Systematic review protocol of the efficacy of prebiotics and probiotics in reducing the colonization and shedding of *Campylobacter* and *Salmonella* in broilers and turkeys

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ABSTRACT

Use of prebiotics and probiotics as an alternative to antibiotics is increasing in poultry production. However, there is still uncertainty about their efficacy in controlling the spread of human pathogens in poultry. This paper describes the protocol for a systematic review assessing the efficacy of prebiotics and probiotics in reducing the colonization and shedding of *Campylobacter* and *Salmonella* in broiler chickens and turkeys. The objective of this protocol is to document the methodology that will be used for the systematic review *a priori.*

Keywords: broilers, turkeys, *Campylobacter*, *Salmonella*, systematic review

INTRODUCTION

Rationale

Prebiotics are food ingredients that can be used by beneficial microorganisms present in the host, while probiotics are live beneficial microorganisms administered to the host. Both products have the potential to modulate the gut microbiome (Markowiak and Slizewska, 2018) and might be used to exclude pathogens from colonizing the gut or to prevent events of dysbiosis, i.e., disturbance of the microbiome due to inflammation. Several studies have shown positive activity of different prebiotics and probiotics towards inducing changes in the gut microbiota or in reducing colonization of pathogens important in public health, such as *Campylobacter* and *Salmonella*. For instance, *Lactobacillus johnsonii* was shown to significantly reduce *Campylobacter jejuni* colonization following alteration of the gut microbiome (Mañez-Lázaro et al., 2017), while *Bacillus amyloliquefaciens* did not alter *Lactobacillus* or *Bacillus* populations, but significantly reduced *Escherichia coli* counts (Ahmed et al., 2014). *Bacillus licheniformis* normalized the ileal microbiota in broilers affected with necrotic enteritis (Xu et al., 2018). In a small study by Smialek et al. (2018), a probiotic containing *Lactococcus lactis*, *Carnobacterium divergens*, *Lactobacillus casei*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae* showed a significant reduction of *Campylobacter* in the chicken gut and in the environment. Furthermore, life-long administration of prebiotics (xylooligosaccharide, fructooligosaccharide, and galactooligosaccharide) extracted from *Bifidobacterium* have been shown to have a significant effect in reducing the colonization of *C. jejuni* in poultry (Baffoni et al., 2012, Baffoni et al., 2017). Despite many reports of positive impacts of prebiotics and probiotics in poultry, many contradictory studies also exist in this field. For example, Geeraerts et al. (2016)

showed that *B. amyloliquefaciens* did not protect against necrotic enteritis regardless of the supernatant of the culture showing high antibacterial activity against *Clostridium perfringens in vitro*. Because of conflicting results in the literature regarding the efficacy of prebiotics and probiotics in poultry, there is a need to synthesize the evidence on this topic.

Systematic reviews are a method to rigorously synthesize current knowledge, and they are extensively used to summarize evidence from randomized controlled trials estimating the clinical efficacy of an intervention (Sargeant and O'Connor, 2020). Compared to narrative reviews, systematic reviews implement a standardized process while evaluating all available evidence on the question at hand (Williams-Nguyen et al., 2016). The systematic review process encompasses an *a priori* established protocol defining the review question, the search strategy, the selection of publications relevant to the review question from the identified pool of publications, the extraction of data and risk of bias assessment of the relevant references, and the synthesis of the overall evidence (Williams-Nguyen et al., 2016). Establishing and publishing a protocol before conducting systematic reviews assures transparency and has been highlighted by Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and PRISMA for protocols (PRISMA-P) (Moher et al., 2009, Moher et al., 2015).

Objectives

This protocol describes a method to thoroughly assess the evidence for the following review question: what is the efficacy of prebiotics and probiotics, compared with no pre-harvest

intervention, in reducing the colonization and shedding of *Campylobacter* and *Salmonella* in broilers and turkeys?

The overall objective of this systematic review is to synthesize the current knowledge on the review question while identifying knowledge gaps. The objective of this protocol is to document the methodology that will be used for the systematic review *a priori*.

METHODS

Eligibility criteria

The review question follows the PICO framework (Population, Intervention, Comparison group, and Outcome) (Sargeant and O'Connor, 2020). The study characteristics derived from this framework are displayed in Table 1. The full list of inclusion/exclusion criteria to be used during the selection of studies is shown in Table 2.

Sources of information

The search will be conducted in the following databases: PubMed/MEDLINE, CAB Abstracts, Scopus, and Agricola.

Search strategy

For PubMed/MEDLINE, the search string is *("Chickens"[Mesh Terms] OR "Turkeys"[Mesh Terms]) AND ("Campylobacter"[Mesh Terms] OR "Salmonella"[Mesh Terms]) AND (("probiotics"[Mesh Terms] OR "probiotics"[All Fields]) OR ("prebiotics"[Mesh Terms] OR "prebiotics"[All Fields] OR "Dietary Carbohydrates"[All Fields] OR "Dietary Fiber"[All Fields] OR "fructo-oligosaccharides"[All Fields] OR "galacto-oligosaccharides"[All Fields] OR "yeast cell wall"[All Fields]))* where 'Mesh Terms' indicates the appropriate Medical Subject Heading term.

Both CAB Abstracts and Agricola will be searched using the OVID search interface, and the search string is the same for both: *((chicken* or turkey*) and (Campylobacter OR Salmonella) and (probiotic* or prebiotic* or "Dietary Fiber" or "fructo-oligosaccharides" or "galacto-oligosaccharides" or "yeast cell wall")).af.* where 'af' indicates all record fields.

Lastly, for Scopus, the search string is: *TITLE-ABS-KEY ((chicken* OR turkey*) AND (campylobacter OR salmonella) AND (probiotic* OR prebiotic* OR "Dietary Fiber" OR "fructo-oligosaccharides" OR "galacto-oligosaccharides" OR "yeast cell wall"))*, where all terms are searched for in the Title, Abstract, and Keyword fields respectively.

Record management

All identified citations (titles and abstracts) will be uploaded to Endnote X9 (Thomson Reuters) to remove duplicates. Title and abstract screening (first stage of selection) will be performed in R

3.6.2 (R Core Team, 2019) with the use of the package revtools (Westgate, 2019), which will be modified to add the inclusion/exclusion criteria in the tool. The revtools package provides support for documenting the screening of titles and abstracts. The output of revtools will be stored as a comma-separated values (csv) file. Conflicts between independent reviewers will be detected in Stata 16 (StataCorp, 2019), and the resolution decision will be recorded in the same file. Materials and methods screening (second stage of selection) will be performed in EndNote X9 using the retrieved pdf of the references that were retained from the first screening process. Data extraction will be performed manually or with the help of the package metaDigitise (Pick et al., 2018) in R 3.6.2 to extract data from figures if needed. Data extraction and risk of bias assessment will be recorded using REDCap (Harris et al., 2009). Deduplication of data extraction and risk of bias assessment entries and resolution of conflicts will be undertaken using REDCap as well. Statistical analyses will be performed in Stata 16.

Selection of studies

Citation records will be screened in two stages. In the first stage, all titles and abstracts will be screened using the inclusion/exclusion criteria by at least two independent reviewers using the package revtools in R 3.6.2. A reference will be excluded when there is evidence that one or more inclusion criteria are not met. Otherwise, the retained references will be further screened using the materials and methods section of the full text by at least two independent reviewers. The agreement among the reviewers will be determined in a pilot trial prior to each stage with 10% of citations randomly selected from the total pool of citations entering each stage. Any disagreements will be resolved through discussion.

Data extraction

At least two independent reviewers will extract the following items from the articles retained from the previous screening: year of publication, geographical area where the study was performed, study design, characteristics of the animals (species, age, breed), pathogens (*Campylobacter* and/or *Salmonella*), type of infection (natural infection or experimental challenge), type of outcome (colonization and/or shedding), type of intervention (probiotics and/or prebiotic), characteristics of the intervention (bacterial strains used, concentration, route, etc.), effects of the intervention (mean differences, ratio of geometric means), the measure of variability of the estimates (95% confidence interval or standard errors), P values, sample sizes, and covariates used for adjustment. If there are figures in the article that need to be used for data extraction, the package metaDigitise in R 3.6.2 will be used. Any disagreements among reviewers will be discussed and resolved.

Risk of bias assessment

The risk of bias of each selected study will be assessed using a qualitative scale (low, unclear, high) pertaining to 3 domains (selection bias, information bias, confounding). The overall risk of bias will be determined using the highest category obtained for the domains. As an example, if a study was considered to have low risk of selection bias, a high risk of information bias, and an unclear risk of confounding, the overall risk of bias would be high. Risk of bias assessment will be performed by at least two independent reviewers who will document and substantiate their assessment. Any disagreements will be discussed and resolved. Similar to the selection process, a

pilot trial will be conducted before data extraction and risk of bias assessment to evaluate reviewers' agreement and the adequacy of the documentation process.

Selection bias

Reviewers will assess the risk of selection bias in each study. Selection bias is the systematic bias incurred when selection into the study is associated with the exposure/treatment and the outcome, either directly or through a cause of exposure/treatment and/or a cause of the outcome. Selection bias is also referred to as collider stratification bias (Hernan and Robins, 2020). There is another type of selection bias due to an association between an effect modifier and selection into the study, but it will not contribute to the risk of bias assessment in this systematic review. This is because the second type of selection bias only affects the external validity, i.e., the generalizability of the results to a population with a different distribution of such effect modifier.

Low risk of selection bias can be exemplified by an experimental study with poultry farms randomized into treatment (probiotics) or control groups, in which access to all farms was not possible during three time points due to severe weather conditions. Additionally, the proportion of missing data was the same in both treatment arms, resulting in a non-differential loss to follow-up. If the proportion of missing data per treatment arm in the aforementioned case is not reported in the paper, the risk of selection bias of the study would be categorized as unclear. By contrast, if the proportion of missing data was different in both types of farms, resulting in differential loss to follow-up, the risk of selection bias of the study would be categorized as high.

Information bias

Reviewers will evaluate the risk of information bias in each study. Information bias is an error arising from the systematic differences in the means by which information on exposure/treatment and/or outcome was obtained (Aschengrau and Seage, 2020).

Low risk of information bias can be exemplified by the documentation of quality assurance and control measures in the paper, e.g., blinding of laboratory personnel, blinding of personnel collecting the samples, blinding of the statistician or data analyst, etc. An example of unclear risk of information bias is the absence of the documentation of quality assurance and control measures in the paper. By contrast, an example of high risk of information bias is when quality assurance and control measures were not taken to reduce the risk of information bias, for instance, laboratory personnel were not blinded to the assignment/use of probiotics at the poultry farm, potentially leading to differential measurement error.

Confounding

Reviewers will also evaluate the risk of confounding in each reference. Confounding occurs due to mixing of effects between an exposure, an outcome, and a third extraneous variable known as a confounder (Aschengrau and Seage, 2020).

Low risk of confounding can be exemplified by an experimental study with poultry farms randomized into treatment (probiotics) or control groups, in which the number of farms included in the study is large (>30) or matching was performed. An example of unclear risk of

confounding is a cohort study with some poultry farms using probiotics and others not using probiotics, in which authors reported that several potential confounding variables were included in the analysis. However, important confounders (e.g., age of the flock) were not included in the analysis, and as a result, residual confounding might still be present. By contrast, an example of high risk of confounding is a cohort study with some poultry farms using probiotics and others not using probiotics, in which authors did not adjust for potential confounding variables during analysis.

Evidence synthesis

The evidence will be synthesized narratively, unless studies with low risk of bias and with very similar characteristics are detected, in which case a random effects meta-analysis will be performed. If the heterogeneity of the effect estimates is at least moderate ($I^2 \geq 30\%$) and the number of studies to analyze is greater than ten (Deeks et al., 2019), then a meta-regression will be undertaken to explain the heterogeneity between the studies. Additionally, publication bias will be evaluated as part of the meta-analysis. The confidence in the cumulative evidence will be assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach (Guyatt et al., 2011).

CONCLUSIONS

After the completion of this systematic review, the current evidence on the efficacy of prebiotics and probiotics in reducing the colonization and shedding of *Campylobacter* and *Salmonella* in broilers and turkeys will be synthesized. An overall appraisal of the quality of the evidence will be provided, which could show knowledge gaps and areas in need of further research. The results of this systematic review could inform veterinarians and producers about the effectiveness of these interventions in broilers and turkeys when used as pre-harvest control measures.

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Table 1. Study characteristics from PICO framework for the systematic review on the efficacy of prebiotics and probiotics in reducing the colonization and shedding of *Campylobacter* and *Salmonella* in broilers and turkeys.

Study characteristic	**Description**
Population	Commercial broilers and turkeys
Intervention	Probiotics or prebiotics (feed, water, or gavage)
Comparison group	No pre-harvest interventions against *Campylobacter* or *Salmonella*
Outcome	Shedding of *Campylobacter* (counts measured in feces or cloacal swabs), shedding of *Salmonella* (counts measured in feces or cloacal swabs), colonization of *Campylobacter* (counts measured in intestinal contents), colonization of *Salmonella* (counts measured in intestinal contents). Counts can be estimated directly as colony forming units (CFUs) or indirectly using molecular tools, such as quantitative PCR

313 Table 2. Inclusion and exclusion criteria for the systematic review on the efficacy of prebiotics
314 and probiotics in reducing the colonization and shedding of *Campylobacter* and *Salmonella* in
315 broilers and turkeys.
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Criterion	**Inclusion**	**Exclusion**
Population	Broiler chickens and/or turkeys	Non-commercial chickens and turkeys, laying hens, breeders, quail, geese, ducks
Pathogens of interest	*Campylobacter* spp., *Campylobacter jejuni*, *Campylobacter coli,* and/or *Salmonella*	*Campylobacter* other than *C. jejuni* and *C. coli*. *E. coli*, *Clostridium*, *Eimeria*
Intervention	Probiotics and/or prebiotics orally (feed, water, gavage)	Vaccines, organic acids, litter remediation, antibiotics, probiotics *in ovo*
Comparison group	No pre-harvest interventions against *Campylobacter* or *Salmonella*	Use of other probiotics or prebiotics, antibiotics (such as in non-inferiority trials), vaccines, organic acids, litter remediation

Criterion	**Inclusion**	**Exclusion**
Source	Primary articles and conference proceedings	Reviews, thesis, book chapters, systematic review, scoping review, government/companies' documents
Study design	*In vivo* experimental studies, cohort studies, case-control studies, cross-sectional studies, ecological studies	*In vitro* experimental studies, *in silico* studies (simulations), case series, case descriptions
Outcome	Shedding of *Campylobacter* (as measured in feces or cloacal swabs), shedding of *Salmonella* (as measured in feces or cloacal swabs), colonization of *Campylobacter* (measured as counts in intestinal contents), colonization of *Salmonella* (measured as counts in intestinal contents). Both shedding and colonization could be detected by culture or molecular techniques	*Campylobacter* or *Salmonella* in litter, in carcass at processing plants, seroconversion to *Campylobacter* or *Salmonella,* prevalence or incidence of *Campylobacter* or *Salmonella*

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