Efficacy of antibiotic and non-antibiotic interventions in preventing and treating necrotic enteritis in broiler chickens: a protocol for a systematic review

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Running title: Non-antibiotic interventions for necrotic enteritis in broiler chickens

Abstract

Necrotic enteritis is one of the most common and economically important bacterial diseases affecting the broiler industry. Limitations on the use of antimicrobials have highlighted the need to evaluate the efficacy of non-antibiotic alternatives and management strategies. However, the available evidence on the efficacy of non-antibiotic interventions for necrotic enteritis has not been systematically synthesized. Here we present a protocol to conduct a systematic review of the literature to assess the efficacy of non-antibiotic interventions compared to antibiotic interventions in preventing and treating necrotic enteritis cases in broiler chickens.

Keywords: poultry, antibiotics, *Clostridium spp*, systematic review, enterotoxemia

Introduction

Rationale

Necrotic enteritis (NE) is a poultry disease that mostly affects broiler chickens between 3-4 weeks of age, and is primarily caused by types A and C toxins produced by pathogenic strains of *Clostridium perfringens* (Mwangi et al., 2019, Singer and Porter, 2019, Opengart and Boulianne, 2020). There are two presentations of NE: a clinical form, characterized by acute onset, mortality rates of up to 50%, and necrosis of the small intestine mucosa; and a subclinical form, characterized by reduced weight gain, decreased digestion, and increased feed conversion ratio (Yegani and Korver, 2008, Opengart and Boulianne, 2020). This disease has a large impact on the poultry industry, with estimated economic losses of $2 billion worldwide annually (McReynolds et al., 2004, Moore et al., 2016, Cooper and Songer, 2016).

There are several risk factors that have been associated with NE outbreaks. Incidence or co-infection with coccidiosis is a main predisposing factor, but diets that are poorly digestible or high in protein, as well as slow gastrointestinal tract transit time can also increase the risk of NE (Yegani and Korver, 2008, Moore et al., 2016, Khalique et al., 2020, Opengart and Boulianne, 2020). Therefore, reducing the exposure to these risk factors may help prevent and control necrotic enteritis.

The broiler industry has historically used antibiotics and coccidiostatic drugs to control NE and to improve the overall health and growth of chickens (Dibner and Richards, 2005, McEwen and

Fedorka-Cray, 2002, Opengart and Boulianne, 2020). In fact, NE is one of the two most important diseases of chickens requiring antimicrobial therapy in the U.S. (Singer and Porter, 2019). However, due to rising concerns about potential emergence of antimicrobial resistance and its public health implications, veterinarians and poultry producers have made changes to their antimicrobial use practices. In addition, changes to antimicrobial policy have been instituted at the national level. For instance, the European Union eliminated the use of antimicrobial growth promoters (AGP) in 2006 (Cooper and Songer, 2016). More recently, the United States Food & Drug Administration (FDA) made several key changes to antimicrobial policy. In FDA Guidance for Industry (GFI) documents #209 and #213 (FDA, 2012, FDA, 2013), medically important antimicrobials in food-producing animals were limited to those situations deemed necessary for assuring animal health. Drug sponsors voluntarily removed label claims relating to production uses (growth promotion/feed efficiency) of medically important antimicrobials, thereby eliminating the use of medically important antimicrobials for growth promotion in U.S. animal agriculture.

After antimicrobial growth promoters (AGP) were eliminated in the E.U. in 2006, the incidence of NE increased significantly in some European countries (Cooper and Songer, 2016). With changes in antimicrobial use practices, the U.S. industry will likely face new challenges to control diseases as well. Therefore, it is critical to investigate the efficacy of non-antibiotic alternatives as they compare to the efficacy of antibiotics in preventing, treating and controlling infectious diseases in broiler production, including NE. To date, such an assessment has been done to evaluate the efficacy of antibiotics (but not the efficacy of non-antibiotic alternatives) to control colibacillosis in broilers in the form of a systematic review. In that study, the authors did

not find compelling evidence for or against the efficacy of antibiotics (Sargeant et al., 2019). However, as the authors note, there were only nine studies included in the final analysis (out of more than 3,000 publicly available papers evaluated), and these studies had poor reporting of key design and methodological features that are essential to evaluate the internal validity of the research, making it unfeasible to draw any sound conclusions (Sargeant et al., 2019). For necrotic enteritis specifically, there is anecdotal evidence of the potential utility of non-antibiotic alternatives such as prebiotics, probiotics, and organic acids to prevent, treat, and control the disease in broilers (Caly, 2015, Cooper and Songer, 2016, Khalique, 2020). However, a comprehensive critical appraisal of the evidence is still lacking.

Systematic reviews (SR) are rigorous scientific methods of research synthesis conducted in a transparent and reproducible manner (Sargeant and O'Connor, 2020). In addition, SR are useful in identifying knowledge gaps pertaining to a specific research question. We will conduct a systematic review to evaluate the efficacy of non-antibiotic interventions as they compare to antibiotic interventions as a disease management approach for necrotic enteritis in broiler chickens.

Objectives

Our goal is to conduct a systematic review of the literature to critically evaluate the available evidence on the following research question: *What is the efficacy of non-antibiotic interventions, compared to antibiotic interventions, in preventing and treating necrotic enteritis in broiler chickens?* The purpose of this protocol is to describe a methodology to perform a systematic review that addresses this question. The methodology follows the guidelines from PRISMA-P (Moher et al., 2015).

Methods

An appropriate review team will be composed of six members and will include expertise on poultry disease management, microbiology, epidemiologic methods, systematic review methods, and indexing and databases.

Eligibility criteria

The criteria used for eligibility for this systematic review will be based on the PICOS (Population, Intervention, Comparator, Outcome, Study design) framework (Sargeant and O'Connor, 2020), and the details can be found in Table 1.

Information sources

The following electronic databases will be searched with no language or date restrictions: PubMed/MEDLINE, CAB Abstracts, Agricola, and Scopus. The search strategy to be conducted for each database is detailed below. In addition to the electronic databases, hand searches of key review papers as well as gray literature on necrotic enteritis will be conducted.

Search strategy

For PubMed/MEDLINE, the search string will be: *("Chickens"[Mesh]) AND ("Enteritis/diet therapy"[Mesh] OR "Enteritis/drug therapy"[Mesh] OR "Enteritis/prevention and control"[Mesh])*, where 'Mesh' represents the appropriate Medical Subject Heading term.

For CAB Abstracts and Agricola, both of which can be searched with the OVID search interface, the search string will be: *(chicken* and enteritis and (protection or control or treatment)).af.*, where 'af' represents all fields of the record.

Lastly, for Scopus: *chicken* AND enteritis AND (protection OR control OR treatment)*.

Study records

Data management

Database records of the articles retrieved by the searches will be imported into EndNote X8 (Thomson Reuters) and duplicate records will be removed. Relevance screening, design screening, data extraction, and risk of bias assessment will be recorded in Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). Statistical analyses (if needed) will be performed in R 3.6.2 (R Core Team, 2019).

Selection process

There will be two stages of screening for the citations: 1) relevance screening and 2) design screening. During the first stage, titles and abstracts will be screened by two independent reviewers, and articles that are not relevant to the review question will be excluded. Conflicts between the two reviewers will be discussed until consensus is achieved, or with the help of a third reviewer. Relevance screening questions will be as follows:

a) Does the title/abstract describe a primary research study or a conference proceeding (as opposed to reviews, theses, and book chapters)?

b) Does the title/abstract refer to necrotic enteritis or to *Clostridium perfringens*?

c) Does the title/abstract refer to broiler chickens?

Answers to these questions will be 'Yes', 'No', or 'Unclear' ('Unclear' will be used when the answer to the relevance screening questions cannot be determined solely by the title/abstract). Articles will be excluded if the answer to at least one of the relevance screening questions is 'No'. Articles where the answers to all the questions are 'Yes', 'Unclear', or a combination of

131 'Yes' and 'Unclear' will be included and evaluated in the second screening stage. The full text of
132 all the articles included during relevance screening will be retrieved. For any conference
133 proceedings, an effort will be made to contact the corresponding author to obtain a copy of the
134 article if the full text cannot be retrieved.

135 During the second screening stage, design screening, only the methods section of the full-text
136 will be reviewed. Two independent reviewers will screen these studies, and discrepancies will be
137 resolved between the two reviewers, or with the help of a third reviewer. Design screening
138 questions will be as follows:

139 a) Is the study design an *in vivo* experiment or an observational study of these designs: cross-
140 sectional, cohort, case-control, ecological? *In vitro* and *in silico* (simulations) studies, case series,
141 and case reports will be excluded.

142 b) Does the study have a comparison group?

143 c) Does the study include quantifiable outcomes associated with NE disease in broiler chickens?

144 Answers to these questions will be 'Yes', 'No', or 'Unclear'. Studies will be included only if
145 they receive 'Yes', 'Unclear' or a combination of the two to all questions. Studies for which the
146 answer to at least one of the questions is 'No' will be excluded. Both stages of the screening
147 process (relevance and design screening) will be tested *a priori* with a random group of articles
148 that result from the search. An effort will be made to translate full-text of articles that are not in
149 English within the review team capabilities. If the full text cannot be translated into English, it
150 will be excluded.

151 **Data extraction**

Data from the final set of included studies will be captured in a spreadsheet form and will include the following information: general study characteristics (geographic location, year the study took place), study design details including sample size, animal characteristics (breed, age, housing/experimental conditions), intervention type and level of the intervention (farm, house, individual), statistical methods, any covariates used for confounding adjustment, effect measure(s) (or test statistics), measures of variability, *P* values, necrotic enteritis detection/diagnostic method(s), and outcome(s) information. Two reviewers will independently extract data from the included studies, and a third reviewer will validate the data entry. The data extraction form will be pretested by all reviewers and improved accordingly.

Risk of bias assessment

All included studies will be evaluated for threats to internal validity in three domains: information bias, selection bias, and confounding. Two independent reviewers will assign a qualitative rubric of low, high, and unclear to each one of the domains for each one of the studies, using their judgment. An overall risk of bias based on the individual domain scores will be determined by assigning the worst risk of bias in any of the domains. For example, if a study were considered to have a high risk of information bias, unclear risk of selection bias, and low risk of confounding, the overall risk of bias would be high risk. Any disagreements between reviewers will be discussed until they reach consensus, or with the help of a third reviewer. This approach is being adapted from the Cochrane Collaboration Risk of Bias Tool used to assess risk of bias in randomized trials in human subjects (Sterne et al., 2019).

Results for the risk of bias will be recorded in a spreadsheet form, which will be tested *a priori* by the reviewers and improved accordingly. Broadly, low risk of bias refers to studies where bias is unlikely to alter the results of that study; unclear risk of bias refers to studies where there is not

enough information to determine if bias would influence the results; and high risk of bias refers to studies where it is very likely that the results would be altered due to bias. What follows is a brief definition of each type of bias that will be evaluated with examples for each one of the qualitative categories (low, unclear, and high).

Information bias is an error that arises from the systematically different way the exposure(s) and outcome(s) are measured between the groups that are being compared (Aschengrau and Seage, 2020). Examples of low, high, and unclear risk of information bias follow.

Low risk of information bias: in a randomized trial, farm personnel were blinded to treatment allocation.

High risk of information bias: in a randomized trial, farm personnel were not blinded to treatment allocation and *Clostridium perfringens* isolation and identification from chicken samples was conducted in two different laboratories.

Unclear risk of information bias: researchers did not provide information on the blinding process of the farm personnel, and there was no information about quality assurance in the paper.

Selection bias (also referred to as collider stratification bias, Hernan and Robins, 2020) results from systematic differences between characteristics of the subjects available for analysis and the population from which they were drawn (Thrusfield, 2018). Examples of low, high, and unclear risk of selection bias follow:

Low risk of selection bias: broilers were randomly selected for an experimental study where one group received a nutritional supplement (treatment group) and the other group did not (control group). Both groups were selected from the same poultry farm and had the same exact baseline characteristics as they were selected into the study (age, weight, husbandry conditions).

High risk of selection bias: broilers were randomly selected for an experimental study where one group received nutritional supplement (treatment group) and the other did not (control group). The treatment group was selected from one poultry company and the control group was selected from a different poultry company. These companies had different husbandry conditions.

Unclear risk of selection bias: broilers were randomly selected for an experimental study where one group received nutritional supplement (treatment group) and the other did not (control group). There were no details about the target population that generated the experimental groups.

Confounding occurs due to a failure of the comparison group to reflect the counterfactual ideal of the exposed group (Aschengrau and Seage, 2020). Examples of low, high, and unclear risk of confounding follow:

Low risk of confounding: randomized trial with broiler chicken farms randomized into treatment (prebiotic) or control (antibiotic) groups. The number of farms in the study was more than 30.

High risk of confounding: cohort study of two broiler chicken farms, where Farm A administered prebiotics and was compared to Farm B, which administered antibiotics. Farm A vaccinated against coccidiosis while Farm B did not. Researchers did not adjust for this other factor in their analyses.

Unclear risk of confounding: cohort study of broiler chicken farms with some farms using prebiotics and other farms using antibiotics. Researchers adjusted in their analyses for potential confounders such as nutritional supplement given in some farms and not others, but did not mention or included other potentially important confounders (e.g. season).

Evidence synthesis

The available evidence addressing the research question will be synthesized narratively, and a meta-analysis will be conducted to provide summary effect measures if there are sufficient data from quantitative and homogeneous studies to permit such a statistical analysis. Additionally, publication bias will be evaluated using funnel plots where possible (Sargeant and O'Connor, 2020).

Discussion

The current protocol lays out the methodology that will be used to conduct a systematic review of the literature to assess the efficacy of non-antibiotic alternatives in the management of necrotic enteritis in broiler chickens. Any deviations from this protocol will be stated in the final systematic review manuscript. The results from the systematic review will prove useful for poultry veterinarians and managers, as it will summarize the available evidence as well as the information gaps on this important topic.

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300 **Table 1**. Eligibility criteria for this systematic review following the PICOS framework

301 (Sargeant and O'Connor, 2020).

Population (P)	Broiler chickens
Intervention (I)	Administration of non-antibiotic interventions to prevent or treat necrotic enteritis (vaccines, probiotics, nutritional management)
Comparator (C)	Administration of antibiotics to prevent or treat necrotic enteritis
Outcome/s (O)	Mortality, clinical or subclinical NE
Study design (S)	*in vivo* experimental studies, observational studies

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