

Fifty years of sheep red blood cells to monitor humoral immunity in poultry: a scientometric evaluation

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ABSTRACT Sheep red blood cells (SRBC) are commonly employed by scientists to address humoral immune responses in poultry. While SRBC are closely related to the study of humoral immunity in poultry, the initial purpose of much research did not focus on the mechanisms involved. Here, we provide a qualitative approach and utilize scientometric techniques, including trend analyses, scientific collaborations and mapping, and word co-occurrence evaluations, to summarize the role of SRBC in the poultry studies. First, a search strategy on Web of Science (WoS) was conducted to find publications that included SRBC in the poultry studies. Publications were partitioned into 4 categories: nutrition, genetics, microbiology, and physiology. For scientometric evaluation, scientific maps and networks were produced to clarify the occurrence of SRBC in the poultry studies. Data used included 702 publications over a period of 50 y (1968-2018) that were retrieved from the WoS database. About 95% of the publications were published in English language. Indigenous,

experimental, and commercial chickens, quail, and medicinal plants field/topics were the main subjects of publications. In recent years, authors have used SRBC to study humoral immune response as a secondary aim of their research, especially when poultry production/performance was studied. This was especially the case in recent decades for studies in poultry nutrition. Analysis of keywords co-occurrence showed that the phrase SRBC mostly occurred with chickens, immune response, and especially with broilers. Moreover, the “medicinal plants” are becoming important especially for research on broilers and the reduced use of antibiotics in feed. Consequently, in addition to studying the medicinal plants, finding antibiotic replacements, and/or growth performance in the birds, humoral immunity is suggested to be investigated using SRBC. Moreover, interdisciplinary studies with the cooperation of scientists from agriculture, veterinary, immunology, biochemistry and molecular biology, and toxicology will develop in the future.

Key words: chicken, humoral immunity, immune responses, SRBC, broilers, medicinal plants

2020 Poultry Science 99:4758–4768

<https://doi.org/10.1016/j.psj.2020.06.058>

INTRODUCTION

In response to increased global demand, poultry meat production has dramatically risen 300% during the last 3 decades and in 2018 was estimated to be more than 128 million tons (FAOSTAT, 2018). Like poultry meat, egg production and consumption have increased as well (FAOSTAT, 2018). Globalization of commercial

broilers and laying hens, plus turkeys, quail, and even indigenous chickens in recent years, have demonstrated attractive markets for poultry meat and eggs. Additionally, with increased global demands for poultry products, research in this field has augmented. There is a significant coordination between global poultry production and the rate of research (Scanes, 2007). Often, poultry scientists have conducted investigations to improve cost-effective profitability (Kanakri et al., 2017; Purandaradas et al., 2018), whereas reproduction and poultry well-being (health and immunity) have not received adequate attention (Mohammadi-Tighsiah et al., 2018).

The immune system provides numerous ways to protect poultry against different types of pathogens

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Received December 9, 2019.

Accepted June 19, 2020.

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(Davison, 2014). The immune response of the poultry could be conveniently partitioned to innate and adaptive immunity in which the adaptive immunity is divided into 2 classes, including cell-mediated and humoral responses. Humoral immunity (B-cell adaptive immune responses) is usually referred to as the antibody titers against pathogens/antigens (Yang et al., 2017). Types of the pathogen/antigen are considered to be one of the more important factors in antibody production; however, experiments are not just designed to study immune responses to specific pathogens/antigens (Siegel et al., 2006).

Based on resource allocation theory, finite resources are allocated for maintenance, growth, reproduction, and health to ensure survival of the individual (Siegel and Honaker, 2009). However, in recent years, intense selection for increased production in meat and layer chickens may have adversely affected the health status and/or immune system performance of the birds (van der Most et al., 2011). Rapid growth in broilers (Geiger et al., 2018) and long-term egg production in laying strains (Wolc et al., 2013) have enhanced profitability, which is desirable most of the commercial breeding programs. Although, studying health status (immune system performance) of the birds alongside the main focus of research have become common in recent years, the primary aim of the research was not to study the immune system per se.

Because the primary aim of some studies in poultry was not immune responses per se, using a noninfectious pathogen (a safe antigen) to investigate humoral immunity has merit. Sheep red blood cells (SRBC) have been identified over the last 50 y for this purpose. Considering its availability and easy access, SRBC is applied in different research fields such as poultry genetics, physiology, and nutrition; however, the primary aim of those studies was not necessarily studying the effect of SRBC on humoral immunity. With little or no impact on other performance criteria (in particular growth and reproduction), SRBC could independently stimulate humoral immunity. Even though, the main focus subject of the research might be nutrition (Dunnington et al., 1996; Parmentier et al., 1997; Qorbanpour et al., 2018), genetics (Pinard et al., 1992; Bovenhuis et al., 2002; Zhou et al., 2003; Parmentier et al., 2017), microbiology (Taheri et al., 2010; Rostami et al., 2015), and/or physiology (Albrecht et al., 2012; Blevins et al., 2012), responses to SRBC was a measurement criterion.

As a specific research field, scientometric techniques are important tools which can be used in quantitative evaluation of the knowledge structures of the science and dynamics of the research community and the intellectual environment of the field (Schubert, 2001). Scientometrics is considered to be a method of text mining. Recently, scientometrics have become common in medicine (e.g., Fricke et al., 2013), but they are rarely considered in the poultry science (Ducrot et al., 2016). Based on the nature of the research, different scientometric techniques are available. The aims of the current study were to provide a qualitative approach (content

analysis) and using scientometric techniques (research topics, and trends analyses, and articles indices) to study the contribution of SRBC in poultry science during last 50 y.

MATERIAL AND METHODS

Search Strategy

The current study aimed to explore the role of SRBC in poultry science through a content analysis and scientometric techniques and indicators. We focused on domestic poultry in Web of Science database and found 702 publications before April, 2019. We used the following search strategy as: “TS= ((Chicken OR chick OR hen OR broiler OR rooster OR cock OR Quail* OR Duck* OR goose OR geese OR pigeon* OR ostrich* OR partridge* OR bird* OR pheasant* OR turk* OR emu OR avian* OR aves OR Guinea fowl OR “Guinea fowl”) AND (“Sheep Red Blood Cell*” OR SRBC OR “Sheep Erythrocyt*”)”. Then, all the publications including each domestic poultry in addition with each of SRBC, “Sheep Red Blood Cell”, or “Sheep erythrocyte” were retrieved.

Content Analysis

Research Fields For content evaluation (qualitative analysis), research topics, poultry species, and time periods were categorized. Closely related topics were put into the same category. According to *Poultry Science* sections, topics were categorized as animal well-being and behavior, genetics and genomics, immunology, health and disease, management and production, metabolism and nutrition, microbiology and food safety, molecular and cellular biology, physiology and reproduction, and processing and products. Here, all the publication items were partitioned into 4 categories (topics), including nutrition, genetics, microbiology, and physiology (Table 1). Because we focused mainly on

Table 1. Categorization of publications based on scientific topics.

Topics	Examples
Nutrition	Nutrient requirement, feed additives, manipulated feeding ¹ , prebiotic and probiotic consumption, vitamin/mineral supplementation, medicinal plants feeding, metabolism, mycotoxins in nutrition
Genetics	Estimation of genetic parameters, QTL mapping, gene expression studies, selection, line-breeding, cross-breeding, GWAS, genomic evaluation, association analysis, molecular biology
Microbiology	Gut microbiology, infectious microbiology, virology, antimicrobial, antibacterial
Physiology	Immunology, environmental stress ² , histological studies, pathology, lighting, hematology, noninfectious diseases, aging, reproduction, molting, endocrinology

¹Manipulated feeding defined as force feeding or restricted feeding.

²Environmental stress such as heat or cold stress and stocking density.

published title, abstract, and/or keywords, some studies might have been categorized into more than one field.

Poultry Species/Strains To explore publications based on poultry species and species-based trends during the last 5 decades, all the publications were classified according to species/strain. Hence, the poultry categories were turkeys, quail, laying hens, dual-purpose chickens, chickens, and broilers. The word chicken totally related to domestic chicken (*Gallus gallus*). Thus, when chicken strain (broiler, laying hen, indigenous chicken, or dual-purpose chicken) was not clear, an additional search was made on type of chicken. Moreover, because frequencies of some species/strains were limited, some of the birds were categorized together. Therefore, the other birds' species/strains categories were wild birds as well as other poultry. According to the web-based search for SRBC with poultry species, some publications were retrieved that were not relevant to our research aims and thus categorized as mammals or irrelevant.

Time Periods To our knowledge, the phrase SRBC first occurred in the literature in Solomon (1968). Also, because the number of publications in 2019 was 2, they were excluded from the final analyses. Therefore, a 50-y period was included, and 4 consequent time-periods were considered as before 1988 (<<1988), 1989 to 1998, 1999 to 2008, and 2009 to 2018.

Scientometric Evaluations

Publication data preparation was conducted through Bibexcel software (Bibexcel, 2013). To Microsoft Excel was employed to illustrate data descriptive characteristics (Microsoft Corporation, Redmond, WA). Type of publications, language, number of authors, and publications with/without citations were extracted. Moreover, scientific collaborations (countries, authors, and organizations) were retrieved to draw scientific networks through NetDraw (version 2.153) and UCInet (6.581 release) softwares (Borgatti et al., 2002). A minimum of 2 co-authors were involved for drawing the countries and authors co-cited networks.

The VOSviewer software was employed to draw the keywords co-occurrence analysis in the mentioned field (Van Eck and Waltman, 2010). Density drawings were prepared to visualize "hot" topics and trends of SRBC in poultry by the keywords in the retrieved publications. Because there was variety between keywords with the same concept, we preprocessed and assessed the keywords and made them unique before the visualization. Moreover, recommendations of poultry scientists have been utilized to the keywords homogenization, classification, and analyses. For example, to prepare final maps, the phrase SRBC was considered instead of "Sheep Red Blood Cell(s)", "Sheep RBC", and "sheep erythrocyte(s)". Moreover, different species/variety of medicinal plants, different kinds of viruses, different classes of antibodies and immunoglobulins, and different classes of antibiotics were converted to "medicinal plants", virus, antibody, and antibiotics, respectively.

Therefore, closer phrases in the map reflect the more frequent co-occurrence keywords. Moreover, as frequency of keywords increased, the phrase letter became bigger, and the color of area in the map tended to become red, against less frequent keywords which would be seen in blue context. Yellow and green colors are referred to as the intermediate frequencies.

RESULTS AND DISCUSSION

Content Analyses

Frequency of publications that used SRBC in poultry researches is shown in Figure 1. To our knowledge, the title of the first paper that used SRBC in the field of poultry science was "Ontogeny of cells producing hemolytic antibody or immunocyto-adherence to Sheep Erythrocytes in chicken" (Solomon, 1968). Published items which have studied humoral immune responses using SRBC in poultry could be conveniently partitioned in 2 periods, before and after 1990 (Figure 1). Before 1990, frequency of publications was approximately constant and maximally reached to 5 items/y. However, during the last 3 decades, the frequency of publications have increased, following a relatively constant pattern with approximately 21 article/y. Most of the publications were published at 2012 (36 publications).

To better assess the publications including SRBC in poultry researches, research fields were evaluated in 4 consequent time-periods, including before 1988 (<<1988), 1989 to 1998, 1999 to 2008, and 2009 to 2018 decades (Figure 2). Before 1988, most of the studies on SRBC in poultry were initially in the field of physiology (23 publications in physiology vs. 13 items in the other 3 fields). During the 1989 to 1998 decade, physiology with 82 publications was the most usual field; however, there was a subsequent decrease in the last 2 decades (63 and 62 publications in 1999-2008 and 2009-2018 decades). Publications in microbiology were almost constant across decades and never exceed 10 publications/decade. While investigating SRBC in poultry in the genetic studies were relatively equal at 1989 to 1998 (78 publications) and 1999 to 2008 decades (75 publications), it declined approximately 2 times in recent years (41 publications).

Between 4 scientific topics, the most remarkable increase occurred in the field of nutrition, whereas publications were 3, 45, 70, and 152 items in 4 consecutive time-periods, respectively (Figure 2). In fact, studies including SRBC in the field of nutrition grew more than 50 times during the studied time-period. Ignoring scientific topics, the paucity of publications before 1990 may be because of several reasons. Insufficient coverage of the scientific databases on all of the existing publications, relatively lower importance of the birds' immune system performance compared with other economic traits in poultry breeding companies (Arthur and Albers, 2003), and lower importance of poultry as a food-producing animals in comparison with swine and beef (FAOSTAT, 2018), have been mentioned as the

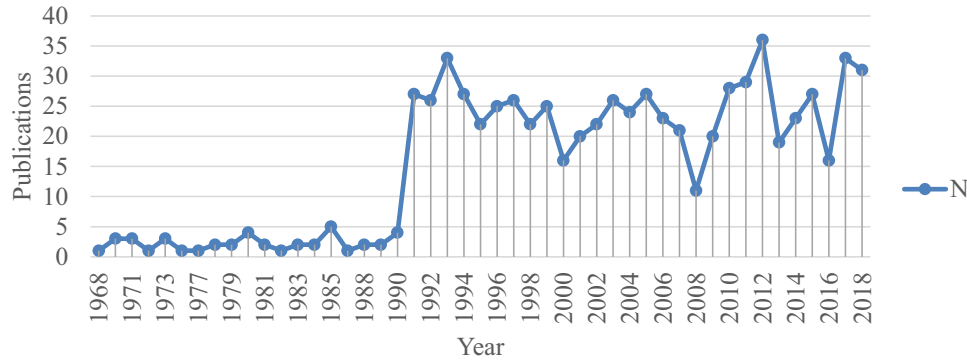


Figure 1. Number of publications including SRBC in poultry over 50 y (from 1968 to 2018). Abbreviation: SRBC, sheep red blood cells.

reasons for this lack of studies. While research on broilers are becoming the most prominent field in poultry science (Figure 3), improved attention to immunity after 1990 may be because of the importance of the broilers' higher market weight, whereas it is emphasized on negative genetic correlations between growth and immunity (e.g., Mohammadi-Tighsiah et al., 2018). Therefore, higher genetic selection pressure for body weight enhancement have adversely influenced birds' immune responses. Balance between the diet ingredients ensures maximizing the birds' genetic potential. In other words, breeding and rearing broilers are closely tied to nutrition. In commercial state, broilers are heavily fed which may suppress their immune system performance referring to the resource allocation theory (Siegel and Honaker, 2009; van der Most et al., 2011). Therefore, using SRBC as a noninfectious antigen to study humoral immune responses in poultry nutrition has become the main topic in recent years (Kidd, 2004; Rasouli-Hiq et al., 2017; Khanipour et al., 2019). As seen in Figure 1, simultaneous study of poultry nutrition and using SRBC to appraise the humoral immune status of the birds may likely remain important in the future.

Influence of SRBC on humoral immune responses in wild birds were reported in 42 publications (Figure 3). Humoral immunity of wild birds has been influenced by climate changes, migration, season, reproductive

performance, and age. These factors have emerged as interesting areas of study for wild avian species (Kilpimaa et al., 2007; Martyka et al., 2011; Grzedzicka, 2017). Other poultry species were totally assigned in 15 publications that included studying SRBC in ducks (5 studies), pheasants (3 studies), dwarf chickens, geese, guinea fowl, ostrich, partridges, and pigeons (each with 1 study). Mostly, the domestic chicken was divided into broilers, laying hens, and dual-purpose or indigenous strains/breeds. However, in some studies, based on their title, abstract, and/or key words, the strain/breed of chickens was not provided, and thus the common noun "chicken" was used.

Most of the 27 studies including "chicken" were published before 1988, and before the 1990s, differences among breed/strain types received little attention. In recent years, using the word "chicken" in the scientific publications has become rare (Figure 3), perhaps because of the differentiation between broilers, laying hens, indigenous chickens, and dual-purpose chickens. While, publications of SRBC on dual-purpose chickens have been reduced in recent years, those on quail (12), indigenous chickens (15), and broilers (132 publications) have increased. In case of broilers, occurrence of SRBC in publications during <<1988, 1989 to 1998, 1999 to 2008, and 2009 to 2018 decades were 1, 26, 65, and 132, respectively. While those on laying hens for the

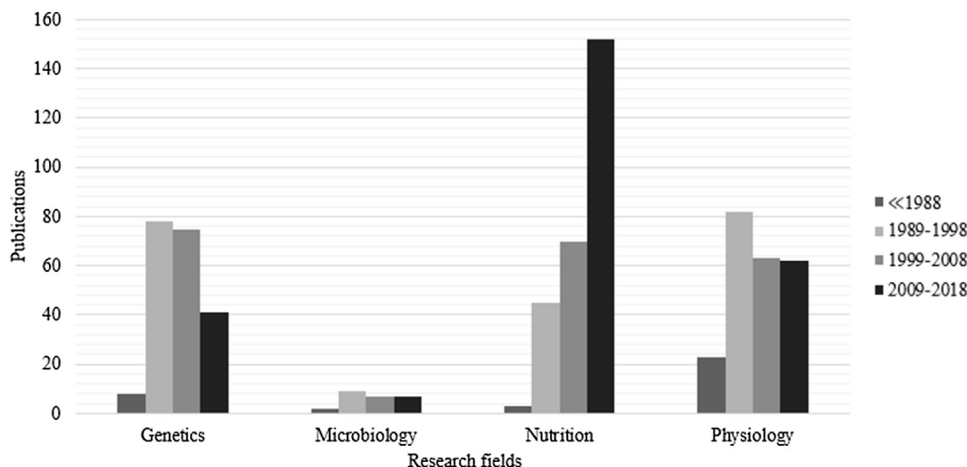


Figure 2. Number of publications on SRBC in poultry by research fields (from 1968 to 2018). Abbreviation: SRBC, sheep red blood cells.

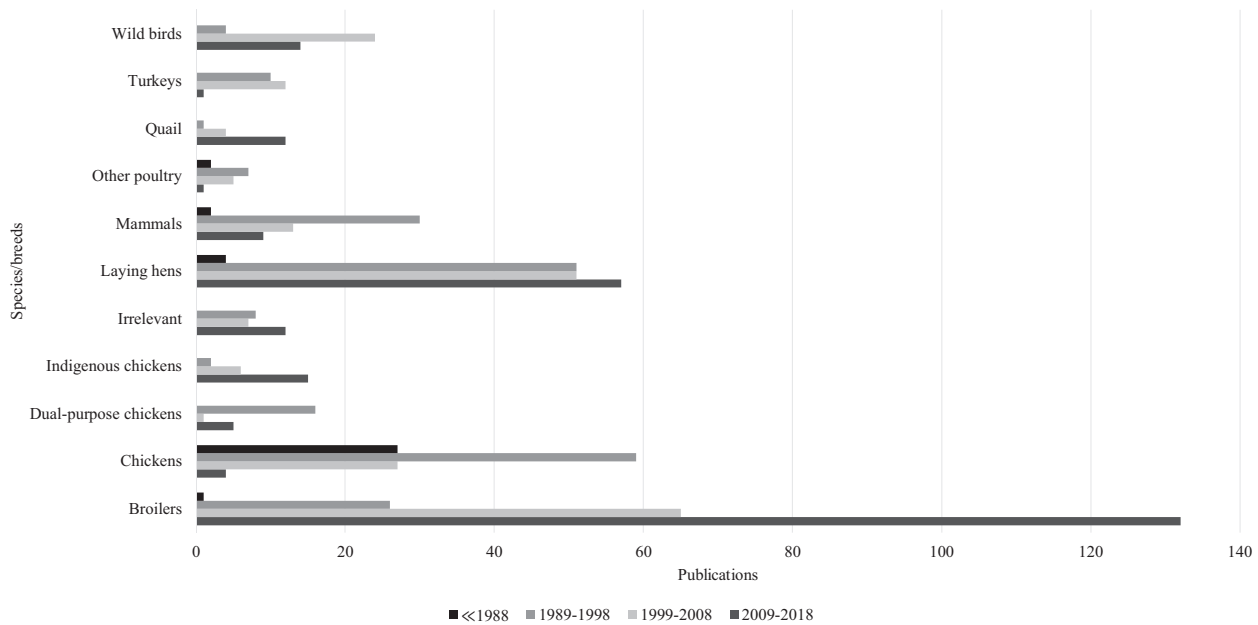


Figure 3. Categorization of publications on SRBC in poultry based on species/breed (from 1968 to 2018). Abbreviation: SRBC, sheep red blood cells.

1989 to 1998 decade were twice that for broilers (51 vs. 26), during the last 2 decades those for broilers were more than those for laying hens, especially for the 2009 to 2018 decade (132 vs. 57). Except for studies before 1988, studying SRBC in laying hens were relatively constant. Unlike broilers, relatively constant number of publications on using SRBC to monitor humoral immunity of laying hens may be a response to the importance of the well-being health in laying hens breeding industry (Futlon, 2004). In other words, the laying hens' immunity is a challenging trait in breeding programs; therefore, layers have less immunological problems than broilers, and less attention has been paid to studying the performance of the laying hens' immune system. Moreover, there has been only 1 publication involving turkeys during the last decade. This means that the study of turkey immune system using SRBC has not received much attention.

Scientometric Evaluation

Types of publications from the 702 retrieved publication items included research articles, conference proceedings, research notes, conference abstracts, review articles, letters, and book sections with 668, 16, 12, 12, 4, 1, and 1 publications, respectively. Moreover, publications ($n = 702$) were written in 9 different languages including English, Russian, Hungarian, and Turkish, with 668, 4, 2, and 2 publications, respectively, followed by Czech, Ukrainian, Japanese, German, and Portuguese languages each with 1 publication.

Scientific Mapping To illustrate country and author collaboration networks, each node (circle) is representative of a country/author, and the lines between the nodes refer to the connection strength between nodes.

Higher publications associated with a country/author resulted in wider diameter of the corresponding circle. Moreover, more collaboration between countries/authors resulted in thicker connecting lines between nodes (Figure 4 and Figure 5). Totally, 54 countries contributed in retrieved publications in which USA, Iran, and India were the most prolific countries with 209, 109, and 62, respectively. Other countries were the Netherlands, Japan, China, Poland, Pakistan, Turkey, and Canada with 48, 32, 25, 20, 18, 18, and 17 publications, respectively. Iranian and Italian researchers had the most scientific collaboration with 8 publications followed by USA–France researchers (7), Belgium–Netherlands researchers (6), and USA–China researchers (6).

Among studied poultry, meat-type chickens and layer hens' immunity are assumed major importance than other breeds/strains (Figure 3). Thus, we consider the chicken meat and egg production as the major parts of the global poultry production. The United States with 209 publications is ranked first and third in terms of global production of the chicken meat and egg, respectively (FAOSTAT, 2018). Therefore, it is assumed an applicable harmony between publications of USA poultry scientist and national commercial poultry industry to study chicken humoral immunity. China was the sixth country contributing to retrieved publications which is ranked third and first in terms of global production of the chicken meat and egg, respectively. Surprisingly, Brazil is ranked second and sixth in chicken meat and egg production, whereas had rank of 17 with 8 scientific publications. Besides, Brazilian researchers had no international collaborations. Results showed that compared with yearly chicken meat and egg production, researchers in China and Brazil had less contribution with industry to study chicken immunity through

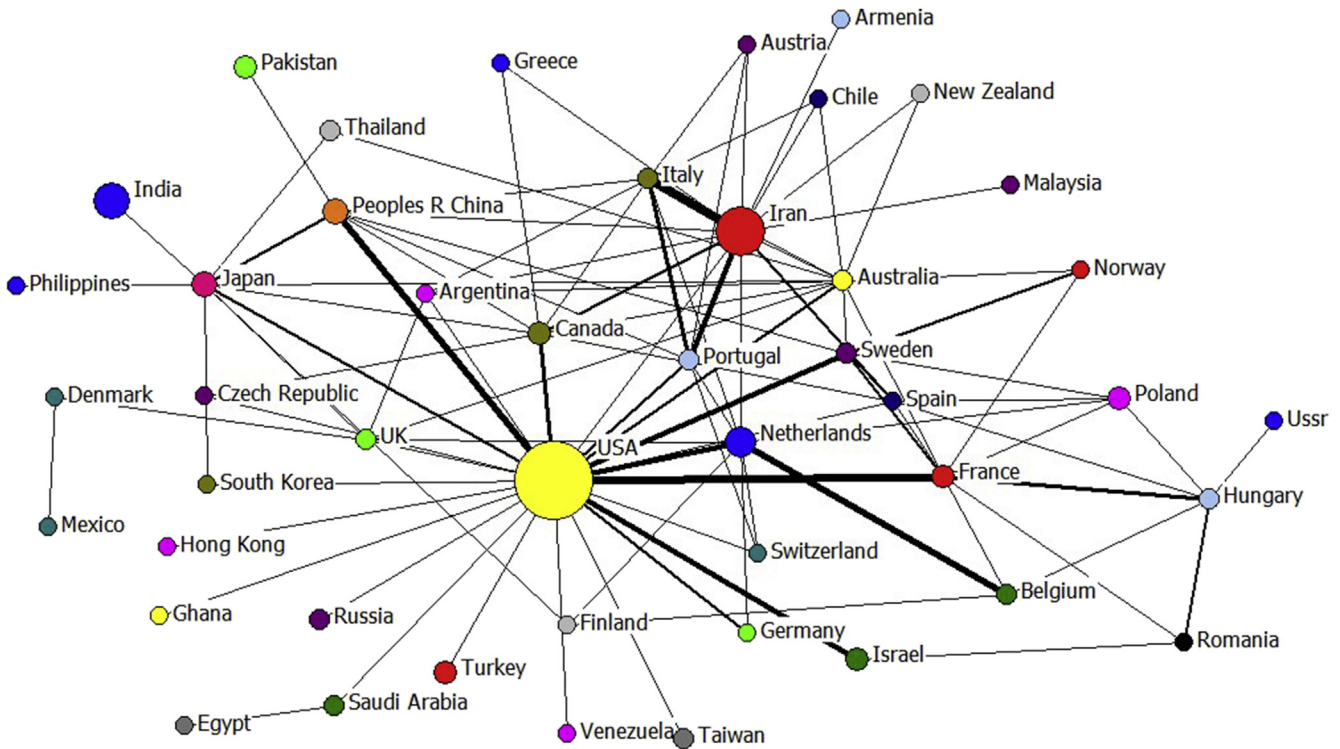


Figure 4. Scientific collaborations among countries involving SRBC in poultry (threshold at least 2 joint publications). Abbreviation: SRBC, sheep red blood cells.

challenging with SRBC than those of USA researchers. Importance of other traits as growth and reproduction, lesser institutional funds for studying chicken immunity and lower health problem of poultry in China and Brazil may be the main reasons. Regarding the publication frequencies, Russia, Mexico, and Indonesia are among top

10 countries for chicken meat and egg production, which surprisingly are not placed among the highest prolific publication countries. Researchers in USA have contribution with 24 countries, followed by Iranian and Chinese researchers with 15 and 8 countries, respectively. Unlike, considering scientific publications, Iran was the

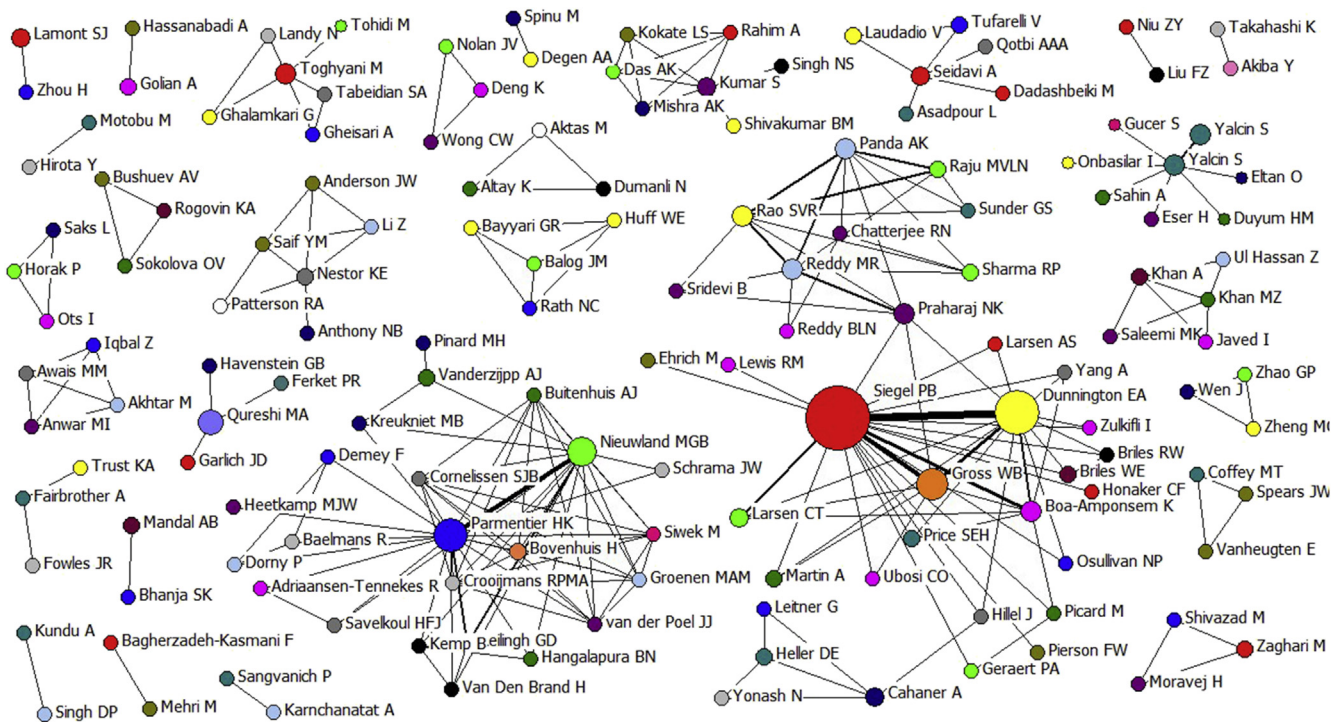


Figure 5. Scientific collaborations among authors involving SRBC in poultry (threshold at least 2 co-authorship). Abbreviation: SRBC, sheep red blood cells.

Table 2. Reference journals with highest number of publications in which data for SRBC in poultry were measured.

Rank	Journal name	Document no.	%	IF (2019)	Country
1	Poultry Science	155	22.08	2.027	USA
2	British Poultry Science	32	4.56	1.096	UK
3	Veterinary Immunology and Immunopathology	21	2.99	1.846	The Netherlands
4	Archiv Fur Geflugelkunde	19	2.71	0.568	Germany
5	Avian Diseases	17	2.42	1.306	USA
6	Indian Journal of Animal Sciences	17	2.42	0.227	India
7	Asian-Australasian Journal of Animal Sciences	15	2.14	1.227	South Korea
8	Journal of Applied Animal Research	14	1.99	1.092	UK
9	Livestock Science	13	1.85	1.376	The Netherlands
10	Journal of Applied Poultry Research	10	1.42	0.808	USA

Abbreviation: SRBC, sheep red blood cells.

in [Table 3](#). The most cited article was written by [Cook et al. \(1993\)](#) with 376 citation. The most recent highly cited article is a meta-analysis to study the trade-off between growth and immunity in commercial broilers with 118 citation ([van der Most et al., 2011](#)).

CONCLUSION

Scientometrics indicators provide a valuable mirror to monitor certain research areas in the literature. Accordingly, our data provide new insights about how research history is associated with SRBC. Considering SRBC as a secondary tool to study humoral immunity, it was revealed that measuring the humoral immune responses in broiler is precursor in recent years. Moreover, scientometrics showed that the poultry nutrition compared

with the other research fields is more related to SRBC-induced immune responses, particularly in recent years. Therefore, in addition to studying the medicinal plants, finding antibiotic replacements, and growth performance, it is suggested that the birds' humoral immunity be investigated using SRBC. Moreover, interdisciplinary studies with the cooperation of scientists from fields of agriculture, veterinary, immunology, biochemistry and molecular biology, and toxicology will develop in the future.

ACKNOWLEDGMENT

The authors thank Prof. Paul B. Siegel for comments involving this manuscript. This work was supported by the Vice Chancellor for Research and

Table 3. Most cited articles where SRBC in poultry science were measured.

Article title	Authors/year	Citation
Immune modulation by altered nutrient metabolism - nutritional control of immune-induced growth depression	(Cook et al., 1993)	376
Production and persistence of antibodies in chickens to sheep erythrocytes.1. directional selection	(Siegel and Gross, 1980)	295
Antigen recognition strength regulates the choice between extra follicular plasma cell and germinal center B cell differentiation	(Paus et al., 2006)	245
Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress	(Bartlett and Smith, 2003)	177
Costs of immunity: Immune responsiveness reduces survival in a vertebrate	(Hanssen et al., 2004)	168
A tradeoff between immunocompetence and sexual ornamentation in domestic fowl	(Verhulst et al., 1999)	162
Long-term divergent selection for 8-wk body weight in White Plymouth Rock chickens	(Dunnington and Siegel, 1996)	157
Production and persistence of antibodies in chickens to sheep erythrocytes. 2. Resistance to infectious-diseases	(Gross et al., 1980)	154
A comparison of the immune performance of a 1991 commercial broiler with a 1957 random-bred strain when fed typical 1957 and 1991 broiler diets	(Qureshi and Havenstein, 1994)	132
Trade-off between growth and immune function: a meta-analysis of selection experiments	(van der Most et al., 2011)	118

Abbreviation: SRBC, sheep red blood cells.

Technology, University of Zabol, Zabol, Iran (Grant No. UOZ-GR9618-47).

Conflict of Interest Statement: The authors did not provide any conflict of interest statement.

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