Disease and viruses as negative factor prohibiting the growth of broiler chicken embryo as research topic trend: a bibliometric review [version 1; peer review: awaiting peer review]

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Abstract

Background
Broiler chickens have properties as meat-producing poultry and produce meat with soft fiber quality. Broiler's embryo needs to be given attention, especially against disease. The purpose of the study was to observe the trend of the research topic and external factors which could affect the embryo's growth of broiler.

Methods
This study used meta-data from Scopus. There were 162 samples started from 2006-to 2022. The data were analyzed using a bibliometric method with two software, Vosviewer, and Biblioshiy from R studio.

Results
It was found that the study of broiler chicken embryos had developed well, especially those related to genetics, disease, and also the immunity system. But the result showed that topics about the disease, viruses, and bacteria were more popular than topics about the gene. The result also showed that based on the importance and development of topics, the words such as “chicken”, “genes”, “development”, “effect”, growth”, and “control” had importance for the study and developed well in research. It means that genes influenced the growth of the embryo of a chicken. But still, even though a chicken gene was predetermined, the genetic engineering of chicken insemination to produce a superior breed with a fast-growing rate of the embryo could be used.

Conclusions
It could be stated that disease, especially research about the virus is one of the main determinants that could affect the growth of the embryo of broiler chicken.
Keywords
Broiler, disease, embryo, growth, virus

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**Introduction**

Broiler chickens are meat-producing chickens that are kept until the age of 6–7 weeks with a weight of 1.5–2 kg. Broiler chicken meat is used as a source of animal protein (Puspita et al., 2021). Broilers have dominant characteristics as meat-producing poultry and produce meat with soft fiber quality. (Puvacà et al., 2019).

The characteristics of broiler chickens are calm, large body shape, fast chicken growth, white chicken skin, and low egg production. In addition, broiler chickens have a weakness that is very sensitive to changes in environmental temperature and infection of various diseases (Qi et al., 2018).

Embryoid eggs as a dynamic biological system are expected to describe in vivo conditions. The intended in vivo condition is the continuous metabolism and development of embryonic cells in the egg. Chemicals, including antiviral agents, can also be inoculated into eggs (Wilson et al., 2003). The effect of these substances on viruses and embryos is influenced by the age of the embryo, the application of the route of administration to parts of the egg (embryo, allantois, yolk sac, air sac, and amnion), the ability to absorb substances by the embryo, and the pharmacological structure of the substance itself. Other chemical substance which was consumed through the hen also could become the factor in deciding the growth of the embryo (van den Brand et al., 2021).

Besides viruses and chemical substances, the temperature limit that will kill the developing embryo is -1.7°C to -1.1°C for 70–95 minutes. Younger embryos are more resistant to these cold temperatures and require a longer exposure time to cause death. In addition, the temperature required to cause embryo death ranged from 41.1°C to 48.3°C. Within this high-temperature range, higher temperatures are required to cause death in older embryos (Sato et al., 2006).

The most ideal air temperature for an incubator is 37.5°C. This temperature range is very important for temperature selection to test the potential for manipulation of the sex ratio of broilers. (Stern, 2018). The aim of the study was to analyze broiler embryos based on the Scopus database, as well as the most important topics that need to be paid attention to related to the embryo of broiler chicken.

**Methods**

**Data collection**

Data gathered from Scopus website with subscription service. Subscription service was different from free service in Scopus because by paying the subscription fee to Scopus, that institution would receive access to all kinds of metadata related to all papers published in journals indexed by Scopus, while free access cannot (Harzing & Alakangas, 2016). This study limited the data related to the embryo of broiler chicken and focused on the analysis of the data on disease and viruses which could affect the growth rate and survivability of the embryo. The data were collected using the keyword of “EMBRYO”, “BROILER CHICKEN”, and “VIRUS”. The duration of data gathering was 2006–2022 with all types of publications included. There were 1348 samples found.

The keywords were typed into advanced search in Scopus website. Those keywords were SRCITLE (EMBRYO), (BROILER CHICKEN), (VIRUS). This study only included papers using English as the samples. The source type were open access articles, and document type were abstract, review article and research article. After being scrutinized to only related to “BROILER CHICKEN”, the remaining samples were 162. The collected samples are then exported to specific files, so that they could be analyzed by software.

**Data analysis**

The analysis of the data from a CSV file would be divided into two steps. The first step of analysis used Vosviewer version 1.6.16 to see the affiliation of the authors and keywords related to the study of the embryo of broiler chicken. The affiliation included the name of the institution, city, and country where the author resides. It also included the number of citations from their affiliations. The data were presented in a table. For the analysis of the keywords, Vosviewer could visualize the keywords related to the intended topics along with their connection to each other, shown by the strings attached. It also could show the keywords that had become the trend in a certain year indicated by color (Polley, 2016).

The second step of the analysis was using Biblioshiny feature from R studio software. Biblioshiny was a powerful tool for analyzing the metadata of publications using the bibliometric method. Using biblioshiny software that could provide many forms of analysis in bibliometrics, this study analyzed the data using four features. First was the “word cloud” feature to see the important word from the abstracts of each paper. The second was the “trend topic” feature to see what topic that was trend each year from 2013 to 2022. The third was the “thematic map” feature to see the importance and how well a keyword was developed in the research field. The fourth was the “thematic evolution” feature to know the frequency of keywords included in papers used as samples and their relationship with the countries and authors of the papers.

**Results**

Table 1 showed that three universities in France had publications with high citations. The country with publications with the highest citation was Canada, but only in one university. Other countries with more than one university that had publications related to the topic of the embryo of broiler chicken with high citations were Netherland and Germany. It means that the trend of publication related to the intended topic was more popular in European countries than in Asia or America.

Figure 1 showed that there were some major keywords used in published papers such as “animal”, “article”, “animal tissue”, “chicken”, “controlled study”, and “genetic embryo”. The keyword “embryo” was connected to some keywords, such as “controlled study”, “genetics”, “immunology”, “poultry disease”, “chicken”, and “unclassified drug”. The appearance
Table 1. List of affiliation of authors’ written papers related to the embryo of broiler chicken.

<table>
<thead>
<tr>
<th>Organization</th>
<th>Documents</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>division of pathobiology, University of Guelph, Guelph, Canada</td>
<td>2</td>
<td>87</td>
</tr>
<tr>
<td>division of animal biochemistry and biotechnology, UTP University of Science and Technology, Bydgoszcz, Poland</td>
<td>2</td>
<td>78</td>
</tr>
<tr>
<td>division of animal science, Iowa State University, Ames, IA, United States</td>
<td>2</td>
<td>76</td>
</tr>
<tr>
<td>inra, ur83 recherches avicoles, Nouzilly, France</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>adaptation physiology division, Wageningen University and Research, Wageningen, Netherlands</td>
<td>3</td>
<td>45</td>
</tr>
<tr>
<td>national engineering laboratory for animal breeding, moa key laboratory of animal genetics and breeding, college of animal science and technology, china agricultural university, Beijing, China</td>
<td>2</td>
<td>33</td>
</tr>
<tr>
<td>college of veterinary medicine, school of veterinary and life sciences, Murdoch University, Murdoch, Australia</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>université françois rabelais de tours, tours, France</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>IFCE, Nouzilly, France</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>institute of poultry diseases, division of veterinary medicine, Freie Universität Berlin, Berlin, Germany</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>institute of veterinary anatomy, division of veterinary medicine, Freie Universität Berlin, Berlin, Germany</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>experimental zoology division, Wageningen University and Research, Wageningen, Netherlands</td>
<td>2</td>
<td>15</td>
</tr>
</tbody>
</table>

Source: Data processed using Vosviewer

Figure 1. Index keyword topics related to the topics of the embryo of broiler chicken. Source: Data processed using Vosviewer.

of those keywords means that several factors could affect the development of the embryo of broiler chicken, such as immunity against disease, some drugs given to hen, and genetics.

Figure 2 showed that the word “genes” and “chicken” were the major words used as the topic in published papers. At the right side of the word “chicken”, there was a word “messenger Ribonucleat acid”, “cell”, and “data”. It means that mRNA also had some kind of influence on the embryo. There were some words, such as “day”, “heat”, and “liver”. It means that besides daylight and heat that could affect the growth of the embryo, the liver condition of the hen of broiler chicken also contributed to the growth of the embryo too.

Figure 3 showed that topics of “chickens” had the biggest frequency come out from the web-based search than other...
topics used in published papers. It was because the chicken was the main topic of this study, which means that it had the biggest one. Besides the topic “chicken”, other topics that had a frequency of more than 100 were “genes”, and “expression”. Interestingly, there were topics like “virus” and “response” that had 100 frequency of occurrence from 2014 to 2016.

Figure 4 showed there were 4 areas of theme based on how important and how well some words developed in research fields. Those words were taken from abstract papers that became samples of this study. The motor theme area was only for words that have the important aspect and are well developed in the research field. Contrary to the motor theme, emerging or declining themes was an area where the keywords were neither developed well enough nor important for their research fields. The niche and the basic theme were different from each other. While Niche themes area for the topics which were not important to the research field but well developed, basic themes were the opposite of niche themes (Radha & Arumugam, 2021).

In the motor theme, there were some words such as “chicken”, “genes”, “development”, “effect”, “growth”, and “control”. It means that genes influenced the growth of the embryo of a chicken. But still, even though a chicken gene was predetermined, the genetic engineering of chicken insemination to produce a superior breed with a fast-growing rate of the embryo could be used. Other factors such as disease and types of feeders also had a significant effect on embryo growth.

Figure 5 showed that the word “chicken” was still the one that has grown most than other words used in published papers. The word “gene” was also the second most growing word. It means
Figure 4. Thematic map of the major words in abstract. Source: Data processed using biblioshiny from R studio.

Figure 5. Word growth from abstract of published papers. Source: Data processed using biblioshiny from R studio.
that the gene of the chicken itself, whether from hen or cock of broiler-type determines the growth rate of the embryo along with how resistant its embryo against disease. Because broiler chicken was the type of chicken raised for its egg and meat, it would grow faster than other types of chicken, so the output rate of meat production would be faster too.

Broiler chicken was one of the main sources of meat in many countries. The demand for meat from chicken, especially from fast-food chain restaurants caused many breeding houses and farms to raise more broiler because broiler could grow fast. Figure 5 showed that words besides “chicken”, such as “genes” and “growth” had raised higher than other types of words included in publications published by which was indexed by Scopus. It means that genes and growth become the major concern of authors around the world who were interested in observing embryos of broiler chickens.

Discussion

Figure 1 showed some keywords such as “chicken”, “embryology”, and “immunology”. In particular, the chicken has uniqueness in the process of regulation of B cell production by B-cell receptor complex (BCR). Marrow Bone does not play a role in the formation of B cells in fowl, but the bursa of Fabricius, a GALT organ specifically, in which there is B-cell lymphopoiesis. However, the surface expression of BCR has been maintained as an important checkpoint both on mammals and birds amidst variations in the microenvironment (Liu et al., 2019). After colonization of the bursal follicle by B cells expresses the prediversified slg receptor, program induced gene conversion to diversify the VDJH gene and VJL through gene conversion. It is at this stage that the repertoire premutation is generated. Some evidence suggests that this repertoire is generated in an independent manner antigens. Most convincingly, this evidence comes from repertoire development among B cells supported by messenger T cell receptors. (Puspita et al., 2021).

In addition, the use of different pseudogenes in these B cells are correlated closely related to the use of pseudogenes in development normal B cells (Wirth et al., 2017). It also supports a model in which primary repertoire development is encouraged by the availability of pseudogene sequences without selection substantial for certain V gene sequences. Obviously, kind this analysis will not be able to detect subtle differences in the repertoire that may occur as consequences of negative selection from reactive specificity self. (Patel et al., 2021).

So, in Figure 1, the word “immunology” is also associated with the word “unclassified drug”. Antibiotics in animal feed have been used since 1946 with the aim of increasing livestock productivity and health. In poultry, Antibiotics are used with the aim of increasing feed digestibility, growth and egg production, improve feed conversion, reduce mortality and maintain health conditions. Currently, the use of antibiotics is not only in broilers, but also other intensively reared birds (Gadde et al., 2017; Puvača et al., 2016).

Figure 2 showed that there were some words related to “genes”, “chicken”, “mRNA”, and “cell”. It could be said that different genes of chicken stored in M-RNA played important role in the characteristics of the embryo itself. For example, laying hens are chickens reared to produce lots of eggs and are the final product of purebred chickens and should not be re-crossed (Aengwanich & Suttajit, 2010). Laying hens are adult hens that are specially reared for their eggs. This type of chicken is a species of “Gallus Domesticus”. The first chickens to enter and start breeding in Indonesia were white leghorn laying hens, which were thin and generally turned into chicken pieces after their productive period (Coyne et al., 2020).

Laying hens are divided into three types, namely the light type from the white leghorn breed, the medium type from the Rhode Island Red, and Barred Plymouth Rock, and the heavy type from the New Hampshire, white Plymouth Rock, and Cornish breeds (Puspita et al., 2021). The origin of laying hens is from partridges that have been domesticated and selected so that they lay quite a lot of eggs. The direction of partridge selection is aimed at large production. However, because the jungle fowl can be taken for eggs and meat, the direction of the selection is starting to be specific. Chickens selected for meat production are known as broilers, while for egg production, known as laying hens (Tamburawa et al., 2018).

Figure 2 also showed that there was a word “vaccine” near two words, “body” and “distributed”. It could mean that broiler chicken needs a vaccine to get immunity from disease. One of the viruses caused by birds that had become famous before was bird flu.

Bird Flu (H5N1), classified as orthomyxoviruses that attack the respiratory or nervous system. This bird flu has a mortality rate up to 100%, so it is often called highly pathogenic avian influenza (Butler, 2006). The bird flu virus can cause human death, because the nature of this virus is easy to mutate to form new types of viruses that are dangerous, and death if bird flu meets human flu (Malek & Hoque, 2022).

Transmission of the bird flu virus by means of the salivary glands (saliva) and chicken manure (excreta), and this virus can live to 30 days with temperature of 0°C, but this virus can die at a temperature of 80 °C within 1 minute. This disease can be spread through the respiratory tract, conjunctiva, and feces (excreta) by being transmitted through direct contact between sick and healthy chickens. In addition, it can be through air contact, it can also be through vehicles, equipment, feed, drinking water that have been contaminated with AI. Blood Defecation Disease (Coccidiosis), was protozoa of Eimeria, causes diarrhea and inflammation of the intestines (enteritis). Feeder and drinking water that have been contaminated with AI. Blood Defecation Disease (Coccidiosis), is a disease caused by protozoa of the genus Eimeria which causes diarrhea and inflammation of the intestines (enteritis) (Butler, 2006).
Coccidiosis attacks young chickens and occurs due to warm litter conditions or high humidity (wet litter). This disease disrupts the absorption process of intestinal nutrients so that the metabolic process does not run perfectly which causes impaired growth in chickens (Butler, 2006). Coccidiosis spreads in the form of single cells (oocysts) that are excreted in the feces (excreta) so that they are brownish or blood-red in color due to intestinal inflammation. Coccidiosis is grouped into three, namely “Eimeria Acervulina” which attacks the front intestine, “Eimeria Necratix” which attacks the middle intestine, and “Eimeria Tenella” which attacks the appendix or back intestine (Butler, 2006). “Protozoa Oocysts” can live outside the chicken’s body for 2–4 days and are eaten by chickens into the intestinal tract, then develop and divide with a developmental process of 4–7 days. The clinical symptoms of the affected chickens include poor appetite but high appetite, brownish or reddish excreta, wrinkled and dull skin, and decreased bodyweight of the chickens.

Figure 3 showed that trend topics per year that had high frequency were “genes”, and “expression”, as well as “virus”. It means that the virus also became a concern for a research topic to learn more about the embryo of chicken. It means that response against the virus was important for the hen to create immunity for its embryo.

The main problem which is the toughest challenge in chicken farming is the emergence of disease, so its management needs to be done efficiently and professionally. Diseases that attack chickens are many and often have almost the same symptoms. Chronic Respiratory Disease (CRD), often called snoring, is a disease caused by the bacterium “Mycoplasma Gallisepticum” that infects the respiratory tract. Snoring disease is also known as complex CRD, which is a combination of “Tetelo” disease, bronchitis, or mixed bacteria (Timms et al., 1989).

Symptoms of chickens that are attacked by CRD are mucus in the nostrils that cause blockage and often shaking their heads to remove the mucus blockage, swelling in the eye and face area, drowsiness, decreased appetite, and a snoring sound in breathing. Complex CRD occurs due to “Mycoplasma Gallisepticum” infection which lasts longer and is clearer, and there are wounds in the respiratory tract, inflammation of the lungs (pneumonia), and thickening of the air sacs (airsaculitis). This disease attacks quickly in a long time with a morbidity rate of 70–90%, the incubation period lasts 24–48 hours after being contaminated through the nose (intrasal) or the nasal cavity (intrasal) (Abbas et al., 2018).

Another type of virus that could affect the embryo of chicken was the virulent strain of Newcastle Disease Virus (VND). It caused disturbances in the nervous, digestive, and respiratory systems of poultry, while infection in chicken embryos causes growth disturbances which can lead to death. A study conducted by Purnasari, Adi, and Winaya observed the effect of the APMV-1 virus isolate from Badung-2/AK/2014 on embryo weight and brain histopathological features of chicken embryos. This study used 18 embryonic chicken eggs (TAB) aged 11 days which were divided into two treatment groups, namely Group A and Group B, each of which consisted of nine eggs. Group A was inoculated with Phosphate Buffer Saline (PBS) and group B was inoculated with isolate Badung-2/AK/2014. Three days after inoculation, allantoic fluid from all treatment groups was collected.

To prove that the two groups did not contaminate each other, hemagglutination (HA) and hemagglutination inhibition (HI) tests were carried out. Chicken embryos were removed from the eggshells, then weighed and the average weight of the embryos of groups A and B were analyzed by independent sample T-test. After that, the chicken embryos were necropsied for brain organs and put into 10% Neutral Buffer Formalin (NBF). Subsequently, preparations were made using the Hematoxylin Eosin (HE) staining technique. Histopathological lesions were observed under a microscope and the results were presented descriptively. The results showed that the isolates caused histopathological lesions in the form of vasculitis and perivascular edema in the brain and resulted in weight loss of chicken embryos (Purnasari et al., 2017).

Figure 4 showed some words related to embryo growth such as “growth” and “genes”. According Mukandungutse et al., Aflatoxins are toxic compounds that are mutagenic, teratogenic, and carcinogenic and are generally found in foodstuffs derived from grains such as corn, rice, and beans of poor quality. Its presence in foodstuffs, including food from livestock in Indonesia, has been widely disclosed by various researchers, but research on its toxicity is still very limited.

A study by Mukandungutse et al tried to observe the effect of aflatoxin B1 on chicken embryos. It also observed effect of various doses of aflatoxin B1 on not alive and the ability to hatch the embryos, as well as complementing the previous information. In this study, the dose of AFB1 used was 0; 15.6; 31.2; 62.5; 125; and 250 ng AFB1 per embryonic egg administered via airbag to 25 5-day-old sprouted eggs each. The results obtained showed that the hatchability of embryos up to day 21 was 66, 28, 26, 16, 0 and 0% for dose 0; 15.6; 31.2; 62.5; 125; and 250 ng AFB1. Administration of AFB1 has also caused embryonic abnormality in the form of bleeding, egg yolk malabsorption, dwarfism, weakness, and mild leg defects. the body weight of hatched chicks did not have a significant difference between groups, although there was a lower tendency for giving high doses of AFB1 (Mukandungutse et al., 2020).

Conclusions
Based on article review that the publications about the embryo of broiler chicken had more concern about immunity, virus, and disease as external factors that could impede the embryo’s growth. Moreover, for internal factors, some words have become a trend in publications, such as “gene”. But still, the study about diseases and viruses that could affect an embryo’s growth was more dominant. It could be seen from Figure 2, Figure 3, and Figure 4 that virus and disease became the trending words in publications. It means that the focus on how to prevent disease, the characteristic of viruses or
bacteria, and the incubation period of microorganisms inside the embryo had become the hot trending topic for publications of papers published with the intended topic. Further study suggested focusing more on the nutrition needed for broiler’s hen as one of the external factors in deciding the growth rate of the embryo.

References


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