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Bibliometric Analysis and Science Mapping on RNA-seq and Gene Expression in Sheep

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Abstract: This study aimed to determine the development of research articles on 'RNA-sequencing and gene expression' in sheep between 2011-2023 in the Web of Science (WoS) database by scientific mapping method. In this regard, 205 articles were examined in the first search using the relevant keywords, and 124 articles suitable for analysis. They were analysed with the Shiny web application of the Bibliometrix R package, and VOSviewer. The results determined that the country with the most publications in the relevant field was China, the related institution with the most studies was "The University of Edinburgh", and the journal with the most publications was "PLoS One". According to the keyword analysis, the trend topics started from studies on granulosa cells and showed into research areas such as immune response, growth, pathway and meat quality. In the abstract analysis, the word 'transcriptome' and words such as mammary gland, muscle, fertility, Peripheral Blood Mononuclear Cells (PBMCs), lactation, fat storage were found together, while the words forming the parasitic agents, drug resistance, miRNA studies were clustered in different groups. The scarcity of the articles obtained in the analysed period reveals the openness of the study area. It can be suggested that the researchers who will plan to work on this subject can plan studies on the identification of variants belonging to different sheep breeds, resistance to antiparasitic drugs used in sheep, meat yield, disease resistance, reproductive tissues and organs, as well as designing all these studies as study subjects based on climate change and global warming factor.

Keywords: Bibliometric analysis, gene expression, RNA-sequencing, sheep

Koyun Türünde RNA-Dizileme ve Gen İfadesi Üzerine Bibliyometrik Analiz ve Bilimsel Haritalama

Öz: Bu çalışmada, Web of Science (WoS) veri tabanında 2011-2023 yılları arasında koyunlarda 'RNA dizileme ve gen ifadesi' konulu araştırma makalelerinin gelişiminin bilimsel haritalama yöntemi ile belirlenmesi amaçlanmıştır. Bu kapsamda, ilgili anahtar kelimeler kullanılarak yapılan ilk taramada 205 makale incelenmiş ve 124 makale analize uygun bulunmuştur. Bu makaleler Bibliometrix R paketinin Shiny web uygulaması ve VOSviewer ile analiz edilmiştir. Sonuçlar, ilgili alanda en çok yayın yapılan ülkenin Çin, en çok çalışma yapılan ilgili kurumun "The University of Edinburgh", en çok yayın yapılan derginin ise "PLoS One" olduğunu ortaya koymuştur. Anahtar kelime analizine göre trend konuları granüloza hücreleri üzerine yapılan çalışmalardan başlayarak bağışıklık tepkisi, büyüme, yolak ve et kalitesi gibi araştırma alanlarına doğru ilerlemiştir. Özet analizinde 'transkriptom' kelimesi ile meme bezi, kas, fertilite, PBMC, laktasyon, yağ depolama gibi kelimeler bir arada bulunurken, parazitik ajanlar, ilaç direnci, miRNA çalışmalarını oluşturan kelimeler farklı gruplarda kümelenmiştir. İncelenen dönemde elde edilen makalelerin azlığı çalışma alanının açıklığını ortaya koymaktadır. Bu konuda çalışma planlayacak araştırmacıların farklı koyun ırklarına ait varyantların belirlenmesi, koyunlarda kullanılan antiparaziter ilaçlara direnç, et verimi, hastalıklara direnç, üreme doku ve organları gibi konularda çalışmalar planlayabilecekleri gibi tüm bu çalışmaları iklim değişikliği ve küresel ısınma faktörüne dayalı çalışma konuları olarak tasarlamaları önerilebilir.

Anahtar kelimeler: Bibliyometrik analiz, gen ifadesi, koyun, RNA-dizileme

Introduction

Bibliometric analyses are important for providing a comprehensive evaluation of the publications reported in terms of institutions, authors, sources, citations, countries (quality, quantity and impact) used. Although there are other types of research, such as meta-analyses and systematic reviews, bibliometric analyses require better management and more resources in the field of research and statistical measurement. (Salinas-Ríos, 2022). With the advancement of bioinformatics algorithms, technologies in the field of molecular genetics rapidly advancing and the decreasing costs of methods and curiosity in planning research in different species is increasing day by day. One of these methods is RNA-seq, transcriptome analysis (Kukurba and Montgomery, 2015). There are no bibliometric study compiling studies in the field of gene-expression and RNA-seq in the sheep species.

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In the study planned for this purpose in the manual search conducted with the keywords "RNA-seq and gene expression" in sheep, the first identified study was a composite transcriptome study using RNA-seq analysis in sheep with a delayed bone healing model, which serves as a model organism (Jäger et al., 2011). Because sheep is a model organism for humans, several studies including a study on polycystic ovary syndrome in sheep by Sinha et al. (2020), a study by Dou et al. (2020) analyzing transcriptional regulation in visceral and subcutaneous fat tissues in female sheep exposed to prenatal bisphenol-A. Another study by Quttainah et al. (2022) where RNA-seq analysis was conducted to obtain transcriptomic information following an experimental design created in sheep related to heart failure observed in humans have been conducted on sheep.In addition to these studies on model organisms, studies related to meat and fat metabolism development have also been identified. Armstrong et al. (2018) conducted a study in which gene expression profiles were analysed in 9 different muscle species to explain the high degree of phenotypic differences observed in histochemical and metabolic parameters related to meat quality among different muscles. Bakhtiarizadeh et al. (2019) conducted a study on fat-tail metabolism in fat-tail sheep. Farhadi et al. (2023) reported the examination of gene expression profiles of fat tissue in thin-tailed and fat-tailed male sheep breeds using RNA-seq analysis method. RNA-seq and gene expression studies related to milk yield in sheep are as follows. Wang et al. (2020) stated a mammary gland transcriptome study in lactating and non-lactating Small-tailed Han ewes; Farhadian et al. (2022) stated a transcriptome analysis by RNA-seq method from milk collected at two different stages of lactation in Ghezel ewes. Suárez-Vega et al. (2023) indicated study in which the relationship between high and low feed efficiency and milk yield was determined by milk somatic cell transcriptome. Some studies related to fleece formation and molecular development in sheep; Lv et al. (2022) stated a study in which miRNA and mRNA profiles of hair follicles in skin tissue of Hu sheep were examined by RNA-seq method; Shi et al. (2022) indicated a study in which signaling pathways and key genes associated with wool density in Hetian sheep were determined.

A study by Li et al. (2020) investigated the lung tissue response to experimental Mycoplasma pneumonia infection in Argali hybrid sheep at the transcriptome level. A transcriptomic meta-analysis on unannotated long non-coding RNAs associated with immune response in sheep was performed by Bilbao-Arribas and Jugo (2022). A study by Kyselová et al. (2023); examined the Caseous Lymphadenitis immune response in sheep at the whole blood transcriptome level. Another study also investigated the Caseous Lymphadenitis immune response in sheep at the whole blood transcriptome level (Lins et al., 2023) and reported the RNA-seq analysis of abomasal tissues against Haemonchus contortus resistance in Santa Ines and Ile de France breed dairy lambs.

When previous studies are examined, it has been reported that many studies have been carried out in the field investigated with the reproductive system in sheep. Many studies on the reproductive system of sheep have been carried out. The effect of poor maternal nutrition during the gestational period on prenatal muscle development and growth was determined by RNA-seq analysis in a study performed by Gauvin et al. (2020). Chang et al. (2022) reported a study investigating the FecB genotype associated with fertility in small-tailed Han sheep and transcriptome analysis in thyroid tissue related to different genotypes; Chen et al. (2022) reported a study in which miRNA-mRNA analysis of adrenal glands playing a role in the reproductive system in sheep was determined by RNA-seq method. Sadeghi et al. (2022) investigated the lncRNA-miRNA, mRNA and ceRNA network in Romanov and Baluchi sheep in good and poor genetic value animals to understand the molecular mechanisms responsible for fertility in sheep. Li et al. (2022) investigated the effect of high altitude on alternative splicing and gene expression of ovarian follicle development in Tibetan sheep. In a study of Liu et al. (2023) reproductive system-related genes were identified in 10 different tissues in Xinggao sheep The effect of melatonin on the morula stage of sheep vitrified embryos was examined at the transcriptome level in a study conducted by Ji et al. (2023). In general, the reviewed literatures were determined as studies in the fields of model organism, meat, milk, wool production, reproductive system, and immune system.

Through bibliometric analyses, important trends in the research area have been reported in literature, journals, authors, keyword analyses, and institutions. Especially with the use of integrated software with Scopus and Web of Science (WoS) databases, these analyses can be conducted. The bibliometric development of gene expression and RNA-seq studies in sheep species is not known. Therefore, in this study, it was aimed to identify RNA-seq and gene expression studies in sheep species in the WoS database between 2011-2024 and to analyse these studies by bibliometric analyses on the basis of citation, author, institution, country and keyword.

Material and Methods

Database creation and bibliometric analyses

The Web of Science database search included literature from 01.01.2011 to 01.01.2024. The search question used to analyze the scientific publications was designed as follows: (All Fields (AF) = ("ovine"

OR "sheep" OR "lamb") AND AF= ("gene expression" AND "RNA-seq"). All records and reference information of the identified literatures were converted into a Plain Text File (.txt). Bibliometric analyses were performed using the R-based Bibliometrix package Biblioshiny version 4.1.4 (Aria and Cuccurullo, 2017) and VOSviewer software, version 1.6.20 (van Eck and Waltman, 2010). The WoS search query identified 205 literatures on 'RNA-seq and gene expression' in sheep from 2011-2023. Relevant literatures were analysed for title and abstract content. After eliminating irrelevant literatures (RNA-seq studies on bacteria causing disease in sheep, RNA-seq studies on viruses, goat-related RNA-seq studies not directly related to sheep), a total of 124 studies were analysed. In the Bibliometrix package, articles were filtered by year and language and analyses were initiated on 124 final articles (articles, papers: early access). Among the analyses performed, annual scientific production of countries and institutions, bibliographic merging with sources, Most Cited Articles and Most Influential Authors, trending topics and common word analyses constituted the main data information.

Results

Quantitative analysis of publication

The main data information analyzed in the Bibliometrix program is presented in Table 1.

Table 1. Main data information

Main information about data	
Timespan	2011:2023
Sources (Journals, Books, etc)	58
Documents	124
Annual Growth Rate %	27.23
Document Average Age	4.6
Average citations per doc	12.79
References	6795
Document contents	
Keywords Plus (ID)	648
Author's Keywords (DE)	344
Authors	
Authors	767
Authors of single-authored docs	0
Authors collaboration	
Single-authored docs	0
Co-Authors per Doc	7.94
International co-authorships %	32.26

The graph of the annual increase of the studies from January 2011 to January 2024 is shown in Figure 1. It has been observed that the number of studies has started to increase since 2014. In particular, it was determined that the number of studies produced in 2020 reached the highest level in the examined research area.

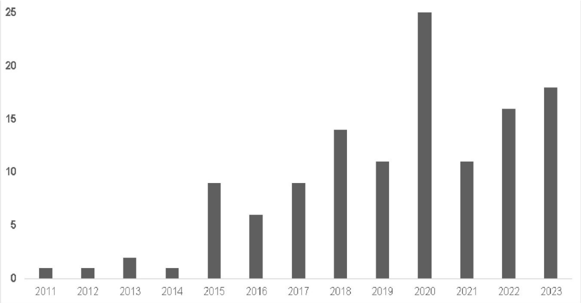

Figure 1: Annually number of publications on gene expression and RNA-seq studies in sheep.

Analysis of countries and institutions

According to the results of the analysis of the publication production of RNA-seq and gene expression research in sheep, there was an increase in countries such as Australia, the United Kingdom, Spain, the USA, and China since 2014, with China being at the highest level in this increase. Considering the most appropriate links identified by the authors of the relevant articles, the University of Edinburgh had the highest number of articles (n=22) which was closely followed by ICAR-Indian Veterinary Research (15 articles) and ICAR-Indian Council of Agricultural Research (13 articles). Figure 2 shows a three-domain plot using the Sankey plot describing the interaction between institutions, countries and journals. The findings indicated that the United Kingdom, India, China, and France were the dominant countries in terms of countries, institutions and resources.

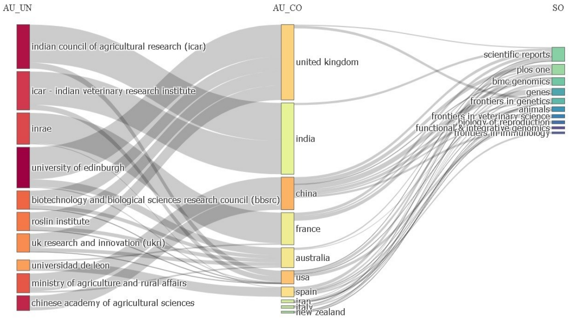

Figure 2: Three-field plot showing the network between institutions (left), countries (middle), and journals (right).

Bibliographic coupling with sources

A total of 62 sources were generated from research articles, and the full counting method was applied with a minimum threshold of 5. Only 6 sources met these criteria. For each of the 6 sources, the total strength of bibliographic coupling links with other sources was calculated (Table 2). The analysis revealed 10 links and total link strength of 530, forming 1 cluster with 5 items. The cluster comprised 5 elements. The source with the highest total link strength was "Scientific Reports", followed by "PLoS One".

The Bradford area reported PLoS One, Scientific Reports, BMC Genomics, Frontiers in Genetics, was identified as the area where the distribution of articles across journals was analyzed and showed the inaugural academic articles relevant to the area under investigation.

Highly cited articles and most influential authors

Table 4 presents the top 5 most cited research articles. "A high-resolution atlas of gene expression in the domestic sheep (Ovis aries)" performed by Clark et al. (2017). Published in 2017 was the most cited article with 90 citations in 5 years in the field of RNA-seq and gene expression in sheep.

Table 2. The top most 10 strong bibliographic coupling with sources

Rank	Sources	Clusters	Links	Total link strength	Documents	Citations
1	PLoS One	1	4	215	11	180
2	Scientific Reports	1	4	257	9	259
3	Frontiers in Genetics	1	4	219	7	53
4	BMC Genomics	1	4	215	7	209
5	Genes	1	4	154	7	51

Among the top 10 journals, "PLoS One" ranked first as the journal with the highest number of published articles. As indicated in Table 4, the number of publications in Frontiers in Veterinary Science, which has the highest Journal Impact Factor (JIF), is still low (Table 3).

Table 3: Top 10 journals with the most papers

Rank	Journals	Documents	JIF Percentile	JIF Quartile
1	PLoS One	11	88.39	Q2
2	Scientific Reports	9	70.5	Q2
3	Animals	7	81.5	Q1
4	BMC Genomics	7	70.6	Q2
5	Frontiers in Genetics	7	65.2	Q2
6	Genes	7	61.7	Q2
7	Frontiers in Veterinary Science	4	92.0	Q1
8	Biology of Reproduction	3	66.1	Q2
9	Frontiers in Immunology	3	78.6	Q1
10	Functional & Integrative Genomics	3	47.1	Q3

JIF: Journal Impact Factor

Table 4. Highly cited articles

Rank	Title	Paper	Total Citations	TC per Year	Normalized TC
1	A high resolution atlas of gene expression in the domestic sheep (Ovis aries).	Clark et al. (2017)	90	11.25	4
2	Effects of early feeding on the host rumen transcriptome and bacterial diversity in lambs.	Wang et al. (2016)	75	8.33	2.76
3	Genome-wide transcriptome analysis of mRNAs and microRNAs in Dorset and Small Tail Han sheep to explore the regulation of fecundity.	Miao et al. (2016)	60	6	1.72
4	Composite transcriptome assembly of RNA-seq data in a sheep model for delayed bone healing.	Jäger et al. (2011)	55	3.92	1
5	Genome-wide mRNA-seq profiling reveals predominant down-regulation of lipid metabolic processes in adipose tissues of Small Tail Han than Dorset sheep.	Miao et al. (2015)	54	5.4	1.55

TC: Total citations

In Figure 3, the most prolific authors in the field of gene-expression and RNA-seq studies are shown based on the number of publications attributed to each author. The size of the circles in the visualization indicates the number of publications authored by a person in a given year, while the density of the circles represents the number of citations received by the author in the same year. As the number of publications and citations increased, the size and density of the circles increased proportionally. In particular, authors such as Arranz JJ, Guteierrez-GIL B, and Suarez-Vega A (Suárez-Vega et al., 2018; Chitneedi et al., 2020; Suárez-Vega et al., 2023) were found to have continuity in their publications from 2015 until 2023. Authors such as Bush SJ, Clark EL, Hume DA and Mcculloch MEB (Clark et al., 2017; Bush et al., 2017; 2018; 2019; 2020; Salavati et al., 2019) were found to be active in terms of publications and citations in 2017 and 2020.

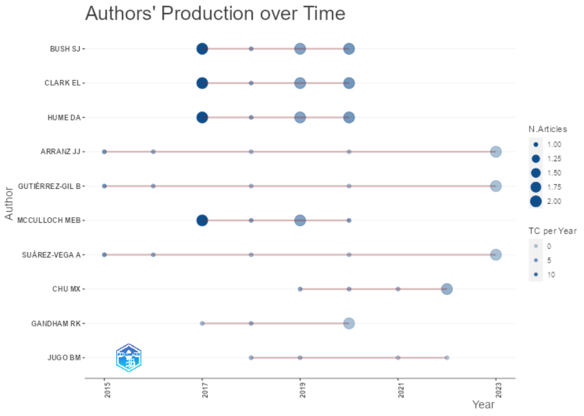

Figure 3. Production of the most productive authors over time.

Keyword analysis

Keyword analysis is crucial for gaining insight into key issues, focus areas, and trends in a research field. Such analyses enable researchers to quickly grasp the most discussed topics and key concepts. The word cloud highlighted the words "rna-seq", "growth", "and expression" but also different terms as identified in Figure 4.

Figure 4. Keyword analysis results for the research area.

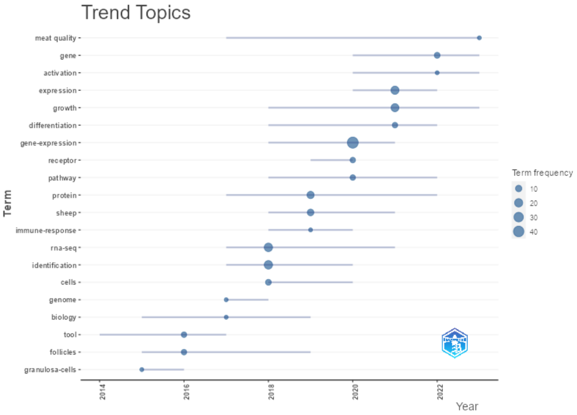

Figure 5. Trend topics across years for RNA-seq and gene-expression studies in sheep.

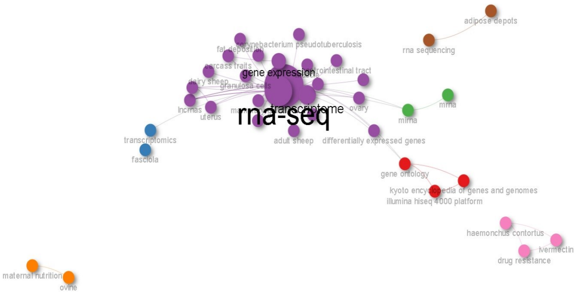

Figure 6. The result of a network of keywords that coword in the field of gene-expression and RNA-seq studies in sheep.

Discussion and Conclusion

Bibliometric analyses are studies in which scientific articles are evaluated both mathematically and statistically. The first study was reportedly conducted by Alan Pitchard in 1969 (Salinas-Ríos, 2022). These analyzes are carried out in many fields of science, but with the development of algorithms, it can be used more effectively in recent years. When the increasing graph of the studies conducted in the investigated field according to years was examined, there was an increase in the studies since 2011 and this increase reached the highest level in 2020. In 2021, it was determined that the decrease in 2023 was temporary and an increase was observed again in 2023. The top 10 journals with the highest number of publications in the field of RNA-seq and gene expression in sheep species were determined as WoS categories; Multidisciplinary Sciences, Agriculture, Dairy & Animals Science, Genetics & Heredity, Veterinary Science, Reproductive Biology, Immunology. The reported research areas can be defined as the intersection of RNA-seq and gene expression studies in sheep.

According to Bradford's law, the top three journals with the most publications were "PLoS One", Frontiers in Genetics", "Animals", while the three most cited journals were "Bioinformatics", "Nucleic Acid Research" and "PLoS One". Clark et al. (2017) was the most cited author in the related research field with 90 citations during the analysis period. When the subject of the study was examined, it was confirmed that a high-resolution gene expression atlas of domestic sheep was created and the researchers benefited from this article as a reference source. It was determined that the authors who produced continuous publications over time were Arranz JJ, Guterrez-Gil B and Suarez-Vega A (Suárez-Vega et al., 2018; Chitneedi et al., 2020; Suárez-Vega et al., 2023; Alonso-Garcia et al., 2023). In addition, Bush SJ, Clarck EL, Hume DA and McCulluch MEB (Clark et al., 2017; Bush et al., 2017; 2018; 2019; 2020; Salavati et al., 2019; 2020; Tsang et al., 2020) were determined as the authors working together with the highest number of local citations. When the fields of study of the most cited researchers were examined, it was determined that they were on early fat storage in sheep, perirenal fat transcriptome (Suárez-Vega et al., 2018; Alonso-Garcia et al., 2023), resistance to gastrointestinal nematodes (Chitneedi et al., 2020), milk transcriptome (Suárez-Vega et al., 2023). Other highly cited researchers worked on lncRNAs (Bush et al., 2018), gastrointestinal tract transcriptome at different developmental stages (Bush et al., 2019), species-specific mammalian macrophage response to Lipopolysaccharide (Bush et al., 2020). It was determined that the identified authors have been studying and publishing in the relevant research field for many years. According to the keyword analyses, the words "rna-seq", "transcriptome", "sheep", "mammary gland", " lncRNA ", "prolificacy" and "gene expression" were highlighted . The United Kingdom, China and India were the top three countries where the most articles were published whereas the top three institutions were University of Edinburgh, Indian Veterinary Research Institute and Indian Council of Agricultural Research (ICAR).

According to the trend subject analyses, it was determined that in the first years, granulosa cell studies were emphasized in the abstract, while in the following years, immune response, pathway, receptor, growth, and meat quality studies came to the fore. It can be recommended that researchers should design their researches in this respect.

Seven different clusters were determined according to the results of keyword analysis in sheep species. These clusters were identified as miRNA, fasciola, drug resistance, maternal nutrition, adipose storage, prolificacy, Corynebacterium pseudotuberculosis, lncRNA, ovary, mammary gland, lactation. In addition to the areas where keywords are specified, studies should be planned by identifying keywords that are not included in the clusters. Studies planned in the reported areas can provide researchers with the opportunity for discussion. Keywords can also shed light on the future in studies to be planned in unreported areas. The results of analyses indicated that the studies conducted in the field of RNA-seq and gene expression in sheep species since 2011 are still in the development stage. It is necessary to carry out gene expression studies specific to different breeds, in different tissues, in different disease or application situations, such as the studies reported by Li et al. (2020) or Li et al. (2022) in the manual review. Especially in cattle species, studies measuring the responses to heat stress have started to be carried out. Research on this subject can also be planned in sheep species (Khan et al., 2020; Czech et al., 2022). Results were also obtained regarding the journals in which the planned studies could be published on Q1 and Q2 WOS cathegories.

This is the first bibliometric analysis planned in the field of RNA-seq and gene expression in sheep with the obtained results, predictions are provided for researchers to plan their studies in this field and they can carry out their planned studies with which institutions and organizations. However, the scarcity of the articles obtained in the analysed period reveals the openness of the study area. It can be suggested that the researchers who will plan to work on this subject can plan studies on the identification of variants belonging to different sheep breeds, resistance to antiparasitic drugs used in sheep, meat yield, disease resistance, reproductive tissues and organs, as well as designing all these studies as study subjects based on climate change and global warming factor. In the light of the present study, it can be recommended that researchers who plan to work in this field should direct their studies and plan their experimental designs in the field of RNA-seq and gene expression in sheep by examining the reported analyses.

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