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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

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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

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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

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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

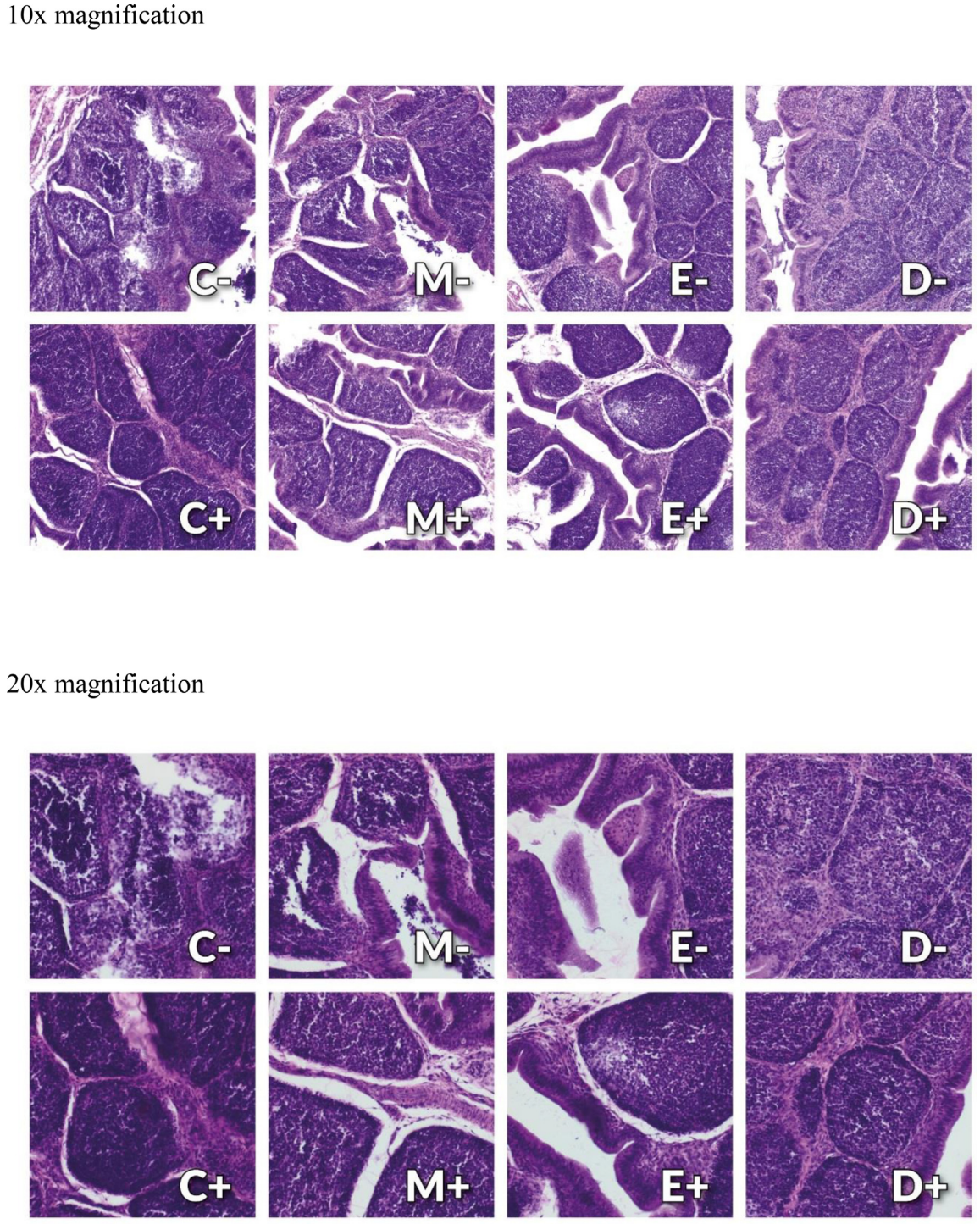

Figure 1. Morphological effects of early administration of antibiotics or feeding a diet containing a coccidiostat and vaccination on the bursa of Fabricius of 7-day-old turkeys. Treatment: (C−) unvaccinated control group; (M−) unvaccinated group treated with monensin; (E−) unvaccinated group treated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

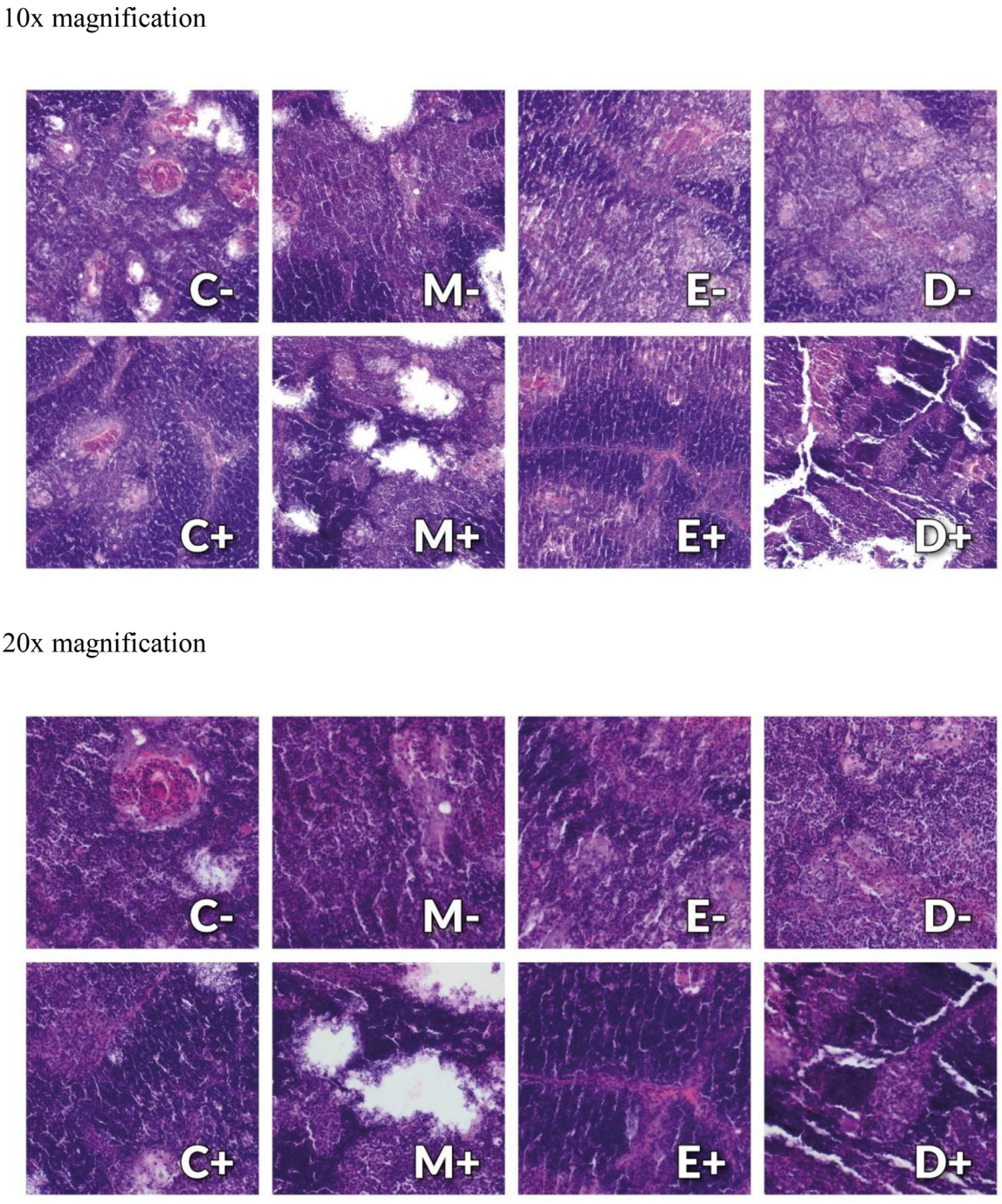

Figure 2. Morphological effects of early administration of antibiotics or feeding a diet containing a coccidiostat and vaccination on the thymus of 7-day-old turkeys. Treatment: (C−) unvaccinated control group; (M−) unvaccinated group treated with monensin; (E−) unvaccinated group treated with enrofloxacin;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

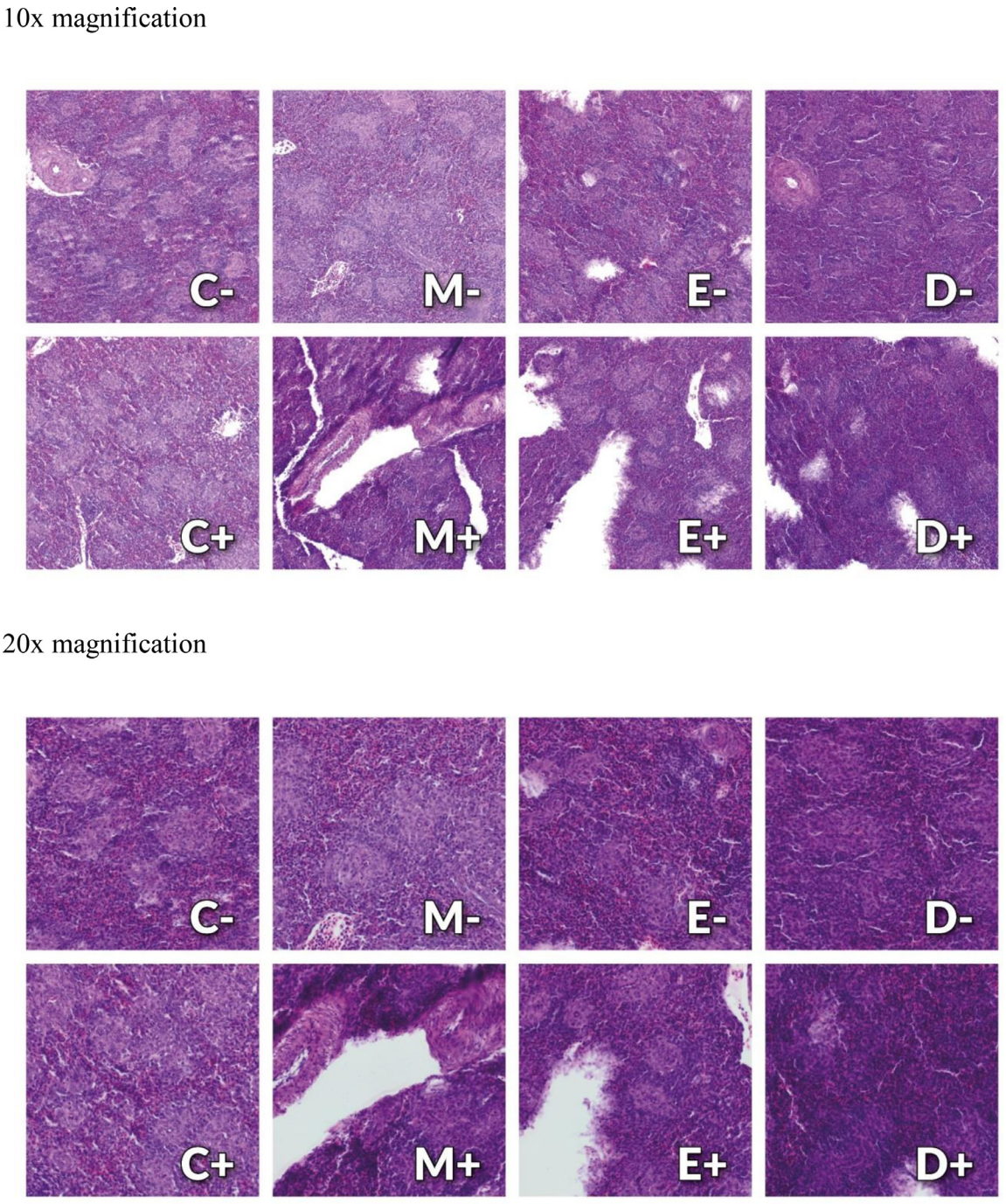

Figure 3. Morphological effects of early administration of antibiotics or feeding a diet containing a coccidiostat and vaccination on the spleen of 7-day-old turkeys. Treatment: (C−) unvaccinated control group; (M−) unvaccinated group treated with monensin; (E−) unvaccinated group treated with enrofloxacin;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

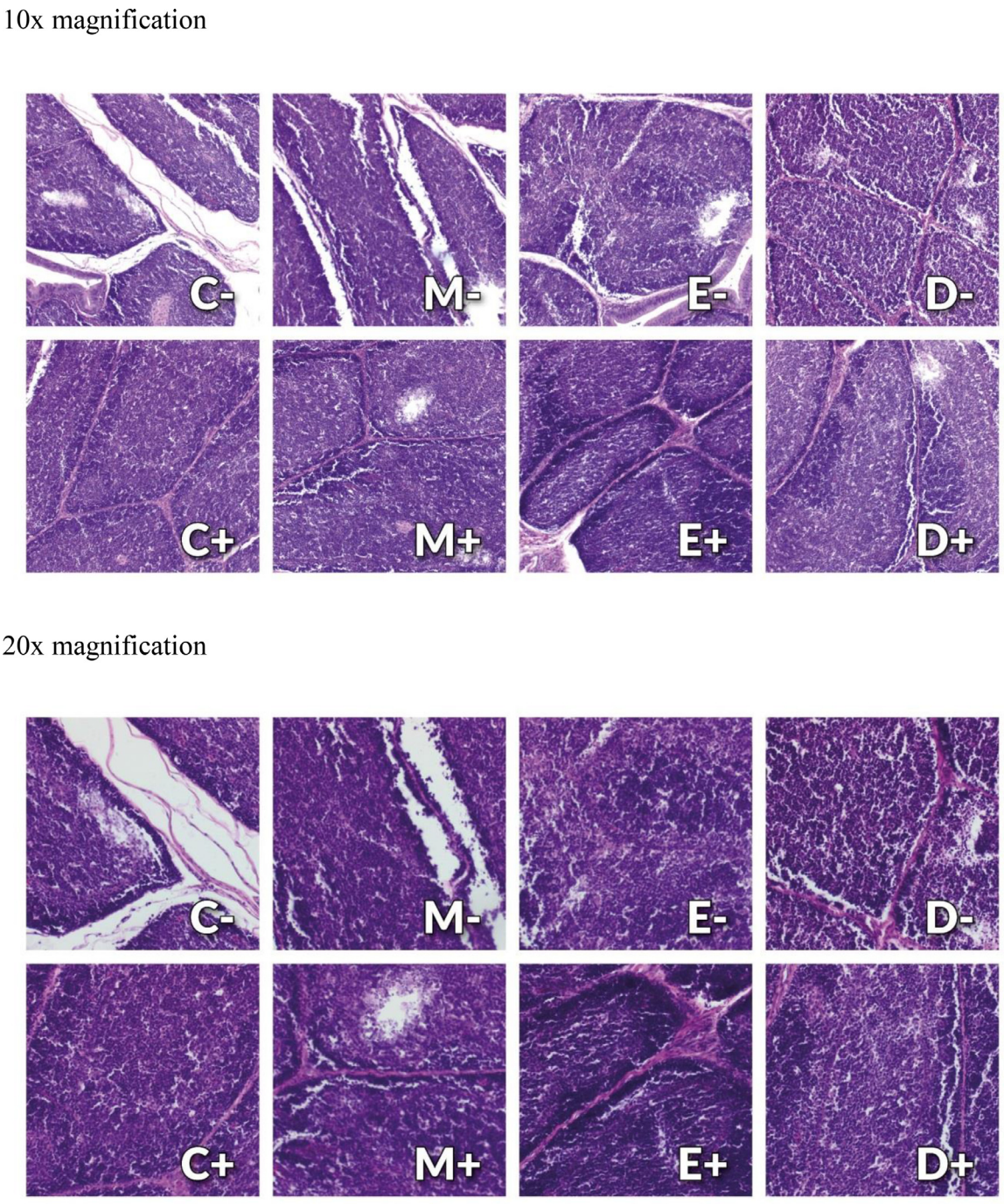

Figure 4. Morphological effects of early administration of antibiotics or feeding a diet containing a coccidiostat and vaccination on the bursa of Fabricius of 56-day-old turkeys. Treatment: (C−) unvaccinated control group; (M−) unvaccinated group treated with monensin; (E−) unvaccinated group treated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

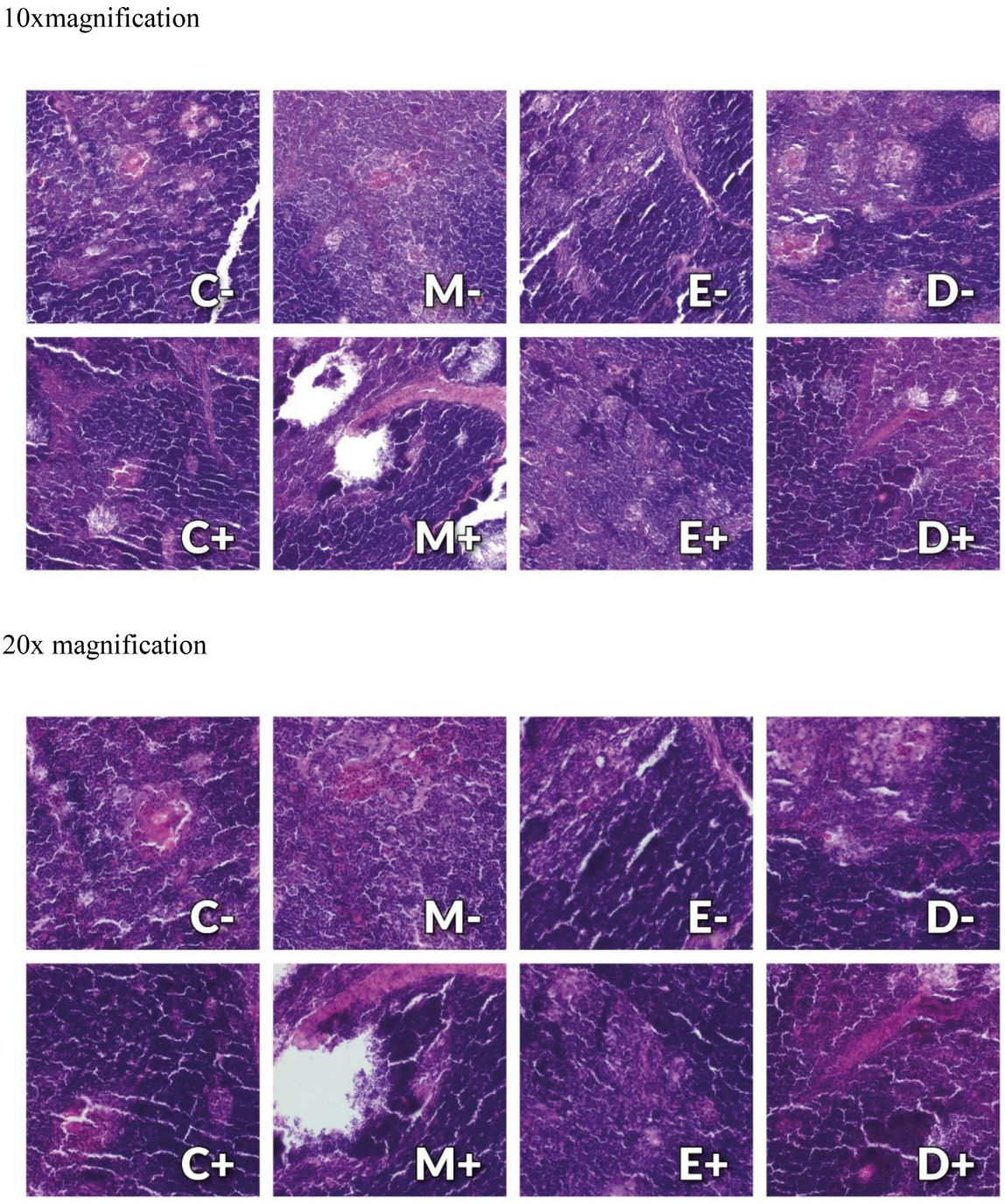

Figure 5. Morphological effects of early administration of antibiotics or feeding a diet containing a coccidiostat and vaccination on the thymus of 56-day-old turkeys. Treatment: (C−) unvaccinated control group; (M−) unvaccinated group treated with monensin; (E−) unvaccinated group treated with enrofloxacin;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

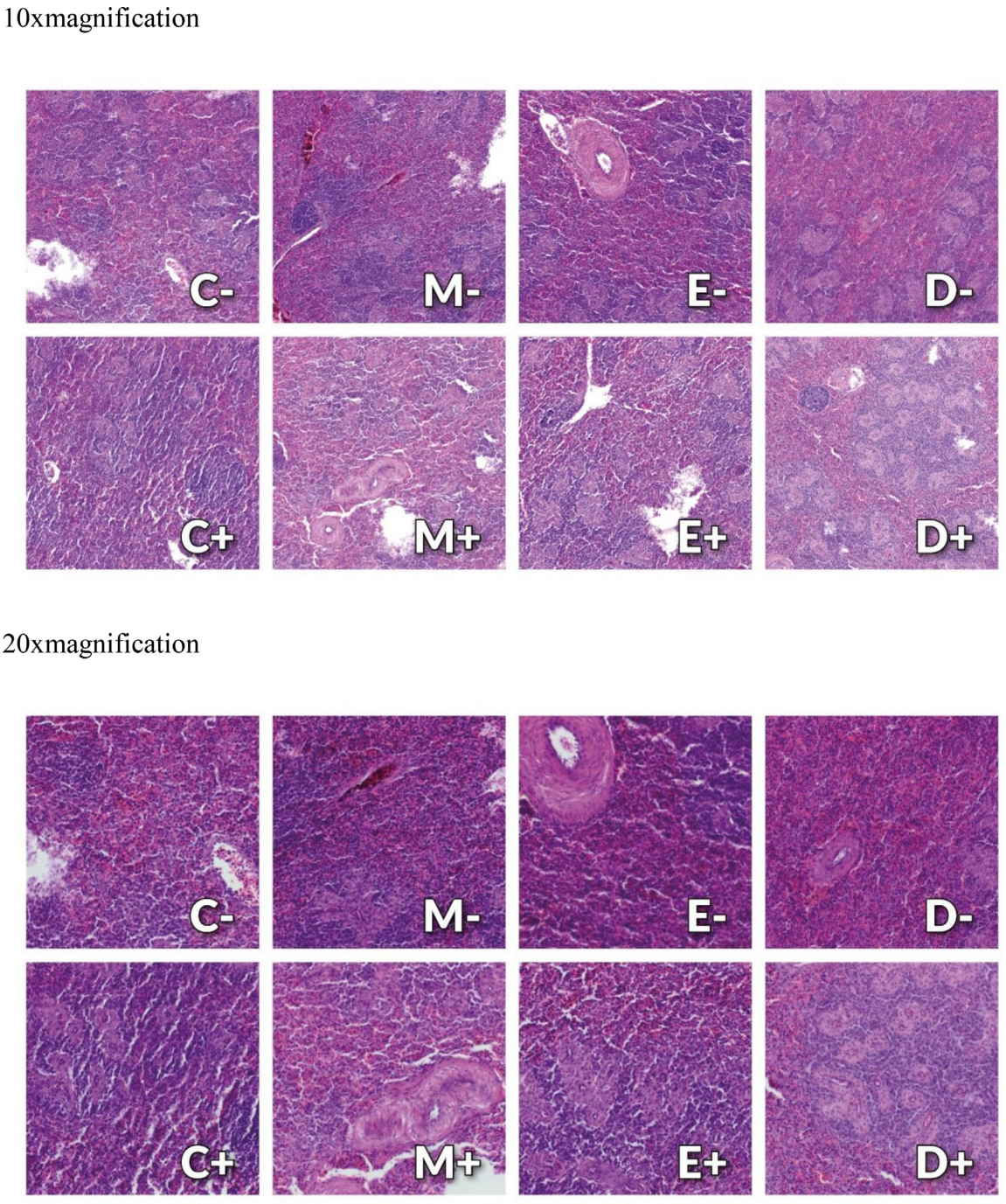

Figure 6. Morphological effects of early administration of antibiotics or feeding a diet containing a coccidiostat and vaccination on the spleen of 56-day-old turkeys. Treatment: (C−) unvaccinated control group; (M−) unvaccinated group treated with monensin; (E−) unvaccinated group treated with enrofloxacin;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

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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.

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