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What unanswered questions remain about the use of molecular markers in beef production?

O que ainda não sabemos sobre o uso de marcadores molecular na produção de carne bovina?

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ABSTRACT

The application of molecular markers in beef production has garnered significant attention in recent years due to its potential to enhance productivity and efficiency. This study conducted a systematic review and bibliometric analysis using the Web of Science (WoS) database to identify and analyze the main molecular markers utilized in beef production. A total of 808 articles published between 2013 and 2023 were retrieved, of which 722 met the inclusion criteria after duplicate removal and filtering by publication year. The bibliometric analysis was performed using the R software and the bibliometrix R package to examine keyword trends and research patterns. The results indicate that Single Nucleotide Polymorphisms (SNPs) and Polymerase Chain Reaction (PCR) were the most frequently cited molecular markers, highlighting their relevance in genetic improvement studies. PCR remains the most widely used technique due to its efficiency in DNA amplification, while SNPs offer a high-resolution approach to detecting genetic variations. Conversely, Amplified Fragment Length Polymorphism (AFLP) marker was the least cited, likely due to its labor-intensive and cost-prohibitive nature. A geographical analysis of the publications revealed that China, the United States, and Brazil were the leading contributors, with China emerging as the most prolific country in this field. Despite being one of the largest beef producers, Brazil lags in scientific contributions, primarily due to limited research funding. The findings indicate an increasing integration of molecular markers in beef production. However, a knowledge gap persists in connecting genetic markers with microbial resistance and meat quality traits. Future research should address these connections to optimize meat production efficiency and quality.

KEYWORDS: animal breeding. beef quality. bibliometric analysis. knowledge gap. molecular genetic. SNP.

RESUMO

O uso de marcadores moleculares na produção de carne bovina tem ganhado destaque nos últimos anos devido a sua contribuição no aumento produtivo e na eficiência. Este estudo realizou uma análise bibliométrica sistemática utilizando a base de dados Web of Science (WoS) para identificar e analisar os principais marcadores moleculares utilizados na produção de carne bovina. Um total de 808 artigos publicados entre 2013 e 2023 foi recuperado, dos quais 722 atenderam aos critérios de inclusão após a remoção de duplicatas e filtragem por ano de publicação. A análise bibliométrica foi realizada utilizando o software R e o pacote bibliometrix para examinar os padrões de pesquisa. Os resultados indicam que os Polimorfismos de Nucleotídeo Único (SNPs) foram os marcadores moleculares mais citados, destacando sua relevância nos estudos de melhoramento genético. A Reação em Cadeia da Polimerase (PCR) continua sendo a técnica mais amplamente utilizada devido à sua eficiência na amplificação de DNA, enquanto os SNPs oferecem uma abordagem de alta resolução para a detecção de variações genéticas. Por outro lado, o marcador AFLP (Polimorfismo de comprimento de fragmento amplificado) foi o menos citado, possivelmente devido ao seu alto custo e à sua complexidade laboratorial. A análise geográfica das publicações revelou que a China, os Estados Unidos e o Brasil foram os principais contribuidores, com a China emergindo como o país mais produtivo nessa área. Apesar de ser um dos maiores produtores de

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carne bovina, o Brasil ainda está atrás em contribuições científicas em nível mundial, principalmente devido ao baixo investimento em pesquisa. Os resultados sugerem uma crescente integração dos marcadores moleculares na produção de carne bovina, embora ainda exista uma lacuna de conhecimento quanto à relação entre marcadores genéticos, resistência microbiana e atributos de qualidade da carne. Pesquisas futuras deverão explorar essas conexões para otimizar a eficiência e a qualidade da produção de carne.

PALAVRAS-CHAVE: melhoramento animal. qualidade da carne. análise bibliométrica. lacuna de conhecimento. genética molecular. SNP.

INTRODUCTION

The primary goal of beef cattle production is to supply meat for the population's consumption. In Brazil, this industry is highly developed and has a broad geographical presence. However, the production of other animals, such as poultry and pigs, is economically significant.

To maintain competitive advantage in the livestock product industry, Brazil's livestock sector has been investing in programs for genetic improvement to enhance production efficiency and standardize animals and their products (GOES et al. 2012).

Phenotypes result from complex interactions between an organism's genotype and its environment. This process involves cellular mechanisms that convert the information encoded in DNA into proteins. DNA carries the instructions needed to produce proteins, which then interact both within the cell and with the surrounding environment. In a controlled setting, the desirable traits will emerge from the genetic information encoded in DNA (REGITANO & COUTINHO 2001).

Molecular markers are valuable tools in biotechnology that help improve production quality while reducing costs. A genetic or molecular marker is a specific segment of DNA that enables the assessment of genetic diversity (ZOLET et al. 2017). These markers are used to identify alleles, which simplifies the identification process. They follow simple Mendelian inheritance patterns, enabling researchers to infer genotype from phenotype. This characteristic facilitates the monitoring of gene marker segregation. To effectively analyze this segregation, it is crucial that there be polymorphism at the marker locus.

Although molecular markers play an increasingly important role in improving meat production, there is a noticeable lack of studies that systematically and critically analyze the development of this field in the scientific literature. In this context, a bibliometric approach becomes relevant to understand the evolution of the area, identify key research trends and existing gaps, and guide future investigations

Molecular markers are becoming increasingly important for enhancing meat production. However, there is a noticeable scarcity of studies that systematically and critically evaluate the development of this field in scientific literature. In this context, a bibliometric approach is useful for understanding the evolution of the area, identifying key research trends and gaps, and guiding future investigations. So, this study aims to investigate the research landscape on the application of molecular markers in meat production through a comprehensive bibliographic review and bibliometric analysis.

MATERIALS AND METHODS

Bibliographic data

For the development of this bibliometric study, we adopted the PRISMA 2020 methodology (Preferred Reporting Items for Systematic Reviews and Meta-Analyses), as proposed by PAGE et al. (2022). A systematic review was conducted, focusing on a well-defined question to identify, select, evaluate, and synthesize the relevant evidence available (GALVÃO & PEREIRA 2014). This type of review enables a broader understanding of the academic output on the topic of interest, allowing us to incorporate a wider spectrum of relevant results rather than limiting our conclusions to just a few articles (SAMPAIO & MANCINI 2007).

Initially, a bibliographic research was conducted in three main phases (TRANFIELD et al. 2003): I) Planning the review: this phase involved identifying the research theme and selecting relevant keywords; II) Conducting a review: we performed a literature search and selected studies that met the established criteria; III) Data extraction: we examined the chosen studies to extract significant data.

The initiation of the planning phase includes the identification of the following research questions: "How can molecular markers enhance productivity and efficiency in beef meat production?", “What are the main molecular markers used in studies related to beef meat production?” and "What are the primary genetic markers utilized for improving the traits related to beef meat?".

In order to answer these questions, the keywords “molecular marker”, “beef”, “meat” and “cattle” were used in the Web of Science (WoS) database, considering the Boolean operators AND, OR, and NOT. For a more refined search, ''advanced search'' was used to consult articles that dealt with the main molecular markers used, such as: SSR (Simple Sequence Repeats), PCR (Polymerase Chain Reaction), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), STS (Sequence Tagged Sites), RAPD (Random Amplified Polymorphic DNA), SNP (Single Nucleotide Polymorphism). All identified studies were definitively included in the analysis.

Regarding the topic covered, only articles and review articles from the years 2013 to 2023 were considered. The search for articles dealing with the use of molecular markers applied to animal genetic improvement returned 808 articles, 59 of which dealt with the use of SNPs, 1 with STS, 28 with RAPD, 233 with AFLP, 24 with RFLP, 372 with PCR and 91 with SSR.

Bibliometric Analysis

The results from WoS were exported using a custom format to include additional data fields. After exporting, the files were generated individually containing each molecular marker and initially saved in "BibTeX" format according to the functions available on the WoS search platform. The inclusion criteria for the documents considered their relevance to the topic being investigated. The selection process prioritized studies that focused on the use of molecular markers to identify superior genotypes for beef production, as well as those related to meat quality.

The initial selection of documents was based on the reading of titles and abstracts, in order to choose those most relevant to the study's research objective. Articles that did not fit within the predetermined research area were excluded. The extraction of raw data was performed directly from the respective database websites and exported in BibTeX format using the export tools provided by Web of Science. The extracted data included information related to the title, authors, abstract, keywords, citations, journals, citation count, access count, affiliations, document type, research areas, cited references, number of cited references, usage count, ID numbers, and language.

The R software (R CORE TEAM 2024) and its packages were the instruments applied in the analysis. The data obtained from the searches was submitted into the bibliometric analysis through the Bibliometrix R package (ARIA & CUCCURULLO 2017) and its tool Biblioshiny, in the WordCloud option for each individually generated file, to check the alignment of the keywords with the content of the article. Subsequently, there was a need to export the files individually to save them in a new format (plain text file). The data was analyzed to remove duplicates and articles that fell outside the year of publication criteria, returning 722 articles.

RESULTS AND DISCUSSION

The Table 1 contains detailed information of the bibliometric survey results. Based on this compilation of data, the documents are well distributed among the 234 different journals, generating an average of three articles per journal.

Table 1. Summary of bibliometric survey results about the use of molecular markers in meat production from the period of 2013 to 2023.

Indicator	Result
Articles	722
Period	2013:2023
Authors	3014
Single-author articles	05
Sources	234
Average co-authorship	**07**

In recent decades, advances in genetic science have significantly enhanced the productivity of animal production systems. This progress has allowed for an increase in both the quantity and quality of products derived from these systems. One of the key biotechnologies contributing to this improvement is the use of molecular markers, which leverage genomic variations to enhance the genetic analysis of individual animals (BORÉM & CAIXETA 2009). This analysis highlights the strong link between science and improvements in productivity and cost-effectiveness within animal production chains. During the last decade, a total of 722 articles were published, which average about 73 articles per year. The publication rate peaked in 2014, with 85 articles released, as illustrated in Figure 1.

During the analysis of the results, it was noted that the keywords "Single Nucleotide Polymorphisms (SNPs)" and "Polymerase Chain Reaction (PCR)" were the most frequently cited in the searches conducted. SNPs are genetic markers that detect variations resulting from the alteration of a single base in the genome. For a variation to be classified as a SNP, it must occur in at least 1% of the population (BORÉM & CAIXETA 2009). This type of marker is less complex but much more abundant, with millions of polymorphisms identified in production animal species, distributed uniformly throughout the genome (CAETANO 2009). In contrast, PCR is the most commonly used method in animal genetic studies because it allows for the exponential amplification of DNA. This makes it a valuable technique when used in conjunction with other markers.

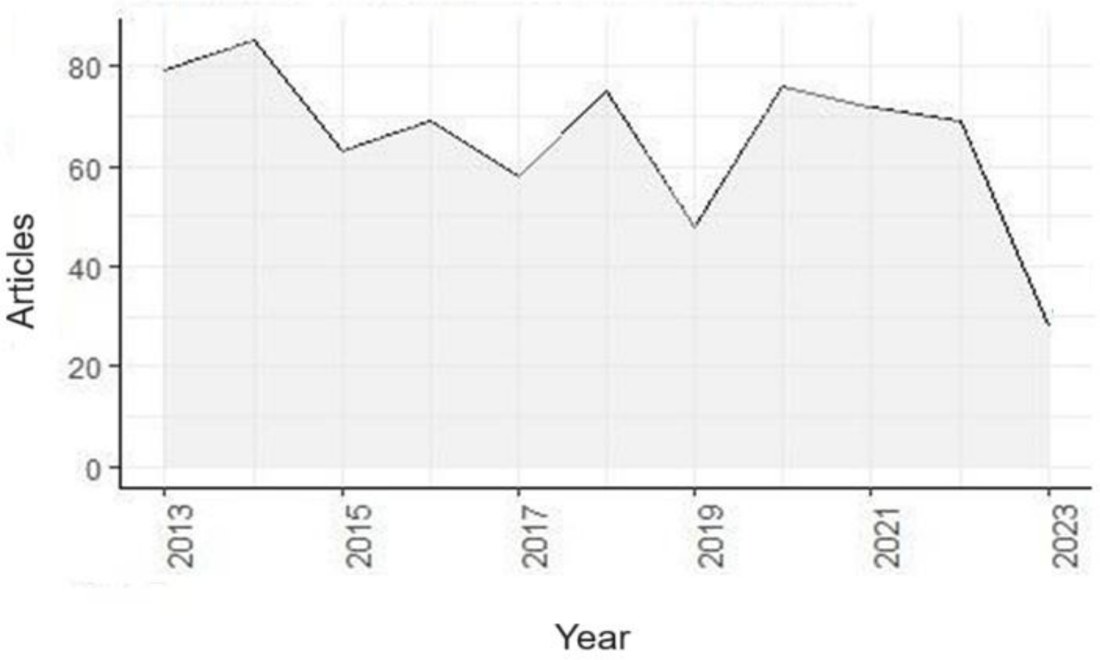


Figure 1. Scientific production about the use of molecular markers in beef meat production from the period of 2013 to 2023.

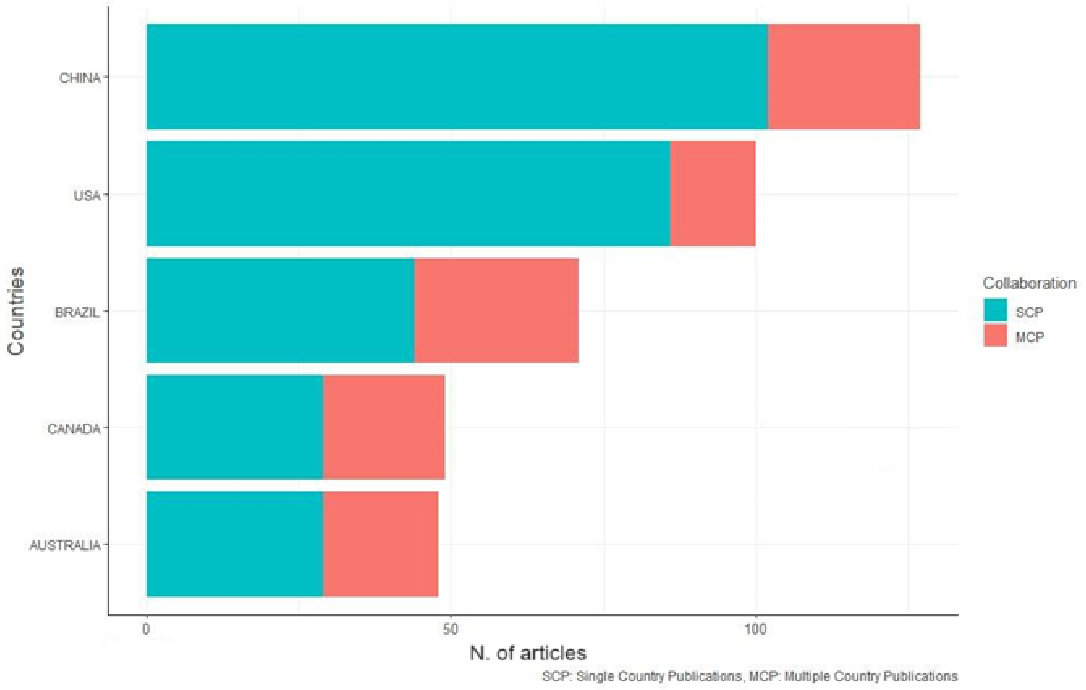
The AFLP genetic marker was found to have the lowest number of citations among the articles reviewed. This technique involves replicating DNA samples through PCR to identify mutations within specific restriction sites of the target DNA (BORÉM & CAIXETA 2009). However, the method is both labor-intensive and costly, requiring a two-step process. It often fails to provide sufficient information about the locus, making it economically inefficient (LANZA et al. 2000).

Over the years, genetic analysis techniques in animal production have evolved from traditional molecular markers to more advanced and precise genomic tools. Classical markers such as RFLPs, AFLPs, microsatellites, and SNPs have historically played a significant role in mapping quantitative trait loci (QTLs) and identifying genetic diversity among livestock populations. Among these, SNPs have become the most widely used due to their abundance and ease of automation (CAETANO 2009). However, while SNPs are useful in association studies, they provide limited insights into gene function.

In contrast, emerging technologies like CRISPR-Cas9 represent a paradigm shift. This technology not only identifies genetic variations but also enables direct gene editing. CRISPR allows for precise alterations in the genome, making it a powerful tool for enhancing traits such as disease resistance, growth rate, and meat quality in a targeted manner (DOUDNA & CHARPENTIER 2014). The integration of CRISPR with traditional molecular approaches marks a new era in genetic improvement programs, where genotyping is no longer just an endpoint but a steppingstone towards functional genomic applications.

Research around the world

The distribution of the 722 articles by country was observed and it was noted that China accounted for 127 of the publications (17.60%), the United States for 100 (13.85%) and Brazil for 71 published articles (9.84%), of which 44 were exclusively Brazilian and 27 were published in collaboration with other countries (Figure 2). To analyze country contribution, the corresponding author's affiliation was considered.



SCP: Single Country Publications, MCP: Multiple Country Publications

Figure 2. Number of articles by country about the use of molecular markers in beef meat production from the period of 2013 to 2023.

In recent years, China has emerged as the leading country in the world for the publication of scientific articles and research, with a total of 898,949 publications in 2022. In contrast, Brazil ranks 12th, having published approximately 74,500 research papers in the same year, a 7.5% reduction from 80,500 publications the previous year

(MARQUES & QUEIROZ 2023, NSB 2024). One key reason for this significant disparity between the two countries is the lack of investment in Brazil's scientific sector by the government. In comparison, China benefits from substantial funding and consistent allocation of resources to its scientific endeavors (SANTOS et al. 2023).

The differences in scientific productivity between Brazil and China are significantly influenced by their respective investments in education and research. Brazil allocates a higher percentage of its Gross Domestic Product (GDP) to education—approximately 5.5%—compared to China's estimated 4.9% (OECD 2023). However, Brazil's investments in research and development (R&D) are considerably lower and less effective. Brazil invests about 1.3% of its GDP in R&D, with more than half of this funding directed toward basic academic research (IPEA 2023). In contrast, China invests around 2.6% to 2.7% of its GDP in R&D, with over 70% of that investment coming from the private sector and focusing on applied research and technological innovation (OECD 2023).

Additionally, the scientific output per million dollars invested is much lower in Brazil than in China; Brazil produces approximately 3.76 articles for every US$1 million invested, which is about half the efficiency rate seen in China (IPEA 2023). These disparities are further exacerbated by ongoing budget cuts in Brazil's science and technology sector. For example, only R$ 89 million of the R$ 690 million approved for the National Scientific and Technological Development Fund (FNDCT) was released in 2021 (SBPC 2022).

As a result of these factors, China has emerged as a global leader in scientific publications, patents, and innovation, while Brazil continues to confront structural and financial challenges that hinder its scientific progress.

China is known for having the highest production of published scientific articles. Figure 3 supports this assertion by showcasing the authors with the highest number of publications. Among the five authors displayed in the graph, three are Chinese scientists: "XU L," "ZHANG L," and "ZAN L." Together, they have a total of 75 publications, which accounts for 59.5% of the publications among the highlighted authors. The remaining 40.5% of the publications are attributed to authors from Canada and the United States.

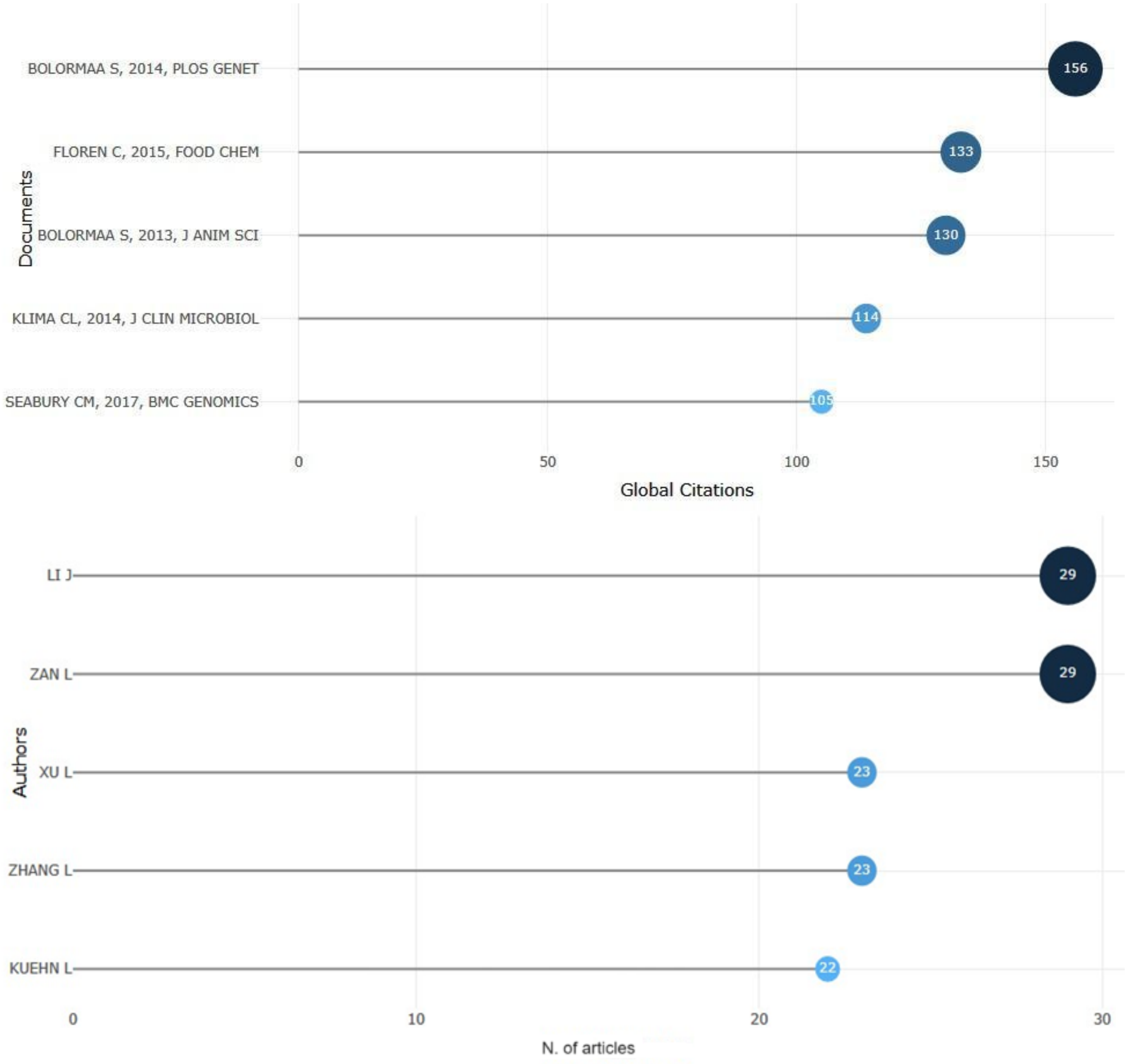


Figure 3. List of Chinese authors with the greatest number of publications about the use of molecular markers in beef meat production.

Authors and Journals

During the analysis of the results, the five publications with the highest citation rates in the scientific field were identified. These publications, each exceeding 100 total citations, are illustrated in Figure 4. Their central theme focuses on the applicability of SNP molecular markers and the use of PCR as a DNA replication technique for diagnosing various pathologies. Among these highly cited publications, two were authored by researchers from Australia, one by an author in Canada, one by a researcher in the United States, and one by an author from England. Notably, of the authors responsible for these frequently cited documents, only Christopher M. Seabury remains active, with his most recent publication dating back to 2022.

Figure 4. The top five most-cited authors in the field of molecular markers used in beef meat production.

When examining the journals with the highest number of publications, it is evident that all of them hold a "Qualis A" classification, as displayed in Table 2. This is the highest rating a scientific journal can achieve. In other words, the publications in these five leading journals undergo careful scrutiny to ensure the accuracy and credibility of the information they contain. Only after this rigorous review process can the publications be approved and subsequently released.

Table 2. The top five of most prolific journals publishing papers in the field of the use of molecular markers in beef meat production.

Journal	Qualis Classification	Number of Articles
Journal of Animal Science	A1	54
Journal of Food Protection	A3	35
Meat Science	A1	34
Plos One	A1	27
BMC Genomics	A2	24

Qualis Periodicals was established in 1988 by the Brazilian Institution CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Coordination for the Improvement of Higher Education Personnel) to evaluate and classify scientific journals in Brazil. The Qualis system includes eight classification levels: A1, A2, A3, B1, B2, B3, B4, B5, and C. Among these, A1 represents the highest classification a journal can achieve, while C indicates a score of 0 (CAPES 2023). This classification information is accessible on the Sucupira platform, providing general knowledge about the ratings of journals.

Topics Covered

Using the WordCloud function available within the Bibliometrix package, the most used keywords in the documents can be obtained, as shown in Figure 5.

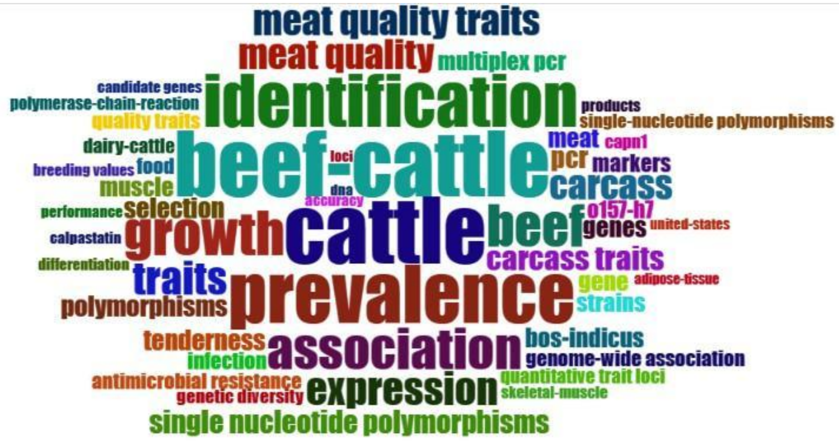


Figure 5. Cloud of words resulted of most used keywords in articles about the use of molecular markers in beef meat production.

Scientific studies that combine molecular markers with meat production primarily focus on producing high-quality beef by examining the expression of specific genes. To conduct a more thorough analysis of the article content, we created a co-occurrence scenario based on the titles of the files. This approach allows us to identify the topics covered and their connections to other relevant areas. The resulting table is presented in Figure 6. The keywords are grouped in two clusters and the thickness of the lines connecting keywords represents the strength of their relationship. The node size is proportional to the frequency of each keyword.

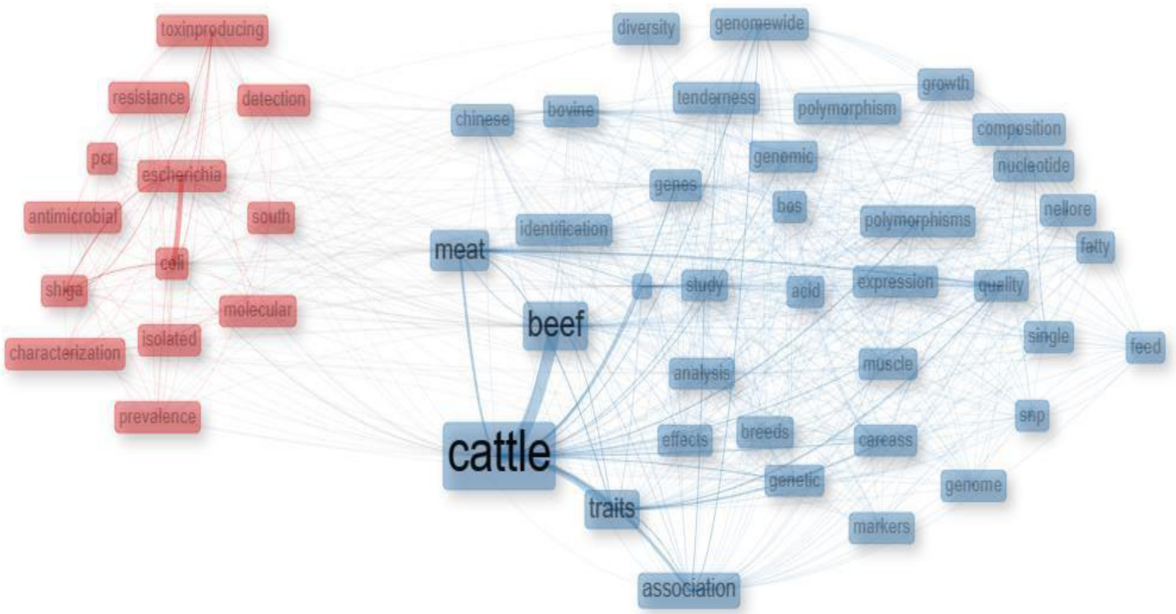


Figure 6. Co-occurrence analysis of the most frequent author keywords in the field of the use of molecular markers in beef meat production.

The highlighted words represent two distinct approaches to studying the use of molecular markers in meat production. The terms in red indicates a line of research focused on food microbiology, utilizing molecular marker tools to examine existing microorganisms and their resistance to toxins. In contrast, the terms in blue are associated with beef cattle production and encompasses aspects such as meat quality, carcass quality, candidate genes, associated polymorphisms, and gene expression. It is proposed that most of the studies focus on beef research utilizing molecular markers. In addition to meat production, the authors have also applied this technology to detect organisms such as *Escherichia coli* and to assist in diagnosing diseases.

Numerous studies in the literature highlight the dual role of molecular markers in food microbiology and meat production. For instance, polymorphisms in genes such as toll-like receptor 4 (*TLR4*) and interleukin 6 (*IL-6*) have been linked to immune responses against pathogenic microorganisms like *Escherichia coli*, as well as variations in meat quality traits, including tenderness and intramuscular fat deposition (PACHECO et al. 2023, PARK et al. 2018, ROUDBARI et al. 2020).

Among the most widely cited genetic markers in beef cattle are calcium-activated neutral protease (*CAPN1*) and calpastatin (*CAST*), which are directly related to meat tenderness because of their involvement in the *postmortem* proteolysis process (KOOHMARAIE & GEESINK 2006, SAUCEDO-URIARTE et al. 2024). These markers have also been examined for their potential interactions with microbial colonization and carcass contamination.

In terms of food safety, *E. coli* O157:H7 strain is not a genetic marker itself, but it is commonly used as a biological indicator of hygienic and sanitary quality in meat production. The detection of this strain through molecular methods, such as PCR targeting the *stx1*, *stx2*, and *eaeA* genes, facilitates the early identification of contamination risks, thereby enhancing quality control processes (BINANDEH et al. 2020, GOMEZ et al. 2014).

There appears to be a knowledge gap regarding the integration of these two areas of research. Could the genes associated with microbial resistance be linked to aspects of meat quality, such as tenderness and marbling? Are the same genetic markers sensitive enough to detect variations in both areas of investigation? Which markers would be most suitable for studying meat quality traits? This dual importance emphasizes the potential for integrated genetic studies that consider both host genotype and pathogen resistance, which currently remains underexplored in research. Future studies should investigate the overlap between immune-related polymorphisms and carcass quality to optimize both animal productivity and food safety.

CONCLUSION

This bibliometric analysis highlights the increasing use of molecular markers in meat production, focusing on areas such as meat quality, gene expression, and the detection of microorganisms. The study identifies the most active authors and countries in this field, particularly China and the United States. Although Brazil is a major meat producer, its scientific output in this area remains modest, primarily due to limited investment in research and development. In addition to their use in genetic breeding, molecular markers have also been applied in disease diagnostics and the detection of pathogens like *Escherichia coli*. However, there is still a notable gap in integrating animal genetics with food microbiology.

This presents opportunities for interdisciplinary research to investigate potential links between microbial resistance genes and meat quality traits. To address these challenges, we recommend strengthening funding policies for research in molecular genetics through both public and private investments. This strategy could enhance the competitiveness of Brazilian meat by not only increasing productivity but also elevating standards of quality and food safety in the global market.

NOTES

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, and formal analysis, João Gabriel Lira da Silva, Camila Pieroni and Déborah Galvão Peixôto Guedes de Araújo; software and validation, João Gabriel Lira da Silva and Camila Pieroni; investigation and methodology, João Gabriel Lira da Silva, Camila Pieroni and Déborah Galvão Peixôto Guedes de Araújo; resources and data curation, João Gabriel Lira da Silva, Camila Pieroni and Déborah Galvão Peixôto Guedes de Araújo; writing-original draft preparation, João Gabriel Lira da Silva and Camila Pieroni; writing-review and editing, Déborah Galvão Peixôto Guedes de Araújo; visualization, João Gabriel Lira da Silva and Camila Pieroni; supervision, Déborah Galvão Peixôto Guedes de Araújo; project administration Déborah Galvão Peixôto Guedes de Araújo. All authors have read and agreed to the published version of the manuscript.

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Not applicable for studies not involving humans or animals.

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available under request.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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