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ORIGINAL PAPER

The effect of early administration of antibiotics or feeding a diet containing a coccidiostat on the level of selected minerals in the yolk sac and blood of turkeys

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ABSTRACT. The study aimed to determine the effect of antibiotic administration and vaccination on the level of selected mineral elements in the yolk sac and blood serum of turkeys. Turkey poults were divided into 8 experimental groups: a control group (C) receiving no antibiotics or coccidiostats; groups receiving either enrofloxacin (E) or doxycycline (D) for the first 5 days post-hatch; and a group administered monensin (M) for 56 days of life. The levels of Ca, P, Mg, Cu, Zn, and Fe were determined in yolk sac and blood serum samples. The results showed that poults utilise minerals from the yolk sac during the first days post-hatch, as indicated by their decreasing levels. Enrofloxacin treatment did not affect yolk sac levels of P and Zn, but it significantly impaired their absorption into circulation, with effects persisting for 7 weeks post-treatment. Both enrofloxacin and doxycycline administration transiently reduced Cu and Fe levels, although compensatory increases in serum concentrations were observed following treatment cessation. Long-term monensin exposure led to clinically relevant reductions in serum calcium levels.

Introduction

The proper development of birds during the embryonic stage and the early post-hatch period depends, among others, on the adequate level of mineral elements. During embryogenesis, the yolk serves as the main source of phosphorus (P), zinc (Zn), copper (Cu), manganese (Mn), and iron (Fe), while the albumen supplies sodium (Na) and potassium (K), along with smaller amounts of Fe, Cu, Mn, and Zn. The eggshell provides essential minerals such as calcium (Ca), magnesium (Mg), and strontium (Sr) (Yair and Uni, 2011). After hatching, the residual yolk sac remains the main source of energy and nutrients until dietary intake begins (van Der Wagt et al., 2020; Wong and Uni, 2021). Insufficient mineral levels during this phase can impair the development of key physiological systems, including skeletal formation, immune function, and circulatory development (Yair and Uni, 2011). Ca and P deficiency can lead to abnormal bone development. Ca also affects blood coagulation, neuronal signalling, and in laying hens, eggshell formation. P, on the other hand, is a structural component of nucleic acids, phospholipids, and cell membranes involved in maintaining acid-base balance and energy transfer (Üstündağ and Özdoğan, 2018; De Carvalho Mello et al., 2012). Mg deficiency in growing

turkeys can negatively affect performance leading to reduced weight gain, muscular dysfunction characterised by poor tone and lack of coordination, lower egg production and feed intake, as well as neurological symptoms such as tremors and seizures (Shastak and Rodehutscord, 2015). Mg is involved in the metabolism of amino acids, fats, and sugars, as well as in the regulation of Ca and vitamin D metabolism in the bones (Nishizawa et al., 2007). Trace elements such as Cu and Zn are cofactors of many enzymes. Cu is specifically involved in the synthesis of haemoglobin, erythrocuprein, and iron transport proteins, with deficiency resulting in anaemia symptoms (Leeson, 2009). Zn plays a role in the proper development and function of the immune system in poultry. Fe, on the other hand, is a component of oxygen-transporting proteins (myoglobin and haemoglobin) and specific redox enzymes. The intestinal absorption and yolk sac bioavailability of these mineral elements are particularly important for proper development in young birds (Houshmand et al., 2011; Abdelqader et al., 2013). Antibiotics can affect the absorption of mineral elements by changing the composition of the intestinal microbiota. In addition, enrofloxacin (a fluoroquinolone) and doxycycline (a tetracycline) are known to form insoluble complexes with divalent cations such as Ca, Fe, Zn, and Cu, thereby reducing their absorption from the gastrointestinal tract (Aguilera et al., 2007; Donaldson and Touger-Decker, 2014). According to Salles et al. (2008), the inclusion of monensin in animal nutrition may alter the bioavailability, absorption, and mineral deposition in tissues, and potentially affect the dietary requirements for these elements in ruminants. Studies demonstrate that monensin administration improves mineral balance under heat stress conditions by increasing retention of P, Na, and K, while reducing faecal calcium excretion and urinary losses of P, Na, K, Mg and Zn (Salles et al., 2008). Additionally, monensin specifically modulates ATP-dependent Ca transport through a mechanism independent of Na-K exchange, indicating that this effect is distinct from its ionophore properties (Lombardini, 1985).

It was hypothesised that early administration of the antibiotics enrofloxacin or doxycycline, as well as prolonged use of monensin during turkey rearing, could disrupt mineral homeostasis. Therefore, the aim of this study was to evaluate the effects of antibiotic administration and vaccination on the levels of selected mineral elements in the yolk sac and blood serum of turkeys.

Material and methods

Ethical statement

The experiment was approved by the Local Ethics Committee for Animal Experiments in Olsztyn, Poland (Decision No. 47/2021), and all procedures were conducted in accordance with the applicable regulations and guidelines consistent with EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Birds and housing

The experiment was conducted on 3080 1-day-old Hybrid Converter turkey hens. The birds were randomly divided into 8 experimental groups (7 replicate pens per group, 55 birds per pen). Turkeys were reared until 8 weeks of age under controlled environmental conditions, adjusted according to age-specific recommendations by Hybrid Turkeys (2020). The initial stocking density was 5.5 birds/m^2. To prevent cross-contamination, vaccinated and unvaccinated birds were housed in separate building sections and handled by different personnel. Environmental parameters were automatically regulated and maintained identically for all turkeys in both sections.

Diets and experimental design

Turkeys were fed complete diets meeting their nutritional requirements at successive rearing stages (Hybrid Turkeys, 2020), with the detailed composition described by Mikulski et al. (2022) and Smagieł et al. (2023). The diets, produced by a local feed mill, were offered as crumbles (days 1–28) and pellets (days 29–84), with *ad libitum* access to feed and water. The turkeys were divided into 8 experimental groups. The control group (C) received neither coccidiostat nor antibiotics. Group M received monensin (Coxidin 200, Huvepharma Polska, Warsaw, Poland) at a dose of 90 mg/kg feed for 56 days. Birds from group E were administered enrofloxacin (Enrofloxacin 10%, Biowet, Drwalew, Poland) added to drinking water at 10 mg enrofloxacin (ENR)/kg body weight (BW) for the first 5 days. Group D received doxycycline (Doxylin CT WSP 433 mg/g, Dopharma Research B.V., Raamsdonksveer, Netherlands) in drinking water at 50 mg doxycycline (DOX)/kg BW for the first 5 days of life.

Each of the experimental groups was further divided into vaccinated (+) and unvaccinated (−) subgroups, resulting in eight experimental groups in total (C+, C−, M+, M−, E+, E−, D+, and D−).

One-day-old turkeys from the vaccinated subgroups (C+, M+, E+, D+) were administered live attenuated vaccines against turkey rhinotracheitis (TRT) (Poulvac TRT; Zoetis, Warsaw, Poland), avian metapneumovirus disease (aMPV) and Newcastle disease (ND) (Nobilis ND clone 30; MSD Animal Health, Isando, South Africa) by coarse spraying. At 28 days of age, the birds were additionally vaccinated subcutaneously with an inactivated ornithobacteriosis (ORT) vaccine (Ornitin, Phibro, Warsaw, Poland). Birds in the unvaccinated subgroups (C−, M−, E−, D−) did not receive any vaccinations.

Sample collection and laboratory analyses

Yolk sacs were collected from 7 birds per group at 1, 3, and 5 days of age. Blood samples were obtained from seven birds per group at 1, 3, 5, 7, and 56 days of age to assess concentrations of selected minerals (Ca, P, Mg, Cu, Zn, and Fe). Yolk sacs were homogenised in phosphate-buffered saline (PBS, pH = 7.4). Blood samples were centrifuged at 3 000 × *g* for 10 min, after which serum was collected for further analysis. Mineral concentrations in yolk sac homogenates and blood serum were determined using Cormay test kits (Cormay, Poland).

Statistical analysis

An individual bird (n = 7) was considered the experimental unit for analyses of mineral levels in serum and yolk sac. Data were analysed using two-way ANOVA with the general linear model (GLM) procedure to assess the main effects of antibiotic treatment (C, M, E, D), vaccination status (vaccinated vs. unvaccinated; V effect), and their interaction. When significant effects were detected, Tukey's HSD test was used to separate treatment means. Results are presented as means with pooled standard errors of the mean (SEM). Statistical analyses were performed using STATISTICA software, version 13.1 (2017) at a significance level of $P < 0.05$.

Results

Significant antibiotic-vaccine interactions were observed for multiple parameters. In turkeys receiving monensin, vaccination reduced Ca levels in the yolk sac of 3-day-old birds, but did not affect serum Ca levels. Conversely, vaccination in doxycycline-treated birds did not alter yolk sac Ca content at 3 days but decreased serum Ca levels. At day 5, vaccination increased Ca levels in the yolk sac of birds treated with either enrofloxacin or doxycycline. However, only enrofloxacin administration caused a decrease in serum Ca levels of vaccinated birds. Moreover, vaccination elevated serum Ca levels in 7-day-old turkeys from the control group and those treated with enrofloxacin or doxycycline during the first 5 days compared to unvaccinated birds (Figure 1).

Vaccination of turkeys from the C group decreased P levels in the yolk sac and blood serum of 3-day-old birds compared to unvaccinated controls. In contrast, vaccination of turkeys from the E and M groups led to a reduction in serum P levels only, with no effect observed in the yolk sac at this stage. At day 5, vaccination of turkeys treated with enrofloxacin resulted in an increase in P concentrations in the yolk sac. However, birds receiving doxycycline showed a decrease in yolk sac P levels compared to unvaccinated counterparts. In the blood serum, vaccination caused a decrease in P levels in both the control and doxycycline (D) groups at the same time point. Following revaccination at 28 days of age, reduced serum P levels were recorded in 56-day-old birds that had received either continuous monensin treatment or early enrofloxacin/doxycycline administration (first 5 days) compared to unvaccinated birds (Figure 2).

Increased Mg levels were observed in the blood serum of 1-day-old vaccinated turkeys receiving monensin, doxycycline, or enrofloxacin compared to unvaccinated birds; however, this increase was not associated with elevated levels of this element in the yolk sac. Conversely, 3-day-old vaccinated turkeys had elevated Mg levels in the yolk sac, while serum Mg levels showed no significant changes (Figure 3).

Although vaccination exerted no effect on Cu levels in the yolk sacs of 3-day-old turkeys, an increase in serum levels of this element was recorded in vaccinated birds receiving monensin, enrofloxacin, or doxycycline. By 5 days of age, vaccinated turkeys administered doxycycline showed reduced copper levels in yolk sacs, though serum levels remained unchanged. However, a decrease in serum Cu concentrations was noted in 5-day-old vaccinated turkeys from the control group, as well as those receiving monensin or enrofloxacin, an effect that persisted until day 7 (Figure 4).

Vaccination of turkeys from the D group increased Zn levels in yolk sacs of 3-day-old birds, accompanied by decreased serum Zn levels. While vaccination did not affect yolk sac Zn levels in other groups (C, M, E), it reduced serum Zn concentrations

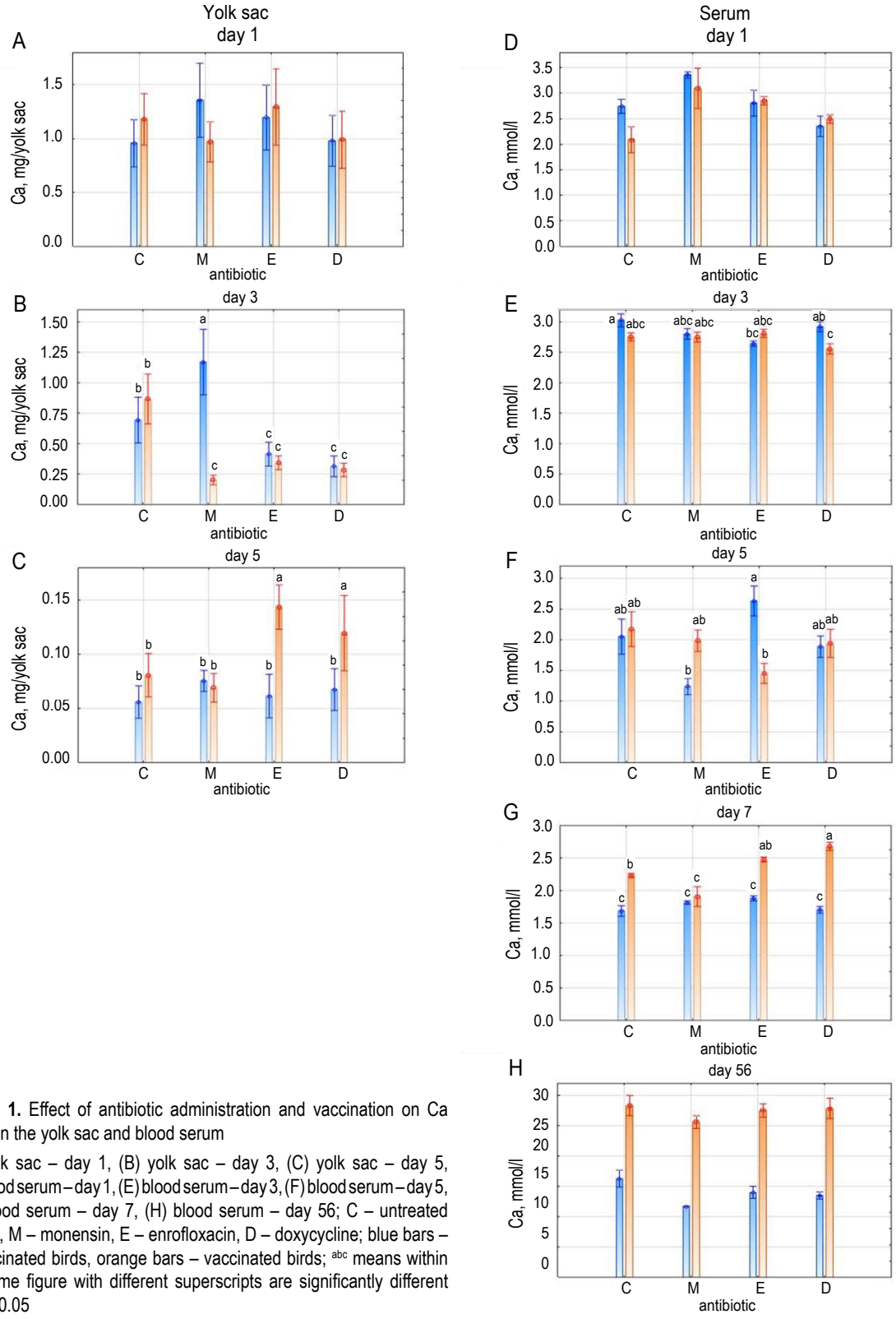

Figure 1. Effect of antibiotic administration and vaccination on Ca levels in the yolk sac and blood serum

(A) yolk sac – day 1, (B) yolk sac – day 3, (C) yolk sac – day 5, (D) blood serum – day 1, (E) blood serum – day 3, (F) blood serum – day 5, (G) blood serum – day 7, (H) blood serum – day 56; C – untreated control, M – monensin, E – enrofloxacin, D – doxycycline; blue bars – unvaccinated birds, orange bars – vaccinated birds; ^{abc} means within the same figure with different superscripts are significantly different at $P < 0.05$

in these birds. Additionally, revaccination at 28 days of age resulted in a decrease in serum Zn levels in 56-day-old birds receiving monensin throughout the rearing period, or enrofloxacin or doxycycline during the first 5 days of life (Figure 5).

Three days of antibiotic administration (monensin, enrofloxacin, or doxycycline) reduced Fe levels in the yolk sac of vaccinated birds without affecting serum iron levels. By day 5, vaccination increased yolk sac iron in enrofloxacin-treated turkeys but decreased it in doxycycline-treated birds. Although

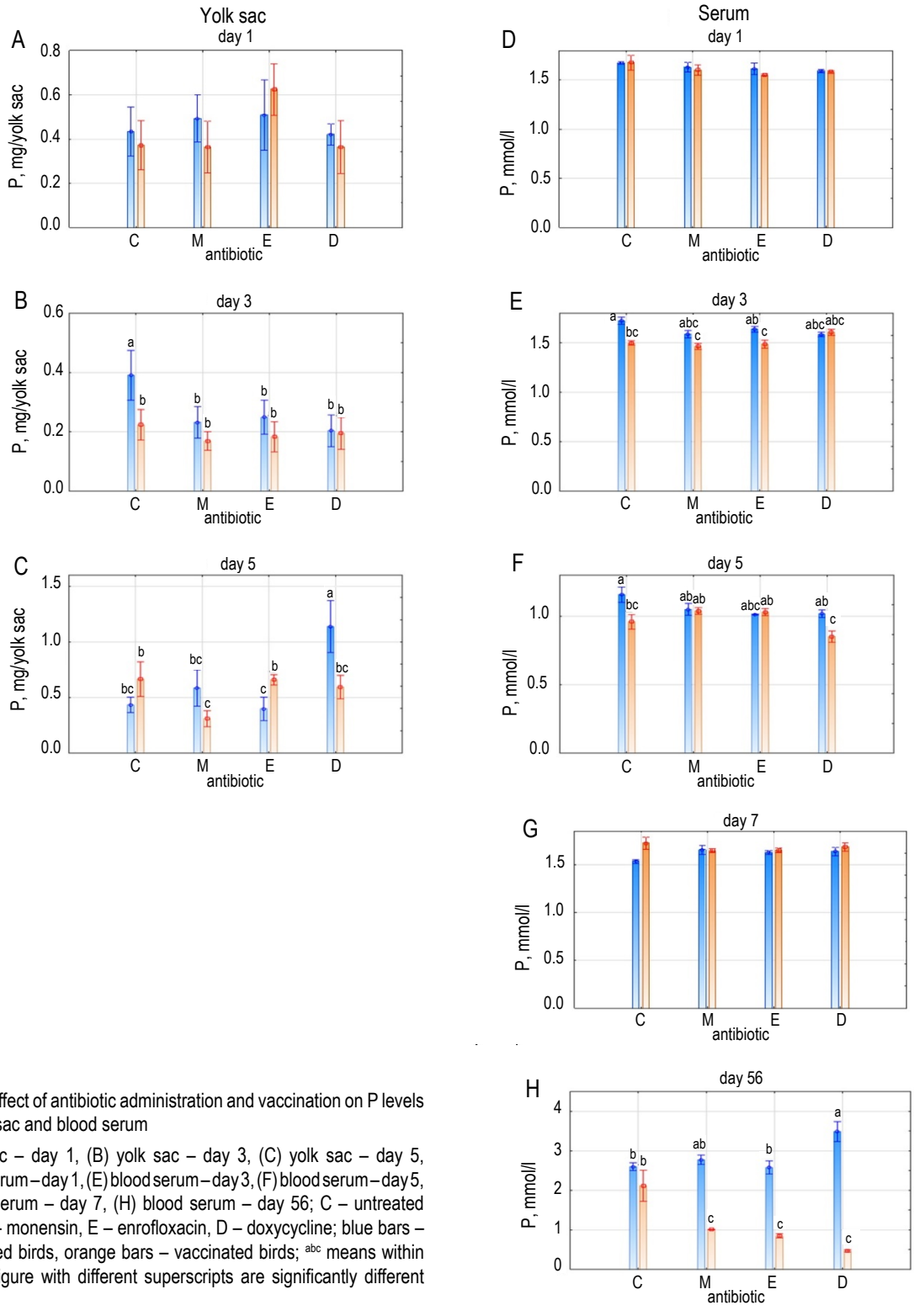

Figure 2. Effect of antibiotic administration and vaccination on P levels in the yolk sac and blood serum

(A) yolk sac – day 1, (B) yolk sac – day 3, (C) yolk sac – day 5, (D) blood serum – day 1, (E) blood serum – day 3, (F) blood serum – day 5, (G) blood serum – day 7, (H) blood serum – day 56; C – untreated control, M – monensin, E – enrofloxacin, D – doxycycline; blue bars – unvaccinated birds, orange bars – vaccinated birds; ^{abc} means within the same figure with different superscripts are significantly different at $P < 0.05$

no changes in yolk sac Fe levels were noted in vaccinated turkeys from C and M groups, a decrease in serum Fe concentration was evident in these groups following vaccination. The reduction in Fe levels in blood serum persisted until day 7 only in the control group. In addition, after vaccination at 28 days of age, 56-day-old doxycycline-treated vaccinated turkeys showed decreased serum Fe levels compared to unvaccinated birds (Figure 6).

Effect of the antibiotic

Significantly decreased levels of P ($P < 0.001$), Cu ($P < 0.001$), Zn ($P < 0.001$), and Fe ($P < 0.001$) were observed in the yolk sac of 3-day-old turkeys

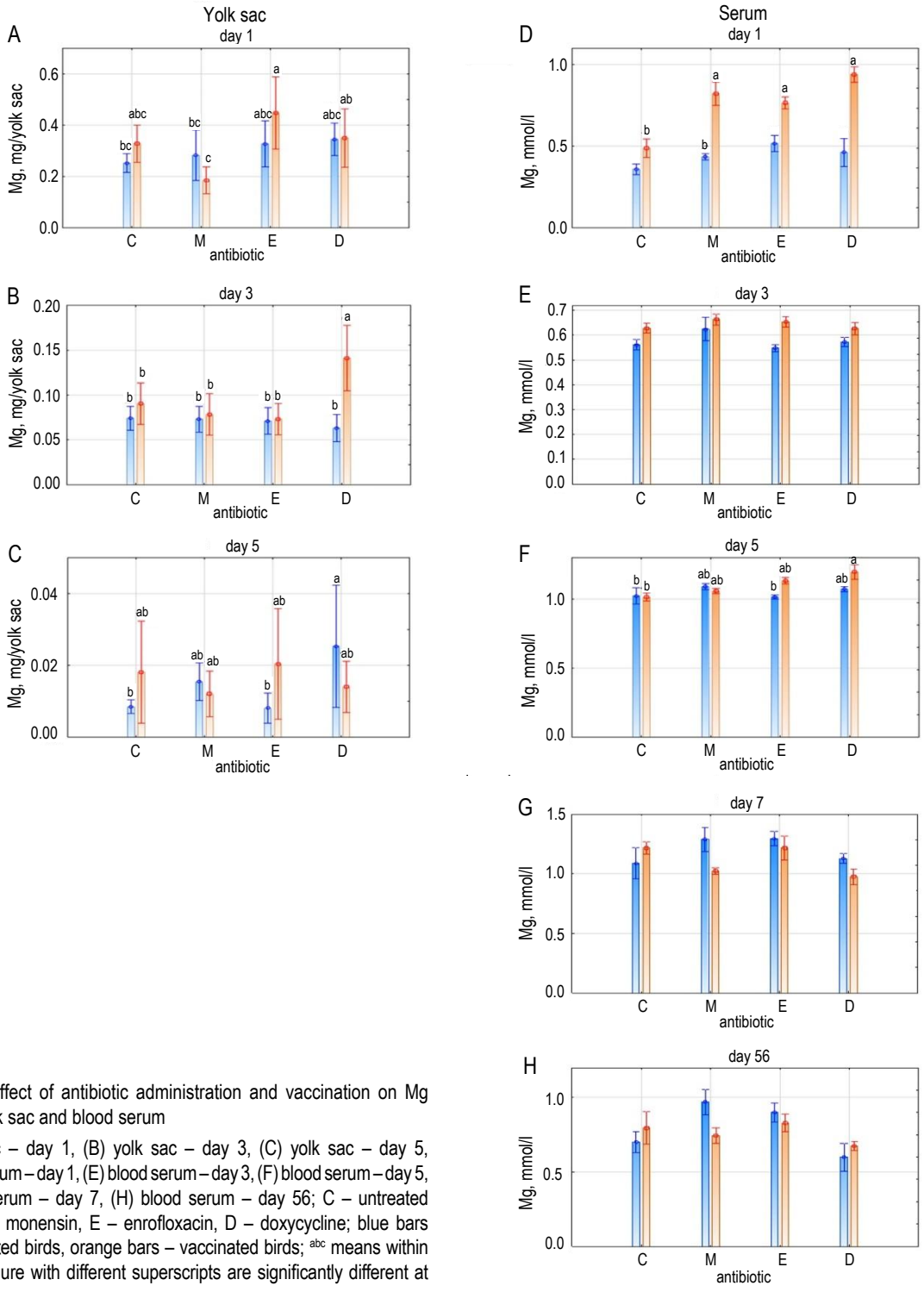

Figure 3. Effect of antibiotic administration and vaccination on Mg levels in yolk sac and blood serum

(A) yolk sac – day 1, (B) yolk sac – day 3, (C) yolk sac – day 5, (D) blood serum – day 1, (E) blood serum – day 3, (F) blood serum – day 5, (G) blood serum – day 7, (H) blood serum – day 56; C – untreated control, M – monensin, E – enrofloxacin, D – doxycycline; blue bars – unvaccinated birds, orange bars – vaccinated birds; ^{abc} means within the same figure with different superscripts are significantly different at $P < 0.05$

treated with monensin, doxycycline, or enrofloxacin. However, a reduction in Ca levels ($P < 0.001$) was found only in the groups administered enrofloxacin or doxycycline. On the other hand, an increase in Ca ($P < 0.001$) and Fe ($P < 0.001$) levels was recorded in the yolk sacs of 5-day-old turkeys receiving enrofloxacin or doxycycline. Additionally, turkeys treated with doxycycline showed elevated levels of P, Cu, and Zn in the yolk sac (Tables 1 and 2).

Three-day-old turkeys treated with doxycycline showed increased serum Zn levels ($P < 0.001$), while all antibiotic treatments (monensin, doxycycline, or enrofloxacin) increased serum Cu levels ($P < 0.001$) compared to controls. Meanwhile, decreased serum levels of P ($P = 0.009$),

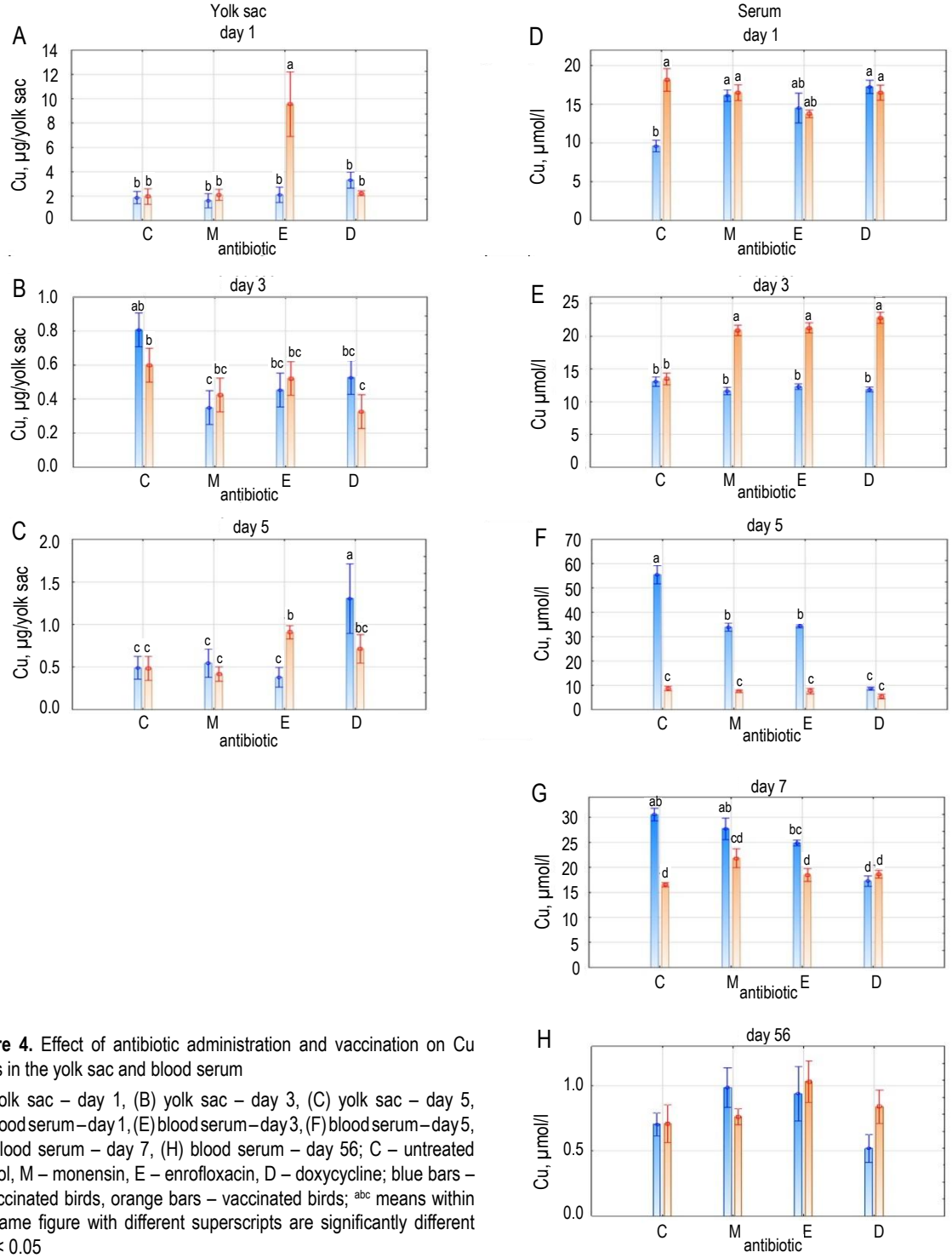

Figure 4. Effect of antibiotic administration and vaccination on Cu levels in the yolk sac and blood serum

(A) yolk sac – day 1, (B) yolk sac – day 3, (C) yolk sac – day 5, (D) blood serum – day 1, (E) blood serum – day 3, (F) blood serum – day 5, (G) blood serum – day 7, (H) blood serum – day 56; C – untreated control, M – monensin, E – enrofloxacin, D – doxycycline; blue bars – unvaccinated birds, orange bars – vaccinated birds; ^{abc} means within the same figure with different superscripts are significantly different at $P < 0.05$

Cu ($P < 0.001$), Fe ($P < 0.001$), and increased levels of Mg ($P = 0.014$) were observed in 5-day-old turkeys receiving doxycycline. Enrofloxacin treatment reduced serum Cu ($P < 0.001$) and Fe ($P < 0.001$) levels, while monensin decreased only Cu ($P < 0.001$) at day 5. At 7 days, doxycycline treatment decreased serum Cu ($P < 0.001$) but increased Ca ($P < 0.001$) and Fe ($P < 0.001$), whereas enrofloxacin increased both Ca ($P < 0.001$) and Fe ($P < 0.001$) levels compared to controls. In the blood serum of 56-day-old turkeys receiving monensin throughout the rearing period, a decrease in Ca levels ($P = 0.031$) and an increase in Fe levels ($P = 0.008$) were observed. In birds that received enrofloxacin during the first 5 days of life, there was a reduction in serum P ($P = 0.010$) and Zn ($P < 0.001$) levels, along with an increase in Fe levels ($P = 0.008$) (Tables 3 and 4).

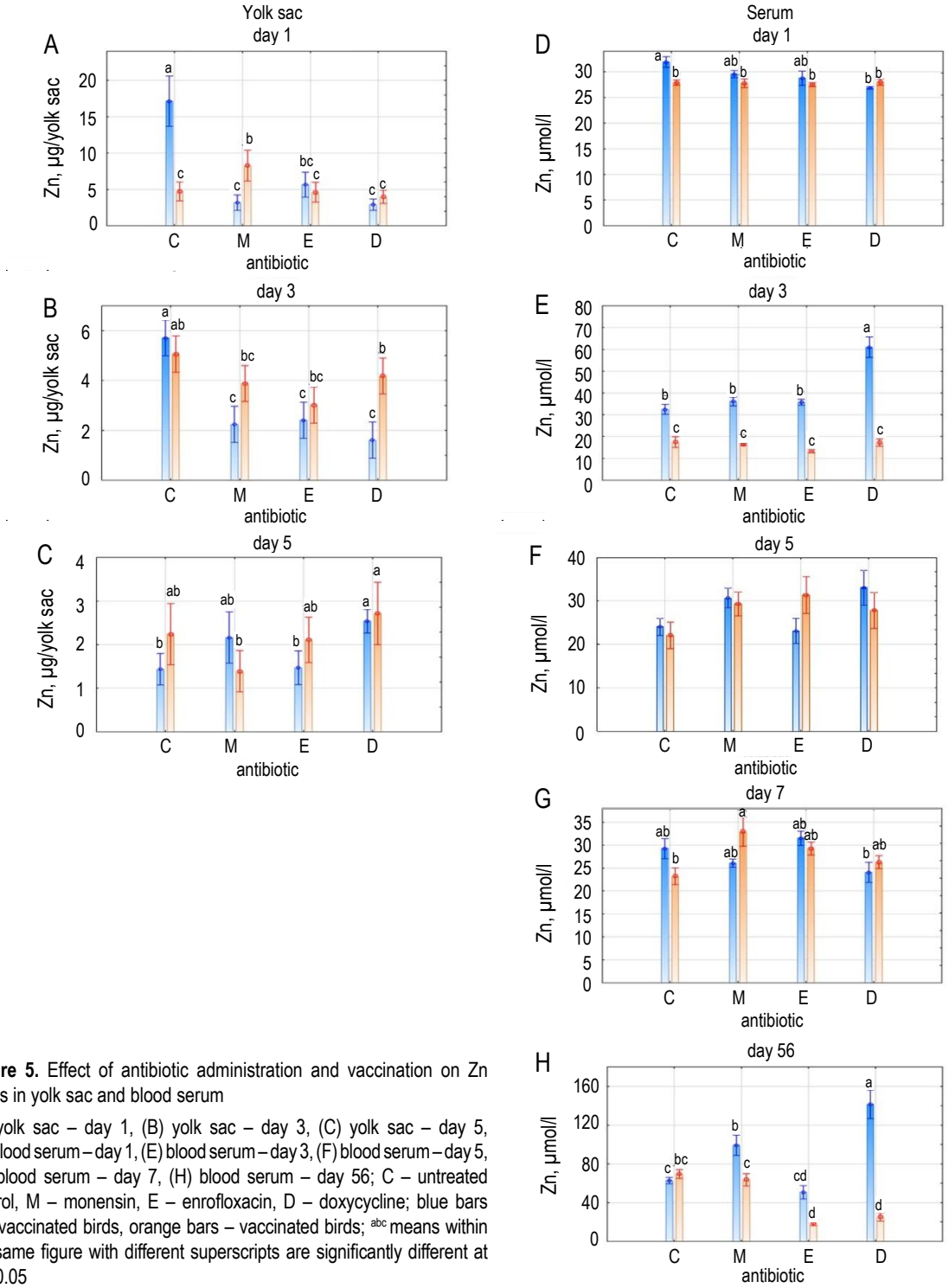

Figure 5. Effect of antibiotic administration and vaccination on Zn levels in yolk sac and blood serum

(A) yolk sac – day 1, (B) yolk sac – day 3, (C) yolk sac – day 5, (D) blood serum – day 1, (E) blood serum – day 3, (F) blood serum – day 5, (G) blood serum – day 7, (H) blood serum – day 56; C – untreated control, M – monensin, E – enrofloxacin, D – doxycycline; blue bars – unvaccinated birds, orange bars – vaccinated birds; ^{abc} means within the same figure with different superscripts are significantly different at $P < 0.05$

Effect of vaccination

Vaccinated 3-day-old turkeys showed increased Mg ($P < 0.001$) and Zn ($P < 0.001$) levels and decreased Ca ($P < 0.001$), P ($P < 0.001$), and Fe ($P < 0.001$) levels in yolk sacs compared to unvaccinated birds. In contrast, by day 5, vaccinated turkeys showed a significant increase in Ca levels ($P < 0.001$) and a decrease in P levels ($P = 0.037$) in yolk sacs compared to the unvaccinated birds (Tables 1 and 2). Vaccination at day 1 also significantly affected serum mineral levels in poults, resulting in increased Mg ($P < 0.001$) and Cu ($P = 0.023$) concentrations, and decreased Zn levels. In the serum of 3-day-old vaccinated birds, reduced levels of Ca ($P = 0.024$), P ($P < 0.001$), and Zn ($P < 0.001$) were found, along with increased Mg ($P = 0.001$) and Cu ($P < 0.001$) levels compared to the unvaccinated groups.

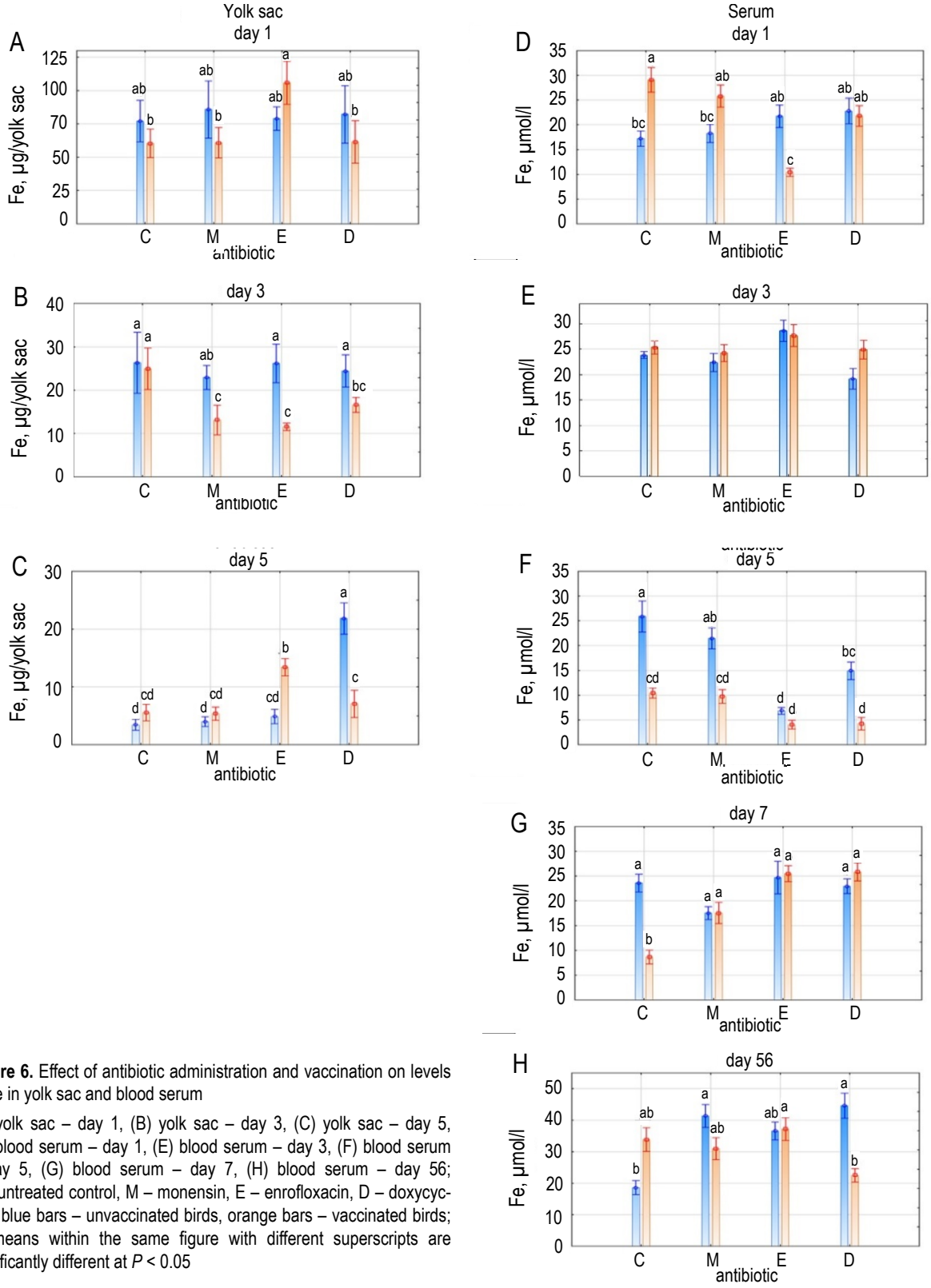

Figure 6. Effect of antibiotic administration and vaccination on levels of Fe in yolk sac and blood serum

(A) yolk sac – day 1, (B) yolk sac – day 3, (C) yolk sac – day 5, (D) blood serum – day 1, (E) blood serum – day 3, (F) blood serum – day 5, (G) blood serum – day 7, (H) blood serum – day 56; C – untreated control, M – monensin, E – enrofloxacin, D – doxycycline; blue bars – unvaccinated birds, orange bars – vaccinated birds; ^{abc} means within the same figure with different superscripts are significantly different at $P < 0.05$

At 5 days of age, vaccinated birds had significantly lower serum levels of P ($P = 0.002$), Cu ($P < 0.001$), and Fe ($P < 0.001$), and higher Mg levels ($P = 0.041$) than their unvaccinated counterparts. By day 7, vaccination was associated with increased serum levels of Ca ($P < 0.001$), P ($P = 0.025$), and Mg ($P = 0.041$), while Cu ($P < 0.001$) and Fe ($P = 0.047$) levels were reduced. Finally, vaccination at both 1 and 28 days of age resulted in higher Ca ($P < 0.001$) and lower P ($P < 0.001$) and Zn ($P < 0.001$) levels in the blood serum of 56-day-old birds (Tables 3 and 4).

Table 1. Content of mineral elements in the yolk sac, mg/yolk sac

Indices	Ca			P			Mg		
	day 1	day 3	day 5	1 day	day 3	day 5	day 1	day 3	day 5
Antibiotic									
C	1.067	0.779^{a}	0.068^{c}	0.403^{b}	0.307^{a}	0.549^{b}	0.290^{bc}	0.082^{ab}	0.013
M	1.162	0.685^{a}	0.072^{bc}	0.429^{b}	0.200^{b}	0.447^{b}	0.234^{c}	0.076^{b}	0.014
E	1.245	0.378^{b}	0.103^{a}	0.567^{a}	0.216^{b}	0.529^{b}	0.388^{a}	0.072^{b}	0.014
D	0.985	0.299^{b}	0.093^{ab}	0.393^{b}	0.199^{b}	0.865^{a}	0.348^{ab}	0.102^{a}	0.020
Vaccine									
-	1.122	0.647^{a}	0.065^{b}	0.464	0.269^{a}	0.639^{a}	0.302	0.070^{b}	0.014
+	1.109	0.423^{b}	0.103^{a}	0.431	0.193^{b}	0.557^{b}	0.328	0.096^{a}	0.016
SEM	0.042	0.047	0.005	0.019	0.012	0.037	0.015	0.004	0.002
P-value									
antibiotic (A)	0.122	<0.001	<0.001	0.002	<0.001	<0.001	0.001	0.005	0.409
vaccine (V)	0.871	<0.001	<0.001	0.322	<0.001	0.037	0.317	<0.001	0.550
A x V	0.053	<0.001	<0.001	0.072	0.011	<0.001	0.026	<0.001	0.025

C – untreated control, M – monensin, E – enrofloxacin, D – doxycycline; (−) – unvaccinated; (+) – vaccinated; SEM – standard error of the mean; ^{abc} means within the same column with different superscripts are significantly different at $P < 0.05$

Table 2. Content of mineral elements in the yolk sac, µg/yolk sac

Indices	Cu			Zn			Fe		
	day 1	day 3	day 5	day 1	day 3	day 5	day 1	day 3	day 5
Antibiotic									
C	1.916^{b}	0.703^{a}	0.487^{b}	10.942^{a}	5.385^{a}	1.837^{b}	68.73^{b}	25.64^{a}	4.492^{c}
M	1.856^{b}	0.387^{b}	0.481^{b}	5.719^{b}	3.060^{b}	1.774^{b}	73.33^{b}	18.02^{b}	4.676^{c}
E	5.826^{a}	0.487^{b}	0.644^{b}	5.133^{bc}	2.709^{b}	1.790^{b}	92.38^{a}	18.87^{b}	9.131^{b}
D	2.764^{b}	0.426^{b}	1.008^{a}	3.435^{c}	2.897^{b}	2.627^{a}	71.78^{b}	20.52^{b}	14.461^{a}
Vaccine									
-	2.226^{b}	0.534	0.679	7.223^{a}	2.991^{b}	1.901	80.99	24.97^{a}	8.525
+	3.955^{a}	0.468	0.630	5.391^{b}	4.035^{a}	2.113	72.12	16.55^{b}	7.855
SEM	0.365	0.025	0.047	0.640	0.217	0.096	2.911	0.938	0.829
P-value									
antibiotic (A)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001
vaccine (V)	<0.001	0.064	0.376	0.001	<0.001	0.170	0.059	<0.001	0.166
A x V	<0.001	0.003	<0.001	<0.001	<0.001	0.003	0.001	0.002	<0.001

C – untreated control, M – monensin, E – enrofloxacin, D – doxycycline; (−) – unvaccinated; (+) – vaccinated; SEM – standard error of the mean; ^{abc} means within the same column with different superscripts are significantly different at $P < 0.05$

Table 3. Mineral levels in the blood serum of turkeys, mmol/l

Indices	Ca					P					Mg				
	day 1	day 3	day 5	day 7	day 56	day 1	day 3	day 5	day 7	day 56	day 1	day 3	day 5	day 7	day 56
Antibiotic															
C	2.416^{b}	2.889	2.112	1.959^{b}	22.25^{a}	1.675	1.612	1.059^{a}	1.628	2.357^{a}	0.424^{b}	0.610	1.019^{b}	1.153	0.749^{ab}
M	3.227^{a}	2.774	1.611	1.862^{b}	18.60^{b}	1.615	1.526	1.044^{a}	1.650	1.891^{ab}	0.629^{a}	0.643	1.074^{ab}	1.157	0.857^{a}
E	2.829^{ab}	2.727	2.041	2.178^{a}	20.71^{ab}	1.581	1.563	1.022^{ab}	1.637	1.713^{b}	0.641^{a}	0.600	1.074^{ab}	1.256	0.864^{a}
D	2.422^{b}	2.738	1.915	2.191^{a}	20.62^{ab}	1.588	1.596	0.935^{b}	1.661	1.978^{ab}	0.701^{a}	0.600	1.134^{a}	1.053	0.637^{b}
Vaccine															
-	2.815	2.847^{a}	1.952	1.772^{b}	13.80^{b}	1.627	1.634^{a}	1.060^{a}	1.612^{b}	2.859^{a}	0.443^{b}	0.576^{b}	1.051^{b}	1.201	0.793
+	2.632	2.717^{b}	1.887	2.323^{a}	27.29^{a}	1.603	1.515^{b}	0.970^{b}	1.676^{a}	1.110^{b}	0.754^{a}	0.642^{a}	1.101^{a}	1.109	0.761
SEM	0.087	0.032	0.090	0.052	1.006	0.015	0.015	0.017	0.015	0.149	0.032	0.010	0.014	0.030	0.029
P-value															
Antibiotic (A)	0.001	0.169	0.118	<0.001	0.031	0.134	0.051	0.009	0.839	0.010	<0.001	0.203	0.014	0.100	0.010
Vaccine (V)	0.229	0.024	0.676	<0.001	<0.001	0.448	<0.001	0.002	0.025	<0.001	<0.001	0.001	0.041	0.110	0.546
A x V	0.234	0.007	0.001	<0.001	0.787	0.889	0.003	0.011	0.069	<0.001	0.013	0.614	0.040	0.098	0.117

C – untreated control, M – monensin, E – enrofloxacin, D – doxycycline; (−) – unvaccinated; (+) – vaccinated; SEM – standard error of the mean; ^{abc} means within the same column with different superscripts are significantly different at $P < 0.05$

Table 4. Mineral levels in the blood serum of turkeys, µmol/l

Indices	Cu					Zn					Fe				
	day 1	day 3	day 5	day 7	day 56	day 1	day 3	day 5	day 7	day 56	day 1	day 3	day 5	day 7	day 56
Antibiotic															
C	13.88^{b}	13.28^{b}	32.05^{a}	23.52^{a}	7.05	29.92^{a}	24.95^{b}	23.05	26.24^{ab}	66.18^{a}	23.15^{a}	24.59^{ab}	18.16^{a}	16.13^{b}	26.26^{b}
M	16.32^{ab}	16.27^{a}	20.66^{b}	24.75^{a}	8.73	28.67^{ab}	26.28^{b}	29.98	29.52^{ab}	81.32^{a}	22.03^{a}	23.35^{b}	15.59^{a}	17.54^{b}	36.14^{a}
E	14.14^{ab}	16.79^{a}	21.02^{b}	21.67^{a}	9.84	28.13^{ab}	24.50^{b}	27.21	30.38^{a}	34.72^{b}	16.07^{b}	28.17^{a}	5.44^{b}	25.10^{a}	36.88^{a}
D	16.89^{a}	17.34^{a}	6.98^{c}	17.94^{b}	6.78	27.45^{b}	39.10^{a}	30.43	25.18^{b}	83.43^{a}	22.30^{a}	22.04^{b}	9.57^{b}	24.39^{a}	33.54^{ab}
Vaccine															
-	14.38^{b}	12.22^{b}	33.10^{a}	25.09^{a}	7.86	29.28^{a}	41.33^{a}	27.70	27.72	88.50^{a}	19.99	23.51	17.27^{a}	22.20^{a}	35.28
+	16.23^{a}	19.62^{a}	7.25^{b}	18.86^{b}	8.34	27.80^{b}	16.09^{b}	27.64	27.95	44.32^{b}	21.78	25.56	7.10^{b}	19.39^{b}	31.13
SEM	0.502	0.651	2.419	0.786	0.501	0.333	2.165	1.194	0.780	5.589	0.987	0.694	1.155	0.983	1.560
P-value															
Antibiotic (A)	0.018	<0.001	<0.001	<0.001	0.101	0.026	<0.001	0.103	0.028	<0.001	0.004	0.008	<0.001	<0.001	0.008
Vaccine (V)	0.023	<0.001	<0.001	<0.001	0.620	0.012	<0.001	0.980	0.868	<0.001	0.225	0.108	<0.001	0.047	0.079
A x V	<0.001	<0.001	<0.001	<0.001	0.275	0.025	<0.001	0.202	0.012	<0.001	<0.001	0.313	0.005	<0.001	<0.001

C – untreated control, M – monensin, E – enrofloxacin, D – doxycycline; (−) – unvaccinated; (+) – vaccinated; SEM – standard error of the mean; ^{abc} means within the same column with different superscripts are significantly different at $P < 0.05$

Discussion

The process of yolk sac resorption typically occurs within 4–5 days after hatching (van der Wagt et al., 2020), during which both dietary intake and residual yolk sac contents serve as mineral sources for poults. Therefore, factors influencing the rate of yolk sac absorption may also indirectly affect mineral availability for young turkeys. Our previous studies on the effects of antibiotics on yolk sac absorption demonstrated that the administration of enrofloxacin or doxycycline to turkeys during the first 5 days of life, as well as the addition of monensin throughout the rearing period, did not inhibit yolk sac resorption (Ognik et al., 2025).

Our research demonstrated that antibiotic administration and vaccination in turkeys affected the levels of mineral elements in both yolk sacs and blood serum. Specifically, administering enrofloxacin or doxycycline decreased yolk sac Ca levels in 3-day-old turkeys, but it did not reduce serum Ca concentrations. This finding is surprising because yolk sac Ca mobilisation typically increases serum Ca concentration during this period. Poults utilise residual nutrients, including minerals stored in the yolk sac, until their diet provides adequate nutrition (Yair and Uni, 2011). In instances where dietary mineral absorption is impaired, compensatory uptake from the yolk sac is expected. Antibiotics such as quinolones, including enrofloxacin, are known to form complexes with multivalent metal cations, such as Mg^{2+}, Ca^{2+}, Mn^{2+}, $Fe^{2+/3+}$, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Al^{3+}, which may alter their pharmacological activity, as well as affect the bioavailability of these minerals (Grabowski et al., 2022; Ftouni et al., 2012; Turel, 2002). For example, Mg^{2+} complexes have been found to reduce the activity of antibiotics (Turel et al., 2000), while Fe^{3+} and Zn^{2+} complexes have been reported to increase microbiological activity (Gao et al., 1995).

Sumano et al. (2004) demonstrated that high calcium (Ca^{2+}) and magnesium (Mg^{2+}) concentrations in drinking water significantly reduced enrofloxacin bioavailability in broilers due to complex formation with these ions. While these complexes retained antibacterial activity, they impaired gastrointestinal absorption, leading to lower plasma concentrations of the antibiotic (Sumano et al., 2004). This suggests that reduced antibiotic absorption may also limit Ca^{2+} and Mg^{2+} uptake. Supporting this notion, Aguilera et al. (2007) confirmed that calcium inhibited enrofloxacin absorption in the gastrointestinal tract. Yan et al. (2022) studied the pharmacokinetics and antibacterial activity of enrofloxacin and the complex of this antibiotic with Ca and found that the combination of Ca ions with enrofloxacin in plasma improved antibiotic efficacy and reduced its toxicity. Our results are consistent with the latter reports, showing elevated serum Ca^{2+} levels in 7-day-old turkeys even after early antibiotic withdrawal (5 days of age). Like enrofloxacin, doxycycline also forms complexes with Ca ions, which may accumulate in bone tissue (Gajda et al., 2024). Therefore, doxycycline is contraindicated in young animals due to potential bone development disorders (Hsiao et al., 2016).

P, like Ca, is crucial for proper bone development. Our research demonstrated that the administration

of enrofloxacin, doxycycline, or monensin increased P absorption from the yolk sac in 3-day-old turkeys; however, despite enhanced yolk sac resorption, the administration of these antibiotics did not significantly alter serum P concentrations. P can form complexes with Ca, Mg, or Fe, which can potentially interact with antibiotics or their metabolites. Interestingly, our findings also showed that antibiotic administration led to an elevated serum Mg concentration in 1-day-old turkeys, which was not associated with higher resorption of this mineral from the yolk sac. Importantly, this effect was observed only in 1-day-old birds, whose primary source of minerals is the yolk sac. In older turkeys, the administration of antibiotics did not affect serum Mg levels.

Cu, Zn, and Fe are essential microelements necessary for the proper physiological functioning and development of turkeys. Our research demonstrated that administering monensin, doxycycline, or enrofloxacin antibiotics increased the absorption of Cu, Zn, and Fe from the yolk sac in 3-day-old birds. However, this increased absorption was reflected in elevated blood serum levels only in the case of Cu. The rise in serum Cu concentrations likely resulted from a combination of enhanced yolk sac resorption and supply from the diet. For Zn, a significant increase in serum concentration was observed only following doxycycline administration, suggesting that while monensin and enrofloxacin may have facilitated greater absorption from the yolk sac, they may have simultaneously inhibited Zn uptake from the feed. In contrast, Fe concentrations in the blood serum of 3-day-old turkeys remained unchanged in all antibiotic treatments. This outcome, despite increased Fe absorption from the yolk sac, implied a compensatory reduction in intestinal Fe absorption. At day 5, when most of the yolk sac is already depleted, dietary intake became the primary mineral source for the growing turkeys.

Enrofloxacin or doxycycline was administered to turkeys for the first 5 days of life. Our previous study showed that the highest levels of these antibiotics were recorded in the liver on day 5. However, after discontinuing the administration, the levels of enrofloxacin and doxycycline rapidly declined to maximum residue limit (MRL) values (Smagieł et al., 2024). The present study revealed reduced serum Cu and Fe levels in 5-day-old birds following antibiotic administration. This effect may result from both direct complex formation between antibiotics and these minerals, potentially altering their absorption, and indirect modulation of intestinal microbiota that could disrupt normal mineral uptake processes.

Monensin, administered to turkeys throughout the rearing period, is an ionophore antibiotic that forms complexes with monovalent cations such as Na^+, K^+, Rb^+, and Li^+. By selectively increasing intracellular sodium concentration, it activates Na^+-Ca^{2+} exchange mechanisms, ultimately elevating cytosolic calcium levels. This mechanism likely explains the reduced serum Ca concentration observed in 56-day-old turkeys in this study.

The available literature contains no evidence of a direct effect of vaccination on the absorption of mineral elements in chickens. However, our findings demonstrate that the simultaneous administration of antibiotics and vaccinations resulted in a decrease in serum P and Zn levels at 3 and 56 days, and Cu levels in 5- and 7-day-old vaccinated turkeys. In contrast, no immediate change in Ca concentration was observed following vaccination of 1-day-old turkeys against TRT and ND. However, by day 7, an increase in serum Ca levels was noted in vaccinated birds from both the control group and the groups receiving enrofloxacin or doxycycline. These observations suggest that the interaction between vaccination and antibiotic therapy may modify intestinal microbiota composition and compromise epithelial barrier function, thereby indirectly affecting mineral absorption (Herrera-Encinas et al., 2021).

Conclusions

Analysis of mineral element levels indicates that, during the first days of life, turkey poults utilise these components stored in the yolk sac, as reflected by the progressive decline in the concentration of these elements within the yolk sac. The dynamics of mineral transfer from the yolk sac to the bloodstream during this period are highly variable and appear to be influenced to a greater extent by the physiological demands of the poults than by the experimental interventions applied.

Although no immediate reductions in serum P and Zn levels were observed during enrofloxacin administration, it impaired their absorption efficiency, with effects persisting up to seven weeks post-treatment. Similarly, both enrofloxacin and doxycycline temporarily reduced Cu and Fe levels during administration, though these deficiencies were subsequently compensated by increased serum concentrations after the end of antibiotic therapy.

Long-term administration of monensin is associated with an unfavourable decrease in serum Ca levels in birds.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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