Quantifying the effect of coccidiosis on broiler performance and infection outcomes in the presence and absence of control methods

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ABSTRACT A systematic review and meta-analysis was conducted to investigate the role of *Eimeria* species, dose and inoculation time, on performance and infection outcomes of different broiler strains infected for different study durations. The meta-analysis addressed *E. acervulina, E. maxima, E. tenella*, and mixed species infections, and involved data from 72 peer-reviewed articles, corresponding to 521 treatments performed on 20,756 broilers. A secondary objective was to investigate the effects of synthetic anticoccidials, ionophores, and vaccination against *Eimeria* on the above outcomes. Performance during infection was scaled (%) to that of the uninfected birds. Infection reduced scaled ADFI and ADG ($P < 0.001$) and increased feed conversion ratio (FCR; $P < 0.05$); there was a significant interaction between dose and species on scaled ADFI and ADG, suggesting that different species affected these variables to different extents ($P < 0.001$). There was a tendency for an interaction between dose and broiler strain on scaled ADFI ($P = 0.079$), and a significant interaction between these variables on scaled ADG ($P < 0.01$). A tendency for an interaction between oocyst dose and *Eimeria* species ($P = 0.067$) on maximum number of oocysts excreted was observed. Lesion scores were significantly affected by dose, species, and their interaction ($P < 0.05$), the latter caused by an increase in the lesion scores during *E. maxima* and *E. tenella* infections. Control methods significantly affected scaled ADG and FCR ($P < 0.05$) and there was an interaction between dose and control methods on ADFI ($P < 0.001$). Synthetic anticoccidial use improved scaled ADG ($P < 0.01$), whereas ionophores improved FCR compared with untreated birds ($P < 0.01$). An interaction between dose and control method on scaled ADFI was caused by the higher ADFI of vaccinated compared to untreated birds, as dose increased. There was a significant effect of control methods on lesion scores ($P < 0.01$). All findings advance our understanding of the factors that influence the impact of coccidiosis and its controls in broilers.

Key words: broiler, coccidiosis, *Eimeria*, meta-analysis, performance

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INTRODUCTION

Coccidiosis is recognized as the major parasitic disease of poultry which is caused by different species of the genus *Eimeria,* causing significant economic consequences to the broiler industry (Allen and Fetterer, 2002). The annual global cost of coccidiosis to the poultry industry is estimated between $7 and $13 billion (Blake et al., 2020). Infection with *Eimeria* leads to reductions in performance, both in terms of growth and feed efficiency, and in the absence of methods of control it may have devastating effects on bird health and welfare. One of the factors contributing to the reduction in performance is the degree of intestinal damage (usually assessed as lesion scores postmortem), as this affects nutrient availability and host metabolism (Allen et al., 1998). Depending on the species, infective dose, and site of infection, coccidiosis can result in limited enteritis resulting in fluid loss and malabsorption of nutrients (*E. acervulina* and *E. mitis*), inflammation of the intestinal wall with pinpoint hemorrhages and sloughing of epithelia (*E. brunetti* and *E. maxima*), or complete villar destruction resulting in extensive hemorrhage and death (*E. necatrix* and *E. tenella*).

Yet, the degree to which the different *Eimeria* species affect broiler performance and infection outcomes is currently poorly understood (Kipper et al., 2013;

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Gilbert et al., 2020). This is largely because there is substantial variation in the experimental conditions of studies performed to address the effect of specific *Eimeria* challenges to a number of specific, usually limited outcomes. Therefore, there is a need to understand the effect of different *Eimeria* species on different infection outcomes and their interaction with a variety of factors such as time and infective dose, broiler strain, study duration, and *Eimeria* species.

Anticoccidial drugs have been in use globally for almost a century to control avian coccidiosis. Despite their acceptance and success in managing this costly and ubiquitous avian disease, the poultry industry has been under constant pressure to reduce its dependence on antimicrobials, including anticoccidial drugs (Tsiouris et al., 2013). The development of resistance or reduced sensitivity of *Eimeria* to chemotherapeutic agents due to long-term exposure has been increasingly reported around the globe (Abbas et al., 2011; Chapman et al., 2013; Noack et al., 2019). Furthermore, public health concerns about the presence of anticoccidial residues in chicken meat and eggs (Bozkurt et al., 2013), emphasize the pressure to explore alternative control methods for coccidiosis, such as vaccination to combat the issues described above. Although there is greater consistency on the experimental conditions under which the efficacy of coccidiosis controls is assessed, there is still great variation on their reported outcomes (Soutter et al., 2020), and uncertainty about the effects of control methods, such as vaccination, on ADFI and ADG (Eckert et al., 2021).

Previous meta-analyses in this field have explored the effect of infection (Kipper et al., 2013), and the influence of anticoccidial drugs and vaccination (Eckert et al., 2021), on the performance of *Eimeria* infected broilers. From these informative meta-analyses, a knowledge gap was identified in the effect of *Eimeria* infection on the severity of infection outcomes, and any possible interactions between infection outcomes and performance characteristics of both treated (i.e., synthetic anticoccidial drugs, ionophores, and vaccinations) and nontreated (i.e., no control methods) broiler chickens. Therefore, this systematic review and meta-analysis was conducted to determine the effect of *Eimeria* infection on broiler performance and infection outcomes in the absence and presence of coccidiosis control methods. We considered a limited number of control methods, namely synthetic anticoccidials, ionophores, and vaccination against *Eimeria*, due to their widespread usage in poultry production systems (Blake et al., 2021). The outcomes of the meta-analysis were expected to provide further understanding of the pathogen- and host-related factors that affect the outcome of coccidiosis, enhance our ability to deal with the infection in a more effective manner and lead to better control methods.

MATERIALS AND METHODS

Ethical approval was not required for this study, as all data were obtained from previous experiments in which ethical approval had already been obtained by the trial investigators.

Throughout this paper, the terms meta-analysis and meta-regression are used interchangeably to describe the statistical methodology utilised in this study. Formally, our analysis constitutes a meta-regression, which is a tool used in meta-analyses to examine the impact of moderator variables on study effect size using regression-based techniques.

Search Strategy

First, a review protocol was developed which outlined the strategies for the systematic review and the subsequent meta-analysis of literature on 2 research objectives: 1) the effect of *Eimeria* infection on broiler growth performance and infection outcomes, the latter defined as excretion of oocysts, lesions scores and mortality; and 2) to determine the consequences of different coccidiosis control methods (synthetic anticoccidials, ionophores, and vaccines) on broiler growth performance and infection outcomes. Next, an initial scoping of the literature was carried out to determine the feasibility of the study and, consequently, multiple, full-scale literature searches were performed. The last literature search was performed on February 9, 2021.

The Web of Science and Scopus databases were selected to identify peer-reviewed articles that were published exclusively between 1990 and 2021, with older articles excluded to account for commercial husbandry and breeding changes. A preliminary screening of studies conducted between 1980 and 1990 suggested that there were major changes in bird strains used prior to 1990. The literature searches were conducted in accordance with the review protocol using a combination of keywords outlined in Table 1. The results of these literature searches were merged and exported into an EndNote library. The search results were then filtered, and duplicate articles were removed as the searches were not mutually exclusive. Each paper was then given its own unique accession number and considered for further analysis.

Table 1. Outline of keyword searches to meet the objective of determining the effect of infection with *Eimeria* species on broiler growth performance and infection outcomes in the presence of absence of control methods.

Components	Keywords
Subject	• *Eimeria* • *Eimeria* AND *maxima* OR *tenella* OR *acervulina* OR • coccidia* OR coccidiosis OR protozoa* • "anti-protozoa" OR "anti-parasite" OR narasin OR zoalene OR nicarbazin OR salinomycin OR monensin OR diclazuril OR lasalocid OR semduramicin OR anticoccidia* OR coccidiostat
Response	• "feed intake" OR anorexia OR fcr OR "BWG" OR "ADG" OR "live weight" OR "body weight gain" OR mortalit* OR "lesion score*" OR oocyst* OR "OPG" OR aci OR "anticoccidial index"
Population	• broiler* OR avian OR poultry OR chick* NOT layer NOT hen NOT sheep NOT camel NOT rabbit* NOT turkey* NOT duck* NOT mammal*

Inclusion and Exclusion Criteria

In the present meta-analysis studies were eligible for inclusion if they met the following criteria:

- Both growth performance (ADFI, ADG, and feed conversion ratio [**FCR**]) and infection outcomes (e.g., oocyst excretion and lesion scores) were presented simultaneously.
- Experiments were carried out on broiler chickens, irrespective of sex.
- At least one of *E. acervulina, E. maxima*, or *E. tenella* were used as the infective species; experiments using other species (e.g., *E. brunetti*) were included only if in a mixed infection using one of the 3 aforementioned species.

Study Selection

A total of 4,600 unique records identified through the literature searches were examined using the aforementioned inclusion and exclusion criteria. The relevance of these studies was assessed in a 3-stage process, largely based on Stewart et al. (2007). Initially, titles and abstracts were inspected by the primary reviewer and the studies deemed irrelevant were discarded. Next, a secondary reviewer was asked to go through a 25% subsample of papers in order to calculate a kappa (k) score. A k score quantifies the strength of agreement between reviewers and can be used to determine the accuracy and reliability of the primary reviewer (Edwards et al., 2002). The k score of 0.70 indicated substantial strength of agreement between the two reviewers (Landis and Koch, 1977). Subsequently, the remaining papers were read in full by the primary reviewer. At this stage of the study selection, the main reason for exclusion was lack of relevant data for either performance or infection outcomes. A detailed summary of study selection procedures is presented in a Preferred Reporting Items for Systematic Reviews and Meta-analyses (**PRISMA**) Flow Diagram in Figure S1.

Data Extraction and Critical Appraisal

The following data, originating from 72 peer-reviewed articles, correspond to 521 treatments performed on 20,756 broiler chickens, were extracted into a purpose-built database in relation to the objectives:

- First author name
- Publication year
- Publication location
- *Eimeria* species
- Oocyst dosage
- Inoculation day
- Study duration in days
- Broiler strain
- Geographical location
- Sample size
- ADFI
- ADG
- FCR
- Macroscopic lesion scores
- Oocyst excretion
- Mortality
- Treatment of groups: untreated, given ionophores, synthetic anticoccidials, or vaccinated
- Standard deviation (**SD**), standard error (**SE**), or standard error of the mean (**SEM**). SEM and SE were converted into SD (Higgins et al., 2008). If only pooled SD, SE, or SEM were reported, these were used as an approximation for all groups. In cases where no measure of variation was provided, the SD was imputed. Since the studies were independent with different experimental designs and authors, it is reasonable to assume that missing values were missing completely at random (Eckert et al., 2021).

Each extracted data point corresponded to the observed mean of a treatment group.

For both dependent and independent factors, there was variation in the number of observations available as not all manuscripts reported data for all factors. Data on as many factors as possible were collected; however, there were some cases where no data were available (e.g., oocyst excretion and mortality). Furthermore, it became apparent that mortality data from the papers were unreliable, likely due to the large number of experiments where low mortality was observed in treatments not using control methods, whereas greater numbers of mortalities were reported in a smaller number of studies assessing the control method treatments. A second explanation for the lower number of mortalities when control methods were not used may reflect the fact that the birds were removed from the treatment as per the humane endpoints outlined in the experiments' ethical approval, and as such are not reported in the subsequent manuscript as mortalities.

In some instances, only 2 performance outputs were presented in the manuscript (e.g., ADFI and ADG); therefore, the third performance output (i.e., FCR) was calculated using the given data. There was substantial variation in the way that oocyst excretion was reported by the authors of different papers. Most studies reported oocyst excretion during specific days of the infection and for this reason both mean and maximum oocyst excretion were calculated to account for the different timepoints oocyst excretion was presented across experiments. To calculate mean oocyst excretion, the data were averaged over the days in which data were presented. The effect of *Eimeria* infection on anticoccidial index was explicitly investigated by 3 studies, with the remaining experiments providing insufficient data for the primary reviewer to calculate this variable. Thus, this variable was not considered further in the analysis.

For each article inserted into the database, the performance variables (ADFI, ADG, and FCR) were expressed as percentage change from their respective

control groups. Scaling the data in this manner allowed the comparison of the effect of *Eimeria* infection on performance across studies because we are able to account for a priori differences in performance between birds of different ages, strains (Abdullah et al., 2009) and between experiments where feed composition may differ (Oikeh et al., 2019). By definition, each of these factors determines the growth trajectories and the growth performance of the birds.

Reported sample sizes and standard errors were recorded in order to provide weights for the meta-analysis and to account for a variable degree of accuracy across studies. In cases where this information was not given, estimated standard errors were derived and used as weights in accordance with the methodology of McPhee et al. (2006).

Articles were critically appraised to quantify any potential sources of bias that may affect the results of experiments using SYRCLE's risk of bias tool (Hooijmans et al., 2014). This tool was adapted from the Cochrane risk of bias tool for randomized controlled trials (Higgins et al., 2011) and is one of the most comprehensive methods used for critical appraisal in animal studies.

Statistical Analyses

Descriptive statistics across studies for the main continuous and categorical factors used in the statistical models can be seen in Table 2 and Supplementary Table 1, respectively. The comparison of adjusted means for ADFI, ADG, and FCR was performed by variance-covariance analysis, with subsequent comparison of means by Tukey test at 5% significance level.

To limit the possibility of obtaining biased parameter estimates in the meta-regression, the existence of random study effects was formally assessed using the likelihood ratio tests (Bolker et al., 2009). The goodness of fit between the null models with performance and infection (with the exception of lesion scores, see below) outputs as a dependent variable and an intercept only were compared with nested models with one added random effects term using a chi-squared distribution. The results of these likelihood ratio tests provided evidence against the null models. Therefore, linear mixed effects regression models were fitted to the data with a random effect associated with each study.

The main fixed effects were chosen from the *a priori* set of variables (*Eimeria* species, broiler strain, study duration, geographical location, and control methods were considered) and all possible two-way interactions between the independent and dependent variables were considered. Moreover, the day of inoculation was included in the model as a covariate, in order to account for the potential effect of age on the development of the infection (Song et al., 2021). Correlations between the main factors were tested, including a Pearson's chi-squared test between continuous and ordinal factors. Although in most cases there were no considerable signs of multicollinearity between the main factors, as their correlations did not exceed 0.60, there was a significant chi-squared test value between geographical location and broiler strain, and therefore the former was excluded from further analyses.

Observations were weighted by the inverse of standard deviation to account for any potential heteroscedasticity which may arise from factors such as differences in sample sizes among studies included in the meta-analysis. LMER model fitting was performed with the nlme package (version 3.1.148) in R (version 4.0.2) by using the restricted maximum likelihood method. Model validity was assessed by examining QQ plots of the standardised residuals against the fitted values generated separately for the fixed and random components. Oocyst excretion data were either collected as back-transformed means or were back-transformed prior to analysis. The diagnostic plots of the back-transformed oocyst excretion revealed that the data were normally distributed and did not violate the LMER model assumptions. However, the diagnostic plots of the performance data expressed as percentage change from the uninfected control birds showed that the data were not normally distributed, therefore, these data were transformed using the logit transformation, at which point the models no longer violated the LMER model assumptions.

As lesion scores are an ordinal variable, a multinomial regression was implemented rather than the LMER detailed above, to examine the relationship between the

Table 2. Descriptive statistics for the main continuous variables included in the meta-analysis (Mean values and standard deviations; medians, minimum and maximum values).

Variables	*n*	Mean	SD	Median	Min	Max
Independent variables						
Oocyst dose	521	115,551	249,226	50,000	0.00	1,681,000
Dependent variables						
ADFI (g/ d)	521	83.2	29.1	83.0	27.4	186
ADG (g/ d)	521	42.6	15.5	42.0	11.3	89.4
FCR	521	2.03	0.59	1.85	1.13	4.43
Maximum oocyst per gram	393	568,397	2,003,424	39,405	0.00	25,400,000
Mean oocyst per gram	339	544,526	1,741,778	15,812	0.00	15,739,000
Scaled ADFI (% of controls)	521	−6.00	9.20	−3.20	−38.4	19.4
Scaled ADG (% of controls)	521	−13.7	17.4	−9.00	−86.5	37.1
Scaled FCR (% of controls)	521	12.0	20.8	4.40	−23.2	143

n represents the number of observations in the dataset.

same independent variables and lesion scores. Model fitting was performed using the ordinal package (version 2019.12.10) in R. Observations again were weighted by the inverse of standard deviation to account for any potential heteroscedasticity. Finally, Pearson correlation analyses were conducted to study the relationship between performance variables and infection outcomes. All data were used to determine these correlations.

RESULTS

Descriptive Analysis

Seventy-two articles describing experiments which fulfilled the inclusion criteria were identified. Six of these articles were published prior to 2000, sixteen articles were published between 2000 and 2009, thirty-seven articles were published between 2010 and 2019 and the remaining 13 articles were from 2020 to 2021. There were 126 treatments with uninfected animals, 15 treatments were infected with *E. acervulina*, 51 were treatments infected with *E. maxima*, 192 treatments were infected with *E. tenella* and 137 treatments were infected with mixed *Eimeria* species. The mean, median, minimum, and maximum oocyst doses are presented in Supplementary Table 2. For *E. acervulina* the oocyst doses ranged from 3,000 to 1,500,000 oocysts per bird, for *E. maxima* the oocyst doses ranged from 3,000 to 175,000 oocysts per bird, for *E. tenella* the oocyst doses ranged from 500 to 200,000 oocysts per bird and the mixed species doses ranged from 5,000 to 1,681,000 oocysts per bird. The breakdown of *Eimeria* species used in mixed infections is also presented in Supplementary Table 2.

Day of inoculation with *Eimeria* species varied between studies from d 1 to d 31 of age, with the majority of studies ($n = 29$) inoculating the birds on d 14 of age. The next most common days of inoculation were day 12 ($n = 7$), 15 ($n = 6$), and 21 ($n = 7$) of age. Geographical locations were grouped into North America, Europe, and 'Other'. Thirty-nine studies were from Europe, 2 from the Middle East, 21 studies were from North America, and the remaining 10 from Latin America or Asia and Pacific. There is scant information on the effect of broiler strain on the outcomes of coccidiosis in terms of broiler performance and infection parameters. Where such data are available, the genetic lines are often presented anonymously (e.g., line A and line B), or presented in insufficient detail. Given the detail provided and the numbers involved, we were not able to consider a greater granularity, other than the commercial broiler breeder, in relation to broiler strain for the purposes of this meta-analysis. Although there was some diversity in the broiler strains used among studies, it was possible to group them into four distinct classes: Group 1 contained Cobb strains ($n = 161$), group 2 included Hubbard strains ($n = 45$), group 3 composed of Ross strains ($n = 126$), and group 4 combined Arbor Acres, Partridge Shank, Huangshi, and other unidentified strains ($n = 189$). Similarly, the study duration varied from 15 to 49 d of age, therefore, these were also separated into 4 distinct groups, which were considered to account for the different stages of infections (see Supplementary Table 1).

In studies evaluating the consequences of different control methods there were insufficient data to assess this for specific control methods, and so these were separated into 4 classes. Group 1 included synthetic anticoccidials (e.g., diclazuril and amprolium, $n = 40$); group 2 constituted of ionophores (e.g., monensin and lasalocid, $n = 55$); group 3 contained different vaccines (e.g., Advent and Paracox, $n = 55$); group 4 were the untreated controls ($n = 371$; Supplementary Table 2). The day the birds were introduced to the different control methods varied substantially across studies, to such an extent that it was not possible to account for this in the subsequent analysis without impeding model convergence.

Adjusted Means of Performance Variables

The adjusted means of performance variables are presented in Table 3. The ADFI of the groups infected with *E. acervulina* and *E. maxima* were not significantly different ($P > 0.05$) from the control group, whereas infection with *E. tenella* or mixed species reduced ADFI by 8 and 10%, respectively compared with the uninfected group ($P < 0.05$). All infected groups had reduced ADG compared with the uninfected group ($P < 0.05$). The greatest reduction in ADG was observed in the group infected with *E. acervulina*, which showed a 32% reduction when compared with the uninfected group. The FCR of the *E. tenella* group was not significantly different from the uninfected group ($P > 0.05$). The remaining infected groups all showed significant increases in FCR compared to the uninfected group ($P < 0.05$), with the greatest increase observed in the mixed species infection group (44%).

Percentage Change in ADFI, ADG, and FCR

The effects of *Eimeria* dose on scaled ADFI, ADG, and FCR estimated from the univariate model are in

Table 3. Adjusted means of ADFI, ADG, and FCR according to treatment groups of broilers experimentally infected with different *Eimeria* species.

Variable	Control	*E. acervulina*	*E. maxima*	*E. tenella*	Mixed species	RSD
ADFI (g/d)	101[b]	94.1[ab]	99.6[ab]	93.0[a]	91.1[a]	5.13
ADG (g/ d)	54.2[b]	36.6[a]	45.8[a]	43.2[a]	40.1[a]	4.55
FCR	1.92[a]	2.46[bc]	2.49[bc]	2.14[ab]	2.76[c]	0.981

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio.
[a-c]Means within a column that do not share a common superscript are significantly different ($P < 0.05$).

Table 4. Main significant fixed effects and their two-way interactions on performance parameters: scaled ADFI, scaled ADG, and scaled FCR, estimated from the univariate model analysis. Performance was scaled to that of the respective uninfected controls (%).

	Probabilities		
Variables	ADFI	ADG	FCR
Oocyst dose	**<0.001**	**<0.001**	**0.049**
Eimeria species	0.436	0.731	0.293
Broiler strain	0.437	0.447	0.890
Study duration	0.275	**0.003**	**0.010**
Oocyst dose × *Eimeria* species	**0.001**	**<0.001**	0.191
Oocyst dose × Broiler strain	**0.079**	**0.002**	0.478
Oocyst dose × Study duration	**0.025**	**<0.001**	0.220

Boldface indicates a significant main effect or interaction ($P < 0.05$) or tendency for a main effect or interaction ($P < 0.10$).

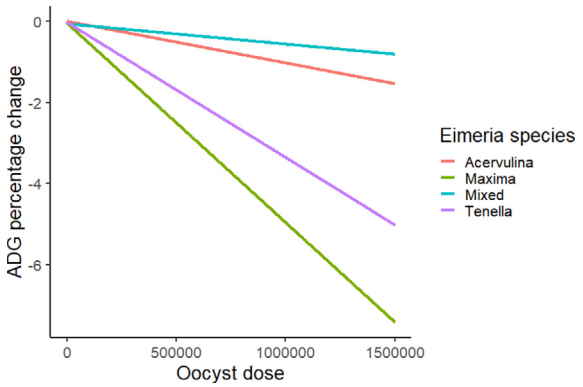

Figure 1. Change in scaled ADG of broilers infected with *Eimeria* species (*E. acervulina, E. maxima, E. tenella*, or mixed species) expressed as percentage change from their uninfected counterparts, to illustrate the interaction between oocyst dose and *Eimeria* species which was identified in the final linear mixed effects regression for ADG. Broiler strain and study duration were fixed in the model predictions. Scaled ADG was logit transformed to obtain normal distribution; the values presented are back transformed.

Table 4; the effects of dosing on the same scaled variables estimated by the final linear mixed effects regression model are in Table 5. The effect of *Eimeria* dose on all scaled performance variables was highly significant (ADFI and ADG, $P < 0.001$) or significant (FCR, $P < 0.05$; Table 4), confirming that increasing the infection dose of the parasite reduced ADFI and ADG, whilst increasing FCR. There was a highly significant interaction ($P < 0.001$) between *Eimeria* dose and species on both scaled ADFI and ADG variables, suggesting that the different species affected these 2 variables to different extents. Increasing the dose of *E. tenella* reduced scaled ADFI and ADG to a greater extent than *E. acervulina,* whereas increasing the dose of *E. maxima* decreased ADG to a greater extent than *E. tenella* (Table 5; Figure 1). Mixed species infections produced the smallest reductions in scaled ADFI and ADG.

There was a tendency for an interaction between oocyst dose and broiler strain on scaled ADFI ($P = 0.079$) and a significant interaction between these

Table 5. Main significant fixed effects and their two-way interactions in the final linear mixed effects regression model for scaled ADFI, scaled ADG, and scaled FCR.

	ADFI			ADG			FCR		
Variables	Estimate (β)	SE	*P*	Estimate (β)	SE	*P*	Estimate (β)	SE	*P*
Intercept	0.01722533	0.05106584	0.737	0.03583403	0.07899665	0.651	−0.1699414	0.2582399	0.512
Oocyst dose	0.00000062	0.00000061	0.315	0.00000017	0.00000105	0.872	0.0000021	0.0000043	0.630
Eimeria Species									
E. acervulina									
E. maxima	0.00027782	0.03707979	0.994	−0.04134551	0.06128056	0.501	0.2268951	0.2017978	0.263
E. tenella	−0.00576320	0.02983493	0.847	−0.02359677	0.05067297	0.642	0.0191954	0.1693535	0.910
Mixed species	−0.01398933	0.03093644	0.652	−0.06500297	0.05139509	0.208	0.1126695	0.1654173	0.497
Study duration (15−19 d)									
Study duration (20−25 d)	−0.00947250	0.04238133	0.824	−0.03148784	0.05900145	0.596	0.2295094	0.2509777	0.364
Study duration (26−33 d)	0.00573412	0.04742616	0.904	−0.01400153	0.06528429	0.831	0.2169083	0.2657199	0.418
Study duration (34−49 d)	−0.01056360	0.03896643	0.787	−0.03011795	0.05545461	0.589	0.0675172	0.2237089	0.764
Broiler strain									
Cobb									
Hubbard	0.00801848	0.03297991	0.809	0.01213835	0.04826391	0.802	0.1241337	0.1765262	0.485
Ross	−0.01043099	0.02226019	0.641	−0.00536181	0.03323094	0.872	−0.0109515	0.1144341	0.924
Other	−0.03948098	0.02532231	0.124	−0.03477788	0.03886901	0.375	−0.0004623	0.1279327	0.997
Oocyst dose × *E. acervulina*									
Oocyst dose × *E. maxima*	0.00000072	0.00000106	0.499	−0.00000390	0.00000161	**<0.017**	0.0000024	0.0000041	0.558
Oocyst dose × *E. tenella*	−0.00000086	0.00000032	**0.084**	−0.00000231	0.00000051	**<0.001**	0.0000026	0.0000019	0.170
Oocyst dose × Mixed species	0.00000013	0.00000007	**0.008**	0.00000053	0.00000013	**<0.001**	−0.0000006	0.0000005	0.167
Oocyst dose × Cobb									
Oocyst dose × Hubbard	−0.00000055	0.00000053	0.307	−0.00000011	0.00000097	0.909	−0.0000021	0.0000049	0.669
Oocyst dose × Ross	−0.00000018	0.00000011	**0.089**	−0.00000036	0.00000020	**0.073**	0.0000004	0.0000007	0.581
Oocyst dose × Other	0.00000003	0.00000008	0.737	0.00000018	0.00000016	0.253	−0.0000002	0.0000005	0.772
Oocyst dose × Study duration (15−19 d)									
Oocyst dose × Study duration (20−25 d)	−0.00000091	0.00000061	0.137	−0.00000109	0.00000104	0.294	−0.0000008	0.0000043	0.847
Oocyst dose × Study duration (26−33 d)	−0.00000074	0.00000061	0.231	−0.00000058	0.00000104	0.582	−0.0000013	0.0000043	0.769
Oocyst dose × Study duration (34−49 d)	-0.00000068	0.00000061	0.264	−0.00000046	0.00000103	0.655	−0.0000018	0.0000043	0.686

Parameter estimates (β) with their standard errors. Performance was scaled to that of the respective uninfected controls (%).
Boldface indicates a significant main effect or interaction ($P < 0.05$) or tendency for a main effect or interaction ($P < 0.10$).

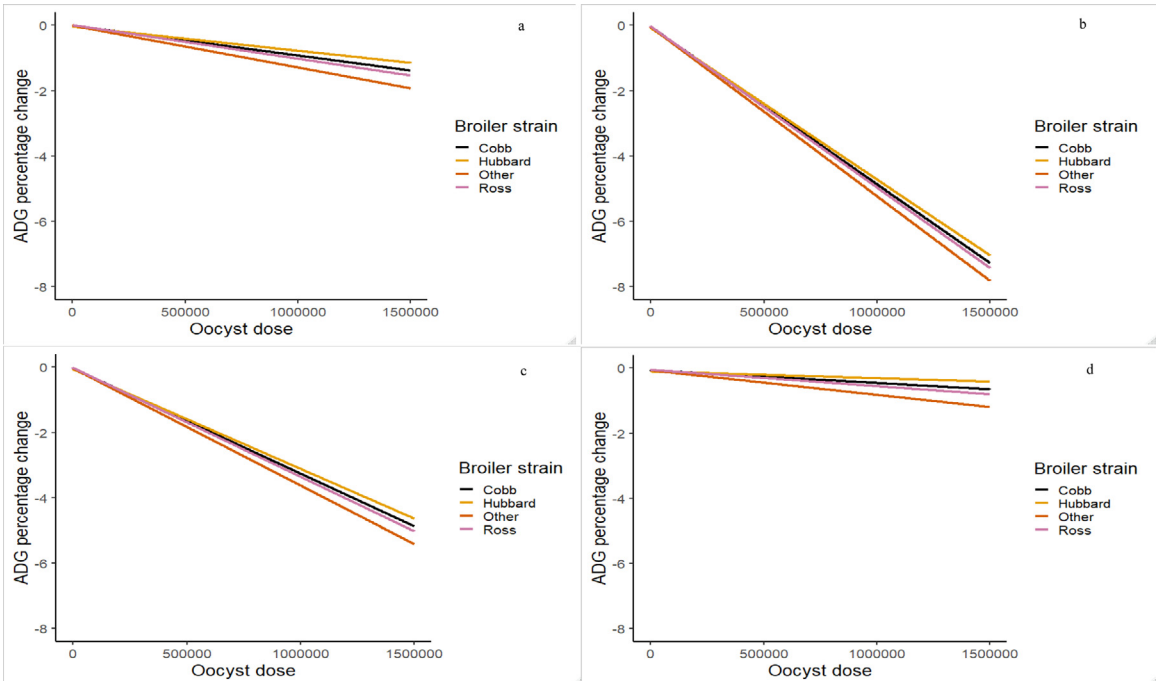

Figure 2. Change in scaled ADG of broilers infected with *Eimeria* species (*E. acervulina* (A), *E. maxima* (B), *E. tenella* (C), or mixed species (D)) expressed as percentage change from their uninfected counterparts, to illustrate the interaction between oocyst dose and broiler strain which was identified in the final linear mixed effects regression for scaled ADG. Broiler strain and study duration were fixed in the model predictions. Scaled ADG was logit transformed to obtain normal distribution; the values presented are back transformed.

2 variables on scaled ADG (Table 4, $P < 0.05$). The interaction was due to the 'Other' broiler strains (group 4) having a greater reduction in their scaled performance than the remaining groups considered (Table 5). However, from the considered strains, the scaled performance of the Ross birds was penalized to a greater extent than both Cobb and Hubbard strains regardless of *Eimeria* species (Table 5 and Figure 2).

Finally, there was an interaction between oocyst dose and study duration on scaled ADFI and ADG (Table 5, $P < 0.05$). Although this interaction was not formally detected by the linear models, the effect of study duration on these 2 variables can be expected since the longer experimental periods were more likely to include both the acute and recovery periods of the infection (Willis and Baker, 1981; Jeurissen et al., 1996; Sakkas et al., 2018), and therefore, the reductions in these 2 variables were attenuated with time.

Table 6. Main significant fixed effects and their two-way interactions on infection outcome parameters from the univariate model analysis.

	Probabilities		
Variables	Mean OPG	Max OPG	Lesion score
Oocyst dose	0.794	0.590	**0.001**
Eimeria species	0.364	0.426	**0.020**
Broiler strain	0.784	0.954	0.242
Study duration	0.198	0.868	0.775
Oocyst dose × *Eimeria* species	0.382	**0.067**	**<0.001**
Oocyst dose × Broiler strain	0.916	0.958	0.111
Oocyst dose × Study duration	0.557	0.559	**0.022**

Boldface indicates a significant main effect or interaction ($P < 0.05$) or tendency for a main effect or interaction ($P < 0.10$).

Infection Outcomes

The effects of *Eimeria* dose on infection outcomes estimated from the univariate model are in Table 6; the effects of dosing on the same variables are in Table 7, these were estimated by the final linear mixed effects regression model (for OPG and OPG max) and the final multinomial regression model (Lesion scores). There was a tendency for an interaction between oocyst dose and *Eimeria* species on max OPG ($P = 0.067$) only (Table 6). This was due to a greater oocyst excretion during parasitism with *E. tenella* than with the other species, which is consistent with the greater fecundity of this species (Williams, 2001). Lesion scores appeared to reflect much better the effects of infection on the parasitized host, as they were significantly affected by *Eimeria* dose, *Eimeria* species and their respective interaction (Tables 6 and 7, $P < 0.05$). The interaction was caused by an increase in the lesion scores during parasitism with *E. maxima* and *E. tenella*, compared to parasitism with *E. acervulina* or mixed infections (Figure 3).

Efficacy of Control Methods on Percentage Change in ADFI, ADG, and FCR

The effects of *Eimeria* dose of treated and untreated birds on scaled ADFI, ADG, and FCR estimated from the univariate model are presented in Table 8; the effects of dosing with the parasite on the same scaled variables estimated by the final linear mixed effects regression model are in Table 9. There was a significant effect of control methods on scaled ADG and FCR (Table 8, $P < 0.05$), and a highly significant interaction between oocyst dose and control methods on ADFI ($P < 0.001$).

Table 7. Main significant fixed effects and their two-way interactions in the linear mixed effects regression model for mean oocyst excretion and maximum oocyst excretion and the multinomial regression model for lesion scores.

	Mean OPG			Max OPG			Lesion scores		
Variables	Estimate (β)	SE	P	Estimate (β)	SE	P	Estimate (β)	SE	P
Intercept	2,159,942	2,358,724	0.363	1,361,905	3,024,605	0.654			
Oocyst dose	−17.500000	30.9	0.573	−33.3	35.5	0.351	0.0000166	0.0000202	0.410
Eimeria species									
E. acervulina									
E. maxima	−990,977	1,477,896	0.505	−201,589	1,725,245	0.907	0.921	1.02	0.369
E. tenella	−306,677	1,191,481	0.798	−69,268	1,445,567	0.962	0.625	0.870	0.472
Mixed species	-1,889,708	1,280,563	0.145	−1,196,674	1,528,609	0.436	0.740	0.858	0.389
Study duration (15−19 d)									
Study duration (20−25 d)	−714,738	2,331,167	0.761	−688,737	2,735,758	0.803	0.195	1.04	0.852
Study duration (2633 d)	-1,642,499	2,627,994	0.536	-1,702,147	3,142,719	0.591	0.759	1.08	0.480
Study duration (34−49 d)	−2,727,135	2,376,505	0.259	-1,726,589	2,833,538	0.546	1.43	0.98	0.145
Broiler strain									
Cobb									
Hubbard	532,373	1,962,209	0.788	53,703	1,965,767	0.978	−1.137	1.046	0.277
Ross	765,814	906,120	0.404	703,051	1,062,666	0.512	−0.677	0.461	0.142
Other	-1,085,514	934,509	0.254	−957,956	1,302,757	0.466	−0.409	0.559	0.464
Oocyst dose × *E. acervulina*									
Oocyst dose × *E. maxima*	31.6	38.9	0.420	24.8	45.3	0.586	0.0000446	0.0000354	**0.011**
Oocyst dose × *E. tenella*	27.6	13.3	**0.041**	44.7	15.5	**0.005**	0.00009007	0.00000858	**<0.001**
Oocyst dose × Mixed species	1.90	3.20	0.553	0.900	3.70	0.817	-0.00000363	0.00000219	**0.098**
Oocyst dose × Cobb									
Oocyst dose × Hubbard	−27.1	110	0.806	−31.2	42.1	0.460	0.0000188	0.0000184	0.307
Oocyst dose × Ross	0.200	8.900	0.982	−5.80	6.40	0.366	-0.00000047	0.00000316	0.882
Oocyst dose × Other	−5.200	8.500	0.542	−5.00	7.00	0.479	−0.00000130	0.00000297	0.661
Oocyst dose × Study duration (15−19 d)									
Oocyst dose × Study duration (20−25 d)	11.5	30.4	0.708	33.3	35.0	0.344	−0.00000701	0.0000200	0.726
Oocyst dose × Study duration (26−33 d)	15.5	30.8	0.617	39.0	34.6	0.263	−0.00000792	0.0000200	0.692
Oocyst dose × Study duration (34−49 d)	23.5	29.7	0.432	38.9	34.5	0.262	−0.0000129	0.0000199	0.517

Parameter estimates (β) with their standard errors.
Boldface indicates a significant main effect or interaction ($P < 0.05$) or tendency for a main effect or interaction ($P < 0.10$).

As expected, the absence of a control method for the *Eimeria* infection decreased scaled ADFI except for the ionophore treatment. Similarly, scaled ADG increased in the groups treated with control methods compared to the absence of control methods. Furthermore, FCR was improved in the groups treated with control methods compared to the absence of control methods (Table 9). Scaled ADG was significantly improved in birds treated with synthetic anticoccidials compared with the untreated birds ($P < 0.01$), whereas FCR was significantly improved by ionophore treatment compared with the untreated birds ($P < 0.01$). The interaction between *Eimeria* oocyst dose and control methods on scaled ADFI (Table 8, Figure 4) was caused by the greater scaled ADFI of vaccinated birds compared to the untreated birds, as oocyst dose increased.

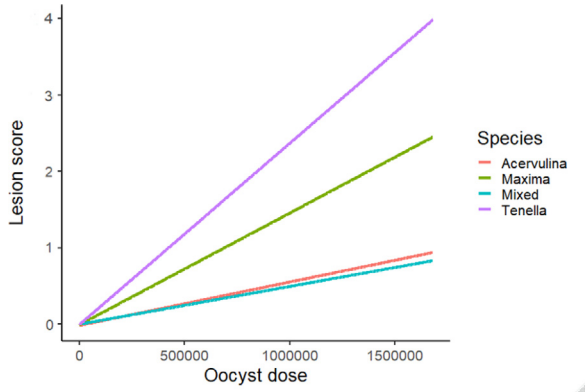

Figure 3. Change in lesion scores of broilers infected with *Eimeria* species (*E. acervulina, E. maxima, E. tenella*, or mixed species), to illustrate the interaction between oocyst dose and *Eimeria* species which was identified in the final linear multinomial regression model for lesion score. Broiler strain and study duration were fixed in the model predictions.

Table 8. Main significant fixed effects and their two-way interactions on performance parameters: scaled ADFI, scaled ADG, and scaled FCR, from the univariate model analysis.

	Probabilities		
Variables	*ADFI*	*ADG*	*FCR*
Oocyst dose	**<0.001**	**<0.001**	**<0.001**
Eimeria species	0.210	0.472	**<0.001**
Broiler strain	0.707	0.642	0.967
Control method	0.348	**0.004**	**<0.001**
Study duration	0.244	**0.001**	**0.006**
Oocyst dose × *Eimeria* species	**<0.001**	**<0.001**	**0.009**
Oocyst dose × Broiler strain	**<0.001**	**<0.001**	**0.006**
Oocyst dose × Control method	**<0.001**	0.210	0.682
Oocyst dose × Study duration	**0.008**	**<0.001**	**<0.001**

Performance was scaled to that of the respective uninfected controls (%). The extracted data used in the models are from studies investigating the efficacy of control methods only ($n = 150$).
Boldface indicates a significant main effect or interaction ($P < 0.05$) or tendency for a main effect or interaction ($P < 0.10$).

Table 9. Main significant fixed effects and their two-way interactions in the final linear mixed effects regression model for scaled ADFI, scaled ADG, and scaled FCR.

	ADFI			ADG			FCR		
Variables	Estimate (β)	SE	P	Estimate (β)	SE	P	Estimate (β)	SE	P
Intercept	-0.01309029	0.06448283	0.839	0.17006656	0.13416732	0.206	−0.522	0.150	**0.001**
Oocyst dose	-0.00000087	0.00000052	**0.092**	-0.00000179	0.00000111	0.109	0.00000370	0.00000149	**0.014**
Eimeria species									
E. acervulina									
E. maxima	0.05953426	0.03479765	**0.088**	-0.14897737	0.07553180	**0.049**	0.39831890	0.08117328	**<0.001**
E. tenella	0.04613055	0.02968440	0.121	-0.01217394	0.06689967	0.856	0.24494920	0.07616606	**0.001**
Mixed species	0.01964922	0.03150528	0.533	-0.12741089	0.06924640	**0.067**	0.41762140	0.07209046	**<0.001**
Study duration (15-19 d)									
Study duration (20−25 d)	0.00858392	0.05456209	0.875	-0.03545712	0.11025866	0.748	0.138236	0.129832	0.288
Study duration (26−33 da)	-0.01834796	0.06054166	0.762	-0.07201633	0.12102943	0.552	0.240892	0.141631	**0.090**
Study duration (34−49 d)	0.00102330	0.05229033	0.984	0.01470628	0.10640364	0.890	0.134157	0.125911	0.287
Broiler strain									
Cobb									
Hubbard	-0.00088632	0.04367032	0.984	-0.03512051	0.08983913	0.697	0.0747432	0.1167238	0.524
Ross	0.00708967	0.02493726	0.777	-0.00307046	0.05124608	0.952	-0.0234238	0.0600374	0.698
Other	-0.03156505	0.03020763	0.300	-0.05438105	0.06214330	0.385	0.0645369	0.0674084	0.342
No control methods									
Ionophores	-0.00814097	0.01376273	0.555	0.03848765	0.03150656	0.223	-0.0897003	0.02925243	**0.002**
Vaccines	0.0321673	0.01872701	**0.087**	0.00319685	0.04722017	0.946	-0.0108003	0.04007378	0.788
Synthetic anticoccidials	0.02903901	0.01905848	0.128	0.11228191	0.03959176	**0.005**	-0.0717925	0.03803701	**0.060**
Oocyst dose × *E. acervulina*									
Oocyst dose × *E. maxima*	-0.00000005	0.00000027	0.866	0.00000083	0.00000059	0.162	-0.00000050	0.00000065	0.403
Oocyst dose × *E. tenella*	-0.00000089	0.00000025	**<0.001**	-0.00000341	0.00000058	**<0.001**	0.00000130	0.00000071	**0.067**
Oocyst dose × Mixed species	0.00000006	0.00000008	0.413	0.00000045	0.00000018	0.012	-0.00000060	0.00000022	**0.004**
Oocyst dose × Cobb									
Oocyst dose × Hubbard	0.00000059	0.00000041	0.145	0.00000327	0.00000093	**0.001**	-0.00000150	0.00000202	0.463
Oocyst dose × Ross	-0.00000009	0.00000009	0.318	-0.00000018	0.00000020	0.375	0.00000040	0.00000028	0.172
Oocyst dose × Other	0.00000007	0.00000007	0.316	0.00000028	0.00000016	**0.074**	-0.00000030	0.00000020	0.098
Oocyst dose × No control methods									
Oocyst dose × Ionophores	0.00000015	0.00000003	0.109	0.00000013	0.00000008	**0.099**	0.0000001	0.00000008	0.501
Oocyst dose × Vaccines	-0.00000019	0.00000012	**<0.001**	-0.00000012	0.00000024	0.630	0.0000003	0.0000002	0.114
Oocyst dose × Synthetic anticoccidials	-0.00000006	0.00000015	0.702	-0.00000033	0.00000028	0.237	0.0000004	0.00000039	0.323
Oocyst dose × Study duration (15-19 days)									
Oocyst dose × Study duration (20-25 days)	0.00000050	0.00000049	0.303	0.00000032	0.00000106	0.760	-0.00000210	0.00000137	0.133
Oocyst dose × Study duration (26-33 days)	0.00000062	0.00000049	0.211	0.00000054	0.00000106	0.613	-0.00000180	0.00000138	0.192
Oocyst dose × Study duration (34-49 days)	0.00000073	0.00000049	0.134	0.00000103	0.00000106	0.330	-0.00000280	0.00000139	**0.042**

Parameter estimates (β) with their standard errors. Performance was scaled to that of the respective uninfected controls (%). The extracted data used in the models are from studies investigating the efficacy of control methods only ($n = 150$).

Boldface indicates a significant main effect or interaction ($P < 0.05$) or tendency for a main effect or interaction ($P < 0.10$).

Efficacy of Control Methods on Infection Outcomes

The effects of *Eimeria* dose on infection outcomes estimated from the univariate model are in Table 10. The effects of dosing on the same variables are presented in Table 11; these were estimated by the final linear mixed effects regression model (for OPG and OPG max) and the final multinomial regression model (Lesion scores). As expected, there was a significant effect of control methods on lesion scores (Table 11, $P < 0.01$), as the use of all control methods caused a reduction in lesion scores. However, there was no effect of control methods on mean OPG or max OPG (Table 10).

Correlations Between Dependent Variables

The correlations between dependent variables (both performance and infection outcomes) are shown on Table 12. As expected, there were high correlations (>0.60) and in the expected direction between performance outcomes, although the correlation between scaled ADFI and FCR was only modest (∼ 0.30). The correlations between the OPG traits and lesion scores were virtually non-existent. The correlations between production and OPG variables were relatively low (<0.20). However, the correlations between production variables and lesion scores were moderate to high (0.30−0.70), and in the expected direction, that is, high lesion scores were associated with reductions in scaled ADFI and scaled ADG, and in increases in FCR.

DISCUSSION

Eimeria infection is a major issue for the poultry industry (Blake et al., 2020) with significant economic losses (Gilbert et al., 2020). Such losses arise from the consequences of infection on performance parameters (e. g., reduced growth rates, pathogen-induced anorexia and inefficient nutrient utilization) and infection outcomes (e.g., mortality and lesion scores; Kipper et al.,

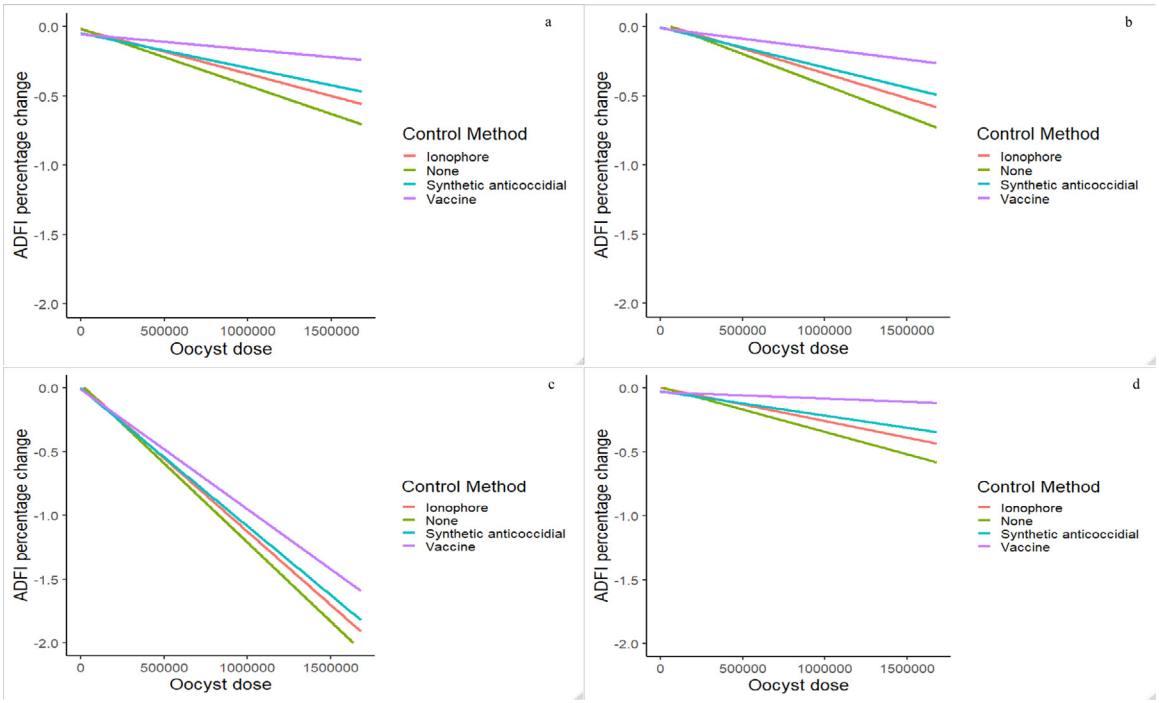

Figure 4. Change in scaled ADFI of broilers infected with *Eimeria* species (*E. acervulina* (A), *E. maxima* (B), *E. tenella* (C), or mixed species (D)), expressed as percentage change from their uninfected counterparts, to illustrate the interaction between oocyst dose and control method which was identified in the final linear mixed effects regression for ADFI. Broiler strain and study duration were fixed in the model predictions. ADFI was logit transformed to obtain normal distribution; the values presented are back transformed. The extracted data used in the models are from studies investigating the efficacy of control methods only ($n = 150$).

2013; Blake et al., 2020). As far as we are aware this is the first meta-analysis that looked simultaneously at the effects of *Eimeria* infections on both performance and infection outcomes in broilers. This inclusion approach had several implications that were reflected in the number of papers considered and the conclusions drawn.

Data Acquisition and Limitations

There were several papers that reported on the effect of coccidiosis on the performance of broilers; these are both small scale-type studies and large-scale ones that measured performance in conditions similar to industry. Most such studies reported at least 2 performance outcomes (most frequently ADG and FCR), so that the third performance outcome could be calculated from them. This, however, was not the case when reporting infectious outcomes.

In most cases, studies reported performance together with only one infectious outcome, most frequently a measurement or measurements of the number of oocysts excreted in the environment (e.g., OPG). What was reported, however, in the literature in relation to this outcome varied significantly between studies: some studies reported a single OPG measurement taken at inconsistent time points postinfection, several studies reported the maximum OPG over the postinfection period, others reported mean OPG again over a very variable period of time, which in some cases considered both acute and recovery stages of infection. This was compounded by the fact that oocyst excretion is highly variable and is affected by a variety of factors, including the method of assessment (Bortoluzzi et al., 2018), parasite fecundity (Soutter et al., 2020), diet composition (Oikeh et al., 2019) and even the time of sampling (Villanúa et al., 2006). As several studies reported oocyst excretion during specific days of the infection both mean and maximum oocyst excretion were calculated to account for the different time points that oocyst excretion was presented across experiments. We considered maximum OPG, a useful infection outcome variable corresponding to the peak of the acute stages of the infection (Cha et al., 2018). The fact that OPG mean and OPG max were highly correlated further justified this choice. However, both OPG variables were only very weakly correlated with performance variables

Table 10. Main significant fixed effects and their two-way interactions on infection outcome parameters from the univariate model analysis.

	Probabilities		
Variables	Mean OPG	Max OPG	Lesion score
Oocyst dose	0.940	0.688	**0.053**
Eimeria species	0.776	0.371	0.554
Broiler strain	0.426	0.680	0.994
Control method	0.945	0.544	**0.006**
Study duration	0.518	0.726	0.578
Oocyst dose × *Eimeria* species	0.856	0.374	0.150
Oocyst dose × Broiler strain	0.910	0.668	0.487
Oocyst dose × Control method	0.951	0.963	0.932
Oocyst dose × Study duration	0.635	0.492	0.469

The extracted data used in the models are from studies investigating the efficacy of control methods only ($n = 150$).

Boldface indicates a significant main effect or interaction ($P < 0.05$) or tendency for a main effect or interaction ($P < 0.10$).

Table 11. Main significant fixed effects and their two-way interactions in the linear mixed effects regression model for mean oocyst excretion and maximum oocyst excretion and the multinomial regression model for lesion scores.

	Mean OPG			Max OPG			Lesion score		
Variables	Estimate (β)	SE	P	Estimate (β)	SE	P	Estimate (β)	SE	P
Intercept	7,996,930	10224186	0.435	1633861	30,95272	0.598			
Oocyst dose	−87.0	122	0.475	−25.2	29.0	0.387	0.0000121	0.0000274	0.658
Eimeria species									
E. acervulina									
E. maxima	−4,089,742	4953035	0.410	−654475	13,90524	0.638	2.533	1.458	**0.082**
E. tenella	−269,540	4258255	0.950	−251924	12,56270	0.841	2.108	0.942	**0.025**
Mixed species	−3,407,891	4448787	0.444	-1111047	12,60304	0.379	3.533	1.523	**0.020**
Study duration (15−19 d)									
Study duration (20−25 d)	−8,124,734	9167530	0.376	-1568016	27,59949	0.570	0.900	2.285	0.694
Study duration (26−33 d)	−8,931,099	10061265	0.376	-1908896	30,05121	0.526	1.379	2.172	0.525
Study duration (34−49 d)	−11,987,767	9470499	0.213	-2596164	28,24397	0.359	1.751	2.240	0.434
Broiler strain									
Cobb									
Hubbard	4,972,164	5980574	0.411	656,795	17,64726	0.711	−1.713	2.123	0.420
Ross	4,495,816	2985784	0.140	914,951	87,1540	0.299	−1.740	0.912	**0.056**
Other	−1,580,615	3223732	0.627	−642500	10,97962	0.561	−0.218	1.001	0.828
No control methods									
Ionophores	−1.986,367	3082630	0.520	-1077661	66,9815	0.109	−2.177	0.853	**0.011**
Vaccines	98,113	3796492	0.979	−326933	87,8922	0.710	−2.802	1.244	**0.024**
Synthetic anticoccidials	18,765	4104193	0.996	−593503	94,8857	0.532	−2.259	1.09	**0.038**
Oocyst dose × *E. acervulina*									
Oocyst dose × *E. maxima*	36.0	41.0	0.377	8.70	10.6	0.410	-0.0000081	0.0000176	0.646
Oocyst dose × *E. tenella*	55.0	42.0	0.199	29.7	12.9	**0.022**	-0.0000035	0.0000142	0.807
Oocyst dose × Mixed species	2.00	14.00	0.888	0.200	3.80	0.952	-0.0000072	0.0000057	0.206
Oocyst dose × Cobb									
Oocyst dose × Hubbard	−72.0	80	0.370	−34.6	20.2	**0.088**	0.0000380	0.0000318	0.232
Oocyst dose × Ross	−20.0	30.0	0.500	−5.80	5.60	0.303	0.00001937	0.0000129	0.134
Oocyst dose × Other	−26.0	29.0	0.381	−5.30	6.20	0.397	0.00000872	0.0000103	0.399
Oocyst dose × No control methods									
Oocyst dose × Ionophores	2.00	26.0	0.929	0.700	1.50	0.663	-0.000001005	0.000005629	0.858
Oocyst dose × Vaccines	−5.00	26.0	0.835	−1.00	4.70	0.835	0.000002539	0.000006176	0.681
Oocyst dose × Synthetic anticoccidials	−17.0	62.0	0.783	1.50	6.70	0.826	0.000006321	0.000009046	0.485
Oocyst dose × Study duration (15-19 days)									
Oocyst dose × Study duration (20-25 days)	73.0	97.0	0.453	27.2	27.8	0.330	-0.00000723	0.0000259	0.976
Oocyst dose × Study duration (26-33 days)	86.0	100	0.390	32.7	27.3	0.232	-0.00001994	0.0000267	0.455
Oocyst dose × Study duration (34-49 days)	98.0	95.0	0.305	32.6	27.3	0.234	-0.0000075	0.0000251	0.764

Parameter estimates (β) with their standard errors. The extracted data used in the models are from studies investigating the efficacy of control methods only ($n = 150$).

Boldface indicates a significant main effect or interaction ($P < 0.05$) or tendency for a main effect or interaction ($P < 0.10$).

(−0.10 to −0.20 for both ADFI and ADG), which is a reflection of the issues raised above.

As far as data regarding lesion scores are concerned, these were reported in a more consistent manner across experiments. This is probably a reflection of the consistent methodology to assess this, as all studies followed the long standing methods of Johnson and Reid (1970), and the scale of assessing these is relatively narrow. The correlations between lesion scores and performance were moderate to high, consistent with the findings of previous studies (Chasser et al., 2020), and reflecting the fact that lesion scores are a consistent measurement of the extent of intestinal damage. However, in contrast to Chasser et al. (2020), lesion scores were not correlated with either OPG mean or OPG max. On the other hand, Soutter et al. (2021) observed a positive correlation between parasite replication (measured by qPCR) and lesion scores. The contrasting results between these 2 studies and our study probably reflect the fact that our meta-analysis covered a wide range of studies, with

Table 12. Correlation coefficients and confidence intervals (in brackets) of the scaled performance and infection outcome variables.

	ADFI	ADG	FCR	OPG mean	OPG max	Lesion scores
ADFI						
ADG	0.628 (0.573, 0.677)					
FCR	−0.296 (−0.373, −0.215)	−0.771 (−0.804, −0.733)				
OPG mean	-0.154 (−0.256, −0.048)	−0.193 (−0.294, −0.088)	0.160 (0.054, 0.263)			
OPG max	−0.111 (−0.208, −0.013)	−0.191 (−0.284, −0.093)	0.194 (0.096, 0.288)	0.772 (0.723, 0.813)		
Lesion scores	−0.383 (−0.454, −0.307)	−0.663 (−0.735, −0.578)	0.336 (0.207, 0.454)	0.042 (−0.132, 0.214)	0.052 (−0.111, 0.212)	

All extracted data were used to analyse the correlations between these variables. Performance was scaled to that of the respective uninfected controls (%).

distinct experimental conditions including oocyst dosage and *Eimeria* species. Furthermore, the correlations between OPG measurements and performance, and lesion scores and performance, suggest that the latter more strongly reflect the impact of *Eimeria* infection on performance. However, as with OPG measurements, there are factors such as *Eimeria* strain (Allen et al., 2005), timing of assessment and management conditions (e.g., floor pens vs. battery cages; Bafundo et al., 2008) which affect lesion severity and require consideration when designing such experiments.

In our initial search we also included mortality as an inclusion criterion since one of the consequences of *Eimeria* infection can be moderate to high mortality in broilers (Williams, 2005; Noack et al., 2019). However, during the process of data selection it became very clear that this variable was associated with several issues. In several, especially small-scale experiments, mortalities were not reported because there were different end points associated with them. As several of such experiments were conducted under regulations for animal welfare, birds were removed before their health deteriorated severely. In such cases, experiments reported zero mortality and at best reported the number of birds removed. This issue exaggerated the relationship between mortality rates and control methods: in a large number of experiments using a small number of birds, low or no mortality was observed in treatments not using control methods, whereas greater numbers of mortalities were reported in a smaller number of studies assessing the control method treatments, due to their focus. As such data heterogeneity was likely to affect the outcomes of the analyses and mortality rates were not considered any further in the meta-analysis.

Our analysis pointed towards several inherent problems associated with data quality, several of which were unnecessary, such as those associated with OPG assessment. Such issues should be carefully considered in future poultry coccidian research, in order for progress to be made, as has been suggested by Eckert et al. (2021).

Performance Outcomes

The primary objective of this meta-analysis sought to determine the effect of *Eimeria* infection, with *E. acervulina, E. maxima, E. tenella* or mixed species on broiler growth performance (ADFI, ADG, FCR). As expected, infection with *Eimeria* penalised the adjusted means of the performance variables (Sakkas et al., 2018; Teng et al., 2020; Eckert et al., 2021). However, the effect of infection with different *Eimeria* species on performance variables was different between our meta-analysis and that of Kipper et al. (2013). We found that ADFI was significantly reduced in birds infected with *E. tenella* and mixed species in comparison to the uninfected birds, whereas Kipper et al. (2013) found the greatest reduction in ADFI in birds infected with *E. acervulina* and *E. tenella*, which was not statistically different from the uninfected birds. Somewhat surprisingly, Kipper et al. (2013) found a significant increase in ADFI and significant reduction in ADG of birds infected with *E. maxima* compared to the uninfected birds. They suggested that this was due to the extensive area of damage (from the duodenum through to the ileum) caused by *E. maxima,* which caused an increase in FCR. The discrepancy between our results and Kipper et al. (2013) may be due to the lower number of *E. maxima* treatments in our meta-analysis (7 vs. 15 treatments, respectively). The difference in the number of experiments selected in our meta-analysis and Kipper et al. (2013) is due to differences in the inclusion/exclusion criteria used. In our study, we were interested in experiments which presented performance variables and infection outcomes simultaneously. Whereas in Kipper et al. (2013), the focus was on performance variables only and as such they were able to include a wider range of *E. maxima* studies. Furthermore, we found a significant increase in FCR across all infected treatments compared to the uninfected birds with the exception of *E. tenella*. As a consequence of the improvements in performance of broilers over the past 30 yr, the adjusted FCR mean (Table 3) was greater than what one might expect of current modern broiler strains. This is a reflection of to the rapid genetic progress in broiler productivity and efficiency over the 30 yr considered in the meta-analysis (Neeteson-van Nieuwenhoven et al., 2013).

In terms of scaled performance, dosing with the parasites reduced ADFI and ADG, while FCR increased. Interactions between oocyst dose and *Eimeria* species influenced the extent of the reduction in performance in broiler chickens. As *E. maxima* and *E. tenella* oocyst dose increased, scaled ADG was penalized to a greater extent than infection with *E. acervulina*. Kipper et al. (2013) have also identified a greater reduction in scaled ADG during infection with *E. maxima* compared with *E. acervulina* and suggested that this reduction was not related to the species effect on ADFI. The findings taken together, seemingly confirm a higher pathogenicity associated with *E. tenella* or *E. maxima* compared with the other *Eimeria* species (Györke et al., 2013; Prakashbabu et al., 2017). The authors appreciate that the pathogenicity and age of an *Eimera* isolate can influence the outcomes of the infection (Ruff et al, 1981). However, this was not accounted for in the meta-analysis as is not frequently provided in published papers.

It should be noted that during the mixed infections the levels of dosing with the individual parasites never reached the high values of dosing during infections with single species, with the exception of Waldenstedt et al. (1999). The fact that oocyst doses were lower in the mixed infections (Supplementary Table 1) may account for the lower impact of infection on performance outcomes compared with the individual *Eimeria* species. Due to the statistical model used, that is metaregression, it was a priori assumed that the relationship between oocyst dose and ADFI would be linear. In reality, however, this will not be the case, as low doses

of a pathogen may not lead to any changes in ADFI and over a wide range of pathogen doses the reduction in ADFI may be constant (Sandberg et al., 2006).

We were unable to investigate any interaction between *Eimeria* species and broiler strain due to low numbers of replications in the dataset, however, we were able to investigate the effect of broiler strain on growth performance during *Eimeria* infection. There was an interaction between broiler strain and oocyst dose on scaled ADG, due to Ross birds showing a greater reduction in scaled ADG on higher *Eimeria* doses than other strains considered.

Infection Outcomes

The secondary objective of this meta-analysis sought to build upon previous meta-analyses (Kipper et al., 2013; Eckert et al., 2021) by also assessing the effect of *Eimeria* infection on infection outcomes (oocyst excretion and lesion scores) and investigate whether there is any correlation between infection outcomes with the effects on performance variables. The interaction between *Eimeria* species and oocyst dose on oocyst excretion suggests that oocyst excretion increased as oocyst dose increased to a greater extent during infection with *E. tenella* compared to the other species. Similarly, lesion scores were more severe in *E. tenella* infections. The effect of *E. tenella* infection on both oocyst excretion and lesion scores are consistent with the greater fecundity (Williams, 2001) and pathogenicity (Györke et al., 2013; Prakashbabu et al., 2017; López-Osorio et al., 2020) of this species.

Interestingly, there was no correlation between oocyst excretion and lesion scores, contrary to recent work which has shown that there was a high positive correlation between oocyst excretion and lesion scores (Chasser et al., 2020). In the experiment by Chasser et al. (2020), oocyst excretion and lesion scores were measured on the same day. Therefore, since we calculated oocyst excretion as mean or maximum in our meta-analysis, the lack of correlation between oocyst excretion and lesion scores may also reflect the fact that oocyst excretion was not analysed as a single time-point measurement. Had we analysed the oocyst excretion from the day at which lesion scores were measured, it is possible that different conclusions could have been drawn. However, this may not be possible within a single experiment, as one is normally interested in the development of the infection over a period of time. Growth performance is considered a measure of tolerance to disease (the ability of a host to maintain performance while infected; Doeschl-Wilson and Kyriazakis, 2012); however, the definition of tolerance can also be extended to limiting the damage of a given parasite burden (Doeschl-Wilson and Kyriazakis, 2012). To that end, it is not surprising that we found moderate to high negative correlations between lesion scores and performance variables since they are a manifestation of the tolerance defence mechanism: the lower the lesion score the higher the ADFI and ADG, and vice versa.

Control Methods

The tertiary objective of this meta-analysis sought to assess the efficacy of various control methods on performance and infection outcomes in broilers infected with *Eimeria*. We considered the following control methods: synthetic anticoccidials (e.g., diclarizul and amprolium), ionophores (e.g., lasalocid and salinomycin), and vaccines (e.g., Advent and Paracox). Eckert et al. (2021) also investigated the efficacy of these control methods, however, we highlighted 2 knowledge gaps from their work: 1) it did not assess the efficacy of control methods across a range oocyst doses, 2) the efficacy of control methods was assessed on performance variables only and not infection outcomes. When broilers were treated with synthetic anticoccidials scaled ADG was significantly improved compared to untreated broilers. Whereas treating broilers with ionophores reduced FCR, but had no significant effect on scaled ADG. The improvements in weight gain of broilers given synthetic anticoccidials likely reflects how such anticoccidials fully prevent the replication of coccidia within the intestine due to their coccidiocidal properties, whereas ionophores only partially reduce the parasite replication (Conway and McKenzie, 2007). This finding is in slight contrast to the results of Eckert et al. (2021) who found that vaccinating or treating birds with synthetic anticoccidials were almost equally effective in limiting the consequences of infection on FCR, although the use of synthetic anticoccidials offered a slight advantage over vaccines. The differences between Eckert et al. (2021) and our meta-analysis may be due to the age at which the birds were challenged in the studies used in both meta-analyses. Only 7 studies challenging birds over the age of 20 d were used by Eckert et al. (2021) compared to 12 studies in this meta-analysis. The advantage of synthetic anticoccidials over vaccines has been attributed to the fact that vaccines cause a low level of intestinal damage (Williams, 2002; Eckert et al., 2021). The interaction between control methods and oocyst dose showed that there was an improvement in scaled ADFI of birds treated with ionophores compared to the untreated birds as a result of the growth promoting effect of ionophores due to their broader action against Gram-positive bacteria (Butaye et al., 2003). Furthermore, there was a tendency for scaled ADFI to increase in vaccinated birds compared to untreated birds, which may be due to efficacy of different application methods of various vaccines (Eckert et al., 2021).

In relation to the infection outcomes, there was no effect of control method on either of the OPG variables considered. However, the control methods may have reduced the viability of the oocysts in the environment, thereby preventing sporulation in the environment and reducing future infections (Beer et al., 2018). On the other hand, there was the expected improvement in

lesion scores due to control methods. We have already accounted for the inconsistencies regarding OPG variables as an infection outcome. These inconsistencies are well demonstrated in the experiments by Parent et al. (2018) and Tsiouris et al. (2021). In the former experiment there were no differences in growth rate or OPG between birds treated with antibiotics and the antibiotic-free birds. Whereas in Tsiouris et al. (2021), growth rate increased and both lesion scores and OPG were reduced when birds were given antibiotics compared to antibiotic-free birds. All control methods considered in this meta-analysis improved lesion scores to the same extent, despite the different mechanisms of each method on resident coccidian populations (Conway and McKenzie, 2007). In our analysis, there were no significant interactions between control methods and oocyst dose on mean or max OPG, or lesion scores. This suggests that the control methods assessed reduce the infection outcomes to the same extent, regardless of oocyst dose. It should be noted that we were unable to assess the effect of the control methods on mortality which would be another important factor to consider when identifying a control method for coccidiosis.

CONCLUSIONS

We conducted a systematic review and meta-analysis to investigate the role of *Eimeria* species, dose of challenge, inoculation day and method of control on performance characteristics and infection outcomes of different broiler strains infected for different study durations. Surprisingly, we found inconsistencies in the way the information was reported regarding the effects of infection on a variety of outcomes. As one of the values of our study lies in the identification of such shortcomings, we recommend the following to guide for conducting poultry *Eimeria* research:

1 ADFI should be measured frequently and ideally measurements should be able to account for the different stages of infection (pre-patent, acute, and recovery Sakkas et al., 2018; Taylor et al., 2021).
2 Similarly, BW should also be assessed frequently during the different stages of the infection, especially to avoid confounding the effects of infection during the acute and recovery stages of infection.
3 Experimenters should present information on the age and strain of the oocysts, as well as management conditions as these factors affect isolate pathogenicity.
4 Experimenters should consider when OPG are measured in relation to their infection protocol. Authors must also ensure transparency and detail in the methods used to measure OPG, including any correction factors used to scale the data.
5 Both the number of birds removed from experiments in accordance with humane endpoints, and the number of mortalities during the postinfection period should be presented.
6 Lesion scores appear to be the most consistent indicator of infection and should be measured when assessing the infectious outcomes of *Eimeria* infection.

Our meta-analysis identified significant differences in how different *Eimeria* species affect performance and infection outcomes, which was the primary motivation for our research. These differences related to the pathogenicity of each parasite species and the mechanisms of affecting performance variables. As far as coccidiosis control methods are concerned, we identified differences in how these methods influenced performance outcomes. For example, we found that birds treated with anticoccidials improved scaled ADG, whereas ionophores improved FCR compared with untreated birds. An interaction between *Eimeria* dose and control methods on scaled ADFI was caused by the higher scaled ADFI of vaccinated compared to untreated birds, as dose increased. We have suggested that these differences arise from the different modes of action of each control on parasite mortality and fecundity. All findings of this study should advance our understanding of the factors that influence the impact of coccidiosis and its controls in broilers.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.101746.

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