.3[ab]	425.3[ab]	388.0[b]	473.7[a]	409.6[b]	15.11	0.047	0.026	0.479
AOPP (ng/mL)	54.21[a]	47.78[a]	46.64[a]	34.98[b]	51.67[a]	40.14[b]	1.857	<0.001	<0.001	<0.001
8-OHdG (ng/mL)	80.01[a]	51.85[c]	66.71[ab]	63.34[bc]	69.12[a]	61.83[b]	2.468	<0.001	0.050	0.001
MPO (U/L)	104.44	97.50	94.44	96.11	114.17[a]	82.08[b]	4.687	0.827	<0.001	0.029
NOX (pg/mL)	392.7[ab]	483.3[a]	419.2[ab]	351.1[b]	438.7[a]	384.5[b]	14.78	0.004	0.031	0.012
GCH1/GTP (ng/mL)	5.307[b]	6.167[ab]	7.421[a]	6.385[ab]	5.722[b]	6.918[a]	0.212	0.001	0.001	0.590
SOD (U/mL)	295.9	288.8	284.5	283.2	294.5	281.7	3.661	0.624	0.092	0.921
CAT (ng/mL)	431.8[a]	412.6[ab]	382.7[b]	387.7[ab]	432.9[a]	374.5[b]	7.723	0.027	<0.001	0.252
OGG1 (pg/mL)	949.5	904.0	873.5	968.7	990.2[a]	857.7[b]	4.079	0.246	0.001	0.234
Casp 3 (pg/mL)	1112.4	1109.9	1108.0	1017.5	1165.9[a]	1008.0[b]	19.76	0.124	<0.001	0.366
Casp 8 (ng/mL)	30.55	32.82	32.84	29.23	32.96[a]	29.76[b]	0.835	0.191	0.025	<0.001
TAS (mmol trolox/L)	1.280	1.336	1.343	1.328	1.297	1.347	0.028	0.804	0.313	0.001

AOPP - advanced oxidation protein products; 8-OHdG - 8-hydroxy-2′ -deoxyguanosine; MPO - myeloperoxidase; NOX - NADPH oxidase; GCH1/GTP - cyclohydrolase 1; SOD - superoxide dismutase; CAT - catalase; OGG1 - oxoguanine glycosylase 1; Casp 3 - caspase 3; Casp 8 - caspase 8; TAS – total antioxidant status.
[1] Treatment: C - untreated control; M - treated with monensin; E - treated with enrofloxacin; D - treated with doxycycline.
[2] Unvaccinated (–) or vaccinated (+).
[a,b,c] Means within the same row with different superscripts differ significantly ($P < 0.05$).

Table 6
The effect of different antibiotics and vaccination on the growth performance of 56 d turkeys (n = 7).

	Antibiotic[1]				Vaccine[2]		SEM	*P*-value		
Item	C	M	E	D	–	+		Antibiotic (A)	Vaccine (V)	A × V interaction
BW 56 d (kg)	4.702[b]	4.808[a]	4.722[ab]	4.676[b]	4.855[a]	4.599[b]	0.022	0.006	<0.001	0.841
Average BWG (kg)	4.632[b]	4.738[a]	4.652[ab]	4.606[b]	4.785[a]	4.529[b]	0.022	0.006	<0.001	0.842
FCR (kg/kg)	1.751[a]	1.703[b]	1.762[a]	1.769[a]	1.752	1.740	0.006	<0.001	0.248	0.148
DFI (g)	142.9	141.8	144.4	143.5	147.3[a]	139.0[b]	0.749	0.305	<0.001	0.331
Mortality (%)	2.99	3.90	2.99	4.94	4.29	3.12	0.507	0.784	0.232	0.358

BWG - BW gain; FCR - feed conversion ratio; DFI - daily feed intake.
[1] Treatment: C - untreated control; M - treated with monensin; E - treated with enrofloxacin; D - treated with doxycycline.
[2] Unvaccinated (–) or vaccinated (+).
[a,b] Means within the same row with different superscripts differ significantly ($P < 0.05$).

Effects of vaccination

It was found that vaccination of turkeys resulted in worse BW gain of turkeys from 1 to 56 days of age ($P < 0.001$). Lower daily feed intake was also found in vaccinated turkeys ($P < 0.001$) (Table 6).

Vaccination of turkeys resulted in an increase in myeloperoxidase and catalase activity ($P < 0.001$, both) and GCH1/GTP levels ($P = 0.032$) in the blood serum of 7-day-old turkeys. At the same time, a decrease in the level of OGG1 ($P = 0.001$) in blood serum was found in vaccinated 7-day-old turkeys (Table 4). As a result of the vaccination of turkeys, a decrease in the level of 8-isoprostanes ($P = 0.026$) was found in the blood serum of 56-day-old turkeys. It was also found that vaccination of turkeys resulted in an increase in GCH1/GTP levels ($P = 0.001$) and a decrease in catalase activity and Casp 3 levels ($P < 0.001$, both), (Table 5).

Discussion

In EU countries, a ban on the use of antibiotics as growth stimulants was introduced in 2006. However, the possibility of continuous use of coccidiostats in the feed, including ionophores, which are also antibiotics, has been left. Constant administration of coccidiostats (antibiotics) in feed mixtures is a method of preventing coccidiosis in birds. Paradoxical situations have arisen where the use of a specific antibiotic as a growth stimulator is prohibited, but monensin is allowed as a coccidiostat.

The conducted research showed that when monensin was constantly administered in the diet of turkeys, birds in the initial period of rearing (1–56 d) grew slightly better. However, during the later rearing period, the BW gains of turkeys from this group were equal to those of the control group, as presented in the publication by Mikulski et al. (2022). Research conducted by Elwinger et al. (1998) showed that the monensin ionophore induces a growth-stimulating effect, which can be attributed to its antibacterial effect. The literature review presented by Rajendran et al. (2018) confirms the pleitropic biological effects of monensin, including antibacterial, antiviral, antiparasitic, and even anticancer properties. Although there are reports of the inability of monensin to improve the growth of healthy birds, in the case of infected birds, the presence of monensin in the diet by modifying the intestinal microflora does not result in worse growth results (Robinson et al., 2019). Early administration of enrofloxacin or doxycycline did not affect the growth performance of turkeys.

Literature data show that this coccidiostat is safe for turkeys and turkey meat consumers, it is quickly biotransformed, which means that after use, only a short withdrawal period of 3–7 days is required for slaughter birds (JECFA, 2021). It is worth noting, however, that the current assessment of the safety of this coccidiostat rarely involved the analysis of redox status indicators at the molecular level. Coccidiostats generally induce the formation of reactive oxygen species, which have killing properties against the pathogen to be combated. Therefore, there is a risk that excessive production of free radicals during the administration of monensin may not only affect the pathogen against which they are directed but also the cells of the host organism, inducing unfavourable oxidative reactions. Available literature indicates that monensin sodium is easily absorbed in the gastrointestinal tract of monogastric animals, metabolised mainly in the liver, and then secreted into the bile and eliminated in the faeces (JECFA, 2021). Anadón and Martínez-Larrañaga (2014) showed that the highest concentrations of monensin residues are detected in the liver and lower concentrations in the fat and muscles of poultry in the period from 0 to 2 days after drug discontinuation. Our research shows that the concentration of monensin in the liver of turkeys gradually increased with increasing the time of its administration to the birds in the diet. Referring to the obtained values to the current values of Maximum Residue Level (**MRL**) – 10 μg/kg in the liver (EMA, 2013), it should be stated that during the period of monensin administration, its level in the liver exceeded three times the MRL value.

It is worth noting, however, that the last measurement of the concentration of this coccidiostat was performed in young birds on the 56th day of life without a withdrawal period, and standard rearing of turkeys for slaughter is carried out for 105–112 days. The liver is the organ where the biotransformation of antibiotics takes place. The recorded comparable values of monensin concentration in the liver of 7- and 56-day-old turkeys indicate the effective biotransformation of this coccidiostat. The withdrawal period for this coccidiostat is approximately 7 days before slaughter. It can therefore be assumed that with an appropriate withdrawal period, the concentration of monensin in the liver will decrease to values consistent with the MRL. There is therefore a need to precisely determine whether the 7-day withdrawal period for administering monensin to turkeys is sufficient, considering that the WHO even allows this period to be shortened to 1 day.

Enrofloxacin is an antibiotic from the fluoroquinolone group that is commonly used to treat bacterial infections in various animal species, including poultry. After oral administration, enrofloxacin is rapidly and almost completely absorbed from the gastrointestinal tract and then distributed throughout the body (Acaröz and Sözbilir, 2020; Slana et al., 2014). Particularly, high concentrations of enrofloxacin are found in the lungs, liver, kidneys, skin, bones, and lymphatic system (Anadon et al., 1995). Interestingly, the concentration of enrofloxacin and its metabolite – ciprofloxacin in tissues is 2–3 times higher than in serum (Knoll et al., 1999). Anadon et al., (1995) based on the results of studies in which chickens were orally administered enrofloxacin at a dose of 10 mg/kg per day for 4 consecutive days, found that although enrofloxacin is slowly removed from the body, on the 12th day after cessation administration, its residues were recorded only in the liver of chickens, and its average concentration in this

organ was only 0.025 +/- 0.003 mg/g. Our research also shows that the level of enrofloxacin in the liver of turkeys increased in the 1st 5 days of life when the birds received this antibiotic (48 μg/kg on the 1st day of life vs 95263.54 μg/kg on the 5th day of life), and then, on the 56th day of life, the level of enrofloxacin in turkey livers was 2.86 μg/kg, which is a very low value to the permissible MRL values for poultry livers of 200 μg/kg. It can therefore be concluded that this antibiotic is very well metabolised in the body and eliminated from it. Therefore, the low content of enrofloxacin in the liver observed on the 56th day of the life of turkeys allows us to assume that its early administration while maintaining an appropriate withdrawal period does not pose a risk to the consumer of turkey meat. It can be assumed that since the content of enrofloxacin in the liver remains very low, and this organ is most exposed to the accumulation of antibiotics as it is responsible for their biotransformation, the content of enrofloxacin in the more frequently consumed skeletal muscles will be even lower and therefore safer than in the liver. This is consistent with the results of the study by Hassan et al. (2019), who showed that the frequency of enrofloxacin residues is much higher in the liver than in the breast muscle, thigh, or stomach of chickens (43.33 vs 13.33, 20, 30%, respectively).

Analogous observations can also be made for doxycycline because, in the livers of turkeys that received doxycycline for the 1st 5 days of life, the level of this antibiotic was 1357.2 μg/kg on the 1st day of life and increased to 49109.61 μg/kg on the 5th day of life. However, the level of this antibiotic in the livers of turkeys decreased in the following days of life, reaching a value of 3.92 μg/kg on day 56, which is not much higher, but even comparable to the level of enrofloxacin in the liver. Moreover, Mestorino et al. (2018) administered doxycycline to broiler chickens at a dose of 10 mg/kg in drinking water for 5 days and also noted that the concentration of this antibiotic in muscles, liver, kidneys and skin/-fat usually falls below the MRL value (i.e. 100 μg/kg for muscles, 300 μg/kg for skin and fat, 300 μg/kg for the liver and 600 μg/kg for the kidneys) already on the 7th day after the end of antibiotic therapy. Due to their chemical structure, ionophores cause Ca^{2+} accumulation in cells and may also promote lipid peroxidation as a result of intensifying oxidative stress, which is undoubtedly one of the unfavourable side effects of its use (Ekinci et al., 2023). Available literature indicates that the ability of monensin to cause oxidative stress is because it affects mitochondria by reducing the mitochondrial membrane potential and disturbing the morphology of mitochondria, thereby inducing excessive production of free radicals (Charvat and Arrizabalaga, 2016). The main molecular mechanism of ionophore toxicity is disruption of the ion concentration gradient in cells, resulting in a change in pH and loss of mitochondrial membrane potential, which ultimately leads to disruption of the electron transport chain, oxidative phosphorylation, and ATP production, as well as increased production of reactive oxygen species (Ekinci et al., 2023; Charvat and Arrizabalaga, 2016). Moreover, Ketola et al. (2010) indicate that the use of monensin induces a transcriptional profile characteristic of the response to oxidative stress. It seems that the cells most sensitive to the adverse effects of reactive oxygen species are cardiac and skeletal muscle cells, which is probably because they are characterised by very high metabolic activity (Ekinci et al., 2023). However, our research does not indicate that the use of monensin worsened the redox status of the tested birds. This may probably be because the level of coccidiostat used was so low that these changes were not visible.

Available literature indicates that antibiotics such as enrofloxacin or doxycycline can also induce the production of reactive oxygen species, which is related to their mechanism of action (Xu et al., 2022; Liu et al., 2023; Badawy et al., 2021; Grabowski et al., 2022; Shan et al., 2022). Enrofloxacin, an antibiotic from the fluoroquinolone group, works by inhibiting bacterial topoisomerase II (DNA gyrase), an enzyme necessary for bacterial DNA replication, transcription, and repair (Grabowski et al., 2022). During this process, enrofloxacin can generate reactive oxygen species that can damage DNA, proteins, and lipids, leading to bacterial cell death, but also deterioration of host cell functioning (Badawy et al. 2021). Studies on carp liver cells have shown that this antibiotic can induce apoptosis of hepatocytes in a mitochondria-dependent manner, negatively affect the level of selected biochemical parameters related to the redox status, such as lactate dehydrogenase and malondialdehyde, and reduce the total potential mitochondrial membrane (DJm), as well as increase the production of reactive oxygen species (at a dose of 200 μg/ml) and reduce the total antioxidant capacity (Liu et al., 2015). Moreover, there is an assumption that the process of deethylation of enrofloxacin to ciprofloxacin by cytochrome P450 microsomal enzymes, which occurs during its biotransformation in the liver, results in the release of free radicals, which may then lead to a decrease in the efficiency of the antioxidant system and increased lipid peroxidation (Giergiel and Posyniak, 2016). The biocidal effect of doxycycline is related to its ability to inhibit protein synthesis by binding to bacterial ribosomes (Chopra et al., 1992). Although the mechanism of its oxidative action is not as good as in the case of monensin or enrofloxacin, it is known that sufficiently high doses of doxycycline may also result in increased induction of free radicals, probably resulting from its ability to interfere with the energy metabolism of the cell (Tan et al., 2017). Interestingly, some reports indicate that the use of pharmacologically appropriate doses of doxycycline reduces oxidative stress, thereby inhibiting lipid peroxidation and inflammatory reactions, thanks to which it can be successfully used in the treatment of many diseases characterised by chronic inflammation (Clemens et al., 2018). The results of our research showed increased catalase activity in the blood of 7-day-old turkeys as a result of doxycycline administration in the first 5 days of life. This enzyme is an important element of the antioxidant defence system of cells of aerobic organisms, and the mechanism of its action involves the degradation of hydrogen peroxide into water and oxygen (Gebicka and Krych-Madej, 2019). It can therefore be assumed that the observed increased catalase activity in the blood of 7-day-old chicks resulted from the increased synthesis of free oxygen radicals and hydrogen peroxide, which required immediate neutralisation. This assumption may be confirmed by the fact that the increase in catalase activity in the blood of young turkeys receiving doxycycline was also accompanied by a decrease in the level of OGG1. OGG1 is an enzyme that plays a key role in repairing DNA damage by excising damaged bases (base excision repair). OGG1 recognises and removes mutagenic bases induced by reactive oxygen species, such as 8-oxoguanine (8-oxoG), replacing them with normal counterparts (Rajendran et al., 2012). Moreover, OGG1 is also involved in regulating the transcription of various oxidative stress response genes (Wang et al., 2021). The binding of OGG1 to its substrate causes DNA bending and induces allosteric DNA change, which facilitates the occupation of Nuclear Factor kappa B and the assembly of the transcription apparatus (Ba and Boldogh, 2018). Reducing the level of OGG1 may indicate an increased synthesis of free oxygen radicals inducing many DNA damages, the need for repair of which led to the depletion of the functional OGG1 enzymatic protein. In the blood serum of 56-day-old turkeys receiving doxycycline in the first days of life, a decrease in the level of 8-isoprostanes, i.e. prostaglandin F2α isomers, which are formed as a result of arachidonic acid peroxidation in a cyclooxygenase-independent reaction, was found. They are considered biomarkers of oxidative stress because their presence in the body is the result of the action of free radicals on the phospholipids of cell membranes. It is assumed that they are a much more sensitive indicator of lipid peroxidation than

malondialdehyde (Milne et al., 2011). The observed reduction in the level of 8-isoprostanes in the blood of older turkeys that received doxycycline at an early stage of life can be treated as a positive effect. As previously mentioned, there are reports that doxycycline used at a sufficiently low dose may have an antioxidant effect. Therefore, it can be assumed that the observed beneficial effect of doxycycline may be related to the fact that in the days following the end of the therapy, its level in the body of the turkey decreased, reaching a level that protected lipids against peroxidation at the end of the experiment.

Similarly to the early administration of doxycycline, the early administration of enrofloxacin also negatively influenced the processes of repairing DNA damaged by the activity of reactive oxygen species in young chicks, as evidenced by the reduced level of OGG1 in the blood of 7-day-old turkey hens. Nevertheless, the unfavourable effect of early administration of enrofloxacin persisted until the end of experimental rearing, as increased levels of GCH1/GTP were found in the blood of older turkeys, along with decreased levels of catalase. GTP cyclohydrolase I (GCH1) is an enzyme that is part of the folate and biopterin biosynthetic pathways. It is responsible for the hydrolysis of guanosine triphosphate (GTP) to the form of 7,8-dihydroneopterin triphosphate (7,8-DHNP-3′-TP, 7,8-NH2-3′-TP) (Kraft et al., 2020). It has been proven that its activity may increase in response to oxidative stress (Latremoliere and Costigan, 2011). The reduction in catalase levels resulting from early administration of enrofloxacin may, in turn, be caused by the depletion of functional enzymatic protein as a result of the need to neutralise excessive amounts of hydrogen peroxide generated during the fight against free radicals generated in large amounts by antibiotic therapy.

Vaccination of birds against various pathogens also seems to have an impact on the redox status. Available literature proves that vaccination of poultry may induce oxidative stress through various mechanisms. Vaccinations, like other medical interventions, may cause stress reactions in animals' bodies, which may then lead to increased production of reactive oxygen species and other free radicals (Wyszyńska et al., 2019). As a consequence, it may also explain the deterioration in the growth performance of vaccinated turkeys observed in our studies. Vaccinations can also induce oxidative stress by inducing an immune response leading to the activation of immune cells such as T cells and macrophages. These, in turn, can produce reactive oxygen species and other free radicals as part of their biocidal activity (Wyszyńska et al., 2019). Our research showed that the vaccinations increased the levels of myeloperoxidase and catalase as well as the levels of GCH1/GTP while reducing the level of OGG1 in the blood serum of 7-day-old turkeys. An increase in the levels of myeloperoxidase and catalase in the blood serum of turkeys after vaccination may indicate increased antioxidant activity. Myeloperoxidase and catalase are enzymes that are involved in the neutralisation of reactive oxygen species, and an increase in their level in the blood may suggest that the turkey's body is responding to oxidative stress induced by vaccination, which is a positive effect because it helps protect cells from damage. An increase in the level of GCH1/GTP may suggest an intensification of the folate and biopterin biosynthetic pathways, which are important for many biological processes, including DNA synthesis and amino acid metabolism. Therefore, the observed increase in the level of this enzyme may suggest that the organism of turkeys is trying to counteract the oxidative stress caused by vaccination by increasing the production of folates and biopterin. Nevertheless, the observed reduction in the level of OGG1, which plays a key role in the repair of DNA damage, is disturbing because it suggests the depletion of the pool of functional OGG1 protein and thus deterioration of the cell's ability to repair DNA damage, which may lead to increased susceptibility to mutations and other DNA damage in young turkeys.

In the blood of 56-day-old turkeys, a decrease in the level of 8-isoprostanes, catalase, and Casp3 and an increase in the level of GCH1/GTP were found due to the vaccination. The obtained results can be interpreted as a positive effect that develops sometime after vaccination. Reducing the level of 8-isoprostanes, which are markers of oxidative stress, as well as catalase involved in the neutralisation of hydrogen peroxide, suggests a reduction in the severity of oxidative stress in the body. Caspase 3 is a key enzyme involved in the process of apoptosis (programmed cell death); hence, its reduction may indicate a lower level of apoptosis, probably resulting from less cellular damage generated by reactive oxygen species. In this case, increasing the level of GCH1/GTP can be treated as a beneficial effect, because GCH1 is an enzyme involved in the synthesis of tetrahydrobiopterin (BH4), which is necessary for the production of some neurotransmitters and is crucial for the proper immune response. In light of the above, it can be assumed that vaccination can improve the long-term health of turkeys by reducing oxidative stress and apoptosis, as well as improving immune functions. However, before the turkey's body reaches the state of adaptation and strengthening of the antioxidant status, a temporary deterioration of the antioxidant response is possible resulting from excessive generation of free radicals in response to vaccination.

Conclusion

Based on the conducted research, it was determined that as a result of administration of enrofloxacin or doxycycline until the 5th day of life, biotransformation of these antibiotics occurred in the liver until the 56th day of life of the turkeys, which was confirmed by their lower level than the MRL. Because the concentration of monensin (a coccidiostat that can be used up to 16 weeks of age) in the liver of turkeys gradually increased with the extension of the time of its administration in the diet, it is probable that discontinuing its addition a day before the slaughter of birds will result in the presence of this coccidiostat in the liver of turkeys. However, despite the accumulation of monensin in the liver of turkeys, this coccidiostat did not increase oxidative reactions in the organism of turkeys.

If it is necessary to use short-term antibiotic therapy in the early rearing period (up to the 5th day of life), it was found that the administration of doxycycline and enrofloxacin intensifies oxidative reactions in the body of turkeys, which is particularly visible in the short period after the end of their administration. Interestingly, the unfavourable effect of inducing oxidative reactions after enrofloxacin administration may persist much longer, even up to 8 weeks of age. However, after administration of doxycycline during the period when its level in the body decreases, there is a beneficial stimulation of the antioxidant system, which eliminates the oxidative reactions induced by this antibiotic.

The research shows that vaccination of turkeys can reduce oxidative reactions and apoptosis in the body. However, the effect of the redox system reaction is different immediately after vaccination (induction of oxidative reactions), which is due to the mechanism of action of the immune system.

If it is necessary to administer an antibiotic in the early rearing period, the effects of doxycycline on the organism's immunity will be less severe than those of enrofloxacin. However, the decision to administer an antibiotic should take into account the fact of a diagnosed disease where the expected health benefits outweigh the risk of weakening immunity.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2024.101321.

Ethics approval

The protocol for this study was approved by the Local Ethics Committee for Animal Experiments in Olsztyn, Poland (Approval No. 47/2021; Olsztyn. Poland).

Data and model availability statement

The data were not deposited in an official repository.

The data that support the study findings are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

Author ORCIDs

R. Smagieł: https://orcid.org/0000-0001-8343-7950.
K. Tutaj: https://orcid.org/0000-0002-3742-3715.
E. Cholewińska: https://orcid.org/0000-0003-0437-4766.
P. Sołek: https://orcid.org/0000-0002-6194-0042.
D. Mikulski: https://orcid.org/0000-0002-6668-9977.
A. Stępniowska: https://orcid.org/0000-0003-2424-8935.
J. Jankowski: https://orcid.org/0000-0002-6250-4031.
K. Ognik: https://orcid.org/0000-0003-4393-4092.

CRediT authorship contribution statement

R. Smagieł: Writing – original draft, Methodology, Formal analysis. **K. Tutaj:** Methodology, Formal analysis. **E. Cholewińska:** Writing – original draft, Formal analysis. **P. Sołek:** Formal analysis. **D. Mikulski:** Methodology, Investigation. **A. Stępniowska:** Writing – review & editing, Formal analysis. **J. Jankowski:** Writing – review & editing, Project administration, Methodology, Funding acquisition, Conceptualization. **K. Ognik:** Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of interest

None.

Acknowledgment

The authors would like to thank Marzena Mikulska from the Department of Poultry Science and Apiculture, University of Warmia and Mazury in Olsztyn for help with statistical calculations.

Financial support statement

This work was supported by the National Science Centre in Poland, Grant No. 2020/39/B/NZ9/00765.

References

Acaröz, D.A., Sözbilir, N.B., 2020. Investigation of enrofloxacin and ciprofloxacin residues by LC-MS/MS in chicken liver marketed in Afyonkarahisar. Ankara Üniversitesi Veteriner Fakültesi Dergisi 67, 137–142.

Anadon, A., Martinez-Larranaga, M.R., Diaz, M.J., Bringas, P., Martinez, M.A., Fernandez-Cruz, M.L., Fernández, M.C., Fernandez, R., 1995. Pharmacokinetics and residues of enrofloxacin in chickens. American Journal of Veterinary Research 56, 501–506.

Anadón, A., Martínez-Larrañaga, M.R., 2014. Veterinary drugs residues: coccidiostats. In: Motarjemi, Y. (Ed.), Encyclopedia of Food Safety, Volume 3: Foods, Materials, Technologies and Risks. Academic Press, San Diego, CA, USA, pp. 63–75.

Ba, X., Boldogh, I., 2018. 8-Oxoguanine DNA glycosylase 1: beyond repair of the oxidatively modified base lesions. Redox Biology 14, 669–678.

Badawy, S., Yang, Y., Liu, Y., Marawan, M.A., Ares, I., Martinez, M.A., Martínez-Larrañaga, M.R., Wang, X., Anadón, A., Martínez, M., 2021. Toxicity induced by ciprofloxacin and enrofloxacin: oxidative stress and metabolism. Critical Reviews in Toxicology 51, 754–787.

Chapman, H.D., 2008. Coccidiosis in the turkey. Avian Pathology 37, 205–223.

Charvat, R., Arrizabalaga, G., 2016. Oxidative stress generated during monensin treatment contributes to altered *Toxoplasma gondii* mitochondrial function. Scientific Reports 6, 22997.

Chopra, I., Hawkey, P.M., Hinton, M., 1992. Tetracyclines, molecular and clinical aspects. Journal of Antimicrobial Chemotherapy 29, 245–277.

Clemens, D.L., Duryee, M.J., Sarmiento, C., Chiou, A., McGowan, J.D., Hunter, C.D., Schlichte, S.L., Tian, J., Klassen, L.W., O'Dell, J.R., Thiele, G.M., Mikuls, T.R., Zimmerman, M.C., Anderson, D.R., 2018. Novel antioxidant properties of doxycycline. International Journal of Molecular Sciences 19, 4078.

Close, B., Banister, K., Baumans, V., Bernoth, E.M., Bromage, N., Bunyan, J., Erhardt, W., Flecknell, P., Gregory, N., Hackbarth, H., Morton, D., Warwick, C., 1997. Recommendations for euthanasia of experimental animals: Part 2. Laboratory Animals 31, 1–32.

Ekinci, İ.B., Chłodowska, A., Olejnik, M., 2023. Ionophore toxicity in animals: a review of clinical and molecular aspects. International Journal of Molecular Sciences 24, 1696.

Elamaran, A., Hariharan, P., Ramesh, S., Vairamuthu, S., 2015. Enrofloxacin induced oxidative stress and its amelioration with antioxidants in liver homogenates of broilers. Indian Journal of Veterinary and Animal Sciences Research 11, 124–128.

Elwinger, K., Berndtson, E., Engström, B., Fossum, O., Waldenstedt, L., 1998. Effect of antibiotic growth promoters and anticoccidials on growth of Clostridium perfringens in the caeca and on performance of broiler chickens. Acta Veterinaria Scandinavica 39, 433–441.

EMA report—EMA/CVMP/78198/2012. 2013. European public MRL assessment report (EPMAR) Monensin (modification of MRLs). Committee for Medicinal Products for Veterinary Use (CVMP), European Medicines Agency. https://www.ema.europa.eu/en/documents/mrl-report/monensin-european-public-maximum-residue-limit-assessment-report-epmar-cvmp_en.pdf [accessed on December 28, 2023].

European Medicines Agency, 2023. Sales of veterinary antimicrobial agents in 31 European countries in 2022 Trends from 2010 to 2022 Thirteenth ESVAC report. https://www.ema.europa.eu/en/documents/report/sales-veterinary-antimicrobial agents-31-european-countries-2022-trends-2010-2022-thirteenth-esvac-report_en.pdf [accessed on May, 28, 20234].

Gbylik-Sikorska, M., Posyniak, A., Sniegocki, T., Sell, B., Gajda, A., Sawicka, A., Olszewska-Tomczyk, M., Bladek, T., Tomczyk, G., Zmudzki, J., 2016. Influence of enrofloxacin traces in drinking water to doxycycline tissue pharmacokinetics in healthy and infected by *Mycoplasma gallisepticum* broiler chickens. Food and Chemical Toxicology 90, 123–129.

Gebicka, L., Krych-Madej, J., 2019. The role of catalases in the prevention/promotion of oxidative stress. Journal of Inorganic Biochemistry 197, 110699.

Giergiel, M., Posyniak, A., 2016. Wpływ stosowania antybiotyków na stres oksydacyjny. Życie Weterynaryjne 91, 908–914 (in Polish).

Grabowski, L., Wegrzyn, G., Węgrzyn, A., Podlacha, M., 2022. Highly different effects of phage therapy and antibiotic therapy on immunological responses of chickens infected with *Salmonella enterica* serovar Typhimurium. Frontiers in Immunology 13, 956833.

Hassan, M.A., Amin, R., Abo Elroos, N., 2019. Enrofloxacin residues in chicken meat and giblets. Benha Veterinary Medical Journal 36, 175–183.

Hybrid Turkeys. 2020. Commercial Management Guide and Nutrient Guidelines for Hybrid Turkeys. Accessed 02 July 2021. http://www.hybridturkeys.com.

JECFA 2021. Toxicological evaluation of certain veterinary drug residues in food: prepared by the eighty-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). WHO Food Additives Series, No. 79. World Health Organization and Food and Agriculture Organization of the United Nations, Geneva, CH.

Kadykalo, S., Roberts, T., Thompson, M., Wilson, J., Lang, M., Espeisse, O., 2018. The value of anticoccidials for sustainable global poultry production. International Journal of Antimicrobial Agents 51, 304–310.

Ketola, K., Vainio, P., Fey, V., Kallioniemi, O., Iljin, K., 2010. Monensin is a potent inducer of oxidative stress and inhibitor of androgen signaling leading to apoptosis in prostate cancer cells. Molecular Cancer Therapeutics 9, 3175–3185.

Knoll, U., Glünder, G., Kietzmann, M., 1999. Comparative study of the plasma pharmacokinetics and tissue concentrations of danofloxacin and enrofloxacin in broiler chickens. Journal of Veterinary Pharmacology and Therapeutics 22, 239–246.

Kraft, V.A., Bezjian, C.T., Pfeiffer, S., Ringelstetter, L., Müller, C., Zandkarimi, F., Merl-Pham, J., Bao, X., Anastasov, N., Kössl, J., Brandner, S., Daniels, J.D., Schmitt-Kopplin, P., Hauck, S.M., Stockwell, B.R., Hadian, K., Schick, J.A., 2019. GTP cyclohydrolase 1/tetrahydrobiopterin counteract ferroptosis through lipid remodeling. ACS Central Science 6, 41–53.

Latremoliere, A., Costigan, M., 2011. GCH1, BH4, and pain. Current Pharmaceutical Biotechnology 12, 1728–1741.

Liu, B., Cui, Y., Brown, P.B., Ge, X., Xie, J., Xu, P., 2015. Cytotoxic effects and apoptosis induction of enrofloxacin in hepatic cell line of grass carp (*Ctenopharyngodon Idellus*). Fish Shellfish Immunology 47, 639–644.

Liu, C., Xu, H., Lu, Y., Pan, K., Song, Y., Jiang, X., 2023. The Effects of Enrofloxacin Exposure on Oxidative Stress, Intestinal Structure and Intestinal Microbiome Community of Largemouth Bass (Micropterus Salmoides). Accessed 01 October 2023 https://doi.org/10.2139/ssrn.4526889.

Madadi, M.S., Bojmehrani, H., Azari, M., 2014. Evaluation of drug interactions and prescription errors of poultry veterinarians in north of Iran. Poultry Science Journal 2, 25–35.

Mestorino, O.N., Zeinsteger, P.A., Buchamer, A.V., Buldain, D.C., Aliverti, F., Marchetti, M.L., 2018. Tissue depletion of doxycycline after its oral administration in food producing chicken for fattening. International Journal of Avian & Wildlife Biology 3, 245–250.

Mikulski, D., Juśkiewicz, J., Ognik, K., Zduńczyk, P., Smagieł, R., Jankowski, J., 2022. Gastrointestinal tract and neuroendocrine system responses of young turkeys to the early administration of antibiotics or feeding a diet containing a coccidiostat. Poultry Science 101, 102098.

Milne, G.L., Yin, H., Hardy, K.D., Davies, S.S., Roberts, L.J., 2011. Isoprostane generation and function. Chemical Reviews 111, 5973–5996.

Rajendran, A., Endo, M., Sugiyama, H., 2012. Structural and functional analysis of proteins by high-speed atomic force microscopy. Advances in Protein Chemistry and Structural Biology 87, 5–55.

Rajendran, V., Ilamathi, H.S., Dutt, S., Lakshminarayana, T.S., Ghosh, P.C., 2018. Chemotherapeutic potential of monensin as an anti-microbial agent. Current Topics in Medicinal Chemistry 18, 1976–1986.

Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. Official Journal of the European Union L 268, 29–43. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32003R1831.

Robinson, K., Becker, S., Xiao, Y., Lyu, W., Yang, Q., Zhu, H., Hua Yang, H., Zhao, J., Zhang, G., 2019. Differential impact of subtherapeutic antibiotics and ionophores on intestinal microbiota of broilers. Microorganisms 7, 282.

Shan, J., Xiaoqian, D., Xia, L., Yu, W., Zhilong, Z., Zhihui, S., Yanjie, Q., 2022. Oxidative stress, autophagy, and apoptosis induced by doxycycline in loach fin cells in vitro. Science of the Total Environment 839, 156379.

Slana, M., Pahor, V., Cvitkovič Maričič, L., Sollner-Dolenc, M., 2014. Excretion pattern of enrofloxacin after oral treatment of chicken broilers. Journal of Veterinary Pharmacology and Therapeutics 37, 611–614.

Smagieł, R., Ognik, K., Cholewińska, E., Stępniowska, A., Listos, P., Tykałowski, B., Mukulski, D., Koncicki, A., Jankowski, J., 2023. The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys. Poultry Science 102, 102876.

Śmialek, M., Konieczka, T., Konieczka, P., Kowalczyk, J., Koncicki, A., Kozłowski, K., Jankowski, J., 2023. Monitoring of antibiotic use in broiler turkey flocks in the Warmia and Mazury province in 2019–2021. Journal of Veterinary Research 67, 243–249.

Splettstoesser, W.D., Schuff-Werner, P., 2002. Oxidative stress in phagocytes -"the enemy within". Microscopy Research and Technique 57, 441–455.

Tan, Q., Yan, X., Song, L., Yi, H., Li, P., Sun, G., Yu, D., Li, L., Zeng, Z., Guo, Z., 2017. Induction of mitochondrial dysfunction and oxidative damage by antibiotic drug doxycycline enhances the responsiveness of glioblastoma to chemotherapy. Medical Science Monitor 23, 4117–4125.

TIBCO Software Inc. 2017. STATISTICA (data analysis software system), version 13. Accessed 12 February 2023. http://statistica.io.

Wang, K., Maayah, M., Sweasy, J.B., Alnajjar, K.S., 2021. The role of cysteines in the structure and function of OGG1. Journal of Biological Chemistry 296, 100093.

WHO FOOD, 2009. Toxicological evaluation of certain veterinary drug residues in food. WHO Food Additives Series, No. 33. World Health Organization and Food and Agriculture Organization of the United Nations, Geneva, CH.

Wyszyńska, A., Kobierecka, P., Jagusztyn-Krynicka, K.E., 2019. Advancements in developing anti-campylobacter vaccine for poultry. Postępy Mikrobiologii - Advancements of Microbiology 58, 385–398.

Xu, Y.H., Wei, X.L., Xu, Y.C., Zhang, D.G., Zhao, T., Zheng, H., Luo, Z., 2022. Waterborne enrofloxacin exposure activated oxidative stress and MAPK pathway, induced apoptosis and resulted in immune dysfunction in the gills of yellow catfish *Pelteobagrus fulvidraco*. Aquaculture 547, 737541.

Ann. Anim. Sci., Vol. 25, No. 1 (2025) 239–257 *DOI: 10.2478/aoas-2024-0063*

sciendo

EARLY ADMINISTRATION OF ANTIBIOTICS TO TURKEY POULTS IMPAIRS MATERNAL IMMUNITY AND POST-VACCINATION ANTIBODY SYNTHESIS*

Katarzyna Ognik[1♦], Bartłomiej Tykałowski[2], Dariusz Mikulski[3], Radosław Smagieł[1], Ewelina Cholewińska[1], Andrzej Koncicki[2], Anna Stępniowska[1], Jan Jankowski[3]

[1]Department of Biochemistry and Toxicology, University of Life Sciences, Akademicka 13, 20-950 Lublin, Poland
[2]Department of Poultry Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Oczapowskiego 14, 10-719 Olsztyn, Poland
[3]Department of Poultry Science and Apiculture, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-719 Olsztyn, Poland
♦Corresponding author: katarzyna.ognik@up.lublin.pl

Abstract

It was assumed that early antibiotic administration can slow down yolk sac resorption and decrease maternal antibody transfer and lysozyme levels in the yolk sac content and serum, thus disrupting the development of humoral immunity in turkeys. The experiment was conducted on female turkeys divided into following group: CON (control) – received no coccidiostat or antibiotics; MON – received monensin in the feed for 56 days; ENR and DOX – received enrofloxacin or doxycycline *per os* for the first 5 days of life. Additionally, half of the birds in each of this group were vaccinated against turkey rhinotracheitis (TRT), the disease caused by avian metapneumoviruses (aMPV) and Newcastle disease caused by Newcastle disease virus (NDV) at the first day of life (*IN*), and against ornithobacteriosis caused by *Ornithobacterium rhinotracheale* (ORT) at 28 days of life (*SC*). On days 1, 3 and 5 of the birds' lives, yolk sacs were collected to assess their resorption. Yolk sac resorption was assessed by calculating yolk sac relative weight based on the measurement of the yolk sac mass and body weight of turkeys. On days 1, 3, 5, 7 and 56, blood was collected to assess anti-aMPV, anti-NDV, anti-ORT antibody titers and immunoglobulin and lysozyme levels. Early administration *per os* of ENR and DOX or feeding diets containing MON did not inhibit yolk sac resorption, but reduced levels of specific maternal anti-aMPV, anti-NDV and anti-ORT antibodies and IgY and IgM in the yolk sac. Enrofloxacin and doxycycline decreased the titers of anti-aMPV and anti-NDV antibodies and the level of maternal IgY and IgM in turkeys, which could be due to the direct effect exerted by antibiotics on maternal antibodies present in the circulatory system of poults and the inhibition of post-vaccination synthesis of specific antibodies. The administration of antibiotics in the early rearing period should only be implemented in situations of clearly confirmed disease states when the expected health benefits outweigh the risk of weakening immunity.

Key words: enrofloxacin, doxycycline, coccidiostat, yolk-sac resorption, immunoglobulins

Normal immune function, involving complex mechanisms of innate (non-specific) and adaptive (acquired) immunity, is required for maintaining body homeostasis. During embryonic development and the early post-hatch period, humoral immunity is conveyed via antigen-specific maternal IgY, IgA and IgM antibodies. They are transferred into the egg content and, subsequently, to the developing embryo and the circulatory system of a newly-hatched chick (Grindstaff et al., 2003; Chrząstek et al., 2011 a; Murai, 2013; Murai et al., 2020). In birds, maternal IgY antibodies are absorbed mostly from the egg yolk into the embryonic circulation and, after hatch, to the chick. The yolk sac, i.e. a membranous sac attached to the embryo, participates in this process. IgY is progressively deposited in the yolk sac by blood transfer through the blood vessel system of every follicle under the follicular hierarchy (F1, F2, F3, F4, F5, ...) (Ulmer-Franco et al., 2012). In contrast, IgM and IgA are mainly transferred to albumen throughout egg transit through each oviduct section (infundibulum, magnum, isthmus and uterus) (Kovacs-Nolan and Mine, 2004). Small concentrations of IgM and IgA antibodies found in the egg yolk come from fallopian tube secretions (Kaspers et al., 1991). Moreover, IgM and IgA antibodies are not transported to the embryonic circulation via the blood vessels of the yolk sac. Instead, they reach the gastrointestinal tract of the developing embryo *per os*, with the amniotic fluid (Rose and Orlans, 1981), and they play an important role in the development of the local immunity over the first few days after hatch (Kowalczyk et al., 2019). In three-day-old chickens, IgY levels ranged from 0.99 to 1.52 mg/ml, accounting for 30% of these antibodies determined in maternal serum (Hamal et al., 2006). In a study by Kowalczyk et al. (2019), the mean transfer of IgY antibodies from turkey breeder hens to poults reached 31.4% over the entire egg production cycle.

The first scientific report on the total concentrations of IgY and IgM antibodies as well IgY antibody titers against the avian metapneumovirus (aMPV), the Newcastle disease virus (NDV), *Ornithobacterium rhinotra-*

*This work was supported by the National Science Centre in Poland, Grant No. 2020/39/B/NZ9/00765.

cheale (ORT) and *Pasteurella multocida* (PM) bacteria in the blood serum of turkey hens and poults, and in the egg yolk and egg white, has been published only recently (Kowalczyk et al., 2019). The results of the cited authors are consistent with the results of previous studies by Gharaibeh et al. (2008) conducted on broiler parent hens, which showed very different levels of pathogen-specific IgY antibodies in both hens and their offspring. The mean transfer of antibodies specific to the avian encephalomyelitis virus (AEV) from hens to their offspring was 4.3%, while the average transfer of antibodies against the infectious bursal disease virus (IBDV) reached 73.6%. Antibody transfer percentage was affected by bird species and hen's age. The transfer of anti-NDV antibodies from broiler breeder hens to chicks ranged from 29.2% to 40.7% (Hamal et al., 2006; Gharaibeh et al., 2008), whereas the transfer of anti-NDV antibodies from turkey breeder hens to poults ranged from 29.38% to 70.66% (51.9% on average), depending on hen's age (Kowalczyk et al., 2019).

A few studies have shown that selected factors (hen's age and breed, incubation and hatching conditions, chick storage and transport conditions, the time between hatching and the first water intake, the physicochemical parameters and microbiological quality of water and feed, stress) affect yolk sac resorption (Jamroz et al., 2004; van der Wagt et al., 2020). In different poultry species, disorders of yolk sac resorption are an important health problem in newly-hatched birds. In turkeys, the content of the yolk sac should be absorbed and disappear, and the involution of yolk sac walls should be completed within four to five days post-hatch (van der Wagt et al., 2020). However, this process is often prolonged or completely inhibited (van der Wagt et al., 2020). In a review article summarizing the results of studies conducted over the last 88 years, van der Wagt et al. (2020) concluded that delayed yolk sac resorption and disorders of content absorption may adversely affect the health status and growth performance of birds. According to the cited authors, there is a paucity of reliable published data concerning yolk utilization at hatch and the relationship with post-hatch performance (van der Wagt et al., 2020). Antibiotics disrupt the composition of gut microbiota, and recovery of the gut microbiome after antibiotic treatment is often slow and incomplete (Hafez and Shehata, 2021; Shehata et al., 2022). Numerous antibiotics exert immunosuppressive effects on birds (Panigrahy et al., 1979; Grondel et al., 1985; Tykałowski et al., 2013), thus directly affecting immune cell subpopulations (Chrząstek et al., 2011 b, 2015).

Lysozyme, one of the key components of non-specific humoral immune mechanisms, is ubiquitous in the animal kingdom (Callewaert and Michiels, 2010). In birds, this enzyme exerts antibacterial and immunomodulatory effects (Ragland and Criss, 2017). One of the layers of bacterial cell walls is a natural substrate for lysozyme. Lysozyme and various substances released to the environment upon bacterial cell lysis modulate immune system activity via pathogen-associated molecular patterns (PAMPs) (Ragland and Criss, 2017). Lysozyme is one of the indicators that can be used to assess non-specific humoral immunity. In birds, the levels of the lysozyme increase in response to infection and immunostimulation, and decrease in response to immunosuppression. In the first days after hatch, large amounts of lysozyme are synthesized by epithelial cells along the length of intestinal villi to protect birds against pathogens entering the host through the digestive tract (Nile et al., 2004). Some antibiotics can compromise the lytic activity of lysozyme (Fernández-Sousa et al., 1977).

In EU countries, in 2006, a ban was introduced on the use of antibiotics as growth stimulants or as prophylaxis. However, there has been no ban on administering antibiotics for therapeutic purposes. Many years of observations have allowed us to conclude that early administration of antibiotics to turkey poults results from peribreeding infections, e.g. *E. coli*, which, in our opinion, may consequently affect the immunity of birds. Early antibiotic therapy may also result in the development of antibiotic resistance, especially if there is a need for repeated antibiotic therapy. Antibiotic resistance is a serious problem for human and animal health. Excessive or inappropriate use of antibiotics can lead to the development of resistant strains of bacteria, which over time makes antibiotics less effective in the long term (Ventola, 2015). Early use of antibiotics in turkey poults poses a risk of hypoglycemia (lowering glucose levels by inducing increased insulin secretion). The development of hypoglycemia in the first days of life of turkeys may pose a risk of other metabolic disorders, which may indirectly contribute to the weakening of immunity (Kennedy et al., 2020).

Therefore, the present study was undertaken to determine whether and how early antibiotic administration to poults or feeding a diet containing an ionophore coccidiostat affect yolk sac resorption and the transfer of maternal antibodies from the yolk sac to the poult as well as lysozyme levels in the yolk sac content and blood serum since lysozyme and maternal antibodies act as the first line of defense against infections in newly-hatched birds. The effect of the administered antimicrobial agents on specific antibody titers after vaccination was also evaluated.

It was assumed that early antibiotic administration can slow down yolk sac resorption and decrease maternal antibody transfer and lysozyme levels in the yolk sac and blood serum, thus contributing to immunosuppression and disrupting the development of active humoral immunity in turkeys.

Material and methods

Ethical 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 (Approval No. 47/2021; Olsztyn, Poland), and the animals were cared for under guidelines comparable to those laid down by EU Directive 2010/63/EU (2010). The study was carried out in compliance with the ARRIVE guidelines. Every effort was made to minimize the suffering of the animals used in the experiment.

Experimental design

The experiment had a two-factorial design, with four treatments (CON, MON, ENR, DOX) and two groups of birds (vaccinated, +, unvaccinated, -). MON birds were administered the coccidiostat monensin (Coxidin 200, Huvepharma Polska, Warsaw, Poland), ENR birds received enrofloxacin (Enrofloxacin 10%, Biowet, Drwalew, Poland), and DOX birds received doxycycline (Doxylin CT WSP 433 mg/g, Dopharma Research B.V., Raamsdonksveer, Netherlands). The antibiotics ENR and DOX were added to drinking water, according to the currently recommended five-day treatment schedule (10 mg ENR/kg body weight (BW) and 50 mg DOX/kg BW, daily). In the MON treatment, the coccidiostat was added to feed (90 mg/kg feed, for 56 d). CON birds served as a control group without any antibiotic treatment or MON administration.

In four experimental groups (CON+, MON+, ENR+, DOX+), one-day-old turkeys were administered live-attenuated vaccines against turkey rhinotracheitis (TRT) (Poulvac TRT; Zoetis, Warsaw, Poland), the disease caused by avian metapneumoviruses (aMPV) and Newcastle disease (ND) (Nobilis ND clone 30; MSD Animal Health, Isando, South Africa) by coarse spray, and 28-day-old birds were administered a subcutaneously injected inactivated vaccine against ornithobacteriosis (ORT) (Ornitin, Phibro, Warsaw, Poland). Turkeys from groups CON-, MON-, ENR-, and DOX- were not vaccinated. Vaccinated birds were kept in a separate area of the same building and were handled by different people to prevent cross-contamination.

Birds and housing

The experiment had a completely randomized design, with 8 groups (a total of 3,080 one-day-old Hybrid Converter female turkeys). Each group consisted of 7 replicate pens bedded with wood shavings, with 55 birds per pen. The replicates (pens) were allocated to groups so as to ensure their uniform (homogeneous) distribution in the house. Stocking density in the initial stage of rearing was identical (5.5 birds/m^2). Vaccinated and unvaccinated birds were kept in separate sections of the building and were handled by different people to prevent cross-contamination. Both identical parts of the building were equipped with forced ventilation, radiant heating and controlled lighting. Environmental conditions were controlled automatically. They were adjusted to the birds' age, consistent with the recommendations of Hybrid Turkeys (2020), and identical for all turkeys in both sections of the building. The trial lasted eight weeks, from 1 to 56 days of age. During two feeding phases (weeks 1−4 and 5−8), birds were fed diets formulated to meet the nutrient requirements of commercial turkeys in a given stage of rearing (Hybrid Turkeys, 2020). The starter diet was offered as crumbles, and the grower diet (29−56 d) was prepared as 3 mm pellets. Throughout the experiment, all birds had unrestricted access to water and feed which was available *ad libitum*.

Sample collection and laboratory analyses

At 1, 3 and 5 days of age, yolk sacs were collected from 21 birds per group (3 birds per pen) to evaluate their resorption. At 1, 3, 5, 7 and 56 days of age, blood was sampled from 21 birds per group to evaluate the level of material antibodies and lysozyme levels (days 1, 3, 5 and 7) and to evaluate the level of antibodies produced and lysozyme at day 56. Yolk sac resorption in turkey poults was evaluated according to the procedure proposed by Chamblee et al. (1992). Yolk sac resorption was assessed by calculating yolk sac relative weight based on the measurement of the yolk sac mass and body weight of turkeys.

In order to determine maternal antibody titers, yolk sacs were weighed and the procedure proposed by Hamal et al. (2006), with certain modifications, was applied. Whole yolk sacs were homogenized in PBS (2 ml of PBS per g of yolk sac). The homogenate was combined with the same volume of chloroform as PBS, and homogenization was continued until a uniform formulation was obtained. The homogenate was centrifuged at 1000 × g at room temperature for 30 min. After centrifugation, the mixture was separated into three distinct layers in the centrifuge tube: an orange-colored solution of lecithin at the bottom, a semisolid emulsion of yolk sac in chloroform in the middle, and an aqueous phase on top. The upper aqueous phase was collected for analyses.

The total levels of IgY and IgM antibodies and lysozyme in the yolk sac and blood serum were determined with the use of diagnostic Qayee ELISA Kits (Shanghai Qayee Biotechnology Co., Ltd., Shanghai, China), and the levels of specific maternal IgY anti-aMPV (TRT), anti-NDV and anti-ORT antibodies were determined with the use of IDEXX (ORT Ab), (aMPV Ab) and (NDV T Ab) ELISA kits (IDEXX Laboratories, Inc., Westbrook, Maine, United States). The procedures were carried out using the Eppendorf epMotion 5075 LH automated pipetting system (Eppendorf Poland Sp. z o.o., Warsaw, Poland) and the BioTek ELx405 washer (Agilent Technologies, Inc. Headquarters, Santa Clara, California, United States). Absorbance was determined with the BioTek Elx800 absorbance microplate reader (Agilent Technologies, Inc. Headquarters, Santa Clara, California, United States). The results were analyzed and calculations were performed using xCheck 3.3 IDEXX software (IDEXX Laboratories, Inc., Westbrook, Maine, United States). The results were expressed for whole

yolk sacs to present actual antibody titers and levels in gradually decreasing yolk sac weight.

Statistical analysis

The values of all the traits and parameters were determined individually in 21 birds per group (3 birds per replicate), whose BW was representative of the average BW in the group. An individual bird (n = 21) was considered as the experimental unit in evaluation of yolk sac resorption, and analysis of antibody and lysozyme levels in yolk sac and serum in turkeys. The data were analyzed by two-way ANOVA with the general linear model (GLM) procedure to examine the main effects of antibiotics used (CON, MON, ENR, DOX), the applied challenge (vaccinated vs. unvaccinated; V effect), and their interaction. When the model was significant Tukey's HSD test was performed to separate treatment means. The results were presented as means and pooled standard errors of the mean (SEM). The presence of maternal antibodies was not detected in a significant high number of yolk sacs and blood serum samples collected from poults. Therefore, the observed frequencies of antibody titers were analyzed using multiway contingency tables and Pearson's chi-squared test. The statistical analysis was performed using STATISTICA software version 13.1 (2021) (StatSoft Corp., Krakow, Poland) at a significance level of $P<0.05$. For birds that did not produce measurable antibodies, a value of 1 was assumed. No experimental data were removed, there were no blank observations, and there was no selective elimination.

Results

Effects of antibiotics and/or a coccidiostat

In all experimental groups, turkeys were characterized by similar BW at 1 and 3 days of age, whereas minor differences in BW were noted between groups on days 5 and 7 (Table 1). On days 5 and 7, turkeys administered DOX had the highest BW, but the difference relative to group CON was not significant. Neither a coccidiostat (MON) included in feed nor early administration of ENR or DOX affected the rate of yolk sac resorption; on days 1, 3, 5 and 7, the total and relative (% BW) weights of yolk sacs did not differ across groups (Table 1).

There were no significant differences in the frequency of anti-aMPV antibodies in the yolk sac and blood serum of turkeys under the influence of MON, ENR or DOX (Table 2). The presence of maternal anti-aMPV antibodies was detected in nearly all yolk sacs (except two in group MON-) and in all serum samples collected from one- and three-day-old poults, regardless of group (Table 3). The frequency of anti-aMPV antibodies in yolk sacs collected on days 3 and 5 decreased ($P<0.001$ and $P = 0.049$, respectively) in response to early administration of antibiotics or a coccidiostat. An analysis of the frequency of anti-aMPV antibodies in yolk sacs collected from three-day-old poults revealed that the administration of MON or ENR and simultaneous vaccination decreased the frequency of anti-aMPV antibodies. Early DOX administration significantly decreased the frequency of anti-aMPV antibodies (Table 2) and anti-aMPV antibody titers (Table 3) in the blood serum of unvaccinated seven-day-old turkeys. Anti-aMPV antibody titers in yolk sacs collected from birds aged 1, 3 and 5 days decreased gradually. The titer of these antibodies in blood serum of poults aged 1 to 3 days was similar in all experimental groups, and early administration of antibiotics or MON had no effect on their titer until day 3. On day 5, anti-aMPV antibody titers were significantly lower in the yolk sacs of poults receiving ENR or DOX ($P = 0.024$), compared with group CON (Table 3).

Table 4 shows that the frequency of maternal anti-NDV antibodies in yolk sacs collected from poults aged 1, 3 and 5 days decreased in response to early administration of ENR and DOX ($P<0.001$, both). A low frequency of anti-NDV antibodies was also observed in the blood serum of 56-day-old turkeys ($P<0.001$) receiving antibiotics or a coccidiostat. The observed interaction in the analysis of the frequency of anti-NDV antibodies in yolk sacs collected from chicks aged 1, 3 and 5 days ($P<0.001$, respectively) results from the fact that the frequency of these antibodies was influenced by both the administration of antibiotics and vaccination against ND in day 1 after hatching (Table 4). Maternal anti-NDV antibody titers in yolk sacs collected from poults aged 1, 3 and 5 days decreased gradually (Table 5). On days 3 and 5, anti-NDV antibody titers were significantly lower ($P<0.001$ both) in the yolk sacs of turkeys receiving MON or antibiotics than in group CON. The titer of anti-NDV antibodies in blood serum of poults aged 1 to 5 days was similar in all experimental groups. On day 7, anti-NDV antibody titers were significantly lower ($P = 0.010$) in the blood serum of turkeys administered DOX for the first five days of life (Table 5). Anti-NDV titers in the blood serum of seven-day-old birds were lower in groups receiving a coccidiostat or antibiotics than in group CON.

The frequency of maternal anti-ORT antibodies in yolk sacs was lower ($P = 0.004$) in three-day-old poults administered ENR or DOX from the first day of life than in group CON (Table 6). Maternal anti-ORT antibody titers in yolk sacs were significantly lower ($P = 0.024$) in three-day-old birds administered MON or DOX than in group CON (Table 7). Anti-ORT antibody titers in yolk sacs collected from poults aged 1, 3 and 5 days decreased gradually. Early DOX administration significantly decreased anti-ORT antibody titers ($P = 0.014$) in the blood serum of three-day-old poults, compared with group CON. On days 5, 7 and 56, anti-ORT antibody titers were similar in all experimental groups (Table 7).

Early administration of antibiotics or a coccidiostat had no influence on total IgY and IgM levels in the yolk sacs of poults aged 1, 3 and 5 days (Tables 8 and 9). However, the administration of MON or DOX led to a significant decrease in serum IgY levels in one- and three-day-old poults ($P<0.001$), and the administration of ENR and DOX contributed to a decrease in serum IgM levels in three-day-old birds ($P<0.001$) (Table 9).

Table 1. Body weight (BW) and yolk sac (YS) weight in turkeys at 1, 3, 5 and 7 days of age

Item	Day 1			Day 3			Day 5			Day 7		
	BW (g)	YS total weight (g)	YS relative weight (% BW)	BW (g)	YS total weight (g)	YS relative weight (% BW)	BW (g)	YS total weight (g)	YS relative weight (% BW)	BW (g)	YS total weight (g)	YS relative weight (% BW)
Antibiotic (n=42)[1]												
CON	67.13	6.184	9.30	93.34	2.339	2.53	132.87 ab	1.030	0.78	183.55 ab	0.116	0.06
MON	67.15	6.505	9.67	97.28	2.177	2.24	129.93 b	0.634	0.50	183.37 ab	0.146	0.08
ENR	68.82	6.906	10.09	98.43	2.006	2.06	132.87 ab	0.620	0.47	176.26 b	0.170	0.10
DOX	67.11	6.688	9.98	96.72	2.113	2.21	137.61 a	0.747	0.56	189.10 a	0.161	0.09
Vaccine (n=84)[2]												
-	66.97	6.503	9.74	96.12	2.223	2.33	136.70 a	0.815	0.61	187.41 a	0.157	0.09
+	68.13	6.638	9.78	96.76	2.094	2.18	129.93 b	0.700	0.55	178.73 b	0.140	0.08
Group (n = 21)												
CON -	67.01	6.218	9.34	93.46	2.050	2.20	136.93	1.167	0.86	189.28	0.120	0.06
CON +	67.25	6.150	9.26	93.22	2.627	2.86	128.81	0.892	0.70	177.82	0.112	0.06
MON -	66.92	6.623	9.87	96.24	2.597	2.69	133.70	0.714	0.54	187.42	0.146	0.08
MON +	67.37	6.386	9.47	98.33	1.757	1.78	126.16	0.554	0.46	179.31	0.145	0.08
ENR -	67.38	6.942	10.37	96.51	2.086	2.20	137.40	0.695	0.52	180.20	0.240	0.14
ENR +	70.27	6.869	9.81	100.35	1.926	1.91	128.34	0.545	0.42	172.31	0.100	0.06
DOX -	66.58	6.229	9.39	98.29	2.160	2.24	138.80	0.683	0.50	192.75	0.121	0.07
DOX +	67.65	7.147	10.56	95.15	2.067	2.18	136.43	0.810	0.63	185.45	0.202	0.11
SEM	0.408	0.147	0.218	0.698	0.088	0.095	0.996	0.064	0.050	1.583	0.017	0.010
P-value												
Antibiotic (A)	0.360	0.367	0.632	0.059	0.588	0.365	0.042	0.092	0.251	0.036	0.708	0.812
Vaccine (V)	0.157	0.650	0.941	0.644	0.457	0.309	0.001	0.375	0.314	0.005	0.625	0.976
A × V interaction	0.651	0.500	0.549	0.312	0.052	0.055	0.601	0.725	0.720	0.964	0.169	0.269

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life.

[2]unvaccinated, - or vaccinated, + (1-day-old turkeys were vaccinated against aMPV (TRT) and NDV).

a, b – means within the same column with different letters differ significantly ($P<0.05$).

Table 2. Observed frequencies of anti-aMPV antibodies in the yolk sac and blood serum of turkeys (%)

Item	Yolk sac			Blood serum				
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 7	Day 56
Antibiotic (n = 42)[1]								
CON	100	88.10 a	42.86 a	100	100	97.62	80.95 bc	59.52
MON	95.24	50.00 b	35.71 ab	100	100	100	95.24 a	50.00
ENR	100	57.14 b	21.43 b	100	100	90.48	92.86 ab	45.24
DOX	100	45.24 b	19.05 b	100	100	97.62	69.05 c	42.86
Vaccine (n = 84)[2]								
-	97.62	69.05 a	29.76	100	100	100 a	91.67 a	9.52 b
+	100	51.19 b	29.76	100	100	92.86 b	77.38 b	89.29 a
Group (n = 21)								
CON -	100	100 a	33.33	100	100	100 a	100 a	23.81 b
CON +	100	76.19 b	52.38	100	100	95.24 ab	61.90 bc	95.24 a
MON -	90.48	61.90 bc	33.33	100	100	100 a	100 a	14.29 bc
MON +	100	38.10 c	38.10	100	100	100 a	90.48 a	85.71 a
ENR -	100	71.43 bd	28.57	100	100	100 a	100 a	0.00 c
ENR +	100	42.86 cd	14.29	100	100	80.95 b	85.71 abc	90.48 a
DOX -	100	42.86 cd	23.81	100	100	100 a	66.67 b	0.00 c
DOX +	100	47.62 bc	14.29	100	100	95.24 ab	71.43 b	85.71 a
P-value								
Antibiotic	0.108	<0.001	0.049	NA	NA	0.101	0.003	0.434
Vaccine	0.155	0.018	1.000	NA	NA	0.013	0.010	<0.001
Group	0.050	<0.001	0.127	NA	NA	0.009	<0.001	<0.001

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life; NA = not analyzed.

[2]unvaccinated, - or vaccinated, + (1-day-old turkeys were vaccinated against aMPV (TRT).

a, b, c – means within the same column with different letters differ significantly (P<0.05).

Table 3. Anti-aMPV (TRT) antibody titers in the yolk sac and blood serum of turkeys

Item	Yolk sac			Blood serum				
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 7	Day 56
Antibiotic (n = 42)[1]								
CON	726.9	520.7	271.07 a	5181.6	5202.3	3487.6	2051.6	353.4
MON	1308.8	491.6	98.55 ab	4981.7	4874.8	4586.9	2108.1	165.9
ENR	1070.3	455.0	24.76 b	4474.0	4008.0	3773.7	1846.4	247.6
DOX	989.0	602.0	22.89 b	5744.4	4662.7	3657.9	1307.5	255.5
Vaccine (n = 84)[2]								
-	543.1 b	663.5	84.53	5018.9	5749.3 a	4824.6 a	2114.9	5.2 b
+	1504.3 a	371.2	124.10	5172.0	3624.6 b	2928.5 b	1541.9	506.0 a
Group (n = 21)								
CON -	479.2	395.2	108.43	5173.8	6629.8	3672.5	2940.5 ab	13.0
CON +	974.5	646.2	433.70	5189.5	3774.8	3302.7	1162.6 bc	693.8
MON -	453.1	625.6	159.02	4047.8	5832.1	5629.6	3212.9 a	6.0
MON +	2164.5	357.7	38.08	5915.6	3917.5	3544.2	1003.4 bc	325.9
ENR -	580.5	535.9	42.14	4849.7	4950.8	5040.8	1696.3 abc	1.0
ENR +	1560.0	374.0	7.38	4098.3	3065.2	2506.6	1996.4 abc	494.3
DOX -	659.6	1097.2	28.53	6004.5	5584.4	4955.5	609.8 c	1.0
DOX +	1318.3	106.7	17.25	5484.4	3741.0	2360.4	2005.2 abc	510.1
SEM	92.95	83.24	33.47	283.0	303.5	297.3	173.8	37.31
P-value								
Antibiotic (A)	0.101	0.933	0.024	0.466	0.539	0.549	0.299	0.227
Vaccine (V)	<0.001	0.078	0.544	0.788	<0.001	0.001	0.083	<0.001
A × V interaction	0.051	0.064	0.082	0.354	0.917	0.492	<0.001	0.260

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life.

[2]unvaccinated, - or vaccinated, + (1-day-old turkeys were vaccinated against aMPV (TRT)).

a, b, c – means within the same column with different letters differ significantly (P<0.05).

Table 4. Observed frequencies of anti-NDV antibodies in the yolk sac and blood serum of turkeys (%)

Item	Yolk sac			Blood serum				
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 7	Day 56
Antibiotic (n = 42)[1]								
CON	100 a	100 a	76.19 a	100	100	100	100	76.19 a
MON	100 a	69.05 bc	47.62 b	100	100	100	88.10	40.48 b
ENR	83.33 b	76.19 b	28.57 bc	100	100	95.24	92.86	33.33 b
DOX	73.81 b	52.38 c	21.43 c	100	100	100	95.24	9.52 c
Vaccine (n = 84)[2]								
-	78.57 b	94.05 a	46.43	100	100	100	100a	35.71
+	100 a	54.76 b	40.48	100	100	97.62	88.10 b	44.05
Group (n = 21)								
CON -	100 a	100 a	76.19 a	100	100	100	100 a	71.43 ab
CON +	100 a	100 a	76.19 a	100	100	100	100 a	80.95 a
MON -	100 a	100 a	47.62 ab	100	100	100	100 a	42.86 bc
MON +	100 a	38.10 c	47.62 ab	100	100	100	76.19 b	38.10 cd
ENR -	66.67 b	100 a	38.10 bc	100	100	100	100 a	23.81 cde
ENR +	100 a	52.38 bc	19.05 c	100	100	90.48	85.71 ab	42.86 bc
DOX -	47.62 b	76.19 b	23.81 bc	100	100	100	100 a	4.76 e
DOX +	100 a	28.57 c	19.05 c	100	100	100	90.48 ab	14.29 de
P-value								
Antibiotic	<0.001	<0.001	<0.001	NA	NA	0.108	0.137	<0.001
Vaccine	<0.001	<0.001	0.436	NA	NA	0.154	0.001	0.270
Group	<0.001	<0.001	<0.001	NA	NA	0.050	0.003	<0.001

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life; NA = not analyzed.

[2]unvaccinated, - or vaccinated, + (1-day-old turkeys were vaccinated against ND).

a, b, c, d, e – means within the same column with different letters differ significantly ($P<0.05$).

Table 5. Anti-NDV antibody titers in the yolk sac and blood serum of turkeys

Item	Yolk sac			Blood serum				
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 7	Day 56
Antibiotic (n = 42)[1]								
CON	285.1	212.2 a	107.86 a	1667.1	1424.4	710.6	443.4 a	76.8 a
MON	245.2	104.1 b	25.41 b	1601.2	1245.9	671.5	388.0 ab	16.7 b
ENR	173.7	113.7 b	12.10 b	1267.6	1265.5	746.3	284.7 ab	20.3 ab
DOX	185.1	56.9 b	50.13 ab	1549.7	1015.7	977.1	195.4 b	9.7 b
Vaccine (n = 84)[2]								
-	111.5 b	113.2	69.55 a	1571.4	1592.8 a	851.8	380.8	8.1 b
+	333.1 a	130.3	28.20 b	1471.4	882.9 b	701.0	275.0	53.6 a
Group (n = 21)								
CON -	149.7	122.4 b	149.14	1524.7	1675.5	783.9	576.9 a	19.8
CON +	420.5	302.1 a	66.59	1809.5	1173.2	637.4	309.8 ab	133.8
MON -	153.2	136.4 b	28.38	1726.5	1653.1	803.8	529.3 a	6.0
MON +	337.1	71.9 b	22.45	1475.9	838.7	539.2	246.8 ab	27.3
ENR -	62.3	116.0 b	14.02	1346.5	1708.8	752.0	265.3 ab	5.2
ENR +	285.2	111.3 b	10.17	1188.7	822.3	740.7	304.0 ab	35.4
DOX -	80.8	78.0 b	86.66	1688.1	1333.9	1067.4	151.5 b	1.6
DOX +	289.5	35.8 b	13.60	1411.4	697.4	886.7	239.4 ab	17.9
SEM	22.85	13.61	8.83	79.19	74.09	53.14	29.32	8.298
P-value								
Antibiotic (A)	0.215	<0.001	<0.001	0.302	0.221	0.174	0.010	0.010
Vaccine (V)	<0.001	0.498	0.013	0.530	<0.001	0.157	0.060	0.004
A × V interaction	0.907	0.003	0.183	0.566	0.757	0.863	0.028	0.096

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life.

[2]unvaccinated, - or vaccinated, + (1-day-old turkeys were vaccinated against ND).

a, b – means within the same column with different letters differ significantly (P<0.05)

Table 6. Observed frequencies of anti-ORT antibodies in the yolk sac and blood serum of turkeys (%)

Item	Yolk sac			Blood serum				
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 7	Day 56
Antibiotic (n = 42)[1]								
CON	100	100 a	69.05	100	100	100	100	88.10 a
MON	100	100 a	66.67	100	100	100	100	73.81 ab
ENR	100	90.48 b	50.00	100	100	100	100	59.52 b
DOX	100	83.33 b	61.90	100	100	100	100	54.76 b
Vaccine (n = 84)[2]								
-	100	100 a	72.62 a	100	100	100	100	38.10 b
+	100	86.90 b	51.19 b	100	100	100	100	100 a
Group (n = 21)								
CON -	100	100 a	71.43 ac	100	100	100	100	76.19 b
CON +	100	100 a	66.67 ab	100	100	100	100	100 a
MON -	100	100 a	76.19 a	100	100	100	100	47.62 b
MON +	100	100 a	57.14 ab	100	100	100	100	100 a
ENR -	100	100 a	61.90 ab	100	100	100	100	19.05 c
ENR +	100	80.95 b	38.10 b	100	100	100	100	100 a
DOX -	100	100 a	80.95 a	100	100	100	100	9.52 c
DOX +	100	66.67 b	42.86 bc	100	100	100	100	100 a
P-value								
Antibiotic	NA	0.004	0.280	NA	NA	NA	NA	0.004
Vaccine	NA	0.001	0.004	NA	NA	NA	NA	<0.001
Group	NA	<0.001	0.042	NA	NA	NA	NA	<0.001

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life; NA = not analyzed.

[2]unvaccinated, - or vaccinated, + (28-day-old turkeys were vaccinated against ORT).

a, b, c – means within the same column with different letters differ significantly ($P<0.05$).

Table 7. Anti-ORT antibody titers in the yolk sac and blood serum of turkeys

Item	Yolk sac			Blood serum				
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 7	Day 56
Antibiotic (n = 42)[1]								
CON	2485.8	1801.6 a	538.3	8118.4	8825.5 a	6549.3	3140.9	6681.7
MON	2290.7	917.8 b	701.6	7380.7	7017.4 ab	6145.1	3405.7	6294.5
ENR	2730.3	1585.4 ab	311.8	8303.3	8286.7 ab	5731.8	2841.7	6340.8
DOX	2111.6	893.6 b	716.0	8368.2	6290.9 b	6543.3	2568.2	6800.9
Vaccine (n = 84)[2]								
-	1727.0 b	1194.3	681.9	8891.3 a	8598.1 a	6246.0	2887.2	32.0 b
+	3082.2 a	1404.9	452.0	7194.0 b	6612.1 b	6238.7	3091.0	13027.0 a
Group (n = 21)								
CON -	1804.3	1465.9	615.0	8318.1	10706.8	7060.0	3910.6 ab	72.4
CON +	3167.3	2137.4	461.7	7918.7	6944.3	6038.5	2371.3 ab	13291.1
MON -	1710.7	1248.2	923.4	8015.1	7972.0	6637.6	4058.0 a	36.7
MON +	2870.7	587.5	479.9	6746.4	6062.8	5652.6	2753.4 ab	12552.4
ENR -	1940.4	1276.2	404.0	9366.3	9229.5	5597.8	2141.6 ab	9.2
ENR +	3520.1	1894.7	219.7	7240.3	7343.8	5865.8	3541.8 ab	12672.3
DOX -	1452.7	786.9	785.3	9865.8	6484.2	5688.7	1438.7 b	9.7
DOX +	2770.5	1000.2	646.8	6870.6	6097.6	7397.8	3697.6 ab	13592.1
SEM	162.2	131.7	77.14	292.6	318.8	272.8	213.6	533.5
P-value								
Antibiotic (A)	0.537	0.024	0.219	0.600	0.014	0.679	0.511	0.697
Vaccine (V)	<0.001	0.415	0.138	0.004	0.001	0.989	0.622	<0.001
A × V interaction	0.972	0.236	0.884	0.421	0.274	0.248	0.002	0.704

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life.

[2]unvaccinated, - or vaccinated, + (28-day-old turkeys were vaccinated against ORT).

a, b – means within the same column with different letters differ significantly ($P<0.05$).

Table 8. Total IgY antibody levels in the yolk sac and blood serum of turkeys

Item	Yolk sac (mg/total yolk sac)			Blood serum (mg/mL)				
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 7	Day 56
Antibiotic (n = 42)[1]								
CON	184.4	43.59	13.90	13.55 a	13.42 a	10.90	17.87	18.50
MON	162.6	39.67	10.07	12.15 bc	12.63 b	10.78	18.02	17.98
ENR	179.5	39.18	8.92	12.94 ab	12.05 bc	13.04	17.33	17.63
DOX	159.1	37.15	11.77	11.40 c	11.85 c	9.97	17.78	17.41
Vaccine (n = 84)[2]								
-	173.8	41.50	10.93	13.25 a	12.60	10.65	18.73 a	18.62 a
+	169.0	38.30	11.40	11.77 b	12.37	11.69	16.77 b	17.14 b
Group (n = 21)								
CON -	216.0 a	43.21	12.91	14.53	13.58 a	10.69	18.78	19.25
CON +	152.8 b	43.96	14.89	12.56	13.26 ab	11.11	16.96	17.74
MON -	160.1 ab	45.52	9.64	12.98	12.23 bc	10.60	19.31	18.70
MON +	165.0 ab	33.82	10.51	11.31	13.02 ab	10.96	16.74	17.26
ENR -	167.1 ab	39.79	10.63	13.11	12.24 bc	10.73	18.53	18.05
ENR +	191.8 ab	38.57	7.20	12.77	11.87 c	15.35	16.14	17.22
DOX -	151.9 b	37.46	10.56	12.35	12.38 bc	10.58	18.31	18.50
DOX +	166.3 ab	36.85	12.99	10.44	11.32 c	9.35	17.26	16.33
SEM	5.294	1.629	1.135	0.188	0.101	0.592	0.181	0.263
P-value								
Antibiotic (A)	0.231	0.569	0.446	<0.001	<0.001	0.299	0.495	0.479
Vaccine (V)	0.643	0.331	0.840	<0.001	0.178	0.378	<0.001	0.005
A × V interaction	0.013	0.516	0.796	0.285	0.003	0.340	0.366	0.842

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life.

[2]unvaccinated, - or vaccinated, +).

a, b, c – means within the same column with different letters differ significantly ($P<0.05$).

Table 9. Total IgM antibody levels in the yolk sac and blood serum of turkeys

Item	Yolk sac (µg/total yolk sac)			Blood serum (µg/mL)				
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 7	Day 56
Antibiotic (n = 42)[1]								
CON	1212.7	546.7	232.2	128.4	124.5 a	68.51	68.91	66.65
MON	1150.9	589.0	183.7	124.1	120.0 ab	71.71	67.82	63.04
ENR	1197.4	560.1	172.6	119.9	111.2 bc	70.63	66.40	63.63
DOX	1251.9	553.0	211.6	119.5	104.0 c	72.65	64.49	68.15
Vaccine (n = 84)[2]								
-	1177.9	627.7 a	208.6	125.1	125.4 a	75.27 a	73.12 a	68.26 a
+	1228.5	496.7 b	191.4	120.8	104.4 b	66.48 b	60.70 b	62.47 b
Group (n = 21)								
CON -	1357.2 ab	543.3	201.7	126.9	122.3 a	75.51 bc	74.83 ac	72.28
CON +	1068.2 b	550.1	262.7	129.9	126.8 a	61.51 d	63.00 b	61.03
MON -	1070.7 b	730.3	195.4	129.3	124.3 a	86.37 a	77.44 a	66.38
MON +	1231.0 ab	447.8	172.1	119.0	115.6 a	57.06 d	58.21 b	59.71
ENR -	1205.6 ab	649.7	217.0	122.1	129.4 a	73.73 bc	73.21 ac	66.72
ENR +	1189.1 b	470.4	128.1	117.7	93.0 b	67.52 cd	59.60 b	60.54
DOX -	1078.2 b	587.6	220.3	122.2	125.7 a	65.44 cd	67.00 bc	67.68
DOX +	1425.6 a	518.4	202.8	116.7	82.2 b	79.86 ab	61.98 b	68.61
SEM	35.65	21.43	18.90	1.471	1.809	1.111	0.899	1.014
P-value								
Antibiotic (A)	0.785	0.895	0.683	0.105	<0.001	0.379	0.174	0.207
Vaccine (V)	0.472	0.002	0.653	0.140	<0.001	<0.001	<0.001	0.004
A × V interaction	0.013	0.078	0.588	0.444	<0.001	<0.001	0.010	0.183

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life.

[2]unvaccinated, - or vaccinated, +).

a, b, c – means within the same column with different letters differ significantly (P<0.05).

Table 10. Lysozyme levels in the yolk sac and blood serum of turkeys

Item	Yolk sac (ng/total yolk sac)			Blood serum (ng/mL)				
	Day 1	Day 3	Day 5	Day 1	Day 3	Day 5	Day 7	Day 56
Antibiotic (n = 42) [1]								
CON	10043.4	2442.7	541.4	411.7	323.6 a	546.6 ab	468.2	403.5
MON	6718.5	1779.1	300.3	353.7	262.8 b	526.7 b	444.3	375.9
ENR	7416.7	1898.5	301.3	383.2	307.2 ab	589.6 a	444.9	371.8
DOX	6642.5	2122.7	370.5	335.4	262.1 b	541.0 b	439.3	368.8
Vaccine (n = 84)[2]								
-	8668.1	2370.9 a	415.2	431.0 a	341.9 a	561.1	475.1 a	405.7 a
+	6742.5	1750.5 b	341.6	311.0 b	236.0 b	540.8	423.2 b	354.4 b
Group (n = 21)								
CON -	13692.8	2987.6	411.4	425.9	342.2 a	554.7	491.8	422.4 a
CON +	6394.1	1897.7	671.4	397.5	305.0 ab	538.4	444.6	384.7 ab
MON -	7094.1	2225.2	419.5	440.4	318.8 ab	533.6	440.8	369.7 ab
MON +	6343.0	1332.9	181.1	266.9	206.8 c	519.7	447.9	382.1 ab
ENR -	6791.1	2127.9	337.3	459.2	371.7 a	607.8	487.8	415.3 a
ENR +	8042.2	1669.1	265.4	307.2	242.7 bc	571.5	402.0	328.3 b
DOX -	7094.2	2142.9	492.7	398.4	334.7 a	548.3	480.1	415.4 a
DOX +	6190.7	2102.4	248.4	272.5	189.4 c	533.7	398.4	322.2 b
SEM	616.3	153.9	54.62	14.16	10.72	6.969	12.20	7.427
P-value								
Antibiotic (A)	0.130	0.435	0.364	0.087	0.004	0.008	0.835	0.167
Vaccine (V)	0.096	0.046	0.503	<0.001	<0.001	0.122	0.037	<0.001
A × V interaction	0.058	0.620	0.329	0.110	0.044	0.913	0.507	0.010

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life.

[2]unvaccinated, - or vaccinated, +).

a, b, c – means within the same column with different letters differ significantly (P<0.05).

Lysozyme levels were highest in yolk sacs collected from one-day-old poults, and they decreased on days 3 and 5 (Table 10). Early administration of a coccidiostat or antibiotics had no effect on lysozyme levels in yolk sacs. In turn, serum lysozyme levels were significantly lower (P = 0.004) in three-day-old birds receiving MON or DOX than in group CON. On day 3, an antibiotic × vaccine interaction (P = 0.044) had a significant effect on serum lysozyme levels. The interaction resulted from the fact that vaccination of turkeys in the control group had no effect on the level of lysozyme, while vaccination of turkeys that received antibiotics resulted in a decrease in the level of lysozyme in the blood serum.

Effect of vaccination

Turkeys vaccinated against TRT and ND had lower BW at 5 and 7 days of age (P = 0.001 and P = 0.005, respectively) (Table 1) than their unvaccinated counterparts. Yolk sac weight was similar in vaccinated and unvaccinated birds.

In comparison with unvaccinated birds, the presence of maternal anti-aMPV antibodies was not detected in a significantly high number of yolk sacs collected from vaccinated three-day-old poults (P = 0.018) and in a significantly high number of blood serum samples collected from vaccinated poults aged 5 and 7 days, (P = 0.013 and P = 0.010, respectively). The frequency of anti-aMPV antibodies (P<0.001) in blood serum was higher in vaccinated than unvaccinated turkeys only at 56 days of age (Table 2). Maternal anti-aMPV antibody titers in blood serum were considerably lower on days 3 (P<0.001) and 5 (P = 0.001) in turkeys vaccinated against TRT and ND on day 1 post-hatch, compared with unvaccinated birds. Anti-aMPV antibody titers were higher (P<0.001) in the blood serum of 56-day-old turkeys that were vaccinated against TRT and ND (on day 1 post-hatch) and against ORT (on day 28 post-hatch) than in their unvaccinated counterparts (Table 3).

Compared to unvaccinated birds, a higher frequency of maternal anti-NDV antibodies was found in the yolk sacs of one-day-old chicks vaccinated on day 1 of life against TRT and ND (P<0.001). Then, until about the 7th day of life of turkey poults vaccinated against TRT and ND, the frequency of anti-NDV was lower in yolk sacs (P<0.001) and in blood serum (P = 0.001) (Table 4). Maternal anti-NDV antibody titers in yolk sacs were higher in vaccinated than unvaccinated poults on day 1 (P<0.001), but they were lower in yolk sacs collected on day 5 and in blood serum samples collected on day 3 (P = 0.013 and P<0.001, respectively) from vaccinated birds (Table 5).

The frequency of maternal anti-ORT antibodies in yolk sacs collected on days 3 and 5 was lower (P = 0.001 and P = 0.004, respectively) in turkeys vaccinated against TRT and ND than in unvaccinated birds. The frequency of anti-ORT antibodies in blood serum was higher (P<0.001) in poults vaccinated against TRT and ND (on day 1 post-hatch) and against ORT (on day 28) than in unvaccinated birds only on day 56 (Table 6). Vaccination against TRT and ND contributed to an increase in maternal anti-ORT antibody titers in yolk sacs collected on day 1 (P<0.001). Vaccination against TRT and ND contributed also to a decrease in anti-ORT antibody titers in blood serum samples collected on days 1 and 3 (P = 0.004 and P = 0.001, respectively). Vaccination against ORT induced an increase in anti-ORT antibody titers in blood serum samples collected on day 56 (P<0.001) (Table 7).

In turkeys vaccinated against TRT and ND, total IgY levels decreased (P<0.001) in yolk sacs collected on day 3 (P = 0.002) and in blood serum samples collected already on day 1, and this trend was also observed on days 7 (P<0.001) and 56 (P = 0.005) (Table 8). Total IgM levels in blood serum decreased on days 3, 5, 7 (P<0.001, respectively) and 56 (P=0.004) in vaccinated poults, compared with their unvaccinated counterparts (Table 9).

In comparison with unvaccinated birds, turkeys vaccinated against TRT and ND were characterized by lower lysozyme levels in yolk sacs collected on day 3 (P = 0.046) and in blood serum samples collected on days 1 and 3 (P<0.001 both). Vaccination against TRT and ND contributed to a decrease in lysozyme levels in seven-day-old poults (P = 0.037), whereas vaccination against ORT induced a decrease in lysozyme levels in birds aged 56 days (P<0.001) (Table 10).

Discussion

The yolk sac is a rich source of nutrients for the developing embryo. According to Mikec et al. (2006), approximately 60% of the yolk sac content is utilized on the first day post-hatch of chicks. On the second day, 40% of the first day resorption is used, 35% on the third day, and 25% on the fourth day. The yolk sac content is a primary source of not only nutrients but also humoral factors involved in the development and function of the immune system in birds (Brudnicki et al., 2015). Jamroz et al. (2004) found that unabsorbed yolk sacs were present in 30% and 10% of chickens on days 7 and 16 post-hatch, respectively, whereas Buhr et al. (2006) reported that unabsorbed yolk sacs were detected in 31% chickens aged 6–8 weeks. In a study by Brudnicki et al. (2015), average yolk sac weight accounted for around 6.4% of the total BW in one-day-old chickens. However, yolk sac weight can be higher, accounting for up to 20% of the bird's BW (Brudnicki et al., 2015). The rate of yolk sac resorption can vary depending on incubation temperature, the availability and type of nourishment and water after hatch, and the presence of infectious agents (Khan et al., 2004; Lourens et al., 2007). To the best of our knowledge, the potential effects of other factors, including early antibiotic administration, on yolk sac resorption in birds have not been investigated to date. In the present study, in one-day-old poults, unabsorbed yolk sacs accounted for 100%, and their weights accounted for approximately 9.7% of the total BW. The hypothesis that

yolk sac resorption can be inhibited by the administration of antibiotics over the first few days after hatch or feeding diets containing a coccidiostat was not validated in this experiment, because the rate of yolk sac resorption was similar in all poults.

The unabsorbed content of the yolk sac is a source of nutrients for pathogens and can weaken the immune system of young turkeys, thus increasing their first-week mortality (Panda and Reddy, 2007). According to Rose and Orlans (1981) and Li et al. (1998), the immune system of embryos cannot produce antibodies before hatch because it is not sufficiently mature, therefore antibodies are not synthesized *de novo* by the embryo. In consequence, the ability of chicks to protect themselves against pathogens in the first week post-hatch is determined mostly by the transfer of specific maternal antibodies released from the yolk sac directly to the intestinal lumen, and by their innate immunity (Grindstaff et al., 2003; Bar-Shira and Friedman, 2006; Chrząstek et al., 2011 a). The diversity of maternal antibodies transferred to the offspring depends on the mother's exposure to antigens, which is closely linked to past infections and immunoprophylaxis (Kowalczyk et al., 2019). Breeder turkey flocks are vaccinated with live and/or inactivated vaccines several times to produce pathogen-specific antibodies that protect turkey hens against infections and are transferred to their progeny. If maternal antibodies are not absorbed from the yolk sac, chicks may be susceptible to infections, and the development and function of their immune system may be compromised. The results of the first scientific report on the titers of specific anti-aMPV, anti-NDV, anti-ORT and anti-PM antibodies and the total concentrations of IgY and IgM antibodies in the blood serum of turkey hens and poults, and in the egg yolk and egg white (Kowalczyk et al., 2019), corroborate the findings from earlier experiments performed on broiler breeder hens (Gharaibeh et al., 2008). According to the cited authors, if the levels of pathogen-specific IgY antibodies vary in the hen, they will also vary in the chick, as maternal antibodies are transferred to the yolk, then to the embryo and subsequently to the chick's circulatory system. They also found that antibody transfer percentage was affected by bird species (Gharaibeh et al., 2008; Kowalczyk et al., 2019).

This study confirmed the assumption that specific maternal anti-aMPV, anti-NDV and anti-ORT antibodies would be present in yolk sacs collected from one-day-old poults, and their titers would be comparable in all groups regardless of the applied experimental factor, i.e. the coccidiostat or antibiotic used and vaccinations. Our research shows that early administration of antibiotics (DOX, ENR and MON) reduced maternal antibody titers in the yolk sacs of turkey poults. This suggests that antibiotics as chemicals directly inactivated maternal antibodies accumulated in some yolk sacs. Our observations also show that from those yolk sacs in which maternal antibodies were not inactivated by antibiotics, their transfer to the blood serum of turkey poults was normal until the yolk sac was absorbed (approx. 5 days of age). It is worth emphasizing that in the case of the assessed frequency and titer of anti-NDV and anti-aMPV antibodies, their level was also influenced by vaccination of one-day-old turkey poults against avian metapneumoviruses (APV) and Newcastle disease (ND). Early administration of ENR and DOX also resulted in the absence of anti-ORT antibodies in the yolk sacs of 3-day-old birds in approximately 10% of turkey poults in each group. Moreover, only in the case of DOX administration, the reduced frequency of this antibody in the sac resulted in a lower anti-ORT titer in the blood serum of 3-day-old birds. A surprising result is the equalization of the titer of this antibody in the blood serum of 5-day-old turkey poults of all experimental groups, as a post-vaccination effect cannot be expected in this case, as vaccination against ORT was performed only on the 28th day of life. Anti-aMPV and anti-NDV antibody titers in the blood serum of birds decreased immediately after the administration of ENR and DOX; anti-NDV antibody titers decreased also in response to MON. Unlike the decrease in anti-ORT antibody titers, the decrease in anti-aMPV and anti-NDV antibody titers in the blood serum of birds receiving antibiotics was long-lasting, and it was still observed at 56 days of age. Ellakany et al. (2007) confirmed the adverse effect of enrofloxacin administered to chickens at a dose 10 times higher than the recommended dose (i.e. 100 mg/kg) on the titer of anti-NDV HI antibodies (decrease of antibody titer). Similarly, Paningrahy et al. (1979), Nagi et al. (1984), Afifi (1987) and Rzedzicki et al. (1991) found the adverse effect of antibiotics such as chloramphenicol, nitrofurans, erythromycin, tylosin and chlortetracycline on antibody titers and phagocytosis.

In the present study, total IgY and IgM levels in the blood serum of poults also decreased during and immediately after DOX treatment. However, similarly to the decrease in anti-ORT antibody titers, this was a short-term effect, because on day 56, serum IgY and IgM levels were comparable in all experimental groups. According to Tokarzewski (2002), ENR may negatively affect the levels of specific IgY antibodies in the blood serum of hens and in the egg yolk.

In the current experiment, early administration of antibiotics or a coccidiostat had no influence on lysozyme levels in the yolk sacs of poults, and the decrease in serum lysozyme levels in three- and five-day old birds administered MON or DOX resulted from the interaction between antibiotic administration and simultaneous vaccination against TRT and ND, noted on day 3. Vaccination stimulates non-specific defense mechanisms, mostly phagocytic activity and the synthesis of non-specific defense factors such as lysozyme and interferon gamma (Marshall et al., 2018). Antibiotic treatment may negatively affect vaccine effectiveness because antibiotics alter the metabolism of immune cells and influence the secretion of cytokines and lysozyme (Khalifeh et al., 2009; Munir et al., 2007). Vaccination of newly-hatched birds with live vaccines, when matAb titers are high, may

be ineffective because pathogens contained in vaccines may be neutralized by these antibodies. Vaccination during antibiotic treatment may affect both the transfer of specific maternal antibodies from the yolk sac and the production of antibodies after vaccination (Khalifeh et al., 2009). According to Khalifeh et al. (2009) and Tokarzewski (2002), the immune system of birds may be unable to effectively fight infectious agents when the transfer of specific maternal antibodies and endogenous (post-vaccination) synthesis of specific antibodies are inhibited. Knowledge about the transfer of maternal antibodies from hens to their offspring and the durability of antibody responses is required to develop effective immunoprophylaxis programs targeting selected diseases (Al-Natour et al., 2004; Ahmed, 2015). According to Wallach (2010), IgY antibodies produced by a mother who has been immunized with live parasites are transmitted through the egg yolk and can provide up to 100% protection to chicks against primary *Eimeria* infection. Research shows that the efficacy of ND vaccination during five-day antibiotic treatment was low because antibiotics decreased the levels of specific anti-NDV antibodies in chickens, which may consequently lead to immunosuppression. Moreover, antibiotics exerted immunosuppressive effects on immune cells responsible for the development of active immunity (Khalifeh et al., 2009).

In turkeys that were vaccinated against TRT and ND (on day 1 post-hatch) and simultaneously treated with antibiotics, the presence of anti-aMPV, anti-NDV or anti-ORT antibodies was not detected in a high number of yolk sacs or blood serum samples collected on days 3–7. In addition, anti-aMPV and anti-NDV antibody titers in yolk sacs and blood serum samples were lower than in unvaccinated birds. The above observations may result from the fact that administration of live aMPV and NDV vaccine causes maternal antibodies contained in the yolk sac and blood serum to react with these viruses, bind to them and their titer decreases. Unvaccinated birds did not "use" their antibodies to inactivate the vaccine viruses, therefore they had higher titers of these antibodies. It should be stressed that the titers of anti-aMPV, anti-NDV and anti-ORT antibodies in blood serum were higher in vaccinated 56-day-old turkeys than in their unvaccinated counterparts. Antibody titers against ORT were much higher compared to the antibody titers against NDV and aMPV because the oil-adjuvanted inactivated vaccine was used. The aMPV and NDV vaccines were live attenuated and give low antibody titers but stimulate a cell-mediated response better, while the inactivated vaccines stimulate a strong humoral response (Clem, 2011). Anti-ORT titers were expected to be higher because birds were vaccinated against ORT at 28 days of age, i.e. the time interval between antibiotic treatment and vaccination was relatively long. In 56-day-old turkeys, the titers of anti-NDV and aMPV antibodies were lower in the groups receiving the tested antibiotics compared to the control group, but these differences were not always statistically significant. However, the titers of these antibodies in 56-day-old turkeys vaccinated once on the first day of life against TRT (aMPV) and NDV will always be lower than the titers of these antibodies in 1–7-day-old chicks where there are maternal antibodies. They will not grow close to ORT because the ORT vaccine was inactivated and adjuvanted. In the first days of life, i.e. up to the 3rd day of life, the frequency of anti-aMPV antibody in the yolk sacs was lower in the vaccinated group, which was consequently reflected in the blood serum. This result indicates that the frequency in blood serum resulted from the transfer of maternal antibodies. However, the high frequency of anti-aMPV antibody in the blood serum of 56-day-old turkeys is probably due to the antibodies produced after vaccination. However, it is worth noting that despite the high frequency of this antibody in the blood, the time that has passed since vaccination against avian metapneumovirus resulted in the level of this antibody in 56-day-old turkeys being several times lower than that recorded in the first days after administration of the vaccine.

Maternal IgY present in the yolk sac can be transferred to the embryonic circulation without degradation or digestion. Since immunoglobulins are accumulated predominantly in the yolk, a higher rate of yolk resorption can accelerate their transfer, in both the pre-hatch and post-hatch period (Murai, 2013). This process is disrupted in case of yolk sac retention or infection (Linden and Roth, 1978; Li et al., 1998). In the present study, yolk sac resorption was not inhibited by antibiotic treatment at the time of vaccination, but the growth performance of turkeys was compromised. Moreover, vaccination of one-day-old poults against TRT and ND during antibiotic treatment induced a decrease in total serum IgY, IgM and lysozyme levels, both in the first days post-hatch and at 56 days of age. Khalifeh et al. (2009) administered antibiotics (tilmicosin, florfenicol, ENR) to chickens at the time of ND vaccination and found that the synthesis of specific anti-NDV antibodies decreased and cell-mediated immunity weakened under the influence of antibiotic treatment. According to the cited authors, antibiotics probably modified the abundance and diversity of gut microbiota, which contributed to shorter durability of vaccine-induced immunity (Khalifeh et al., 2009). It is possible that also in the current study, early antibiotic administration induced changes in gut microbiota whose stable composition and diversity were not yet established in young turkeys, which consequently resulted in the above-mentioned reduction in antibody levels. The above undesirably altered humoral and cell-mediated immune responses in birds.

Conclusions

Our studies did not support part of the hypothesis that early administration of antibiotics inhibits yolk sac resorption. Nevertheless, they confirmed the assumption that early administration of antibiotics, through their direct effect on the titer of antibodies present in the unabsorbed yolk sac, reduces the titer of maternal antibodies

in the body of several-day-old turkeys. In addition to the fact that enrofloxacin and doxycycline had a direct effect on maternal antibodies present in the chicks' circulatory system, they additionally inhibited the endogenous (post-vaccination) synthesis of specific antibodies. Due to the above, the administration of antibiotics in the early rearing period should only be implemented in situations of clearly confirmed disease states, when the expected health benefits outweigh the risk of weakening immunity.

References

Afifi M.A.A. (1987). Studies on some immunosuppressives interfering with vaccination in chickens. M.V.Sc. Thesis, Birds and rabbit diseases, Fac. Vet. Med., Cairo Univ., Egypt.

Ahmed A.S. (2015). Assessment of maternal immunity against Newcastle disease in offspring chicks as affected by parents genetic crossing. Asian J. Anim. Vet. Adv., 10: 35–42.

Al-Natour M.Q., Ward L.A., Saif Y.M., Stewart-Brown B., Keck L.D. (2004). Effect of different levels of maternally derived antibodies on protection against infectious bursal disease virus. Avian Dis., 48: 177–182.

Bar-Shira E., Friedman A. (2006). Development and adaptations of innate immunity in the gastrointestinal tract of the newly hatched chick. Dev. Comp. Immunol., 30: 930–941.

Brudnicki A., Szymeczko R., Bednarczyk M., Głowińska B., Pietruszyńska D. (2015). Assessment of the effect of alpha-galactosides on yolk sac resorption rate in broiler chicken. J. Anim. Plant Sci., 25: 387–391.

Buhr R.J., Northcutt J.K., Richardson L.J., Cox N.A., Fairchild B.D. (2006). Incidence of unabsorbed yolk sacs in broilers, broiler breeder roosters, white Leghorn hens, and Athens-Canadian randombred control broilers. Poultry Sci., 85: 1294–1297.

Callewaert L., Michiels C.W. (2010). Lysozymes in the animal kingdom. J. Biosci., 35: 127–160.

Chamblee T.N., Brake J.D., Schultz C.D., Thaxton J.P. (1992). Yolk sac absorption and initiation of growth in broilers. Poultry Sci., 71: 1811–1816.

Chrząstek K., Wieliczko A. (2015). The influence of enrofloxacin, florfenicol, ceftiofur and E. coli LPS interaction on T and B cells subset in chicks. Vet. Res. Commun., 39: 53–60.

Chrząstek K., Wozniak A., Wieliczko A. (2011 a). Maternal antibodies in young birds. Med. Weter., 67: 250–252.

Chrząstek K., Madej J.P., Mytnik E., Wieliczko A. (2011 b). The influence of antibiotics on B-cell number, percentage, and distribution in the bursa of Fabricius of newly hatched chicks. Poultry Sci., 90: 2723–2729.

Clem A.S. (2011). Fundamentals of vaccine immunology. J. Glob. Infect. Dis., 3: 73–78.

Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. OJEU L276, 20.10.2010, pp. 33–79.

Ellakany H.F., Abu El-Azm I.M., Bekhit A.A., Shehawy M.M. (2007). Studies on the effects of enrofloxacin overdose on different health parameters in broiler chickens. Proc. 5th Scientific Conference, BS Vet. Med. J., pp. 176–186.

Fernández-Sousa J.M., Gavilanes J.G., Municio A.M., Rodriguez R. (1977). On the inhibition of hen egg-white lysozyme activity by aminoglycosidic antibiotics. Biochem. Biophys. Res. Commun., 75: 895–900.

Gharaibeh S., Mahmoud K., Al-Natour M. (2008). Field evaluation of maternal antibody transfer to a group of pathogens in meat-type chickens. Poultry Sci., 87: 1550–1555.

Grindstaff J.L., Brodie E.D., Ketterson E.D. (2003). Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. Proc. Biol. Sci., 270: 2309–2319.

Grondel J.L ., Angenent G.C., Egberts E. (1985). The influence of antibiotics on the immune system III. Investigations on the cellular functions of chicken leukocytes *in vitro*. Vet. Immunol. Immunopathol., 10: 307–316.

Hafez H.M, Shehata A.A. (2021). Turkey production and health: current challenges. Ger. J. Vet. Res., 1: 3–14.

Hamal K.R., Burgess S.C., Pevzner I.Y., Erf G.F. (2006). Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. Poultry Sci., 85: 1364– 1372.

Hybrid Turkeys (2020). Commercial Management Guide and Nutrient Guidelines for Hybrid Turkeys. Accessed July 2021, http://www.hybridturkeys.com

Jamroz D., Wertelecki T., Wiliczkiewicz A., Orda J., Skorupinska J. (2004). Dynamics of yolk sac reabsorption and posthatching development of the gastrointestinal tract in chickens, ducks, and geese. J. Anim. Physiol. Anim. Nutr., 88: 239–250.

Kaspers B., Schranner I., Lösch U. (1991). Distribution of immunoglobulins during embryogenesis in the chicken. Zbl. Vet. Med. A., 38: 73–79.

Kennedy K.E., Teng C., Patek T.M., Frei C.R. (2020). Hypoglycemia associated with antibiotics alone and in combination with sulfonylureas and meglitinides: an epidemiologic surveillance study of the FDA Adverse Event Reporting System (FAERS). Drug Safety, 43: 363–369.

Khalifeh M.S., Amawi M.M., Abu-Basha E.A., Yonis I.B. (2009). Assessment of humoral and cellular-mediated Immune response in chickens treated with tilmicosin, florfenicol, or enrofloxacin at the time of Newcastle disease vaccination. Poultry Sci., 88: 2118–2124.

Khan K.A., Khan S.A., Aslam A., Rabbanil M., Tipu M.Y. (2004). Factors contributing to yolk retention in poultry. A review. Pakistan Vet. J., 24: 46–51.

Kovacs-Nolan J., Mine Y. (2004). Avian egg antibodies: basic and potential applications. Avian Poult. Biol. Rev., 15: 25–46.

Kowalczyk J., Śmiałek M., Tykałowski B., Dziewulska D., Stenzel T., Koncicki A. (2019). Field evaluation of maternal antibody transfer from breeder turkey hens to egg yolks, egg whites, and poults. Poultry Sci., 98: 3150–3157.

Li X., Nakano T., Sunwoo H.H., Paek B.H., Chae H.S., Sim J.S. (1998). Effects of egg and yolk weights on yolk antibody IgY. production in laying chickens. Poultry Sci., 77: 266–270.

Linden C.D., Roth T.F. (1978). IgG receptors on foetal chick yolk sac. J. Cell Sci., 33: 317– 328.

Lourens A., van den Brand H., Heetkamp M.J.W., Meijerhof R., Kemp B. (2007). Effects of eggshell temperature and oxygen concentration on embryo growth and metabolism during incubation. Poultry Sci., 86: 2194–2199.

Marshall J.S., Warrington R., Watson W., Kim H.L. (2018). An introduction to immunology and immunopathology. Allergy Asthma Clin. Immunol., 14: 49.

Mikec M., Biđin Z., Valentić A., Savić V., Amšel Zelenika T., Raguž Đurić R., Lukaè Novak I., Baleńovic M. (2006). Influence of environmental and nutritional stressors on yolk sac utilization, development of chicken gastrointestinal system and its immune status. World Poult. Sci. J., 62: 31–40.

Munir K., Muneer M.A., Tiwari A., Chaudhry R.M., Muruganandan S. (2007). Effects of polyether ionophores on the protective immune responses of broiler chickens against Angara disease and Newcastle disease viruses. Vet. Res. Comm., 31: 909–929.

Murai A. (2013). Maternal transfer of immunoglobulins into egg yolks of birds. J. Poult. Sci., 50: 185–193.

Murai A., Hamano T., Kakiuchi M., Kobayashi M., Horio F. (2020). Evaluation of a receptor gene responsible for maternal blood IgY transfer into egg yolks using bursectomized IgY-depleted chickens. Poultry Sci., 99: 1914–1920.

Nagi S.A., Sahin N., Wagnar G., Williams J. (1984). Adverse effects of antibiotics on the development of gut associated lymphoid tissues and serum immunoglobulins in chickens. Am. J. Vet. Res., 45: 1425–1429.

Nile C.J., Townes C.L., Michailidis G., Hirst B.H., Hall J. (2004). Identification of chicken lysozyme *g2* and its expression in the intestine. Cell. Mol. Life Sci., 61: 2760–2766.

Panda A.K., Reddy M.R. (2007). Boosting the chicks immune system through early chick nutrition. Poult. Inter., 47: 22–26.

Panigrahy B., Grumbles L.C., Millar D., Naqi S.A., Hall C.F. (1979). Antibiotic-induced immunosuppression and levamisole-induced immunopotentiation in turkeys. Avian. Dis., 23: 401–408.

Ragland S.A., Criss A.K. (2017). From bacterial killing to immune modulation: Recent insights into the functions of lysozyme. PLoS Pathog., 13: e1006512.

Rose M.E., Orlans E. (1981). Immunoglobulins in the egg, embryo and young chick. Dev. Comp. Immunol., 5: 15–20.

Rzedzicki J., Cybulska R., Trawinska B., Jaworska-Adamu J. (1991). Effect of some antibacterial agents on the immune response and intestinal mucosa of chickens (in Polish). Zesz. Nauk. Akad. Rol. Wrocław, Weterynaria, 49: 179–189.

Shehata A.A., Attia Y., Khafaga A.F., Farooq M.Z., El-Seedi H.R., Eisenreich W., Tellez-Isaias G. (2022). Restoring healthy gut microbiome in poultry using alternative feed additives with particular attention to phytogenic substances: Challenges and prospects. Ger. J. Vet. Res., 2: 32–42.

Tokarzewski S. (2002). Influence of enrofloxacin and chloramphenicol on the level of IgY in serum and egg yolk after immunostimulation of hens with *Salmonella* Enteritidis antigens. Pol. J. Vet. Sci., 5: 151–158.

Tykałowski B., Stenzel T., Śmiałek M., Pestka D., Choszcz D., Koncicki A. (2013). Influence of the oral administration of oxytetracycline on selected parameters of cellular immunity in turkeys (in Polish). Med. Weter., 69: 432–435.

Ulmer-Franco A.M., Cherian G., Quezada N., Fasenko G.M., McMullen L.M. (2012). Hatching egg and newly hatched chick yolk sac total IgY content at 3 broiler breeder flock ages. Poultry Sci., 91: 758–764.

van der Wagt I., de Jong I.C., Mitchell M.A., Molenaar R., van den Brand H. (2020). A review on yolk sac utilization in poultry. Poultry Sci., 99: 2162–2175.

Ventola C.L. (2015). The antibiotic resistance crisis: part 1: causes and threats. Pharm. Therap., 40: 277.

Wallach M. (2010). Role of antibody in immunity and control of chicken coccidiosis. Trends Parasitol., 26: 382–387.

Received: 9 XI 2023
Accepted: 8 V 2024

ANNALS OF ANIMAL SCIENCE
ISSN: 2300-8733, https://sciendo.com/journal/AOAS

ACCEPTED AUTHOR VERSION OF THE MANUSCRIPT:

Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency?

DOI: 10.2478/aoas-2025-0125

Radosław Smagieł[1], Katarzyna Ognik[1♦], Ewelina Cholewińska[1], Przemysław Sołek[1], Dariusz Mikulski[2], Bartłomiej Tykałowski[3], Jan Jankowski[2], Krzysztof Tutaj[1♦]

[1]Department of Biochemistry and Toxicology, University of Life Sciences in Lublin, 20-950 Lublin, Poland
[2]Department of Poultry Science and Apiculture, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland
[3]Department of Poultry Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland

♦Corresponding author: katarzyna.ognik@up.edu.pl; krzysztof.tutaj@up.edu.pl

Received date: 13 May 2025
Accepted date: 29 October 2025

To cite this article: (2026). Smagieł R., Ognik K., Cholewińska E., Sołek P., Mikulski D., Tykałowski B., Jankowski J., Tutaj K. (2026). Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency?, Annals of Animal Science, DOI: 10.2478/aoas-2025-0125

This is unedited PDF of peer-reviewed and accepted manuscript. Copyediting, typesetting, and review of the manuscript may affect the content, so this provisional version can differ from the final version.

Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency?

Radosław Smagieł[1], Katarzyna Ognik[1♦], Ewelina Cholewińska[1], Przemysław Sołek[1], Dariusz Mikulski[2], Bartłomiej Tykałowski[3], Jan Jankowski[2], Krzysztof Tutaj[1♦]

[1]Department of Biochemistry and Toxicology, University of Life Sciences in Lublin, 20-950 Lublin, Poland

[2]Department of Poultry Science and Apiculture, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland

[3]Department of Poultry Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland

♦Corresponding author: katarzyna.ognik@up.edu.pl; krzysztof.tutaj@up.edu.pl

DOI: 10.2478/aoas-2025-0125

Abbreviated title: Early medication impacts poultry immune development?

*This work was supported by the National Science Centre in Poland, Grant No. 2020/39/B/NZ9/00765.

Abstract

The research aimed to determine whether the yolk sac resorption rate, the maternal antibody levels, as well as the efficiency of the immune system of growing turkeys may be affected by early administration of antibiotics and feeding a diet containing a coccidiostat. The experiment was conducted in a 3 × 2 factorial design, which included 3 groups of birds (CON, ENR, DOX), each of which was fed with or without monensin (+, -). The CON groups did not receive antibiotic supplementation (control groups), while those in the ENR groups received enrofloxacin at a dose of 10 mg/kg body weight (BW) for the first 5 days of life, and those in the DOX groups received doxycycline at a dose of 50 mg/kg BW for the first 5 days of life. The CON-, ENR-, DOX- groups did not receive monensin in their diet, while those in the CON+, ENR+, DOX+ groups received monensin at a dose of 90 mg/kg of feed. Adding monensin to the diet significantly decreased the feed conversion ratio (FCR) ($P < 0.01$) and body weight in 3-day-old and 7-day-old turkey hens ($P < 0.05$). Early enrofloxacin administration increased anti-ORT antibody titers in 1-day-old turkeys ($P < 0.05$), while monensin supplementation decreased several antibody titers such as anti-MPV, anti-NDV, and anti-ORT ($P < 0.05$). Immunoglobulin levels (IgY and IgM) and T cell percentages ($CD4^+$, $CD8^+$) were also significantly reduced in young turkeys fed monensin ($P < 0.01$ to $P < 0.05$). Monensin disturbs passive and specific immunity, especially in young turkeys. Early enrofloxacin or doxycycline administration to young turkeys may lead to undesirable changes in the developing immune system, including impaired humoral immunity in later life.

Enrofloxacin administration to turkeys in the first five days of life and feeding them a diet containing the coccidiostat monensin strongly stimulated the immune system, which in consequence caused changes in the immune system, indicating immunosuppression in the period distant from the enrofloxacin administration. The early administration of the antibiotics enrofloxacin or doxycycline with a diet without coccidiostat or containing the coccidiostat monensin did not affect the turkeys' growth performance.

Key words: turkeys, immune system, maternal antibody, yolk, antibiotics

The first days after hatching are a critical period in a poults life, marked by intensive physiological changes, including immune system maturation and the development of functions essential for protection against pathogens later in life. In this early crucial development period, chicks mainly use passive immunity, which is acquired in the form of antibodies (mainly IgY) transferred to them by the mother via the yolk sac (Noy & Sklan, 1999; Ognik et al., 2025). Therefore, effective yolk sac resorption within the first days after hatching is necessary for optimal immune system programming (Prabakar et al., 2016). Effective resorption of the yolk sac within the first 3–5 days after hatching is crucial for the proper transfer of maternal antibodies and the maturation of immune organs, ensuring optimal development of the chick's immune system. Delays in this process lead to impaired immune stimulation and increased mortality risk during the first week of life (Wang et al., 2020).

Over the last few decades, there has been a significant intensification of poultry production. As a result of selective breeding, various parameters like body weight gain, feed consumption, and slaughter efficiency of poultry have been significantly improved. However, the unfavourable effect of these practices has been the simultaneous weakening of immunity, which may result in higher susceptibility to infections and increased bird mortality (Rubio, 2019). In Poland, prophylactic administration of antibiotics has not been used for many years, due to the ban introduced in 2006 by the European Union. It is only permissible to administer antibiotics to confirmed infections, and the pharmacological intervention must always be carried out in a strict therapeutic regime with appropriate withdrawal periods. Due to the increased susceptibility to infections in high-production poultry, it is sometimes necessary to implement therapeutic antibiotic therapy even in the first days of the poultry life. However, using coccidiostats such as monensin as a feed additive is permitted almost throughout the entire rearing period (Ognik et al., 2025). Antibiotics such as enrofloxacin or doxycycline are commonly used in clinical veterinary practice to treat poultry infections. However, our previous studies have shown that early administration of the enrofloxacin or doxycycline recommended dose to turkeys (for the first 5 days of life), similarly to feeding a diet with monensin throughout the rearing period, reduced the specific maternal IgY and IgM antibodies levels both in the yolk sac and blood, which may potentially limit passive immune protection inherited from the mother (Ognik et al., 2025). We have also shown that early administration of these antibiotics may inhibit the innate immune response in turkeys vaccinated against ND (Newcastle Disease) and TRT (Turkey Rhinotracheitis) and causes adverse changes in the morphology of

immunocompensatory organs. However, the most adverse effect on the morphology of these organs is due to a constant use of monensin as a dietary additive. Moreover, this active substance was significantly less effective in inhibiting the post-vaccination inflammatory response in turkeys than enrofloxacin or doxycycline. (Smagieł et al., 2023). Schokker et al. (2017) also noted that antibiotics given to chickens in the early days of their life may adversely modulate the profile of intestinal microbial colonization, which can disrupt systemic immune responses. The gut microbiota plays a fundamental role in shaping the immune competence of birds. The intestines of newborn poultry are rapidly colonized by a diverse microbiota, which is an integral part of the development and regulation of the gut-associated lymphoid tissue (GALT) and the entire immune system of birds (Zenner et al., 2021; Rodrigues et al., 2021). Therefore, early administration of antimicrobial agents may disrupt the natural succession of the gut microbiome leading to dysbiosis and potentially reduce the stimulation necessary for the immune cells and tissues maturation, which may consequently translate into a permanent reduction in its efficiency (Broom & Kogut, 2018, Rodrigues et al., 2021). Therefore, it is worth emphasizing that early exposure to antimicrobials (antibiotics, coccidiostats) may impair both humoral and cellular immune responses, which may have long-term effects on the poultry's health and productivity.

In breeding practices, it sometimes happens that apart from adding the commonly used coccidiostat monensin to the diet, it is necessary to simultaneously administer antibiotics (enrofloxacin or doxycycline) for therapeutic purposes. However, there is no data on the additive effect of these active substances on the yolk sac absorption, the maternal antibodies transfer, and the turkey's immune system general functioning, especially when administered to them at a key phase of the immune system development and maturation. Therefore, the present experiment was designed to enable verification of the research hypothesis, assuming that early administration of antibiotics, especially during feeding a diet containing a coccidiostat, may limit the yolk sac absorption rate and reduce the antibody levels, and consequently worsen the functioning of the immune system of growing turkeys. The research aimed to determine whether the yolk sac resorption rate, the maternal antibody levels, as well as the efficiency of the immune system of growing turkeys may be affected by early administration of antibiotics and feeding a diet containing a coccidiostat.

Material and methods

Experimental animals and housing conditions

The experiment was conducted on young Hybrid Converter Novo turkey hens purchased as one-day-old poults (total 1152 birds) from the commercial hatchery Grelavi in Kętrzyn. The hatching day was considered zero, and the following day was the first day of life. All birds were vaccinated with live, attenuated vaccines against APV (Avian metapnumoviruses, Poulvac TRT vaccine; Zoetis) and NDV (Newcastle Disease Virus, Nobilis ND clone 30 vaccine; MSD Animal Health) on the first day of life using the macromolecular spray method, and against ORT (Ornithobacterium rhinotracheale) on the 28th day of life using the inactivated vaccine Ornithine Phibro, Poland) administered by a subcutaneous injection. The birds were reared until the 12th week of life (from 1st to 84th day). Turkey rearing was carried out in 48 pens (replicates) in the Department of Poultry Science and Apiculture in the Olsztyn experimental building. Pens

with an area of 4 m^2 each were lined with wood shavings. The density in the initial rearing phase was 6 pcs/m^2. The environmental conditions were controlled automatically as adjusted to the age of the birds and following the recommendations of Hybrid Turkeys (2020), were identical for all turkeys. In three rearing phases (weeks 1-4, 5-8, and 9-12), the birds were fed with complete mixtures following the nutritional requirements for utility turkeys at an adequate stage of rearing (Hybrid Turkeys, 2020). The component composition and nutritional value of the feed mixtures in the individual feeding periods are presented in Table 1. The mixtures were fed in crumbles (1-28 days) and 3 mm granules. Throughout the experiment, all birds had unlimited access to water and feed, which was available *ad libitum*.

The 1152 one-day-old turkey hens were allocated to 6 experimental groups: CON-, ENR-, DOX-, CON+, ENR+, DOX+ (see Table 2). The experiment was conducted in a 3 × 2 factorial design, which included 3 groups of birds (CON, ENR, DOX), each fed feed with or without the monensin addition (+, -). Turkey hens from the CON groups did not receive the antibiotic supplement (control groups), while those from the ENR groups received enrofloxacin (Enrofloxacin 10%, Biowet, Drwalew, Poland) at a dose of 10 mg/kg BW for the first 5 days of life, and those from the DOX groups received doxycycline (Doxylin CT WSP 433 mg/g, Dopharma Research B.V., Raamsdonksveer, Netherlands) at a dose of 50 mg/kg BW for the first 5 days of life. The antibiotics enrofloxacin and doxycycline were added to drinking water. Turkey hens from the CON-, ENR-, DOX- groups did not receive monensin in their diet, while those from the CON+, ENR+, DOX+ groups received monensin (Coxidin 200, Huvepharma Polska, Warsaw, Poland) at a dose of 90 mg/kg of feed. The groups within the experiment were formed by 8 pens, each with 24 birds, being a replicate within the group. The replicates (pens) were assigned to the groups in a way that ensured their even (homogeneous) distribution in the building.

Assessment of yolk sac resorption and maternal antibody transfer

During the experiment, the body weight of turkeys, feed consumption, and mortality were assessed in groups, at times determined by the experimental factors used. On the 1st, 3rd, and 5th day of life, yolk sacs and blood were collected from 8 individuals from each group to assess yolk sac resorption and transfer of maternal antibodies. Blood from poults was collected during decapitation into tubes without anticoagulant to obtain serum. Blood samples were incubated at room temperature (approximately 22°C) for 1–2 hours in an upright position to allow for complete clotting and then centrifuged in a tabletop centrifuge for 10 minutes at 3000 × g at 4°C. The resulting serum was separated and stored at –80°C until analysis. Simultaneously, after opening the abdominal cavity, yolk sacs were aseptically collected, placed in sterile tubes, and stored at –80°C until further analysis. Yolk sac resorption in turkey poults was evaluated according to the procedure described by Chamblee et al. (1992). At predetermined time points after hatching, poults were individually weighed and then euthanized. The residual yolk sac was carefully removed and blotted to remove excess fluid, after which its weight was recorded. Yolk sac resorption was expressed as relative yolk sac weight, calculated as the ratio of yolk sac weight to body weight × 100 %. This method allows for quantitative assessment of yolk sac utilization during the early post-hatch period. To assess the transfer of maternal antibodies from yolk to poult, the general level of IgY (cat. no. MBS760369; sensitivity 0.938 ng/mL; CV<10%) and IgM antibodies (cat. no. MBS2020626;

sensitivity 19.27 pg/mL; CV<12%) was determined in the yolk sac and blood serum using diagnostic MyBioSource ELISA Kits – (MyBioSource, Inc., San Diego, CA, USA), and the level of specific maternal IgY antibodies to avian metapneumovirus (anti-aMPV), Newcastle disease virus (anti-NDV), and ornithobacterium rhinotracheale (anti-ORT) were measured using Idexx kits (IDEXX ORT Ab Test; cat. no. 06-43600-06), (IDEXX APV Ab Test; cat. no. 06-44300-06), and (IDEXX NDV Ab Test; cat. No. 06-01096-16) using ELISA (IDEXX Laboratories, Inc. Westbrook, ME, USA). All phases of the assay were performed automatically using the Eppendorf EpMotion 5075LH pipetting station (Eppendorf SE, Hamburg, Germany) and the BioTek ELx405 washer (Agilent Technologies Inc., Santa Clara, CA, USA). Absorbance reading was performed using the BioTek ELx800 plate reader (Agilent Technologies Inc., Santa Clara, CA, USA), and data analysis and calculations were performed in the IDEXX xCheck software environment.

Assessment of the immune system response, including non-specific cellular immunity and specific humoral and adaptive immunity

Blood was collected from 8 individuals from each group at 7 and 56 days of life post-hatch via venipuncture of the wing vein. Blood for immunological studies was collected both for clotting and for the anticoagulant heparin or EDTA. Next, birds were euthanized, and immunocompetent organs were also collected (spleen and bursa of Fabricius to determine their weight). Biopsies taken from these organs were subjected to assessment of the immune system response, including nonspecific cellular immunity and specific humoral and adaptive immunity.

Mononuclear cells isolated from blood and spleen were counted and viability determined using a Vi-Cell XR counter (Beckman Coulter, Brea, CA, USA), and then one million of them were labeled using Mouse Anti-Chicken monoclonal antibodies (AbD Serotec) (cat. no. MCA2164F, Bio-Rad, Kidlington, UKand Goat Anti-Chicken polyclonal antibodies (AbD Serotec) (cat. no. MCA2166PE, Bio-Rad, Kidlington, UK) specific for CD4 and CD8a T cell receptors and sIgM B cell receptors to determine their percentage among lymphocytes. Immunophenotypic analysis of cells was performed using a BD FACSAria II flow cytometer (Becton Dickinson (BD), Franklin Lakes, NJ, USA).

The levels of Toll-Like receptor 4 (TLR-4; cat. no. MBS743084; sensitivity 0.1 ng/mL; CV<12%), interleukin 6 (IL-6; cat. no. MBS2021018; sensitivity 5.5 pg/mL; CV<12%), interleukin 2 (IL-2; cat. no. MBS2022032; sensitivity 6.1 pg/mL; CV<12%), interleukin 4 (IL-4; cat. no. MBS2020672; sensitivity 6.3 pg/mL; CV<12%), interleukin beta (IL-1β; cat. no. MBS778170; sensitivity 1.0 pg/mL; CV<15%), interleukin 8 (IL-8; cat. no. MBS700701; sensitivity 0.78 pg/mL; CV<10%), interleukin 12 (IL-12; cat. no. MBS2031963; sensitivity 6.1 pg/mL; CV<12%), interleukin 13 (IL-13; cat. no. MBS451617; sensitivity 5.3 pg/mL; CV<12%), tumor necrosis factor alpha (TNFα; cat. no. MBS2031870; sensitivity 3.1 pg/mL; CV<12%), C-reactive protein (CRP; cat. no. MBS2024111; sensitivity 0.134 ng/mL; CV<12%), interferon gamma (IFN-γ; cat. no. MBS2020832; sensitivity 5.5 pg/mL; CV<12%), nuclear transcription factor (NF-κB; cat. no. MBS2020695; sensitivity 0.055 ng/mL; CV<12%) and total immunoglobulin Y (IgY; cat. no. MBS760369; sensitivity 0.938 ng/mL; CV<10%), immunoglobulin M (IgM; cat. no. MBS2020626; sensitivity 19.27 pg/mL; CV<12%) and immunoglobulin A (IgA; cat. no. MBS2023753; sensitivity 32 pg/mL; CV<12%) were determined in the blood plasma of turkeys by immunoenzymatic methods, using ELISA

diagnostic kits from MyBioSource (MyBioSource, Inc.,San Diego, CA, USA). These kits are adapted from chicken assays.

Assessment of Immune-Related Gene Expression

Blood was collected from 8 individuals from each group at 7 and 56 days of life post-hatch via venipuncture of the wing vein. Blood for gene expression studies was collected for the anticoagulant EDTA.

To analyze the expression of *IgY*, *IFN-γ*, and *IL-6* genes, total RNA was isolated from 200 µL blood using the Syngen Blood/Cell RNA Mini Kit (cat. no. SY301012; Syngen Biotech Sp. z o.o., Wrocław, Poland) according to the manufacturer's instructions. The RNA samples were quantified spectrophotometrically (Nabi UV-VIS spectrophotometer, MicroDigital Co. Ltd., Gyeonggi, Republic of Korea), and an equal amount of 2 µg was reverse-transcribed into cDNA using the NG dART RT Kit (cat. no. E0801-02; EURx Sp. z o.o., Gdańsk, Poland). Quantitative gene expression analysis was performed using Real-Time PCR with SG qPCR Master Mix (2x) (cat no. E0401-02; EURX Ltd., Gdańsk, Poland) and gene-specific primers (Table 2) using a QuantStudio™ 7 Pro Real-Time PCR System (Thermo Fisher Scientific Inc., Waltham, MA, USA). The transcript levels of the target genes (*IgY*, *IFN-γ*, *IL-6*) were analyzed using the relative expression method, with three reference genes: *ACTB*, *GAPDH*, and *18S rRNA*. The PCR reaction was carried out with the following thermal profile: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, primer-specific annealing temperature for 30 seconds, and elongation at 72°C for 45 seconds, with a final extension at 72°C for 5 minutes. For each sample, the mean threshold cycle (CT) value was determined for both target and reference genes. The ΔCT value was calculated as the difference between the average CT of the target gene and the average CT of the reference genes, enabling normalization of gene expression levels and reduction of inter-sample variability.

Statistical analysis

Each pen was a replicate in the statistical analysis of the rearing results. The values of the remaining traits were determined individually in one bird from each replicate (eight birds from each group), with a body weight close to the group mean. The collected data were subjected to statistical analysis using STATISTICA software version 13 (StatSoft Inc., 2013) and two-way ANOVA, followed by Tukey's test. The data variability was expressed as standard deviation (σ) and standard error of the mean (SEM), and a P value < 0.05 was considered statistically significant.

Results

Growth performance parameters

The monensin effect

Adding monensin to the diet for turkeys decreased the FCR ($P < 0.01$, Table 4). Moreover, this treatment decreased BW ($P < 0.05$) in 3-day-old turkeys (Table 5). It was also found that feeding a diet with monensin addition contributed to obtaining a lower BW ($P < 0.05$) in 7-day-old turkey hens (Table 11).

Antibody titers

The antibiotic effect (enrofloxacin or doxycycline)

Early administration of enrofloxacin increased the titer of anti-ORT antibodies ($P < 0.05$) in the blood of 1-day-old turkey hens (Table 8).

The monensin effect

Adding monensin to the diet for turkeys decreased the anti-MPV titer ($P < 0.05$) in the blood of 5-day-old birds (Table 6). Adding monensin to the diet for turkeys also decreased anti-NDV titer in the yolk sac of 5-day-old turkeys ($P < 0.05$) and the blood of 56-day-old turkeys ($P < 0.05$; Table 7). In the groups of 5-day-old turkey hens receiving the addition of monensin in the diet, a lower anti-ORT titer ($P < 0.05$) in the blood was noted (Table 8).

Effect of simultaneous use of monensin in the diet and early administration of antibiotics - enrofloxacin or doxycycline

Early enrofloxacin administration in the group of turkey hens fed a diet with added monensin increased the anti-ORT titer ($P < 0.05$) in the yolk sac on the 5th day of life, which was not observed in the case of using monensin alone or its combination with doxycycline (Table 8).

Other immune parameters

The antibiotic effect (enrofloxacin or doxycycline)

Early administration of enrofloxacin decreased IgY level ($P < 0.01$) in the blood of 56-day-old turkey hens (Table 9). Early doxycycline administration initially (5^{th} day of life) decreased IgY level in the blood ($P < 0.05$), after which the level of this immunoglobulin increased on the 7th day of life ($P < 0.05$), while on the 56th day of life, IgY level was decreased again ($P < 0.01$) (Table 9). Early enrofloxacin or doxycycline administration decreased IgM levels in the yolk sacs of 3-day-old turkey hens ($P < 0.01$). In turn, early doxycycline administration initially (on the 3rd day of life of the turkey) decreased the IgM level in the blood ($P < 0.01$) after which, on the 7th day of life, there was an increase in this immunoglobulin level ($P < 0.01$; Table 10). Early doxycycline administration also increased IL-8 level ($P < 0.01$) in the blood of 7-day-old turkey hens (Table 14). Early enrofloxacin or doxycycline administration increased the IL-2 level ($P < 0.05$) in the blood of 56-day-old turkey hens (Table 15).

The monensin effect

The use of a diet with monensin addition decreased IgY level in yolk sacs ($P < 0.01$) and blood ($P < 0.05$) of 1-day-old turkeys (Table 9). In the blood of 3-day-old turkey hens receiving a diet with monensin addition, a decrease in IgM level ($P < 0.01$) was noted (Table 10). It was also found that feeding a diet with monensin addition contributed to obtaining a lower bursa of Fabricius total mass ($P < 0.05$) in 7-day-old turkey hens (Table 11). Moreover, adding monensin to the diet for turkeys decreased $CD4^+$ ($P < 0.05$) and $CD8^+$ ($P < 0.05$) cell percentages in the blood of 7-day-old turkey hens (Table 12). Turkey hens fed a diet with monensin addition had lower $CD4^+$ $CD8^+$ cells percentage ($P < 0.01$) in the spleen at the 7th

day of life, while they had higher IgM+ cells percentage ($P < 0.01$) in this organ at the 56th day of life (Table 13) compared to control group receiving a diet without monensin supplementation. In the groups of turkey hens receiving a diet with added monensin, a higher IL-8 level ($P < 0.01$) in the blood was found at the 56th day of life compared to those fed a diet without monensin addition (Table 15).

Effect of simultaneous use of monensin in the diet and early administration of antibiotics - enrofloxacin or doxycycline

An increase in the IgY level in the yolk sacs of 3-day-old turkey hens ($P < 0.05$) was observed only in the group fed a diet with added monensin, while in the groups fed a diet with added monensin, which received enrofloxacin or doxycycline in the first five days, such an effect was not observed (Table 9). Early doxycycline administration to turkeys fed a diet supplemented with monensin resulted in increased blood IgM levels ($P < 0.05$) at 1 day of life, which was not observed when monensin alone or monensin with enrofloxacin were used (Table 10). Early enrofloxacin administration to turkeys fed a diet supplemented with monensin decreased the $CD4^+$ ($P < 0.01$) and $CD8^+$ ($P < 0.05$) cell percentage in the blood of 56-day-old birds. Such an effect was not observed when doxycycline was administered in the early stage of life of turkeys fed a diet with monensin added (Table 12). An increase in the $CD4^+$ cells ($P < 0.01$) percentage was observed in the blood of 56-day-old turkeys fed a diet supplemented with monensin, (Table 12). Early enrofloxacin or doxycycline administration to turkeys fed a diet with monensin added increased the percentage of $CD8^+$ cells ($P < 0.01$) in the spleen of 56-day-old birds, which was not observed in the group fed a diet supplemented with monensin. Similarly, early doxycycline administration to turkeys fed a diet with monensin added increased the percentage of $CD4^+CD8^+$ cells ($P < 0.05$; Table 13). In the blood of 7-day-old turkeys, a decrease in the level of TLR-4 ($P < 0.01$), IFN-γ ($P < 0.01$), and NF-kβ ($P < 0.01$) was observed as a result of feeding a diet with monensin added, which was not observed in the case of this feeding combined with early administration of enrofloxacin or doxycycline. Early enrofloxacin administration to turkey hens fed a diet with monensin added reduced the IL-6 ($P < 0.05$), IL-12 ($P < 0.01$), and CRP ($P < 0.01$) levels in the blood of 7-day-old turkeys. In the case of early doxycycline administration and simultaneous feeding with a diet with monensin, an opposite effect was noted for the IL-12 ($P < 0.01$) and CRP ($P < 0.01$) level, and this treatment additionally increased the IL-2 ($P < 0.01$) and IL-1ß ($P < 0.05$) level (Table 14). Compared to the group of turkey hens fed a diet without monensin, the addition of this coccidiostat increased the TLR-4 ($P < 0.01$), IL-12 ($P < 0.01$), IL-13 ($P < 0.01$), IL-1ß ($P < 0.01$), IFN-γ ($P < 0.01$), NF-kβ ($P < 0.01$), and CRP ($P < 0.01$) level in the blood of 56-day-old turkey hens. Early enrofloxacin administration to turkey hens receiving monensin in the diet reduced the IgA level ($P < 0.05$) in the blood at the 56th day of life. In turn, early doxycycline administration to turkey hens receiving monensin in the diet decreased the IL-4 ($P < 0.05$), IL-12 ($P < 0.01$), and TNF-α ($P < 0.05$) level in the blood of 56-day-old birds (Table 15).

Immune–related gene expression

The antibiotic effect (enrofloxacin or doxycycline)

Early enrofloxacin or doxycycline administration decreased IgY gene expression level in the blood of both 7 ($P < 0.05$) and 56 ($P < 0.05$) day-old birds. In turn, early enrofloxacin administration increased the IL-6 gene expression level ($P < 0.01$) in the blood of 7-day-old turkey hens, while early doxycycline administration increased this gene expression level ($P < 0.01$) in the blood of 56 day-old birds. Early enrofloxacin administration increased the IFN-γ gene expression level ($P < 0.01$) in the blood of 7-day-old turkeys, but decreased it ($P < 0.05$) in the blood of 56-day-old birds (Table 16).

Discussion

The results of our previous studies have shown that enrofloxacin or doxycycline administration in the first five days of turkeys' life reduces the anti-MPV and anti-NDV titers and the maternal IgY and IgM levels, which may be due to the direct antibiotic effect on maternal antibodies present in the circulatory system of poults and inhibition of the specific post-vaccination antibodies synthesis (Ognik et al., 2025). In turn, the results of the current study did not confirm the effect of erofloxacin and doxycycline on changes in the anti-MPV and anti-NDV levels. The results of this study indicate that early administration of enrofloxacin to young turkey hens (in the first 5 days of life) had a modulating effect on the immune response development, with the observed effects being both time-limited and differentiated in terms of the type of response (innate vs. adaptive). Early enrofloxacin administration for the first 5 days of turkeys' life increased the anti-ORT titer in the blood of 1-day-old birds, while in the following days of life their level did not differ significantly between the groups. This indicates that the observed effect was short-term and did not result from an active humoral response. Under the influence of enrofloxacin, there was probably an increased absorption of maternal immunoglobulin present in the yolk sac of the poult, and not its production of this antibody. Khalifeh et al (2009) showed that enrofloxacin administered to laying hens reduced the post-vaccination humoral immune response against NDV and beneficial effect on the cellular immune response. At the same time, our present study have shown that this treatment also decreased the IgM level in the yolk sacs of 3-day-old turkey hens with no differences on days 1st and 5th, which may suggest that enrofloxacin causes a transient disturbance in the metabolism of immunoglobulins or the transport of IgM from the yolk sac to the blood or its earlier use. On the one hand, early enrofloxacin administration for the first 5 days of turkey hens' life caused early stimulation of the immune system, which was manifested as an increase in the expression of the IL-6 and IFN-γ genes in 7-day-old birds. The fact of stimulation of a non-specific immune reaction may be the body's response to the disruption of the intestinal microbiota caused by antibiotic therapy (Willing et al., 2011; Smagieł et al., 2023). This statement is confirmed by our previous studies, which showed that the microbiota of the cecum of turkeys is sensitive to the effects of antibiotics such as enrofloxacin or doxycycline to a greater extent than to the effects of monensin (Mikulski et al., 2022). The increased interleukin IL-2 level in the blood of 56-day-old turkeys noted in our study may indicate the Th1-type cellular immune response activation, and Jarosz et al. (2018) indicate such a mechanism. At the same time, as a result of early enrofloxacin administration, a significant reduction in the IgY gene expression was observed both immediately after the end of antibiotic therapy (7th day of life) and in the later period of rearing (56th day of life), which translated into a decrease in the

IgY level in the blood of 56-day-old turkeys. This indicates a long-term impairment of B lymphocyte maturation or their secretory function, despite the current activity of cytokines stimulating the cellular response. The observed effect of immunosuppression under the influence of early enrofloxacin administration occurs with a delay, which may indicate an impact on the immune system development and affect the immunity of birds later in life, and not necessarily only a temporary activation of the immune system (Smagieł et al., 2023). Similarly, Jankowski et al. (2022) observed a decrease in IgY level in the blood serum of chickens receiving enrofloxacin in early life. According to Sureshkumar et al. (2013), enrofloxacin, although effective against various bacteria, may have an immunosuppressive effect because it causes a decrease in the lymphocyte level in the bursa of Fabricius and spleen, although these effects may be reversible.

Early doxycycline administration significantly affected the development of the immune response in turkey hens. A transient decrease in IgM levels was observed in the yolk sacs and blood of 3-day-old turkey hens, with no differences between the 1st and 5th day of life. Similar to the effect of enrofloxacin, doxycycline may also temporarily interfere with the transport or metabolism of immunoglobulins, especially since the IgM level in 7-day-old turkey hens increased after the antibiotic was discontinued. Additionally, an increase in the IgY and IL-8 levels in the blood immediately after the end of antibiotic therapy (7th day of life) suggests the activation of a non-specific immune response during this period. Despite the transient activation of the immune system shortly after the end of doxycycline administration, a long-term decrease in the IgY level in the blood was observed on the 5th and 56th day of life and in the expression of its gene on the 7th but also the 56th day of life, which may indicate that doxycycline, like enrofloxacin, may inhibit the maturation of B lymphocytes or their secretory activity (Jankowski et al., 2022). The increase in the IL-6 gene expression and the IL-2 level in the blood of 56-day-old turkeys indicates a possible secondary immune system activation, which does not necessarily mean infection, but may be only a manifestation of adaptation. Initially, antibiotics change the microflora, and in the following weeks, the microbiota may be reprogrammed, which activates the immune system newly (Simon et al., 2016). Importantly, doxycycline did not affect the level of anti-ORT, anti-NDV, or anti-aMPV antibodies at any of the time points studied, which suggests that the specific response was not significantly impaired. The results of the conducted studies imply that despite the temporary activation of the immune system, doxycycline may negatively affect the development of the humoral component of immunity.

Monensin, an ionophore antibiotic used as a coccidiostat in poultry production, affects the immune system through various mechanisms. It modulates the immune system development after hatching, affecting immune cell populations and cytokine expression (Lee et al., 2012). However, its effect on inflammatory responses may be less pronounced than other antibiotics and may cause histological changes in immunocompetent organs, especially in combination with vaccination (Smagieł et al., 2023). Our studies show that monensin added to the diet of turkey hens significantly affected their immune development, especially in the first weeks of life. A decrease in the IgY and IgM levels in yolk sacs and blood, as well as a decrease in the titers of specific antibodies against MPV, NDV, and ORT, indicates a disturbance of both passive and adaptive immunity. The reduction of the bursa of Fabricius mass and a decrease in the number of T lymphocytes ($CD4^+$, $CD8^+$, $CD4^+CD8^+$) on 7-day-old birds additionally

confirm the potential inhibition of the maturation of the immune system, especially in the cellular response associated with the function of T lymphocytes. A decrease in the subpopulation of $CD4^+$ cells in the intestinal endothelial lymphocytes of broiler chickens receiving monensin as a coccidiostat in the diet was noted by Lee et al. (2012). Although our studies show that in the later period of life (day 56) there was an increase in the IgM+ cells percentage in the spleen and an increase in the IL-8 level in the blood, which may indicate the activation of the non-specific response, these changes do not compensate for the earlier deficits in immunity caused by the administration of monensin in the diet of young turkeys. It appears that monensin may have an immunosuppressive effect, especially during the critical period of primary immunity development (Simon et al., 2016; Wisselink et al., 2017).

Some reports indicate that feeding birds with a feed supplemented with monensin and simultaneously using other antimicrobials modulates the immunity development, changing lymphocyte populations and cytokine expression (Lee et al., 2012). Our studies show that the enrofloxacin administration in the first days of turkeys life and simultaneous feeding with a diet with monensin added, which is also an antibiotic, despite the short-term stimulation of specific passive immunity (increased anti-ORT antibodies titer in the yolk sac of 5-day-old turkey hens) and a visible anti-inflammatory effect (reduction of IL-6, IL-12 and CRP levels immediately after antibiotic therapy). The decrease in the number of $CD4^+$ and $CD8^+$ T lymphocytes in peripheral blood, with a simultaneous increase in the $CD4^+CD8^+$ cells percentage in the spleen, observed in the study, may indicate the development of immunosuppression in turkeys under the influence of the simultaneous administration of enrofloxacin and monensin. Both substances are known for their antibacterial activity, but their effect on the functioning of the immune system is complex and may lead to immunomodulatory or immunosuppressive effects. Monensin, as an ionophore, disturbs the intracellular ionic balance, which may lead to disturbances in signal transduction, as well as apoptosis of activated T lymphocytes. Enrofloxacin, a fluoroquinolone, has not only antibacterial but also immunomodulatory effects, affecting, among others, the production of cytokines and the functioning of mitochondria (Lee et al., 2018). An increase in the number of $CD4^+CD8^+$ lymphocytes in the spleen may suggest an arrest of the T cell maturation process or their local accumulation as a result of altered microenvironmental conditions, e.g., by impaired expression of cytokines or chemokine receptors. $CD4^+CD8^+$ cells are typically considered a transitional population in the thymus, but their presence in peripheral organs, such as the spleen, is sometimes noted in pathological situations, immune activation, or T cell maturation disorders (Lee et al., 2018). From a functional point of view, the described changes may lead to a weakening of the immune competence of the organism. A decrease in functional effector cells ($CD4^+$ and $CD8^+$) in the peripheral circulation may result in a limited ability to eliminate pathogens and respond to new antigens. Therefore, the concomitant enrofloxacin and monensin use in turkeys should be considered a potential immunosuppressive factor.

Administration of doxycycline in early life to turkeys fed a diet with monensin added leads to strong activation of the early non-specific response (increased IgM level already on the 1st day of antibiotic and coccidiostat administration) and increased IL-12, IL-2, IL-1β, and CRP levels immediately after the end of antibiotic therapy with doxycycline. IL-1β is a classic mediator of the acute phase of inflammation. IL-12 is a strong activator of NK and Th1 cells. IL-2 stimulates T lymphocyte proliferation, and CRP is a marker of the acute phase and

systemic inflammatory response (Smagieł et al., 2023). Increased levels of these indicators usually indicate an inflammation developing, which should not usually occur immediately after the end of antibiotic therapy. However, such an effect suggests activation of the inflammatory response, which may indicate a side effect of treatment or excessive stimulation of the immune system. Administration of antibiotics in the absence of infection can cause inflammation by inducing dysbiosis, activating the innate system by DAMPs (Damage-Associated Molecular Pattern) and other metabolites, and modifying the function of immune regulatory cells (Roh et al., 2018; Taitz et al., 2025). The immune homeostasis of the organism is largely dependent on the microbiome and activated signaling pathways regulating the immune system response. It is worth noting that this strong effect of immune system stimulation is the result of the interaction of two active substances: an antibiotic and a coccidiostat. It was particularly noticeable through the increased $CD8^+$ and $CD4^+CD8^+$ cells percentages in the spleen of 56-day-old turkeys, which indicates the stimulation of a cytotoxic immune response. The reduction in the levels of pro- and anti-inflammatory cytokines IL-4 and IL-12 and TNF-α observed in our study in the blood of 56-day-old turkeys, i.e., after a longer time after doxycycline administration, is most likely due to the body's natural tendency to return to a state of immunological rest (Tykałowski et al., 2025).

Madubuike et al. (2020) noticed that early treatment with doxycycline reduced body weight gain and FCR, but did not affect the immune response after vaccination against NDV. In turn, our studies show that early administration of both enrofloxacin and doxycycline did not worsen the growth performance of turkeys. Similarly, Sureshkumar et al. (2013) did not observe any deterioration in the production performance of broiler chickens given enrofloxacin. However, our studies show that feeding a diet with the addition of the coccidiostat monensin resulted in a deterioration in growth in the early rearing period (between 3 and 7 days of life). Despite a reduced feed conversion ratio throughout the rearing period, turkeys fed the feed with the addition of monensin ultimately achieved a body weight similar to the other experimental groups. Our previous studies, in which turkeys were given enrofloxacin or doxycycline or fed feed with added monensin, did not show the effect of these treatments on the growth results of the birds compared to the control group, which was not subjected to any treatments. However, it was found that birds receiving monensin in the diet gained better than those that received doxycycline for five days in the early rearing period (Mikulski et al., 2022; Smagieł et al., 2023). The combined administration of antibiotics (enrofloxacin or doxycycline) with a diet containing the coccidiostat monensin used in the presented studies did not worsen the growth performance of the birds.

Conclusions

Monensin, a coccidiostat approved for use as a feed additive, disturbs passive and specific immunity, especially in young turkeys, as it may negatively affect the maturation of T lymphocyte populations and the development of immunocompetent organs. Early enrofloxacin or doxycycline administration to young turkeys may lead to undesirable changes in the developing immune system, including impaired humoral immunity in later life. This may reduce the effectiveness of the humoral post-vaccination response in the longer term of the birds' lives, especially in vaccination programs against pathogens such as ORT, NDV, and MPV. Enrofloxacin administration to turkeys in the first five days of life and feeding them a

diet containing the coccidiostat monensin strongly stimulated the immune system, which in consequence caused changes in the immune system, indicating immunosuppression in the period distant from the enrofloxacin administration. The immunosuppressive effect was not observed in turkeys that received only monensin or a monensin and doxycycline combination. The early administration of the antibiotics enrofloxacin or doxycycline with a diet without coccidiostat or containing the coccidiostat monensin did not affect the turkeys' growth performance.

References

Broom L.J., Kogut M.H. (2018). The role of the gut microbiome in shaping the immune system of chickens. Vet. Immunol. Immunopathol., 204: 44–51.

Chamblee T.N., Thompson J.R., Thaxton J.P. (1992). Effect of day old vaccination on broiler performance. Poult. Sci., 71 (Suppl 1): 144.

Hybrid Turkeys. 2020. Commercial Management Guide and Nutrient Guidelines for Hybrid Turkeys. Accessed July 2021. http://www.hybridturkeys.com.

Jankowski J., Tykałowski B., Stępniowska A., Konieczka P., Koncicki A., Matusevičius P., Ognik K. (2022). Immune parameters in chickens treated with antibiotics and probiotics during early life. Animals, 12: 1133.

Jarosz Ł.S., Marek A., Grądzki Z., Kwiecień M., Kaczmarek B. (2018). The effect of feed supplementation with a copper-glycine chelate and copper sulphate on selected humoral and cell-mediated immune parameters, plasma superoxide dismutase activity, ceruloplasmin and cytokine concentration in broiler chickens. J. Anim. Physiol. Anim. Nutr., 102: e326-e336.

Khalifeh M.S., Amawi M.M., Abu-Basha E.A., Yonis I.B. (2009). Assessment of humoral and cellular-mediated immune response in chickens treated with tilmicosin, florfenicol, or enrofloxacin at the time of Newcastle disease vaccination. Poult. Sci., 88: 2118–2124.

Lee I.K., Gu M.J., Ko K.H., Bae S., Kim G., Jin G.D., Kim E.B., Kong Y.Y., Park T.S., Park B.C., Jung H.J., Han S.H., Yun C.H. (2018). Regulation of $CD4^{+}CD8^{-}CD25^{+}$ and $CD4^{+}CD8^{+}CD25^{+}$ T cells by gut microbiota in chicken. Sci. Rep., 8: 8627.

Lee K.W., Lillehoj H.S., Lee S.H., Jang S.I., Park M.S., Bautista D.A., Ritter G.D., Hong Y.H., Siragusa G.R., Lillehoj E.P. (2012). Effect of dietary antimicrobials on immune status in broiler chickens. Asian-Austr. J. Anim. Sci., 25: 382–392.

Madubuike K.G., Okoroafor O.N., Asuzu I.U. (2020). Effects of early-life antibiotics administration on the immune response to newcastle disease lasota vaccination and weight indices of broiler chicken. Folia Vet., 64: 74–79.

Mikulski D., Juśkiewicz J., Ognik K., Zduńczyk P., Smagieł R., Jankowski J. (2022). Gastrointestinal tract and neuroendocrine system responses of young turkeys to the early administration of antibiotics or feeding a diet containing a coccidiostat. *Poult.* Sci., 101: 102098.

Noy Y., Sklan D. (1999). Different types of early feeding and performance in chicks and poults. J. Appl. Poult. Res., 8: 16–24.

Ognik K., Tykałowski B., Mikulski D., Smagieł R., Cholewińska E., Koncicki A., Stępniowska A., Jankowski J. (2025). Early administration of antibiotics to turkey poults impairs

maternal immunity and post-vaccination antibody synthesis. Ann. Anim. Sci., 25: 239–257.

Prabakar G., Pavulraj S., Shanmuganathan S., Kirubakaran A., Mohana N. (2016). Early nutrition and its importance in poultry: A review. Indian J. Anim. Nutr., 33: 245.

Rodrigues D.R., Wilson K.M., Bielke L.R. (2021). Proper immune response depends on early exposure to gut microbiota in broiler chicks. Front. Physiol., 12: 758183.

Roh J.S., Sohn D.H. (2018). Damage-associated molecular patterns in inflammatory diseases. Immun. Net., 18: e27.

Rubio L.A. (2019). Possibilities of early life programming in broiler chickens via intestinal microbiota modulation. Poult. Sci., 98: 695–706.

Schokker D., Jansman A. J.M., Veninga G., De Bruin N., Vastenhouw S.A., De Bree F.M., Bossers A., Rebel J.M.J., Smits M.A. (2017). Perturbation of microbiota in one-day old broiler chickens with antibiotic for 24 hours negatively affects intestinal immune development. BMC Genom., 18: 241.

Simon K., Verwoolde M.B., Zhang J., Smidt H., de Vries Reilingh G., Kemp B., Lammers A. (2016). Long-term effects of early life microbiota disturbance on adaptive immunity in laying hens. Poult. Sci., 95: 1543–1554.

Smagieł R., Ognik K., Cholewińska E., Stępniowska A., Listos P., Tykałowski B., Mikulski D., Koncicki A., Jankowski J. (2023). The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys. Poul. Sci., 102: 102876.

Sureshkumar,V., Sarathchandra,G., Ramesh J. (2013). Effect of enrofloxacin on zootechnical performance, behaviour and immunohistopathological response in broiler chicken. Vet. World, 6: 337.

Taitz J.J., Tan J., Ni D., Potier-Villette C., Grau G., Nanan R., Macia L. (2025). Antibiotic-mediated dysbiosis leads to activation of inflammatory pathways. Front. Immunol., 15: 1493991.

Tykałowski B., Kowalczyk J., Ognik K., Mikulski D., Koncicki A., Kozłowski K., Jankowski J. (2025). Early post-hatching effects of antibiotics and ionophore coccidiostat supplementation on immune parameters in turkeys. Poult. Sci., 104: 105782.

Wang J.S., Hu H.J., Xu Y.B., Wang D.C., Jiang L., Li K.X., Wang Y.Y., Zhan X.A. (2020). Effects of posthatch feed deprivation on residual yolk absorption, macronutrients synthesis, and organ development in broiler chicks. Poult. Sci., 99: 5587–5597.

Willing B.P., Russell S.L., Finlay B.B. (2011). Shifting the balance: antibiotic effects on host-microbiota mutualism. Nature Rev. Microbiol. 9: 233–243.

Wisselink H.J., Cornelissen J.B.W.J., Mevius D.J., Smits M.A., Smidt H., Rebel J.M. (2017). Antibiotics in 16-day-old broilers temporarily affect microbial and immune parameters in the gut. Poult. Sci. 96: 3068–3078.

Zenner C., Hitch T.C.A., Riedel T., Wortmann E., Tiede S., Buhl E.M., Abt B., Neuhaus K., Velge P., Overmann J., Kaspers B., Clavel T. (2021). Early-life immune system maturation in chickens using a synthetic community of cultured gut bacteria. mSystems, 6: e01300-20.

Received: 13 V 2025
Accepted: 29 X 2025

Table 1. Ingredient composition and nutrient content of turkey diets (g/100 g, as-fed basis) (presented in Mikulski et al., 2022)

Item	Feeding period, weeks		
	1–4	5–8	9–12
Ingredients			
Wheat	37.00	45.13	46.18
Maize	10.00	10.00	15.00
Soybean meal, 46% CP	42.65	33.95	25.75
Rapeseed meal full fat 20,7% CP	4.00	5.00	6.00
Soybean oil	1.21	1.36	3.00
Sodium bicarbonate	0.15	0.15	0.15
Sodium chloride	0.20	0.20	0.20
Limestone	1.41	1.40	1.28
Monocalcium phosphate	1.87	1.54	1.15
L Lysine HCL	0.52	0.45	0.44
DL Methionine	0.32	0.21	0.21
L-Threonine	0.14	0.08	0.11
Ronozyme P	0.01	0.01	0.01
Ronozyme WX	0.02	0.02	0.02
Vitamin-mineral premix[1]	0.50	0.50	0.50
Calculated nutrient content			
Metabolizable energy, kcal/kg	2750	2850	3050
Crude protein	26.50	23.50	20.50
Lysine	1.75	1.50	1.30
Methionine	0.68	0.54	0.51
Met + Cys	1.12	0.95	0.88
Threonine	1.08	0.90	0.82
Calcium	1.20	1.10	0.95
Available phosphorus	0.58	0.50	0.40
Na	0.14	0.14	0.14
Analysed chemical composition			
DM	89.09	89.56	88.43
Crude protein	26.59	24.16	21.49
Crude fat	4.42	5.41	6.82

[1]Provided per kg diet (feeding periods: weeks 0–4, 5–8, 9–12): mg: retinol 3,78, 3,38 and 2,88, cholecalciferol 0,13, 0,12 and 0,10, α-tocopheryl acetate 100, 90 and 80, vit, K_3 5,8, 5,6 and 4,8, thiamine 5,4, 4,7 and 4,0, riboflavin 8,4, 7,5 and 6,4, pyridoxine 6,4, 5,6 and 4,8, cobalamin 0,032, 0,028 and 0,024, biotin 0,32, 0,28 and 0,24, pantothenic acid 28, 24 and 20, nicotinic acid 84, 75 and 64, folic acid

3,2, 2,8 and 2,4, Fe 64, 60, 56 and 48, 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.

Table 2. Experiment scheme

		Antibiotic		
		CON (without antibiotic)	ENR (enrofloxacin)	DOX (doxycycline)
Monensin	- (without)	CON -	ENR -	DOX -
	+ (present)	CON +	ENR +	DOX +

CON – group without monensin and receiving no antibiotics; CON + group with monensin and receiving no antibiotics; ENR – group without monensin and receiving enrofloxacin; ENR + group with monensin and receiving enrofloxacin; DOX – group without monensin and receiving doxycycline; DOX + group with monensin and receiving doxycycline.

Table 3. Primer sequences and their corresponding Tm values

Target	Sequence (5′→3′)	Tm (°C)
GAPDH	CCCTGAGCTCAATGGGAAGC	55.9
	TCAGCAGCAGCCTTCACTAC	53.8
ACTB	TACCCCATTGAACACGGCAT	51.8
	CTCCTCAGGGGCTACTCTCA	55.9
18S rRNA	CGAAAGCATTTGCCAAGAAT	47.7
	GGCATCGTTTATGGTCGG	50.3
IgY	GAATGGTTGGTGGACGGAGT	53.8
	GCATCCCTTGACGTGATCCT	53.8
IFN-gamma	CTGACAAGTCAAAGCCGCAC	53.8
	AGTCATTCATCTGAAGCTTGGC	53.0
IL-6	AAGGCGTGGATAGAGAAG	48.0
	TGACAGATCGGTAACAGAG	48.9

Table 4. Growth performance parameters of turkeys (from 1 to 85 days of life)

Item	n	BW 1d, g	BW 85d, kg	BWG, kg	DBWG, g	DFI, g	FCR, kg/kg	Mortality birds/%
Antibiotic[1]								
CON	16	60.8	8.045	7.984	90.2	187.7	2.081	0 / 0.0
ENR	16	60.9	8.174	8.113	91.5	192.8	2.109	11 /2.5
DOX	16	60.7	8.226	8.166	92.5	192.8	2.085	2 / 0.4
Monensin[2]								
-	24	60.9	8.129	8.068	91.0	192.4	2.115 [a]	8/ 1.2
+	24	60.8	8.168	8.107	91.8	189.8	2.069 [b]	5 / 0.7
Group								

CON -	8	60.8	8.015	7.955	89.8	189.5	2.110	0 / 0.0
CON +	8	60.8	8.074	8.013	90.5	185.9	2.053	0 / 0.0
ENR -	8	61.2	8.206	8.145	91.5	195.7	2.141	6 / 2.7
ENR +	8	60.7	8.143	8.082	91.4	189.8	2.077	5 / 2.2
DOX -	8	60.7	8.166	8.106	91.6	191.9	2.094	2 / 0.9
DOX +	8	60.8	8.287	8.226	93.3	193.6	2.077	0 / 0.0
SEM		0.139	0.040	0.040	0.492	1.060	0.007	
P value								
Antibiotic (A)		0.895	0.183	0.182	0.181	0.073	0.205	NA
Monensin (M)		0.670	0.637	0.636	0.440	0.204	0.001	NA
A x M interaction		0.624	0.648	0.649	0.778	0.286	0.309	NA

NA = not analyzed.

The tables present the original mean and the pooled standard error of the mean (SEM).

[a-b] Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.

BW-body weight, BWG-body weight gain, DBWG-daily body weight gain, DFI-daily feed intake, FCR-feed conversation ratio.

Table 5. Body weight (BW) of turkeys, total yolk sac weight (TYS), and relative weight of yolk sac (RYS) at 1, 3, and 5 days of life

		1day			3day			5day		
Item	n	BW, g	TYS, g	RYS, % of BW	BW, g	TYS, g	RYS, % of BW	BW, g	TYS, g	RYS, % of BW
Antibiotic[1]										
CON	48	71.02	2.67	3.75	91.15	0.97	1.07	117.8	0.26	0.23
ENR	48	67.53	2.74	4.05	91.24	0.89	0.98	117.8	0.25	0.22
DOX	48	69.40	2.79	4.06	92.79	0.81	0.88	114.4	0.21	0.18
Monensin[2]										
-	72	70.36	2.73	3.91	93.56[a]	0.86	0.93	116.1	0.24	0.21

+	72	68.28	2.73	4.00	89.90[b]	0.92	1.02	117.3	0.24	0.21
Group										
CON -	24	71.65	2.62	3.65	92.05	0.86	0.94	116.2	0.25	0.23
CON +	24	70.39	2.73	3.86	90.25	1.08	1.20	119.5	0.27	0.23
ENR -	24	69.87	2.98	4.27	94.89	0.96	1.02	118.0	0.24	0.21
ENR +	24	65.20	2.50	3.83	87.59	0.82	0.94	117.6	0.26	0.23
DOX -	24	69.56	2.61	3.81	93.75	0.76	0.83	114.1	0.23	0.20
DOX +	24	69.24	2.96	4.32	91.84	0.86	0.93	114.8	0.18	0.16
SEM		0.596	0.010	0.147	0.720	0.036	0.039	1.130	0.031	0.028
P value										
Antibiotic (A)		0.053	0.895	0.677	0.565	0.180	0.174	0.379	0.760	0.884
Monensin (M)		0.075	0.991	0.791	0.010	0.409	0.260	0.587	0.993	0.798
A x M interaction		0.279	0.224	0.336	0.194	0.096	0.276	0.789	0.853	0.844

Relative sac mass percentage values were subjected to the Bliss (arc sine) transformation for statistical comparisons. The tables present the original mean and the pooled standard error of the mean (SEM).

[a-b] Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.

Table 6. Anti-aMPV (TRT) antibody titers in the yolk sac (at 1, 3, and 5 days of life) and blood serum (at 1, 3, 5, 7 and 56 days of life) of turkeys

		Yolk sac			Blood serum				
Item	n	1d	3d	5d	1d	3d	5d	7d	56d
Antibiotic[1]									
CON	48	1101.3	179.8	132.22	3446.4	2024.1	2228.8	1006.2	239.5
ENR	48	1174.8	169.1	233.32	3624.5	1558.4	1954.2	1437.4	154.5
DOX	48	858.0	222.6	54.12	3345.9	1363.5	1810.9	1956.9	117.3
Monensin[2]									
-	72	1119.1	230.9	203.22	3247.3	1689.2	2431.2[a]	1615.5	207.5
+	72	970.3	150.1	76.56	3697.2	1608.2	1564.8[b]	1318.2	133.4
Group									
CON -	24	997.9	112.7	191.82	3051.9	1977.2	2415.3	1281.4	284.7
CON +	24	1204.6	246.9	72.61	3840.9	2071.0	2042.3	731.0	194.3
ENR -	24	1381.2	246.8	320.54	4219.5	1531.7	2446.8	1106.6	197.8
ENR +	24	968.4	91.44	146.09	3029.6	1585.1	1461.6	1768.3	111.2
DOX -	24	978.2	333.1	97.29	2470.7	1558.6	2431.3	2458.5	139.9
DOX +	24	737.8	112.0	10.96	4221.1	1168.4	1190.5	1455.3	94.63
SEM		121.9	37.46	37.02	299.8	159.7	192.6	196.2	39.82
P value									
Antibiotic (A)		0.546	0.826	0.140	0.929	0.229	0.664	0.139	0.409
Monensin (M)		0.546	0.282	0.087	0.455	0.801	0.025	0.445	0.333
A x M interaction		0.570	0.122	0.885	0.129	0.792	0.637	0.198	0.965

The tables present the original mean and the pooled standard error of the mean (SEM).

[a-b] Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.

Table 7. Anti-NDV antibody titers in the yolk sac (at 1, 3, and 5 days of life) and blood serum (at 1, 3, 5, 7 and 56 days of life) of turkeys

		Yolk sac			Blood serum				
Item	n	1d	3d	5d	1d	3d	5d	7d	56d
Antibiotic[1]									
CON	48	262.4	144.75	61.81	967.4	798.8	1221.5	462.9	283.3
ENR	48	537.5	66.66	46.46	1267.6	528.4	915.4	655.3	303.0
DOX	48	264.9	61.21	49.38	1123.8	526.6	1028.8	670.9	376.1
Monensin[2]									
-	72	274.2	105.92	84.15[a]	1064.8	666.2	1217.1	647.9	373.8[a]
+	72	435.6	75.83	20.95[b]	1174.4	569.6	893.3	544.9	267,8[b]
Group									
CON -	24	152.2	150.54	93.17	942.5	776.3	1287.0	456.6	335.3
CON +	24	372.6	138.97	30.45	992.3	821.2	1156.0	469.3	231.3
ENR -	24	410.5	96.17	63.63	1418.4	705.6	1001.8	770.4	335.8
ENR +	24	664.5	37.15	29.29	1116.7	351.2	829.0	540.3	270.3
DOX -	24	260.0	71.05	95.65	833.3	516.6	1362.6	716.8	450.4
DOX +	24	269.9	51.36	3.10	1414.3	536.5	695.0	625.1	301.7
SEM		64.17	17.99	14.65	100.8	69.63	108.1	69.44	27.01
P value									
Antibiotic (A)		0.134	0.108	0.902	0.479	0.188	0.507	0.403	0,335
Monensin (M)		0.208	0.404	0.032	0.587	0.488	0.137	0.463	0.049
A x M interaction		0.700	0.847	0.719	0.202	0.424	0.532	0.777	0.819

The tables present the original mean and the pooled standard error of the mean (SEM).

[a-b] Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.

Table 8. Anti-ORT antibody titers in the yolk sac (at 1, 3, and 5 days of life) and blood serum (at 1, 3, 5, 7 and 56 days of life) of turkeys

		Yolk sac			Blood serum				
Item	n	1d	3d	5d	1d	3d	5d	7d	56d
Antibiotic[1]									
CON	48	459.2	179.0	119.14	2991.8[b]	2120.4	3105.0	1455.7	17649.8
ENR	48	725.5	180.6	329.67	4597.6[a]	1640.2	2535.4	915.6	18348.9
DOX	48	407.2	166.0	181.38	3480.1[ab]	1878.7	2664.0	1191.5	17759.8
Monensin[2]									
-	72	491.9	163.1	178.72	3488.0	2081.7	3303.3[a]	1359.4	18334.7
+	72	569.4	187.3	241.41	3891.6	1677.8	2233.0[b]	1015.8	17504.4
Group									
CON -	24	500.6	201.4	164.60[b]	3464.4	2523.7	3284.7	1465.3	19110.2
CON +	24	417.8	156.5	73.67[b]	2519.2	1717.0	2925.4	1446.0	16189.4
ENR -	24	726.1	216.7	84.06[b]	4025.9	1935.0	3263.3	1047.6	17867.9
ENR +	24	724.9	144.5	575.28[a]	5169.2	1345.4	1807.6	783.7	18830.0
DOX -	24	248.9	71.18	287.49[ab]	2973.8	1786.5	3362.1	1565.2	18025.9
DOX +	24	565.4	260.8	75.27[b]	3986.4	1971.0	1965.9	817.9	17493.6
SEM		75.03	38.95	55.15	262.1	158.7	233.0	108.8	540.0
P value									
Antibiotic (A)		0.182	0.986	0.266	0.035	0.469	0.573	0.126	0.853
Monensin (M)		0.606	0.759	0.563	0.433	0.205	0.022	0.112	0.446

A x M interaction		0.519	0.330	0.020	0.181	0.410	0.553	0.374	0.342

The tables present the original mean and the pooled standard error of the mean (SEM).

[a-b] Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.

Table 9. Total IgY antibody levels in the yolk sac (at 1, 3, and 5 days of life) and blood serum (at 1, 3, 5, 7 and 56 days of life) of turkeys

		Yolk sac (mg/total yolk sac)			Blood serum (mg/mL)				
Item	n	1d	3d	5d	1d	3d	5d	7d	56d
Antibiotic[1]									
CON	48	21.16	10.079	2.953	3.282	5.621	7.594[a]	5.133[b]	5.718[a]
ENR	48	27.23	7.169	2.851	3.606	5.450	6.913[ab]	4.760[b]	3.622[b]
DOX	48	32.02	8.548	2.147	3.865	5.958	6.524[b]	6.174[a]	2.842[b]
Monensin[2]									
-	72	20.67[b]	7.156[b]	2.527	3.190[b]	5.499	7.114	5.213	3,794
+	72	32.93[a]	10.042[a]	2.775	3.979[a]	5.854	6.907	5.498	4,328
Group									
CON -	24	16.88	6.735[b]	3.365	2.839[b]	5.760	8.075	5.185	5.259
CON +	24	25.43	13.424[a]	2.542	3.726	5.482	7.113	5.082	6.178
ENR -	24	23.41	7.046[b]	2.044	3.265	5.474	6.438	4.167	3.095
ENR +	24	31.04	7.292[b]	3.659	3.947	5.427	7.389	5.353	4.149
DOX -	24	21.73	7.688[b]	2.171	3.465	5.262	6.828	6.287	3.028
DOX +	24	42.30	9.409[ab]	2.123	4.264	6.653	6.219	6.060	2.657
SEM		2.355	0.556	0.313	0.160	0.170	0.175	0.187	0.297
P value									

Antibiotic (A)	0.157	0.085	0.522	0.322	0.459	0.038	0.005	0.000
Monensin (M)	0.009	0.007	0.693	0.014	0.294	0.546	0.432	0.345
A x M interaction	0.441	0.037	0.272	0.965	0.095	0.055	0.214	0.524

The tables present the original mean and the pooled standard error of the mean (SEM).

[a-b] Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.

Table 10. Total IgM antibody levels in the yolk sac (at 1, 3, and 5 days of life) and blood serum (at 1, 3, 5, 7 and 56 days of life) of turkeys

		Yolk sac (µg/total yolk sac)			Blood serum (µg/mL)				
Item	n	1d	3d	5d	1d	3d	5d	7d	56d
Antibiotic[1]									
CON	48	264.1	141.07[a]	22.18	48.64	42.50[a]	72.27	61.32[b]	49.45
ENR	48	243.0	90.21[b]	24.84	33.97	40.44[a]	61.32	61.57[b]	52.89
DOX	48	239.7	97.93[b]	15.09	48.69	31.53[b]	64.46	88.28[a]	49.40
Monensin[2]									
-	72	242.8	100.30	19.46	35.62[b]	44.44[a]	70.15	67.77	51.98
+	72	255.1	119.18	21.94	51.91[a]	31.87[b]	61.88	73.00	49.19
Group									
CON -	24	225.5	121.68	26.79	38.59[ab]	47.47	75.14	59.37	51.35
CON +	24	302.7	160.46	17.58	58.70[ab]	37.52	69.41	63.27	47.56
ENR -	24	266.3	93.15	17.94	36.62[b]	46.51	60.14	64.49	50.97
ENR +	24	219.6	87.27	31.73	31.33[b]	34.37	62.51	58.64	54.81
DOX -	24	236.4	86.06	13.67	31.66[b]	39.33	75.18	79.46	53.62
DOX +	24	243.1	109.80	16.51	65.72[a]	23.73	53.73	97.10	45.19

SEM	12.16	6.322	2.897	3.067	1.590	3.665	3.932	1.726
P value								
Antibiotic (A)	0.674	0.001	0.368	0.062	0.006	0.457	0.005	0.643
Monensin (M)	0.612	0.121	0.670	0.006	0.000	0.262	0.495	0.422
A x M interaction	0.117	0.311	0.273	0.022	0.731	0.406	0.454	0.348

The tables present the original mean and the pooled standard error of the mean (SEM).

[a-b] Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.

Table 11. Effects of treatments on immunocompetent organ weights (spleen and Bursa Fabricii) at day 7 and 56 days of turkeys' life

		7 day					56 day				
Item	n	Body weight, g	spleen		Bursa Fabricii		Body weight, g	spleen		Bursa Fabricii	
			Total weight, g	Relative weight, % of BW	Total weight, g	relative weight, % of BW		Total weight, g	Relative weight, % of BW	Total weight, g	elative weight, % of BW
Antibiotic[1]											
CON	16	158.7[ab]	0.113	0.071	0.274	0.17	4320	3.854	0.089	4.458	0.103
ENR	16	168.3[a]	0.124	0.074	0.307	0.18	4356	3.782	0.087	4.311	0.099
DOX	16	154.4[b]	0.112	0.073	0.256	0.16	4274	3.952	0.092	4.296	0.101
Monensin[2]											
-	24	165.1[a]	0.116	0.070	0.300[a]	0.18	4316	3.634	0.084	4.496	0.104
+	24	155.9[b]	0.117	0.075	0.258[b]	0.17	4317	4.091	0.095	4.215	0.097
Group											
CON -	8	162.9	0.106	0.065	0.284	0.17	4286	3.561	0.084	4.410	0.103
CON +	8	154.5	0.120	0.077	0.265	0.17	4354	4.146	0.095	4.507	0.104
ENR -	8	175.2	0.128	0.073	0.344	0.20	4396	3.576	0.081	4.496	0.102

ENR +	8	161.4	0.121	0.075	0.270	0.17	4316	3.989	0.093	4.126	0.095
DOX -	8	157.1	0.114	0.073	0.273	0.17	4266	3.766	0.088	4.581	0.108
DOX +	8	151.7	0.110	0.073	0.239	0.16	4281	4.137	0.096	4.011	0.093
SEM		2.209	0.006	0.003	0.010	0.005	33.21	0.121	0.003	0.109	0.002
P value											
Antibiotic (A)		0.022	0.989	0.989	0.079	0.429	0.622	0.852	0.645	0.804	0.696
Monensin (M)		0.028	0.555	0.555	0.026	0.112	0.990	0.070	0.071	0.211	0.177
A x M interaction		0.690	0.854	0.854	0.452	0.510	0.677	0.931	0.965	0.458	0.515

Relative spleen and bursa Fabricii mass percentage values were subjected to the Bliss (arc sine) transformation for statistical comparisons. The tables present the original mean and the pooled standard error of the mean (SEM).

[a-b] Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.

Table 12. Percentages of T-cell and B-cell subpopulations in the blood of turkeys

Item	n	7 day				56 days			
		$CD4^+$	$CD8^+$	$CD4^+CD8^+$	IgM^+	$CD4^+$	$CD8^+$	$CD4^+CD8^+$	IgM^+
Antibiotic[1]									
CON	16	20.08	1.673	0.533	5.234	23.54[a]	2.675	0.341	7.231
ENR	16	19.01	1.442	0.573	5.144	21.71[a]	2.205	0.319	6.077
DOX	16	21.42	1.801	0.604	5.588	15.49[b]	1.807	0.240	6.039
Monensin[2]									
-	24	23.07[a]	1.885[a]	0.594	5.304	21.13	2.396	0.308	6.789
+	24	17.27[b]	1.393[b]	0.546	5.339	19.36	2.062	0.292	6.108
Group									
CON -	8	26.51	2.103	0.514	5.041	18.80[b]	2.353[ab]	0.290	7.091
CON +	8	13.65	1.243	0.553	5.426	28.28[a]	2.998[a]	0.391	7.370
ENR -	8	21.35	1.493	0.660	5.041	29.34[a]	2.886[a]	0.381	7.198
ENR +	8	16.66	1.391	0.485	5.246	14.08[b]	1.524[b]	0.256	4.956
DOX -	8	21.35	2.059	0.609	5.830	15.26[b]	1.950[ab]	0.253	6.079
DOX +	8	21.49	1.544	0.600	5.345	15.73[b]	1.664[b]	0.228	5.999
SEM		1.378	0.106	0.020	0.099	1.229	0.161	0.029	0.292
P value									
Antibiotic (A)		0.756	0.339	0.325	0.148	0.001	0.064	0.354	0.155
Monensin (M)		0.033	0.018	0.215	0.856	0.318	0.260	0.787	0.231
A x M interaction		0.137	0.309	0.069	0.159	0.000	0.027	0.303	0.151

The tables present the original mean and the pooled standard error of the mean (SEM).

[a-b] Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.

Table 13. Percentages of T-cell and B-cell subpopulations in the spleen of turkeys

Item		7 day				56 days			
	n	$CD4^+$	$CD8^+$	$CD4^+CD8^+$	IgM^+	$CD4^+$	$CD8^+$	$CD4^+CD8^+$	IgM^+
Antibiotic[1]									
CON	16	43.56	13.95	1.577	8.074	36.66	19.30^b	1.917	14.27
ENR	16	46.59	14.20	1.739	8.648	33.69	23.96^a	2.053	15.25
DOX	16	45.93	14.40	1.740	8.626	34.20	24.04^a	2.221	14.60
Monensin[2]									
-	24	46.03	14.97	1.836^a	8.293	35.50	20.75^b	2.021	12.61^b
+	24	44.68	13.39	1.534^b	8.606	34.20	24.12^a	2.106	16.80^a
Group									
CON -	8	45.71	13.76	1.735	7.394	35.00	21.14^{ab}	2.064^{ab}	12.84
CON +	8	41.41	14.14	1.419	8.754	38.31	17.46^b	1.770^b	15.71
ENR -	8	47.15	14.56	1.916	9.006	34.29	20.46^b	2.123^{ab}	13.18
ENR +	8	46.03	13.83	1.561	8.290	33.09	27.46^a	1.984^{ab}	17.31
DOX -	8	45.24	16.59	1.858	8.478	37.20	20.65^b	1.878^b	11.80
DOX +	8	46.61	12.22	1.623	8.775	31.20	27.44^a	2.565^a	17.39
SEM		0.963	0.674	0.050	0.269	0.827	0.806	0.075	0.623
P value									
Antibiotic (A)		0.421	0.964	0.253	0.626	0.275	0.004	0.208	0.774
Monensin (M)		0.493	0.255	0.002	0.568	0.419	0.010	0.541	0.001
A x M interaction		0.499	0.342	0.860	0.309	0.068	0.001	0.013	0.621

The tables present the original mean and the pooled standard error of the mean (SEM).

$^{a-b}$ Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.

Table 14. Immunological parameters in the blood plasma of turkeys at the 7th day of life

Item	n	IgA total, ng/mL	TLR-4, ng/mL	IL-2, ng/mL	IL-4, pg/mL	IL-6, ng/L	IL-8, pg/mL	IL-12, ng/mL	IL-13, pg/mL	IL-1ß, pg/mL	TNFα, pg/mL	IFN-γ, pg/mL	NF-kB, ng/mL	CRP, ng/ml
Antibiotic[1]														
CON	16	630.0	1.506^{a}	5.011	52.78^{ab}	34.46	7.578^{b}	14.77^{b}	20.41	66.56^{b}	382.1	113.19^{a}	2.694	10.680^{a}
ENR	16	593.5	0.847^{b}	4.960	47.53^{b}	36.82	8.234^{b}	18.66^{a}	18.99	73.64^{ab}	382.2	77.70^{b}	2.590	4.918^{b}
DOX	16	521.6	0.900^{b}	5.325	56.44^{a}	38.31	11.192^{a}	16.60^{ab}	19.35	87.36^{a}	371.7	120.59^{a}	2.438	9.130^{a}
Monensin[2]														
-	24	526.4	1.279^{a}	4.717	50.65	38.14	8.851	16.00	18.68	69.12	365.5	112.57^{a}	2.684	9.221^{a}
+	24	637.1	0.889^{b}	5.481	53.85	34.91	9.152	17.35	20.48	82.59	391.8	95.08^{b}	2.465	7.264^{b}
Group														
CON -	8	578.5	2.268^{a}	4.084^{b}	52.07	35.41^{ab}	7.639	13.96^{c}	19.79	66.38^{b}	365.4	142.28^{a}	3.346^{a}	12.520^{a}
CON +	8	681.6	0.744^{b}	5.938^{ab}	53.48	33.50^{ab}	7.518	15.58^{bc}	21.03	66.74^{b}	398.8	84.11^{bc}	2.042^{b}	8.841^{abc}
ENR -	8	568.3	0.807^{b}	5.679^{ab}	49.36	42.61^{a}	7.256	21.19^{a}	18.25	70.67^{b}	383.8	72.01^{c}	2.726^{ab}	8.236^{bc}
ENR +	8	618.7	0.888^{b}	4.241^{b}	45.70	31.03^{b}	9.212	16.12^{bc}	19.73	76.61^{b}	380.5	83.39^{bc}	2.455^{ab}	1.599^{d}
DOX -	8	432.5	0.764^{b}	4.388^{b}	50.52	36.41^{ab}	11.659	12.85^{c}	18.00	70.30^{b}	347.2	123.42^{a}	1.979^{b}	6.906^{c}
DOX +	8	610.8	1.036^{b}	6.263^{a}	62.36	40.21^{ab}	10.725	20.36^{ab}	20.69	104.42^{a}	396.1	117.75^{ab}	2.897^{ab}	11.353^{ab}
SEM		28.42	0.099	0.232	1.496	1.250	0.436	0.628	0.661	2.935	9.300	5.002	0.118	0.632
P value														
Antibiotic (A)		0.277	0.000	0.735	0.037	0.411	0.001	0.006	0.674	0.003	0.873	0.000	0.580	0.000
Monensin (M)		0.052	0.004	0.070	0.249	0.177	0.695	0.152	0.189	0.006	0.170	0.018	0.280	0.016
A x M interaction		0.641	0.000	0.002	0.074	0.035	0.289	0.000	0.896	0.012	0.518	0.001	0.000	0.000

The tables present the original mean and the pooled standard error of the mean (SEM).

[a-d] Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.
IgA -Immunoglobulin A, TLR-4-Toll like receptor 4, IL-2-interleukin 2, IL-4- interleukin 4, IL-6- interleukin 6, IL-8-interleukin 8, IL-12- interleukin 12, IL-13- interleukin 13, IL-1ß- interleukin 1ß, TNFα-turmot necrosis factor, IFN-γ-interferon γ, NF-kB-nuclear factor kappa light chain, CRP- C reactive protein.

Table 15. Immunological parameters in the blood plasma of turkeys at the 56th day of life

Item	n	IgA total, ng/mL	TLR-4, ng/mL	IL-2, ng/mL	IL-4, pg/mL	IL-6, ng/L	IL-8, pg/mL	IL-12, ng/mL	Il-13, pg/mL	IL-1ß, pg/mL	TNFα, pg/mL	IFN-γ, pg/mL	NF-kB, ng/mL	CRP, ng/ml
Antibiotic[1]														
CON	16	654.3	1.179	6.726^{y}	82.45^{a}	47.35	9.694	25.93^{b}	17.14	103.9	223.6^{a}	46.36^{b}	2.806	0.821
ENR	16	673.7	1.144	8.464^{x}	83.64^{a}	45.20	10.583	35.55^{a}	19.99	128.5	171.8^{b}	52.89^{ab}	3.365	0.860
DOX	16	779.8	1.265	8.541^{x}	58.00^{b}	47.62	9.859	30.02^{ab}	19.09	123.7	174.7^{b}	57.66^{a}	2.849	0.850
Monensin[2]														
-	24	795.6^{a}	1.138	7.409	80.52^{a}	44.98	8.448^{b}	30.37	16.84^{b}	97.1^{b}	199.2	42.47^{b}	2.457^{b}	0.905
+	24	609.6^{b}	1.253	8.412	68.87^{b}	48.46	11.642^{a}	30.63	20.64^{a}	140.3^{a}	180.9	62.13^{a}	3.556^{a}	0.783
Group														
CON -	8	773.2^{abc}	0.766^{c}	5.868	79.69^{a}	48.64	7.870	12.64^{b}	11.85^{b}	58.3^{b}	210.4^{a}	21.64^{c}	1.725^{c}	0.615^{b}
CON +	8	535.4^{bc}	1.593^{a}	7.583	85.22^{a}	46.06	11.518	39.22^{a}	22.44^{a}	149.5^{a}	236.9^{a}	71.07^{a}	3.886^{a}	1.028^{a}
ENR -	8	843.9^{a}	1.303^{ab}	8.343	91.31^{a}	46.46	9.562	38.88^{a}	22.05^{a}	114.1^{a}	173.4^{ab}	46.36^{b}	3.335^{ab}	1.048^{a}
ENR +	8	503.4^{c}	0.984^{bc}	8.586	75.97^{a}	43.94	11.604	32.21^{a}	17.93^{ab}	142.9^{a}	170.3^{ab}	59.41^{ab}	3.394^{ab}	0.672^{ab}
DOX -	8	769.6^{abc}	1.346^{ab}	8.015	70.56^{ab}	39.83	7.913	39.58^{a}	16.60^{ab}	118.8^{a}	213.9^{a}	59.40^{ab}	2.309^{bc}	1.050^{a}
DOX +	8	790.0^{ab}	1.183^{abc}	9.067	45.43^{c}	55.40	11.805	20.45^{b}	21.57^{a}	128.7^{a}	135.6^{b}	55.91^{ab}	3.388^{ab}	0.649^{ab}
SEM		31.80	0.059	0.326	3.186	1.822	0.430	1.841	0.951	5.965	8.273	2.726	0.156	0.047
P value														
Antibiotic (A)		0.132	0.558	0.032	0.000	0.832	0.587	0.004	0.359	0.060	0.006	0.023	0.114	0.916

Monensin (M)	0.001	0.225	0.109	0.025	0.334	0.000	0.907	0.025	0.000	0.202	0.000	0.000	0.126
A x M interaction	0.025	0.000	0.620	0.048	0.068	0.549	0.000	0.003	0.001	0.012	0.000	0.003	0.000

The tables present the original mean and the pooled standard error of the mean (SEM).

[a-c] Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.

IgA -Immunoglobulin A, TLR-Toll like receptor, IL-2-interleukin 2, IL-4- interleukin 4, IL-6- interleukin 6, IL-8-interleukin 8, IL-12- interleukin 12, IL-13- interleukin 13, IL-1ß- interleukin 1ß, TNFα-turmot necrosis factor, IFN-γ-interferon γ, NF-kB-nuclear factor kappa light chain, CRP- C reactive protein.

Table 16. Expression of genes in the blood of 7-day-old and 56-day-old turkeys

		7 day			56 day		
Item	n	IgY	IL-6	IFN- γ	IgY	IL-6	IFN- γ
Antibiotic[1]							
CON	16	0.558[a]	0.330[b]	0.975[b]	0.586[a]	0.295[b]	0.989[a]
ENR	16	0.541[b]	0.349[a]	0.995[a]	0.575[b]	0.292[b]	0.975[b]
DOX	16	0.544[b]	0.342[ab]	0.985[ab]	0.577[b]	0.305[a]	0.983[ab]
Monensin[2]							
-	24	0.548	0.338	0.985	0.582	0.299	0.980
+	24	0.547	0.342	0.985	0.577	0.295	0.985
Group							
CON -	8	0.557	0.326	0.972	0.590	0.299	0.987
CON +	8	0.560	0.333	0.978	0.583	0.291	0.991
ENR -	8	0.542	0.347	0.997	0.576	0.296	0.973
ENR +	8	0.539	0.351	0.994	0.575	0.288	0.977
DOX -	8	0.546	0.341	0.988	0.581	0.303	0.980
DOX +	8	0.541	0.343	0.982	0.572	0.307	0.986

SEM	0.003	0.002	0.003	0.002	0.002	0.002
P value						
Antibiotic (A)	0.010	0.002	0.009	0.011	0.003	0.025
Monensin (M)	0.729	0.320	0.887	0.075	0.167	0.291
A x M interaction	0.787	0.897	0.640	0.491	0.174	0.963

The tables present the original mean and the pooled standard error of the mean (SEM).

[a-b] Means within the same column with different superscripts differ significantly ($P < 0.05$).

[1]Treatment: CON, untreated control; MON, treated with monensin at a dose rate of 90 mg/kg feed for 56 days; ENR, treated with enrofloxacin via drinking water at a dose rate of 10 mg/kg BW for the first 5 days of life; DOX, treated with doxycycline via drinking water at a dose rate of 50 mg/kg BW for the first 5 days of life. [2]Denotes without monensin - or with monensin +.

IgY- Immunoglobulin Y, IL-6- interleukin 6, IFN- γ – interferon.

Oświadczenia doktoranta oraz współautorów dotyczących ich wkładu w przygotowanie publikowanych prac naukowych

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

mgr inż. Radosław Smagieł
ul. Akademicka 13
Lublin, 20-950

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu Przyrodniczego w Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Katarzyna Ognik, Bartłomiej Tykałowski, Dariusz Mikulski, Radosław Smagieł, Ewelina Cholewińska, Andrzej Koncicki, Anna Stępniowska, Jan Jankowski, 2025, **Early administration of antibiotics to turkey poults impairs maternal immunity and post-vaccination antibody synthesis.** *Ann. Anim. Sci.* Volume 25 Issue 1 s. 239- 257.

Mój udział polegał na wykonaniu analiz, opracowaniu wyników i zatwierdzeniu ostatecznej wersji publikacji.

2) Radosław Smagieł, Krzysztof Tutaj, Ewelina Cholewińska, Przemysław Sołek, , Dariusz Mikulski, Anna Stępniowska, Jan Jankowski, Katarzyna Ognik, 2024, **The effect of early administration of antibiotics or feeding a diet containing coccidiostats on the level of their accumulation in liver and the redox status of turkeys.** *Animal* Vol. 18 Issue 10 Article number: 101321

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, wykonaniu analiz, opracowaniu wyników, napisaniu publikacji.

3) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Anna Stępniowska, Piotr Listos, Bartłomiej Tykałowski, Dariusz Mikulski, Andrzej Koncicki, Jan Jankowski, 2023, **The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys.** *Poult. Sci.* Vol. 102 Issue 9 Article number: 102876

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, wykonaniu analiz, opracowaniu wyników, napisaniu publikacji.

4) Dariusz Mikulski, Jerzy Juśkiewicz, Katarzyna Ognik, Przemysław Zduńczyk, Radosław Smagieł, Jan Jankowski, 2022, **Gastrointestinal tract and neuroendocrine system responses of young turkeys to the early administration of antibiotics or feeding a diet containing a coccidiostat.**. *Poult. Sci.* Vol. 101 Issue 11 Article number: 102098

Mój udział polegał na wykonaniu analiz, opracowaniu wyników i zatwierdzeniu ostatecznej wersji publikacji.

5) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Przemysław Sołek, Dariusz Mikulski, Bartłomiej Tykałowski, Jan Jankowski, Krzysztof Tutaj, 2026, **Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency?** *Ann. Anim. Sci.,* ahead of print

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, wykonaniu analiz, opracowaniu wyników, napisaniu publikacji.

Radosław Smagieł

Podpis

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

Dr Ewelina Cholewińska
ul. Akademicka 13
Lublin, 20-930

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu
Przyrodniczegow
Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Katarzyna Ognik, Bartłomiej Tykałowski, Dariusz Mikulski, Radosław Smagieł, Ewelina Cholewińska, Andrzej Koncicki, Anna Stępniowska, Jan Jankowski, 2025, **Early administration of antibiotics to turkey poults impairs maternal immunity and post-vaccination antibody synthesis.** *Ann. Anim. Sci.* Volume 25 Issue 1 s. 239- 257.

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

2) Radosław Smagieł, Krzysztof Tutaj, Ewelina Cholewińska, Przemysław Sołek, , Dariusz Mikulski, Anna Stępniowska, Jan Jankowski, ,Katarzyna Ognik, 2024, **The effect of early administration of antibiotics or feeding a diet containing coccidiostats on the level of their accumulation in liver and the redox status of turkeys.** *Animal* Vol. 18 Issue 10 Article number: 101321

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

3) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Anna Stępniowska, Piotr Listos, Bartłomiej Tykałowski, Dariusz Mikulski, Andrzej Koncicki, Jan Jankowski, 2023, **The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys.** *Poult. Sci.* Vol. 102 Issue 9 Article number: 102876

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

4) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Przemysław Sołek, Dariusz Mikulski, Bartłomiej Tykałowski, Jan Jankowski, Krzysztof Tutaj, 2026, **Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency?** *Ann. Anim. Sci.,* ahead of print

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

Ewelina Cholewińska

Podpis

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

Dr hab. Anna Stępniowska
ul. Akademicka 13
Lublin, 20-930

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu
Przyrodniczegow
Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Katarzyna Ognik, Bartłomiej Tykałowski, Dariusz Mikulski, Radosław Smagieł, Ewelina Cholewińska, Andrzej Koncicki, Anna Stępniowska, Jan Jankowski, 2025, **Early administration of antibiotics to turkey poults impairs maternal immunity and post-vaccination antibody synthesis.** *Ann. Anim. Sci.* Volume 25 Issue 1 s. 239- 257.

Mój udział polegał na pomocy w interpretacji wyników i zatwierdzeniu ostatecznej wersji publikacji.

2) Radosław Smagieł, Krzysztof Tutaj, Ewelina Cholewińska, Przemysław Sołek, , Dariusz Mikulski, Anna Stępniowska, Jan Jankowski, ,Katarzyna Ognik, 2024, **The effect of early administration of antibiotics or feeding a diet containing coccidiostats on the level of their accumulation in liver and the redox status of turkeys.** *Animal* Vol. 18 Issue 10 Article number: 101321

Mój udział polegał na pomocy w interpretacji wyników i zatwierdzeniu ostatecznej wersji publikacji.

3) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Anna Stępniowska, Piotr Listos, Bartłomiej Tykałowski, Dariusz Mikulski, Andrzej Koncicki, Jan Jankowski, 2023, **The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys.** *Poult. Sci.* Vol. 102 Issue 9 Article number: 102876

Mój udział polegał na pomocy w interpretacji wyników i zatwierdzeniu ostatecznej wersji publikacji.

Anna Stępniowska

Podpis

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

Prof. dr hab. Katarzyna Ognik
ul. Akademicka 13
Lublin, 20-930

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu
Przyrodniczego w
Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Katarzyna Ognik, Bartłomiej Tykałowski, Dariusz Mikulski, Radosław Smagieł, Ewelina Cholewińska, Andrzej Koncicki, Anna Stępniowska, Jan Jankowski, 2025, **Early administration of antibiotics to turkey poults impairs maternal immunity and post-vaccination antibody synthesis.** *Ann. Anim. Sci.* Volume 25 Issue 1 s. 239- 257.

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, pomocy w interpretacji wyników i zatwierdzeniu ostatecznej wersji publikacji.

2) Radosław Smagieł, Krzysztof Tutaj, Ewelina Cholewińska, Przemysław Sołek, , Dariusz Mikulski, Anna Stępniowska, Jan Jankowski, ,Katarzyna Ognik, 2024, **The effect of early administration of antibiotics or feeding a diet containing coccidiostats on the level of their accumulation in liver and the redox status of turkeys.** *Animal* Vol. 18 Issue 10 Article number: 101321

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, pomocy w interpretacji wyników i zatwierdzeniu ostatecznej wersji publikacji.

3) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Anna Stępniowska, Piotr Listos, Bartłomiej Tykałowski, Dariusz Mikulski, Andrzej Koncicki, Jan Jankowski, 2023, **The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys.** *Poult. Sci.* Vol. 102 Issue 9 Article number: 102876

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, pomocy w interpretacji wyników i zatwierdzeniu ostatecznej wersji publikacji.

4) Dariusz Mikulski, Jerzy Juśkiewicz, Katarzyna Ognik, Przemysław Zduńczyk, Radosław Smagieł, Jan Jankowski, 2022, **Gastrointestinal tract and neuroendocrine system responses of young turkeys to the early administration of antibiotics or feeding a diet containing a coccidiostat.**. *Poult. Sci.* Vol. 101 Issue 11 Article number: 102098

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, pomocy w interpretacji wyników i zatwierdzeniu ostatecznej wersji publikacji.

5) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Przemysław Sołek, Dariusz Mikulski, Bartłomiej Tykałowski, Jan Jankowski, Krzysztof Tutaj, 2026, **Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency?** *Ann. Anim. Sci.,* ahead of print

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, pomocy w interpretacji wyników i zatwierdzeniu ostatecznej wersji publikacji.

Katarzyna Ognik
.....Podpis

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

dr hab. n.wet., dr n. pr. Piotr Listos,
prof. uczelni
ul. Głeboka 30E
Lublin, 20-400

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu
Przyrodniczegow
Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Anna Stępniowska, Piotr Listos, Bartłomiej Tykałowski, Dariusz Mikulski, Andrzej Koncicki, Jan Jankowski, 2023, **The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys.** *Poult. Sci.* Vol. 102 Issue 9 Article number: 102876

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

Piotr Listos

Podpis

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

prof. dr hab. Dariusz Mikulski
ul. M. Oczapowskiego 5
Olsztyn, 10-719

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu
Przyrodniczegow
Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Katarzyna Ognik, Bartłomiej Tykałowski, Dariusz Mikulski, Radosław Smagieł, Ewelina Cholewińska, Andrzej Koncicki, Anna Stępniowska, Jan Jankowski, 2025, **Early administration of antibiotics to turkey poults impairs maternal immunity and post-vaccination antibody synthesis.** *Ann. Anim. Sci.* Volume 25 Issue 1 s. 239- 257.

   Mój udział polegał na prowadzeniu doświadczenia oraz ocenie wyników produkcyjnych.

2) Radosław Smagieł, Krzysztof Tutaj, Ewelina Cholewińska, Przemysław Sołek, , Dariusz Mikulski, Anna Stępniowska, Jan Jankowski, ,Katarzyna Ognik, 2024, **The effect of early administration of antibiotics or feeding a diet containing coccidiostats on the level of their accumulation in liver and the redox status of turkeys.** *Animal* Vol. 18 Issue 10 Article number: 101321

Mój udział polegał na prowadzeniu doświadczenia oraz ocenie wyników produkcyjnych.

3) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Anna Stępniowska, Piotr Listos, Bartłomiej Tykałowski, Dariusz Mikulski, Andrzej Koncicki, Jan Jankowski, 2023, **The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys.** *Poult. Sci.* Vol. 102 Issue 9 Article number: 102876

Mój udział polegał na prowadzeniu doświadczenia oraz ocenie wyników produkcyjnych.

4) Dariusz Mikulski, Jerzy Juśkiewicz, Katarzyna Ognik, Przemysław Zduńczyk, Radosław Smagieł, Jan Jankowski, 2022, **Gastrointestinal tract and neuroendocrine system responses of young turkeys to the early administration of antibiotics or feeding a diet containing a coccidiostat.**. *Poult. Sci.* Vol. 101 Issue 11 Article number: 102098

Mój udział polegał na prowadzeniu doświadczenia oraz ocenie wyników produkcyjnych.

5) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Przemysław Sołek, Dariusz Mikulski, Bartłomiej Tykałowski, Jan Jankowski, Krzysztof Tutaj, 2026, **Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency?** *Ann. Anim. Sci.,* ahead of print

Mój udział polegał na prowadzeniu doświadczenia oraz ocenie wyników produkcyjnych.

Mikulski Dariusz

Podpis

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

prof. dr hab. inż. Jerzy Juśkiewicz
ul. Trylińskiego 18
Olsztyn, 10-683

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu
Przyrodniczegow
Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Dariusz Mikulski, Jerzy Juśkiewicz, Katarzyna Ognik, Przemysław Zduńczyk, Radosław Smagieł, Jan Jankowski, 2022, **Gastrointestinal tract and neuroendocrine system responses of young turkeys to the early administration of antibiotics or feeding a diet containing a coccidiostat.**. *Poult. Sci.* Vol. 101 Issue 11 Article number: 102098

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

Juśkiewicz Jerzy
Podpis

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

dr inż. Przemysław Zduńczyk
ul. Trylińskiego 18
Olsztyn, 10-683

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu Przyrodniczegow Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Dariusz Mikulski, Jerzy Juśkiewicz, Katarzyna Ognik, Przemysław Zduńczyk, Radosław Smagieł, Jan Jankowski, 2022, **Gastrointestinal tract and neuroendocrine system responses of young turkeys to the early administration of antibiotics or feeding a diet containing a coccidiostat.**. *Poult. Sci.* Vol. 101 Issue 11 Article number: 102098

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

Podpis

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

dr hab. Bartłomiej Tykałowski prof, UWM
ul. M. Oczapowskiego 14
Olsztyn, 10-719

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu
Przyrodniczegow
Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Katarzyna Ognik, Bartłomiej Tykałowski, Dariusz Mikulski, Radosław Smagieł, Ewelina Cholewińska, Andrzej Koncicki, Anna Stępniowska, Jan Jankowski, 2025, **Early administration of antibiotics to turkey poults impairs maternal immunity and post-vaccination antibody synthesis.** *Ann. Anim. Sci.* Volume 25 Issue 1 s. 239- 257.

   Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

2) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Anna Stępniowska, Piotr Listos, Bartłomiej Tykałowski, Dariusz Mikulski, Andrzej Koncicki, Jan Jankowski, 2023, **The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys.** *Poult. Sci.* Vol. 102 Issue 9 Article number: 102876

   Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

3) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Przemysław Sołek, Dariusz Mikulski, Bartłomiej Tykałowski, Jan Jankowski, Krzysztof Tutaj, 2026, **Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency?** *Ann. Anim. Sci.*, ahead of print

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

KATEDRA CHORÓB PTAKÓW
UWM w Olsztynie
dr hab. Bartłomiej Tykałowski, prof. UWM
Podpis

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

prof. dr hab. Jan Jankowski
ul. M. Oczapowskiego 5
Olsztyn, 10-719

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu
Przyrodniczegow
Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Katarzyna Ognik, Bartłomiej Tykałowski, Dariusz Mikulski, Radosław Smagieł, Ewelina Cholewińska, Andrzej Koncicki, Anna Stępniowska, Jan Jankowski, 2025, **Early administration of antibiotics to turkey poults impairs maternal immunity and post-vaccination antibody synthesis.** *Ann. Anim. Sci.* Volume 25 Issue 1 s. 239- 257.

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, zatwierdzeniu ostatecznej wersji publikacji.

2) Radosław Smagieł, Krzysztof Tutaj, Ewelina Cholewińska, Przemysław Sołek, , Dariusz Mikulski, Anna Stępniowska, Jan Jankowski, ,Katarzyna Ognik, 2024, **The effect of early administration of antibiotics or feeding a diet containing coccidiostats on the level of their accumulation in liver and the redox status of turkeys.** *Animal* Vol. 18 Issue 10 Article number: 101321

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, zatwierdzeniu ostatecznej wersji publikacji.

3) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Anna Stępniowska, Piotr Listos, Bartłomiej Tykałowski, Dariusz Mikulski, Andrzej Koncicki, Jan Jankowski, 2023, **The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys.** *Poult. Sci.* Vol. 102 Issue 9 Article number: 102876

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, zatwierdzeniu ostatecznej wersji publikacji.

4) Dariusz Mikulski, Jerzy Juśkiewicz, Katarzyna Ognik, Przemysław Zduńczyk, Radosław Smagieł, Jan Jankowski, 2022, **Gastrointestinal tract and neuroendocrine system responses of young turkeys to the early administration of antibiotics or feeding a diet containing a coccidiostat.**. *Poult. Sci.* Vol. 101 Issue 11 Article number: 102098

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, zatwierdzeniu ostatecznej wersji publikacji.

5) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Przemysław Sołek, Dariusz Mikulski, Bartłomiej Tykałowski, Jan Jankowski, Krzysztof Tutaj, 2026**, Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency?** *Ann. Anim. Sci.,* ahead of print

Mój udział polegał na opracowaniu koncepcji badań, nadzorze doświadczenia, zatwierdzeniu ostatecznej wersji publikacji.

Jan Jankowski
Podpis

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

prof. dr hab. Andrzej Koncicki
ul. M. Oczapowskiego 14
Olsztyn, 10-719

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu
Przyrodniczegow
Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Katarzyna Ognik, Bartłomiej Tykałowski, Dariusz Mikulski, Radosław Smagieł, Ewelina Cholewińska, Andrzej Koncicki, Anna Stępniowska, Jan Jankowski, 2025, **Early administration of antibiotics to turkey poults impairs maternal immunity and post-vaccination antibody synthesis.** *Ann. Anim. Sci.* Volume 25 Issue 1 s. 239- 257.

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

2) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Anna Stępniowska, Piotr Listos, Bartłomiej Tykałowski, Dariusz Mikulski, Andrzej Koncicki, Jan Jankowski, 2023, **The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on inflammatory responses and the morphological structure of selected organs of the immune system in young meat-type turkeys.** *Poult. Sci.* Vol. 102 Issue 9 Article number: 102876

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

.....
Podpis

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

dr Przemysław Sołek
ul. Akademicka 13
Lublin, 20-930

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu
Przyrodniczegow
Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Radosław Smagieł, Krzysztof Tutaj, Ewelina Cholewińska, Przemysław Sołek, , Dariusz Mikulski, Anna Stępniowska, Jan Jankowski, ,Katarzyna Ognik, 2024, **The effect of early administration of antibiotics or feeding a diet containing coccidiostats on the level of their accumulation in liver and the redox status of turkeys.** *Animal* Vol. 18 Issue 10 Article number: 101321

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

2) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Przemysław Sołek, Dariusz Mikulski, Bartłomiej Tykałowski, Jan Jankowski, Krzysztof Tutaj, 2025, **Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency?** *Ann. Anim. Science,* ahead of print

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

Podpis

Załącznik nr 7 – Oświadczenie o współautorstwie

Lublin, 02.02.2026

Dr Krzysztof Tutaj
ul. Akademicka 13
Lublin, 20-930

Rada Dyscypliny Zootechnika i Rybactwo
Uniwersytetu Przyrodniczegow Lublinie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

1) Radosław Smagieł, Krzysztof Tutaj, Ewelina Cholewińska, Przemysław Sołek, , Dariusz Mikulski, Anna Stępniowska, Jan Jankowski, ,Katarzyna Ognik, 2024, **The effect of early administration of antibiotics or feeding a diet containing coccidiostats on the level of their accumulation in liver and the redox status of turkeys.** *Animal* Vol. 18 Issue 10 Article number: 101321

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

2) Radosław Smagieł, Katarzyna Ognik, Ewelina Cholewińska, Przemysław Sołek, Dariusz Mikulski, Bartłomiej Tykałowski, Jan Jankowski, Krzysztof Tutaj, 2026, **Does early antibiotic administration to turkeys receiving a coccidiostat in the diet affect the yolk sac absorption rate, the maternal antibody levels, and the immune system efficiency?** *Ann. Anim. Sci.,* ahead of print

Mój udział polegał na wykonaniu wybranych analiz i pomocy w interpretacji wyników.

.....Podpis