Efficacy of prebiotics and probiotics on growth performance in poultry: A protocol for a systematic review

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ABSTRACT

The use of prebiotics and probiotics as dietary additives in poultry has been practiced widely as an alternative to antibiotics and to support gut health. Despite the wealth of products available, there is currently a lack of clear scientific evidence that the use of prebiotics and probiotics beneficially impact growth performance in poultry. The aim of this protocol paper is to document the process of a systematic review addressing the following review question: *Does oral administration of a prebiotic (defined as dietary fiber, dietary carbohydrates,*

oligosaccharides, or yeast cell wall) or probiotic (defined or undefined single or mixed cultures of living bacteria, fungi, and/or yeast) provide beneficial effects on growth performance in broiler chickens and turkeys? The proposed systematic review will provide evidence on the effectiveness of prebiotics and probiotics for enhancing growth performance in poultry, and will help to alleviate high skepticism, criticism, and confusion over the use of prebiotics and probiotics in poultry among poultry producers.

BACKGROUND INFORMATION

Prebiotics and probiotics have been widely used in the poultry industry as a potential alternative to antibiotics, aimed to improve gut development and the overall health of growing birds. Prebiotics have been defined broadly to include any non-digestible food ingredients that selectively promote the growth of bacteria that are beneficial to the gut, and promote changes in composition and activity of gut microbiota (Gibson and Roberfroid, 1995, Gibson et al., 2004, Kolida et al., 2002). In particular, dietary fiber, indigestible oligosaccharides, and yeast cell wall are often labeled as prebiotics (Teng and Kim, 2018). Probiotics are single or mixed cultures of living bacteria, fungi or yeast that, when administered, may provide beneficial health effects to the host in multiple ways, such as improving gut maturation (Gao et al., 2017), competitively excluding pathogenic bacteria (Mountzouris et al., 2009, Ceccarelli et al., 2017), and improving metabolism (Zheng et al., 2016). Lactic acid producing bacteria such as *Lactobacillus* spp. are among the most commonly used probiotic bacteria in addition to *Bacillus* spp., *Enterococcus* spp., and yeast (*Saccharomyces* spp.) (Karaoglu and Durdag, 2005, Yadav and Jha, 2019).

A number of studies have evaluated the effects of prebiotic and probiotic supplementation on growth performance and overall poultry health (Midilli et al., 2008, Torres-Rodriguez et al., 2007, Kim et al., 2011, Çınar et al., 2009). However, no consensus exists among the poultry industry as to whether the use of prebiotics and probiotics provide beneficial effects in the growth performance of poultry. Despite this, there is a wealth of prebiotic and probiotic products available and marketed to the poultry industry. Clear scientific evidence behind these products is mostly lacking, leading to a high degree of skepticism, criticism, and confusion among poultry producers for these products.

OBJECTIVES

This paper describes the protocol for a systematic review that will evaluate the following question: *Does oral administration of a prebiotic (defined as dietary fiber, dietary carbohydrates, oligosaccharides, or yeast cell wall) or probiotic (defined or undefined single or mixed cultures of living bacteria, fungi, and/or yeast) provide beneficial effects on growth performance in broiler chickens and turkeys?* The objective of this systematic review is to critically assess and summarize the presence and quality of available evidence addressing whether prebiotic and probiotic use in poultry provide beneficial effects on growth performance.

METHODS

Eligibility criteria

The PICO (Population, Intervention, Comparison group, and Outcome) framework will be used to develop a review question and eligibility criteria (Higgins and Green, 2011). See Table 1.

Studies with longitudinal experimental trials and observational designs (pen or field setting) and with or without microbiological challenges, will be retained regardless of the random allocation of treatments. Studies with cross-sectional study designs will not be retained because the outcomes of interests cannot be assessed and reported in a non-longitudinal study design.

Primary research studies conducted in broiler chickens and turkeys, including thesis chapters, peer-reviewed articles, and conference proceedings will be included. Research studies conducted

in vitro (i.e. a study evaluating effects of a dietary fiber in chicken intestinal cell lines) and non-research studies, such as reviews, editorials, book chapters, or opinions, as well as non-peer reviewed articles will be excluded.

Information sources

The following databases will be searched for studies relevant to the review question without any limits on language and publication date: PubMed/MEDLINE, CAB Abstracts, Scopus, and Agricola. All searches will be conducted on the same day, and the search strategies used on each database will be recorded.

Search strategy

The following search strings will be used to systematically search the electronic databases mentioned above:

PubMed/MEDLINE:

("Chickens/growth and development"[Mesh] OR "Turkeys/growth and development"[Mesh] OR "Poultry Diseases/mortality"[Mesh]) 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' was the Medical Subject Heading.

Scopus:

TITLE-ABS-KEY ((chicken* OR turkey*) AND (“feed conversion” or “growth rate” or “body weight” or mortality) AND (probiotic* OR prebiotic* OR "Dietary Fiber" OR "fructo-oligosaccharides" OR "galacto-oligosaccharides" OR "yeast cell wall")) where ‘ABS’ represents the record’s abstract field, and ‘KEY’ represents the keyword field.

CAB Abstracts and Agricola:
(chicken* or turkey*).af. and (feed conversion or feed conversion efficiency or growth rate or body weight or mortality).sh. and (probiotic* or prebiotic* or "Dietary Fiber" or "fructo-oligosaccharides" or "galacto-oligosaccharides" or "yeast cell wall").af. where ‘af’ represents all fields in the record.

Data management

EndNote X9 (Thomson Reuters) will be used to manage records retrieved from searches. Duplicates will be identified and removed by EndNote during the file importing process, and then manually by comparing the complete citation. Relevance and design screenings, data extraction, and risk of bias assessment will be performed in Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA). Statistical analyses, if needed, will be conducted in R (R Core Team, 2013).

Selection process

The selection of relevant studies will be performed using a 2-stage screening approach: 1) relevance screening and 2) design screening. Prior to each screening process, thirty studies will be randomly chosen to validate the screening questions. For the relevance screening, the title and

abstract of each study will be reviewed independently by at least two reviewers to identify studies that potentially meet inclusion criteria based on the following questions:

1) Do the title and abstract describe a primary research study reported in a peer-reviewed journal, thesis or conference proceeding?
2) Is the study population a commercial breed(s) of broiler chickens and/or turkeys?
3) Does the study describe and specify one or more treatment groups of prebiotics and/or probiotics?

Possible answers to these questions are 'Yes', 'No' and 'Unclear'. Any disagreement between reviewers will be resolved through discussion. Studies that do not meet the inclusion criteria (those that answer 'No' to any of the three questions) will be excluded. Studies that meet the inclusion criteria (those that answer 'Yes' to all three questions) or those that cannot be determined from the title and abstract will be retained for the design screening process.

Full-text of the articles that pass the relevance screening or those that could not be evaluated as described above will be retrieved. For the design screening process, the materials and methods section will be reviewed independently by at least two reviewers.

Studies will be screened using the following questions:

1) Is the article written in English? If not, is translation deemed practical?
2) Did the study use a commercial breed(s) of broiler chickens and/or turkeys?
3) Does the study describe and specify one or more treatment groups of prebiotics (any mention of extracted or purified dietary fiber, dietary carbohydrates, oligosaccharides or

yeast cell wall) or probiotics (defined or undefined single or mixed cultures of living bacteria, fungi, and/or yeast) that were administered orally (in feed/water or gavaged)?

4) Does the study report an appropriate comparison or control group(s), such as a negative control group where no treatment (prebiotics, probiotics or any other treatments, including antibiotics) was given to bird?
5) Does the study measure any of the three outcome variables (feed conversion ratio, average daily gain, or livability/viability) or include raw/relevant data such that these outcome variables can be computed?

Since searches were not restricted by language, translation of eligible studies in a language other than English will be attempted given the resources and personnel available to the reviewers and collaborators. Those studies that cannot be adequately translated will be excluded. If more than one publication reports the same results from the same study population (i.e. the same results published by the same research group first as a conference proceeding and later as a peer-reviewed article), only the publication with the latest publication date will be included. Any disagreement between reviewers will be resolved through discussion as a team. Studies that meet the inclusion criteria (those that answer 'Yes' to all five questions) will be retained for data extraction and risk of bias assessment.

Data collection process

A standardized data extraction form will be created to extract data from included studies for assessment of quality of studies and evidence synthesis using Microsoft Excel. The data from each study will be extracted independently by two reviewers and validated by a third reviewer.

Prior to the data extraction, reviewers will test the data extraction form using ten randomly chosen studies.

The following data will be extracted from each study, when available:

1) Publication details: Title of a study, author(s), publication year, language, country/geographical area
2) Study design and methods: study details (study design, study set-up, objective/hypothesis), sample characteristics (sample size, breed, age, vaccination status, etc.), details about comparison/control group(s), treatment details (concentration used, frequency, timing and duration of treatment, route of administration), and if performed, details about microbiological challenge (isolate/strain used, inoculation dose, timing and frequency of challenge)
3) Outcome measures: outcome variables (feed conversion ratio, average daily gain, livability/viability), statistical test(s) or model(s) used, test statistics, measure of variability, *P-values*, methods to control for potential confounding or other biases

Outcomes and prioritization

The primary outcome variables will be 1) feed conversion ratio, 2) average daily gain and 3) livability. Feed conversion ratio is a measure of the amount of feed it takes to grow one kilogram of meat. It is also known as the feed-to-gain ratio, and is calculated as the cumulative amount of feed intake over the amount of weight gain. Average daily gain is an average weight gain per day over a given period of time. It is calculated as body weight gain over the number of days in a growth period, where body weight gain is the difference between the final and initial weight of

an animal. Livability or viability is the percent of surviving birds in a flock, and can be calculated as 1-mortality. When outcome variables of interests are not reported, but sufficient relevant data are reported, an effort will be made to extract the data and compute the outcome variables.

Risk of bias assessment

Risk of bias assessment will be performed qualitatively based on the rubric developed by Williams-Nguyen et al (Table 2) (Williams-Nguyen et al., 2016). This rubric system was modified from the Cochrane Collaboration Risk of Bias Tool (Higgins and Green, 2011) and was chosen for its flexibility. This system assesses risk of bias based on domains of bias (selection bias, information bias, and confounding) rather than specific mechanisms to control for bias (random allocation, blinding of researchers/personnel, blinding of outcome assessment, etc.), allowing the use of a single rubric to assess risk of bias with different study designs.

Risk of bias assessment will be based on three domains: sample selection bias, information bias and confounding. Each domain will be classified as low, high, or unclear (insufficient information provided to determine the risk of bias). The overall risk of bias of a study will be determined by combining qualitative scores from each domain. Specifically, the highest qualitative score of the three domains will be assigned as the final risk of bias score (i.e. if a study is assigned unclear for sample selection bias, low for information bias, and high for confounding, the overall risk of bias score is high). Risk of bias will be performed independently by two reviewers. Any disagreement between reviewers will be resolved through discussion as a

team. The final paper will include a table showing the overall risk of bias assessment scores of all included studies.

Sample selection bias

Sample selection bias, arising due to systematic differences in sample characteristics and sampling methods between the comparison groups, will be assessed using the following question: *Other than treatment status, were birds assigned to control and treatment groups systematically different from each other, and did sampling methods differ systematically between the control and treatment groups?*

Examples of low risk of sample selection bias:

-Birds (or broiler houses/farms) were randomly allocated to treatment and control groups;

-Birds used in the study were of the same breed and from the same hatchery;

-Experimental time points (e.g. time of the day at which daily body weight of bird was measured or day at which final body weight was measured) were consistent among treatment and control groups.

Examples of high risk of sample selection bias:

-Birds allocated to control and treatment groups were of different breeds;

-Birds allocated to control and treatment groups came from different hatcheries;

-Experimental time points were not consistent among treatment and control groups.

Risk of sample selection bias will be assigned 'Unclear' if insufficient information about sample characteristics and sampling methods between control and treatment groups is provided to determine the risk of bias.

Information bias

Information bias, which is caused by systematic differences in outcome ascertainment will be assessed using the following question: *Were outcomes of interest ascertained in a way that ensures accuracy across the control and treatment groups?*

Examples of low risk of information bias include:

-Laboratory staff were blinded to treatment allocation;

-Identical methods (e.g. using the same scale to measure body weights of birds throughout the experiment or calibrating the scale each time birds were measured) to measure outcome variables were applied to all samples regardless of treatment status.

Examples of high risk of information bias include:

-Laboratory staff were not blinded to treatment assignment;

-Different methods (e.g. different scales or un-calibrated scales were used to measure body weight of birds) to measure outcome variables were applied depending on treatment assignment.

Risk of information bias will be assigned 'Unclear' if a study does not describe outcome ascertainment procedures in enough detail to determine the risk of information bias.

Confounding

Confounding is a systematic error arising due to mixing of the exposure effect with other additional factors resulting in distortion in the outcome measure (Greenland and Robins, 2009). Risk of bias due to confounding will be assessed using the following question: *Were appropriate measures implemented to reduce potential confounding in the study?*

Examples of low risk of confounding include:

-Treatment was randomly assigned to birds (or broiler farms/houses);

-Geographical region/area, grow-out seasonality, and other characteristics, such as husbandry practices, litter type, and housing conditions of the broiler farms enrolled in a field trial were matched to ensure similar characteristics between control and treated farms;

-The same starter, grower, and finisher diets were fed to all birds;

-Appropriate statistical approaches (e.g. adjusting for potential confounding variables in regression analysis) were used.

Examples of high risk of confounding include:

-Treatment was not randomly assigned to birds (or broiler farms/houses);

-Geographical region/area and grow-out seasonality were matched among enrolled broiler farms, but other farm characteristics that may be considered potential confounding variables were not matched between the control and treatment groups;

-No statistical approaches were applied to control for potential confounding variables.

Risk of bias due to confounding will be assigned 'Unclear' if a study does not describe likely sources of confounding and analytical methods to control for such confounding (or whether the methods were applied correctly) in sufficient detail.

Data synthesis

Evidence from retained studies will be stratified into poultry type (broiler chicken and turkey) then by treatment type (prebiotic and probiotic). Heterogeneity among included studies will be assessed using I^2 statistics. A random-effect meta-analysis will be conducted within strata if there is adequate homogeneity across included studies and I^2 <50% is identified. Otherwise, a qualitative synthesis of the evidence will be undertaken. The presence of publication bias will be assessed within strata using funnel plots.

CONCLUSION

Any deviations to this protocol will be documented in the full review, which will be published in a peer-reviewed journal.

The purpose of this systematic review is to critically examine and summarize the literature on the use of prebiotics and probiotics and their effects on growth performance in broiler chickens and turkeys. The results of this systematic review could help inform poultry producers and veterinarians with an evidence-base for the use of prebiotics and probiotics to improve growth performance in poultry, in addition to highlighting areas where future research is needed.

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352 **Table 1. Eligibility criteria of the systematic review evaluating the efficacy of prebiotics and**

353 **probiotics on growth performance in poultry using PICO framework.**

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	Description
Population	Commercial breeds of broiler chickens and turkeys
Intervention	Oral administration of prebiotic (or dietary carbohydrate, dietary fiber, oligosaccharides, or yeast cell wall) or probiotics (defined or undefined single or mixed cultures of living bacteria, fungi or yeast)
Comparison group	Raising commercial breeds of broiler chickens and turkeys without prebiotics or probiotics
Outcome	Feed conversion ratio (FCR), Average daily gain (ADG) or livability/viability

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Table 2. Qualitative rubric of the overall risk of bias assessment adopted from the Cochrane Collaboration Risk of Bias Tool (Higgins and Green, 2011) and William-Nguyen et al. (2016).

Overall risk of bias score	Criteria
Low risk of bias	The study is judged to be at low risk of bias for all three domains.
Unclear risk of bias	The study is judged to be at unclear risk of bias for one or more domains, but not enough information is provided to be judged at high risk of bias for any domain.
High risk of bias	The study is judged to be at high risk of bias for one or more domains.