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ANIMAL PRODUCTION

SSR and ISSR markers in assessing genetic diversity in *Gallus gallus domesticus*: a quantitative analysis of scientific production

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ABSTRACT: *Poultry meat is a major source of animal protein in the world. Research indicates a high inbreeding rate derived from a relative absence of heterozygous subpopulations of chicken from different suppliers. Molecular markers can provide information for the genetic basis of chicken consumed in rural areas and help establishing a chicken database for product quality and warranty. The bibliometric research, comprises between 1994 and 2018, from five previously selected databases: Google Scholar, PubMed, ScienceDirect, Scopus and Web of Science, using the following descriptors: 'microsatellites', 'SSR', 'ISSR', 'genetic variability' and 'genetic diversity', all of them coupled to 'chicken' and/or 'birds' results in 66 scientific publications. The publications were then categorized according to their titles to the use of ISSR or SSR markers. They were also addressed by countries according first author cited. The publications data appointed that countries with the height production of poultry meat and hens are the most interested in the genetic diversity study of these species. The SSR markers, due to its more specific characteristic, are more frequently applied to genetic diversity assignment, compared to ISSR.*
Key words: *bibliometrics, scientific publications, genetic diversity, domestic chicken, ISSR (Inter Simple Sequence Repeat), DNA microsattelite, SSR (Simple Sequence Repeats).*

Marcadores SSR e ISSR na avaliação da diversidade genética em *Gallus gallus domesticus*: uma análise quantitativa da produção científica

RESUMO: *A carne de frango é uma das principais fontes de proteína animal do mundo. Pesquisas indicam uma alta taxa de endogamia derivada de uma relativa ausência de subpopulações heterozigotas de frango de diferentes fornecedores. Marcadores moleculares podem fornecer informações para a base genética de frango consumido em áreas rurais, e ajudar a estabelecer um banco de dados de frango para qualidade e garantia do produto. A pesquisa bibliométrica compreende entre 1994 e 2018, a partir de cinco bancos de dados selecionados anteriormente: Google Scholar, PubMed, ScienceDirect, Scopus e Web of Science, usando os seguintes descritores: 'microssatélites', 'SSR', 'ISSR', 'variabilidade genética' e 'diversidade genética', todos eles associados a resultados de 'galinha' e / ou 'aves' o que resultou em 66 publicações científicas. As publicações foram então categorizadas de acordo com seus títulos para o uso de marcadores ISSR ou SSR. Eles também foram abordados pelos países, segundo o primeiro autor citado. Os dados das publicações obtidas apontam que os países com grande produção de carnes de frangos são os mais interessados no estudo da diversidade genética dessas espécies. Os marcadores SSR, devido à sua característica mais específica, são frequentemente aplicados à atribuição de diversidade genética, em comparação com o ISSR.*
Palavras-chave: *bibliometria, publicações científicas, diversidade genética, frango doméstico, ISSR (=segmentos entre sequências simples repetidas), microsatélite de DNA, SSR (=segmentos de sequências simples repetidas).*

INTRODUCTION

Gallus gallus domesticus is a major source of animal protein for food in the world and the worldwide chicken population was more than 58 billion in 2011 (FAO, 2013). According USDA Foreign Agricultural Service (2018), United States of American, Brazil, European Union and China are the higher word producers of poultry. Research indicated a high inbreeding rate derived from a relative absence of heterozygous subpopulations of chicken from different suppliers (TADELE et al., 2019; BORTOLUZZI et al., 2018; BOSSE et al., 2019). Besides the fact that its products are healthier for human consumer and contribute for alimentary insurance of village populations (DIKMEN et al**.,** 2016), molecular markers can provide information for the genetic basis of chicken consumed in rural areas; markers can also help

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establishing a chicken database for product quality and warranty (MARCHA et al., 2017; ABEBE et al., 2015; YILMAZ DIKMEN et al., 2016).

Among domesticated animals, this species is ideal for genetic mapping and qualitative trait loci (QTL) analysis since there is a short generation interval. Besides, its genome is small compared to mammals, being 39% the size of the human genome (LEDUR et al., 2007). Increasing productivity has been achieved through intensive directional selection of production traits over tens of generations in purebred populations. Following breeding strategies in the production of livestock and maximizing yield; however, has been the price of reduced immunity, accompanied by a number of undesirable traits. These negative effects may result from gene pleiotropy. Understanding the nature of adaptive forces acting on the chicken genome knowing the genome can provide insight into the complex relationship between production, disease and genes, opening new directions to further improve this animal and global food security (MOST, 2011; QANBARI et al., 2019).

DNA molecular markers represent an important tool to identify and characterize populations through nucleotide sequences, since they contributed to the study of genetic variability, kinship identification, as well as to genetic mapping (AMARANTE; WOMACK, 2004).

The SSR (Simple Sequence Repeats) molecular markers are DNA regions that correspond to a sequence of 1 to 6 nucleotides repeating in tandem (successive nucleotide repetitions). These markers are extensively used for the analysis of genetic structuration due to their codominant characteristic, allowing for us to recognize heterozygous loci of multiallelic nature, promoting simultaneous identification of several alleles (ZUCCHI, 2003), high polymorphism, reproducibility (ZANE; BARGELLONI; PATARNELLO, 2002) and uniform distribution through the genome (SOUZA, 2015). The SSR primers correspond sequence are obtained from DNA sequences that flank tandem nucleotide repetitions, allowing the specific primer for these loci to be synthetized and posteriorly used in the PCR reaction (OLIVEIRA et al., 2006).

The ISSR (Inter Simple Sequence Repeats) is a dominant DNA fragment marker, having between 100 and 300 bases pairs longer. The ISSR allows the analysis of multiple loci in the same reaction (LORENZONI et al., 2014). In genetic diversity studies, ISSR, differently from SSR, can amplify random sequences from the genome, under risk of contamination during the preparation of the PCR reaction (GUIMARÃES et al., 2009). The ISSR not require previous knowledge of the genomic region (FALEIRO, 2007; CANÇADO et al., 2012). Because it is a dominant marker, it is only possible to verify the presence or absence of alleles (TURCHETTO-ZOLET et. al., 2017). The ISSR also is notable for being fast, simple and efficient in obtaining results (REDDY et al., 2002).

In general, molecular markers are useful to distinguish chicken breeds and genetic variation within a population (MARCHA et al., 2017; ABEBE et. al., 2015). Considering the importance of molecular markers to study population's diversity and the concern to conserve the genetic variability of chicken, we aimed to conduct a systematic review on the application of SSR and ISSR markers in the study of genetic diversity.

DEVELOPMENT

The literature review of the use of microsatellite and ISSR markers on the analysis of genetic variability of birds used studies that range from 1994 to 2018. We conducted searches for papers related to this theme during the months of January to March of 2018 from five different previously selected databases: *Scholar Google*, *Pub Med*, *ScienceDirect*, *Scopus* and *Web of Science*.

The following descriptors are defined for searching: 'microsatellites', 'SSR', 'ISSR', 'genetic variability' and 'genetic diversity', all coupled to 'chicken' and/or 'birds'. Our resulting data was plotted in a table with the headers: paper title, author names, country, and year of publication, descriptors and paper characterization. We considered the country of the publication according to the address provided by the first author. This study was reported seven steps according to the guidelines of the Cochrane Collaboration, which follows seven steps: (1) question formulation, (2) study location; (3) critical evaluation of the studies; (4) data collection; (5) analysis and interpretation of data; (6) interpretation of the data; (7) enhancement and update of revision (ROTHER, 2007).

Selection of papers occurred in three steps for each database (Figure 1); on the first one we used the title and abstract as selection criteria (i.e. if the study was about molecular analysis in the study of birds/chicken). The second step was the screening of studies that used molecular markers and we selected papers that dealt with SSR and/or ISSR. During the third step we selected solely researches that used these markers to assess genetic diversity, excluding,

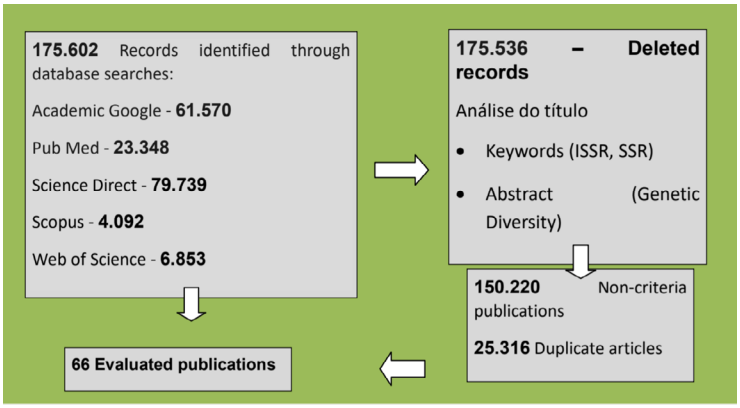

Figure 1 - Exclusion criteria and total articles evaluated for analysis.

thus, researches that only reported isolation and characterization of these markers or that only referred to animal behavior.

After applied the exclusion criteria, 66 papers appears involving application of microsatellite markers or ISSR on the study of genetic diversity of birds (Table 1). Publications were widely distributed around the globe, including 26 countries. The Asian continent presented the highest rate of selected papers, comprising 43.94% of the total papers found in our search, in second, the works of the European continent, with 27% of the articles followed by the American, with 19% of the papers. The lowest rate of selected papers belongs to Africa and Oceania, with only 4% of the papers each (Figure 2).

Within the American continent, three countries stand out regarding the number of publications: Brazil (9), USA (3) and Canada (1). Among European countries, we reporte the following publications: Italy (5), Germany (4), France (3), Netherlands (2), Ukraine, Hungary, Spain and Finland (1). Asia authors are responsible for 29 papers on the subject, been from Iran, China and Japan (5), Israel and India (4), Turkey (2), Syria, Saudi Arabia, Vietnam and Thailand (1). From the African continent, Egypt, Ethiopia and Kenia (1). From Oceania: New Zealand (2) and Australia (1).

Table 1 - Number of articles reported and selected.

Data base	Articles found	Selected articles
Google Acadêmico	61.570	20
Pub Med	23.348	25
Science Direct	79.739	5
Scopus	4.092	6
Web of Science	6.853	10
TOTAL	175.602	66

Regarding the molecular markers used in the searches, we reported 62 papers with SSR and 4 that used ISSR. Out of those, 26 are related to indigenous chicken breed, 27 to commercial poultry and 12 to birds in general and one (1) that dealt with the comparison between chicken and birds. Out of the 26 papers that emphasized indigenous chicken breeds, 24 developed with SSR markers and only two used ISSR.

When it comes to studies involving SSR markers, we observed a higher number of publications during the period from 2007 to 2017 (Figure 3), with ISSR studies were reported few articles starting from 2006. In the period from 1994 to 2018, we only found studies in the African continent starting from 2006 (Figure 4). Of the articles studied, 65% (43) corresponded to genetic studies of diversity and variability. Only 22% (15) corresponded to population studies, and 12% (8) described researched involving population and genetic variability. Search engines in which searches were conducted differed in regard to number of records (Table 2) and demonstrated the predominance of commercial chicken (poultry) as the most studied species.

Results of the present study indicated countries with large production of chickens are the most interested in the genetic study, this may be

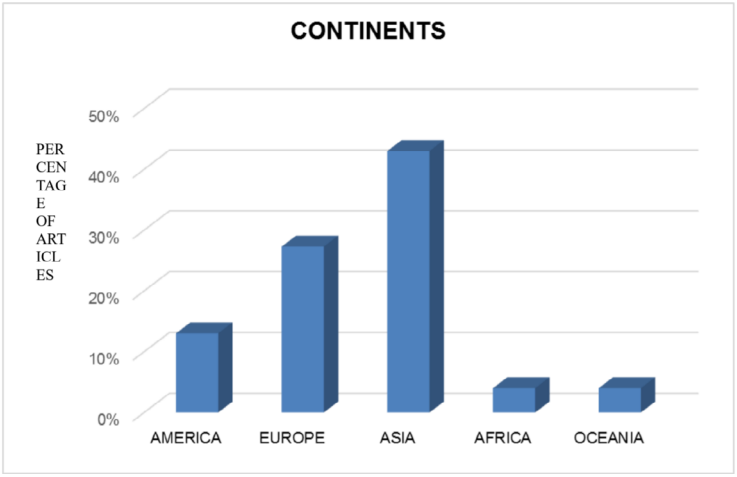

Figure 2 - Scientific articles published per continent during the period 1994 to 2018.

associated with the funding of research related to genetic diversity, as well as chicken meat consumption rates. According to the US Department of Agriculture (USDA), the interest to know the genome may be linked to agro-industrial features (USDA, 2017). In North America, projections for the per capita consumption in the USA may end in 2018 with 42kg, a 15% increase since 2012.

Countries such as China, Japan and Iran, which represented the Asian continent, had a significant number of published papers, propelling Asia as the continent with the largest number of publications in comparison with other continents. Asia is the region where *Gallus gallus* was originally domesticated for human consumption.

The Neotropics presented fewer publications that involved molecular markers, since when we also consider studies of isolation and characterization of markers or their application in studies of animal behavior, Europe stands out in

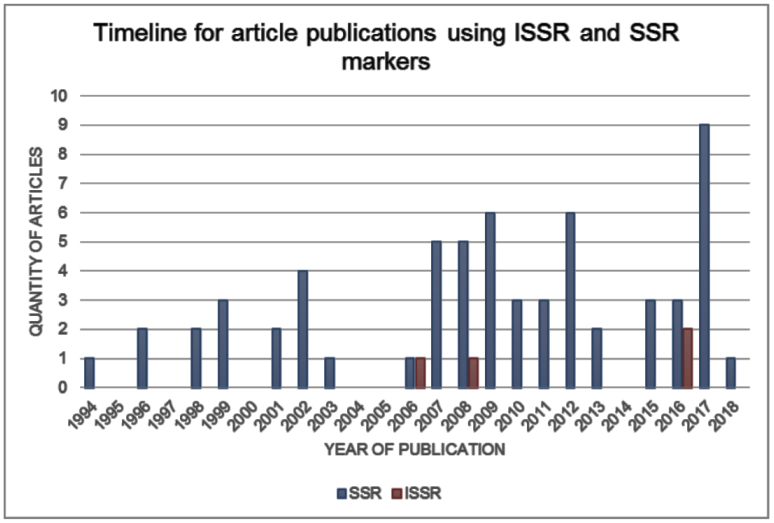

Figure 3 - Timeline for article publications using ISSR and SSR markers.

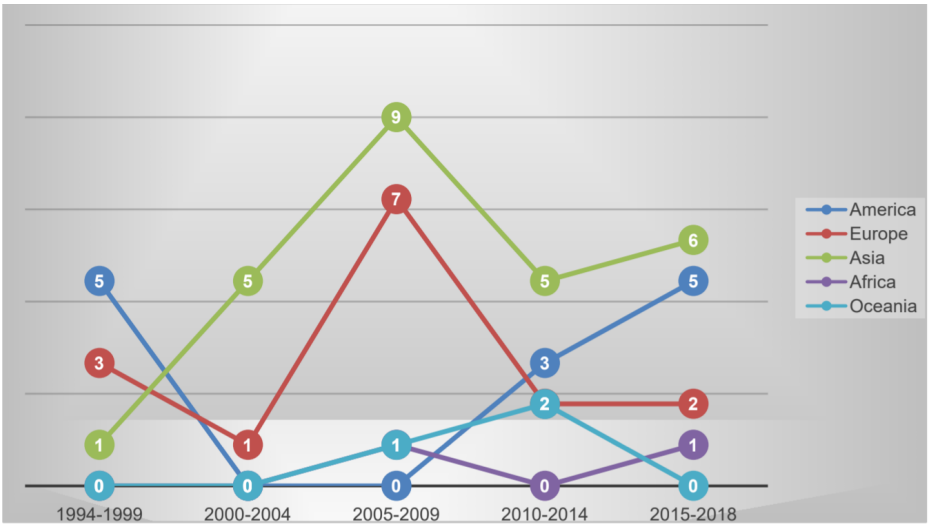

Figure 4 - Publications involving SSR markers in the Americas, Europe, Asia, Africa and Oceania in the period from 1994 to 2018.

number of publications, followed by the United States (MOURA; DAWSON; NOGUEIRA, 2017). However, Brazil rises as the country with the highest number of studies. Besides, it stands out as the country with more publications between the period of 2008 and 2017. This result may be related to the large Brazilian production of chicken meat and rearing of native chicken in several parts of its territory. Brazil is the second largest chicken producer of the world, surpassed only by China, and producing about 12.9 million tons per year, as reported by the *Associação Brasileira de Proteína Animal* (ABPA – Brazilian Association of Animal Protein) of 2017, besides exporting this type of meat to over 150 countries (ABPA, 2017).

Employment of molecular markers is becoming increasingly common in studies, due to their ability of locating quantitative traits loci (QTL), which is ideal for genetic improvement researches due to helping in the identification of genes, and providing a variety of information related to characters that concern production (GUIMARÃES et al., 2009).

Widespread use of molecular markers becomes important, since that across of the genetics information is possible to select parents, allowing more accuracy in crosses that aid in the conservation and increasing of the genetic variability of the chicken populations (MARCHA et al., 2017). With the increasing of the variability, chicken farms increase their productivity due the decreasing of the endogamy and consequently of the loss of alleles linked to the production. Consequently, novel characteristics can be selected as advantageous and maintained in a populational equilibrium, since there would still be surviving specimen adapted to different environmental loads. This reduced loss of productivity, in opposition to when there is little variability, in which any environmental adversity could cause great populational damage to chicken.

The molecular markers have an advantage over morphological markers, because can be detected prematurely in the organism allowing that the genetics information have been assessed with more precision, which justifies their use in several studies. However, this ability can be higher or lower depending on the genetic bases of the marker, taking into consideration the differences between ISSR and microsatellite markers (GUIMARÃES et al., 2009).

Considering this scenario, our investigation reported that the high use of the SSR marker is due its capacity to supply several information population-genetic (LEDUR et al., 2007). The use of SSR is pointed as advantageous by literature due to its feasibility, since several primers are available for the species under study, high polymorphism and good distribution in the genome (EMBRAPA, 2006; LEDUR et al., 2007). Microsatellites are widely used in genetic analyses due to their abundance throughout

Table 2 - Main articles on chickens and chickens, with respective objectives and markers.

	Molecular Markers	Species	Purpose of the study	Reference	Species	Purpose of the study	Reference
WEB OF SCIENCE	ISSR	Commercial chicken	Comparison of genetic variations among native and some local chicken populations.	TUNCA et. al., 2016	Local Chicken	Analyze a large number of expressed sequence tags (ESTs) to test the possibility of using EST-derived microsatellite markers for investigating the *Gallus gallus* genome.	BAKHTIARIZADEH et. al.,2012
			Genetic variability and phylogenetic relationship establishes distinctness of chicken using 24 microsatellite markers.	SHARMA et. al., 2017			
			Evaluation of the number of SNPs needed to reach the same differentiation power as 29 SSRs to classify animals into eight chicken populations.	GARKE et. al., 2012		Genetic diversity and population structure analysis using 25 microsatellite markers.	KUMAR et. al., 2015
SCOPUS	SSR	Local Chicken	Evaluation of genetic diversity of naked neck and frizzle genotypes based on microsatellite markers.	ABOU-EMERA et. al., 2017	Commercial chicken	Develop PolySSR for the identification of polymorphic SSRs by means of EST, potato, tomato, rice, Arabidopsis, Brassica and chicken sequences available publicly.	TANG et. al., 2008
			Identify SSRs and transposable elements (TEs) for understanding the distributions of replicates in all chromosomes in the red jungle fowl.	GUIZARD et. al.,2016			
			Estimation of genetic diversity using SSR and RAPD markers.	AL-JALLAD; CHOUMANE; HMESHE, 2012		Characterization the genetic diversity, genetic relationship and population structure of Denizli chicken subpopulations using 19 microsatellite markers.	OZDEMIR; CASSANDRO, 2017
PUBMED	SRR	Local Chicken	Analysis a large number of expressed sequence tags (ESTs) to test the possibility of using EST-derived microsatellite markers for investigating the *Gallus gallus* genome.	BAKHTIARIZADEH et. al., 2012	Commercial chicken	Analysis in three generations of fat strains (FL) and lean line (LL) of broilers chickens..	BAI et.al., 2002
			Determine the number of SNPs needed to obtain the same differentiation power of a standard set of microsatellites. Eight chicken breeds were genotyped for 29 SSRs and 9216 SNPs. After filtering, only 2931 SNPs remained.	GARKE et. al., 2012		Evaluation the genetic diversity and relationships of 5 Saudi native chicken populations and a White Leghorn (L) strain as an exotic breed, using 25 microsatellite markers.	FATHI et. al., 2017
ACADEMIC GOOGLE	SSR	Commercial chicken	Evaluation the genetic variability of Brazilian hens from blue eggs of Dois Lajeados - RS using 15 microsatellite markers.	FONTEQUE, 2011	Commercial chicken	Research on the genetic diversity of these nine Hungarian chicken populations using 29 microsatellite markers.	BODZSAR et. al. 2009
			Evaluation the genetic structure of 65 chicken populations by means of 29 SSR loci.	GRANEVITZE et. al., 2009		Characterize the diversity of a set of traditional chicken breeds from two distant regions (France and Taiwan).	BERTHOULY et. al., 2008
			Evaluation of intra-population variation in 1970 chickens from 64 populations using 29 autosomal microsatellites.	GRANEVITZE, 2007		Evaluate genetic diversity, conduct genetic characterization, and evaluate the utility of an individual assignment test for 12 commercial chicken lines using 40 microsatellite markers.	TADANO et. al.., 2007

the eukaryote genome and highly polymorphic being adequate to characterize and estimate genetic distance between population, allowing the comparison between races and the comprehension of the nature of avian biodiversity (ABOU-EMERA et al., 2017).

The ISSR marker is of simple use, besides being cheaper than SSR, don't need previous knowledge of the genome and might be more frequently used in the future on research of chicken or birds in general due to these advantages (TUNCA & TASKIN, 2016). However, ISSR is characterized by being a dominant marker and the presence of amplified fragment might mean a dominant homozygous or heterozygous, while its absence represents a recessive homozygous, because is lowest informative than SSR; uhowever, been useful for characterization of genetic structure, mainly in preliminary studies (COSTA et al. 2015).

Another factor we observed in our research, and can to be determinant to the rare use of the ISSR marker with free-range chicken and birds, is the fact that this type of marker to be universal and to have problems with contamination by microorganism as well as with unspecific amplification that can to compromise the results, besides it don't be feasible to detect the heterozygosities degree (main estimator of genetic diversity) (GUIMARÃES et al., 2009; KESAWAT, 2009). Due to these characteristics of the ISSR marker and, because of the chicken to be an animal of economic interest, the results generated by the ISSR marker do not manifest as a research focus, with the SSR marker being the main focus, as seen in table 2.

The table 2 also demonstrated that researches involving molecular markers and chicken aimed to assess the level of genetic variability in populations from different places, highlighting any possible distinction between chickens under different environments. These studies are essentials when the aim is to maintain genetic variability to be useful in future crosses (TUNCA & TASKIN, 2016).

CONCLUSION

Our current review revealed that SSR and ISSR markers are useful for conservation and genetic improvement, mainly the SSR due the fact to be more effective and informative in the studies of population characterization. Thus, due the importance given to the production of chickens, the countries of the Asian and American continents were that had greater highlight scientific production with molecular markers compared to others.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

JWGOF aproved and supervised IFPI research grant. MSFSS Jr, AASB and SRNS carried out research and prepared the draft manuscript. AMA coordinated experimental genetic resources chicken local nucleus from Embrapa. GRS and VGM performed bibliometric data analysis. ACSD, AMA and JWGOF revised the manuscript. All authors critically revised and approved of the final version.

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