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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-*

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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.

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