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MAURÍCIO SÉRGIO FERREIRA SOARES DA SILVA JÚNIOR

POLYMORPHISMS IN GENES ASSOCIATED WITH EGG PRODUCTION IN CANELA-PRETA CHICKENS

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2025

MAURÍCIO SÉRGIO FERREIRA SOARES DA SILVA JÚNIOR

POLIMORFISMOS EM GENES ASSOCIADOS A PRODUÇÃO DE OVOS EM GALINHAS DA RAÇA CANELA-PRETA

Tese apresentada ao Programa de Pós-Graduação em Zootecnia Tropical do Centro de Ciências Agrárias da Universidade Federal do Piauí, como parte das exigências visando a obtenção do título de Doutor em Zootecnia Tropical.
Área de concentração: Produção Animal.

Orientador: Prof. Dr. Fábio Barros Britto

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MAURÍCIO SÉRGIO FERREIRA SOARES DA SILVA JÚNIOR

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Sumário

Polymorphisms in genes associated with egg production in Canela-Preta chickens

ABSTRACT: For the bibliographic analysis, a search was carried out in the Web of Science and Scopus databases with the descriptor “Snps and/or Chicken” from 2008 to 2024, using the "bibliometrix" package in the R platform to analyze data exported in BibTeX format. The search results showed 1668 articles, with the year 2024 having the highest number of publications (137), China having the highest number of published articles (723) and the most cited genes GH, IGF, MHC, CORT, PRL, MC1R and VIP. We conclude that genetic polymorphisms have great potential for application in chicken genetic improvement programs, focusing on production, reproduction and disease resistance characteristics. The research highlights the growth in the use of SNP markers, reinforcing their importance for the sustainability and efficiency of global poultry farming, especially in developing countries such as China and Brazil. Forty individuals were analyzed, and DNA was extracted using the Quick-DNA/RNA™ MagBead kit. Five polymorphisms in the PRL and VIPR1 genes (named PRL1, PRL2, PRL3, VIPR1A and VIPR1B) associated with egg production and brooding behavior in hens were analyzed. SNP detection was performed by ARMS-PCR tetraprimers and gel electrophoresis. Allele and genotypic frequencies were calculated and Hardy-Weinberg equilibrium was verified using GenAlEx software. After genotyping, the results showed a low observed heterozygosity (Ho) of 0.287 and an expected heterozygosity (He) of 0.492. The PRL1 *locus* showed the lowest heterozygosity (Ho = 0.077), with a high fixation index (F = 0.846), indicating high inbreeding. GLM analysis revealed that the PRL3-CG genotype had a significant negative effect on APELVE ($p = 0.0491$), while PRL2-TC, VIPR1A-CT and VIPR1B-CT showed suggestive effects. For CHOCO, the PRL3-CG and PRL3-GG genotypes showed significant negative effects, and the C allele (PRL3) was positively associated with increased broody frequency ($p = 0.042$). No significant association was identified between the genotypes and cloacal temperature, although time (week 32) influenced this variable. The findings reinforce the potential use of PRL and VIPR1 markers in genetic selection programs aimed at productivity and reproductive behavior in Canela-Preta chickens.

Keywords: PRL, VIPR1, Bibliometric analysis, tetra*primers*.

Polimorfismos em genes associados a produção de ovos em galinhas da raça Canela-Preta

RESUMO: Inicialmente, foram realizadas análises bibliométricascom buscas busca nos bancos de dados Web of Science e Scopus com o descritor “Snps and/or Chicken” no período de 2008 a 2024, utilizou-se o pacote "*bibliometrix*" na plataforma R para analisar dados exportados no formato BibTeX os resultados das buscas mostraram 1668 artigos, sendo o ano de 2024 com maior número de publicações (137), a China com maior número de artigos publicados (723) e os genes mais citados GH, IGF, MHC, CORT, PRL, MC1R e o VIP. Concluímos que os polimorfismos genéticos apresentam grande potencial para aplicação em programas de melhoramento genético de galinhas, com foco em características de produção, reprodução e resistência a doenças. A pesquisa evidencia o crescimento no uso de marcadores SNPs, reforçando sua importância para a sustentabilidade e eficiência da avicultura global, especialmente em países em desenvolvimento como China e Brasil. Em um segundo momento, foram analisados 40 indivíduos, sendo o DNA extraído usando o kit *Quick-DNA/RNA™ MagBead*. Foram analisados cinco polimorfismos nos genes PRL e VIPR1 (denominados PRL1, PRL2, PRL3, VIPR1A e VIPR1B) associados à produção de ovos e comportamento de choco em galinhas. A detecção dos SNPs foi realizada por tetra*primers* ARMS-PCR e eletroforese em gel. As frequências alélicas e genotípicas foram calculadas e o equilíbrio de Hardy-Weinberg foi verificado com o software GenAlEx. Após a genotipagem, os resultados mostraram baixa heterozigose observada (Ho) média de 0,287 e uma heterozigose esperada (He) de 0,492. O *locus* PRL1 apresentou a menor heterozigose (Ho = 0,077), com um índice de fixação elevado (F = 0,846), indicando alta endogamia. A análise GLM revelou que o genótipo PRL3-CG teve efeito negativo significativo sobre APELVE (p = 0,0491), enquanto PRL2-TC, VIPR1A-CT e VIPR1B-CT mostraram efeitos sugestivos. Para CHOCO, os genótipos PRL3-CG e PRL3-GG apresentaram efeitos negativos significativos, e o alelo C (PRL3) foi positivamente associado ao aumento da frequência de choco (p = 0,042). Não foi identificada associação significativa entre os genótipos e a temperatura cloacal, embora o tempo (semana 32) tenha influenciado essa variável. Os achados reforçam o potencial de uso dos marcadores PRL e VIPR1 em programas de seleção genética voltados à produtividade e comportamento reprodutivo em galinhas Canela-Preta.

Palavras-chave: PRL, VIPR1, Análise bibliométrica, tetra*primers*.

1 INTRODUÇÃO GERAL

A avicultura é um setor de grande importância para a segurança alimentar global, fornecendo carne, ovos e outros produtos de origem animal para milhões de pessoas em todo o mundo. A demanda por alimentos está em constante crescimento devido ao aumento da população mundial e às mudanças nos padrões alimentares (FAO, 2023). A Organização das Nações Unidas para Agricultura e Alimentação (FAO) estima que a produção de alimentos deve crescer em torno de 60% até 2050 para atender às necessidades alimentares de uma população global que deve alcançar aproximadamente 9 bilhões de pessoas (OECD, 2022). Esse crescimento demanda não apenas a expansão das áreas de cultivo e produção animal, mas, também a melhoria da eficiência e sustentabilidade dos sistemas de produção agrícola e pecuária.

No Brasil, a produção de carne de frango atingiu 14,4 milhões de toneladas em 2023, ocupando o segundo lugar no ranking mundial, atrás apenas dos Estados Unidos (ABPA, 2023). A crescente demanda por carne de frango impulsiona a necessidade de aumentar a eficiência da produção avícola, classificada como principal fonte de proteína no mundo (Ledur, 2007;ABPA, 2023). Já a demanda por ovos tem aumentado a cada ano sendo 260 ovos/habitante/ano em 2024 e houve um aumento de aproximadamente 13,04% no consumo de ovos no Brasil nos últimos 5 anos (ABPA, 2024).

A genética animal emerge como uma ferramenta crucial para o desenvolvimento de aves com características superiores em termos de crescimento, produção de ovos, resistência a doenças e bem-estar animal (FAO, 2023). No Brasil, a demanda por aves caipira e ovos caipira está aumentando, principalmente por um grupo específico de consumidores que busca produtos caipiras, onde os animais são criados em ambientes com o maior conforto possível (Queiroz *et al.* 2014).

Na região Nordeste do Brasil os desafios na criação avícola são ampliados por fatores climáticos e socioeconômicos específicos. O Nordeste brasileiro enfrenta um clima tropical, caracterizado por altas temperaturas e longas secas, que impactam significativamente a agricultura e a produção animal (FIDA, 2016). A irregularidade das chuvas e a elevada evaporação reduzem a disponibilidade de água e afetam a qualidade das pastagens, contribuindo para a baixa produção das atividades agropecuárias na região (de Brito Cavalcanti *et al.* 1999). Além disso, a desigualdade socioeconômica e a falta de infraestrutura adequada agravam ainda mais esses desafios, limitando o acesso a tecnologias e práticas de manejo eficientes (Hissa-Teixeira, 2018).

No caso da criação de galinhas caipiras de raças locais, os problemas são multifacetados. A falta de recursos para a nutrição adequada das aves, a escassez de água, a ausência de vacinas e medicamentos resultam em doenças, como deficiências vitamínicas e minerais, afetando a saúde das galinhas (EMBRAPA, 2007). Portanto, é essencial desenvolver estratégias de melhoramento que considerem esses fatores ambientais.

Na produção de ovos, que é a atividade mais praticada pelo produtor rural (Du *et al.* 2020a), esses problemas de natureza socioambientais são evidentes. As condições ambientais adversas, como altas temperaturas e umidade relativa insuficiente, afetam diretamente a saúde das galinhas e a eficiência produtiva. As galinhas submetidas a estresses térmicos tendem a apresentar menores taxas de postura, qualidade reduzida dos ovos e maior incidência de problemas de saúde, como a mortalidade e doenças metabólicas, essas condições podem levar a uma diminuição significativa na produção e qualidade dos ovos, prejudicando a segurança alimentar e a economia local (Henrique *et al.* 2023).

Em galinhas caipiras da raça Canela-Preta o desafio se amplia visto que trabalhos têm demonstrado dificuldades com a produção de ovos e choco (Araújo de Carvalho *et al.* 2020). Logo após a postura dos ovos, as galinhas costumam chocar, que é uma característica definida como a cessação da produção de ovos. As aves chocas param sua postura durante aproximadamente 21 dias, o que se caracteriza como um problema atrelado à produção para avicultores rurais (Dou *et al.* 2022). A expressão do comportamento de choco na galinha doméstica consiste em nidificação persistente, cacarejo característicos, agressividade ou defesa do ninho, aumento na temperatura corporal, diminuição da ingestão de ração, água e parada na produção de ovos, em galinhas nativas e com pouca ou nenhuma seleção genética essa característica tende a ser predominante (Santos, 2023).

Para enfrentar esses desafios, a utilização de marcadores genéticos, em especial marcadores do tipo SNP (*Single Nucleotyde Polymorphisms*), se apresenta como uma estratégia promissora. Os marcadores genéticos são ferramentas poderosas para a seleção e melhoramento genético das aves, permitindo a identificação de indivíduos com características desejáveis, como maior resistência a condições adversas e melhor desempenho produtivo (Kang *et al.* 2012). No contexto da produção de ovos, a aplicação de marcadores genéticos pode facilitar a seleção de galinhas com maior capacidade de postura e melhor adaptação ao ambiente, contribuindo para a sustentabilidade e eficiência da avicultura.

Devido à alta ve*loci*dade com que as informações são geradas na atualidade e à grande densidade de dados, um levantamento bibliográfico com ferramentas bibliométricas sobre marcadores genéticos em galinhas permite que os pesquisadores sintetizem e análisem os estudos mais recentes sobre o tema, oferecendo uma visão global e atualizada sobre o uso de

marcadores SNPs na genética de galinhas. Isso é fundamental, já que a área de genética molecular e melhoramento genético está em constante evolução.

O uso de marcadores SNPs tem mostrado grande potencial para a seleção genética em diversas espécies, incluindo as galinhas. Ao revisar os estudos existentes, é possível identificar os principais avanços na identificação de SNPs associados a características importantes, como produção de ovos, resistência a doenças e qualidade da carne (Drobik-Czwarno *et al.* 2018; Kim *et al.* 2023; Rusnadi; Dewi; Sriyani, 2022). Além disso, é possível comparar as metodologias utilizadas e as melhorias tecnológicas que permitiram avanços mais rápidos nesse campo.

Um trabalho de revisão bibliográfica sobre SNPs em galinhas é crucial para consolidar os conhecimentos existentes, identificar tendências, orientar futuras pesquisas e melhorar as práticas de melhoramento genético. Além disso, possibilita a integração de avanços científicos ao setor produtivo, aumentando a eficiência e a sustentabilidade da produção avícola.

Dentre os principais genes estudados, o PRL (Prolactina) e VIPR-1 (Receptor de Peptídeo Inibitório Vasoativo 1) demonstram associação direta com a produção de ovos em galinhas. O gene PRL é fundamental na regulação hormonal da reprodução e, portanto, na produção de ovos (a prolactina, hormônio associado à lactação e à reprodução, tem mostrado influência significativa na produção de ovos em aves, afetando diretamente a taxa de postura e a qualidade dos ovos (Cheng *et al.* 2023).

O VIPR-1, por sua vez, está envolvido na regulação do crescimento e desenvolvimento das glândulas reprodutivas. Estudos indicam que os polimorfismos no gene VIPR-1 podem impactar a eficiência reprodutiva e a adaptação das aves às condições ambientais adversas como também na produção de ovos (El-Halawani *et al.* 2000).

A investigação dos genes PRL e VIPR-1 e seus polimorfismos oferecem uma oportunidade para compreender melhor os mecanismos genéticos que influenciam a produção de ovos das galinhas da raça Canela-Preta às condições do Nordeste brasileiro. Esse conhecimento pode contribuir para o desenvolvimento de estratégias de melhoramento genético que promovam a sustentabilidade e a eficiência da produção de ovos na região.

Neste trabalho, objetivou-se elaborar uma revisão bibliográfica com ferramentas bibliométricas e identificar polimorfismos associados a produção de ovos e ao choco em galinhas da raça Canela-Preta no gene prolactina (PRL) e no gene receptor de peptídeo intestinal vasoativo 1 (VIPR-1).

A Tese é apresentada em Capítulos, de acordo com as Normas do Programa de Pós-Graduação em Zootecnia Tropical da UFPI. A estrutura é apresentada da seguinte forma:

Referencial Teórico - Composto pelos tópicos de aspectos gerais das galinhas caipira Canela-Preta, investigação de SNP's em galinhas caipiras e técnicas de genotipagem, genes candidatos e polimorfismos associados a produção de ovos.

Capitulo 1 - Levantamento global de estudos com marcadores SNPs em galinhas (*Gallus gallus domesticus*): Uma abordagem bibliométrica, onde é abordado sobre a aplicação de marcadores de polimorfismos de nucleotídeo único (SNPs) em galinhas, utilizando dados da Coleção Principal da Web of Science (WoS) e Scopus (Este capitulo foi elaborado segundo as normas da Associação Brasileira De Normas Técnicas - ABNT. NBR 6023).

Capitulo 2 - Genes pleiotrópicos PRL e VIPR-1 associados a características de produção de ovos em galinhas nativas Canela-Preta. Onde a foi analisado a associação de polimorfismos nos genes Prolactina (PRL) e Receptor de Peptídeo Intestinal Vasoativo 1 (VIPR-1) com a produção de ovos (temperatura cloacal, abertura dos ossos pélvicos e choco) em galinhas da raça Canela-Preta. Foram analisados 40 indivíduos pelo método de tetra*primers* ARMS-PCR (Este capitulo foi elaborado segundo as normas da Associação Brasileira De Normas Técnicas - ABNT. NBR 6023).

2. REFERENCIAL TEÓRICO

2.1 ASPECTOS GERAIS DAS GALINHAS CAIPIRAS CANELA-PRETA

As galinhas caipiras de raças locais representam um segmento distinto dentro da avicultura brasileira, sendo tradicionalmente criadas em sistemas de manejo extensivo ou semiextensivo. Este tipo de criação está associado a práticas mais rústicas a uma produção em menor escala, o que confere à sua carne características organolépticas diferenciadas, valorizadas por consumidores que buscam produtos mais autênticos e saudáveis (SEBRAE, 2019). Além do apelo ao bem-estar animal, as galinhas caipiras são uma importante fonte de renda para pequenos agricultores, contribuindo para a segurança alimentar e a economia rural no Brasil. Estudos demonstram que a carne de galinha caipira possui um perfil nutricional superior, com maior teor de ácidos graxos insaturados e menor quantidade de gordura total em comparação à carne de frango convencional (Coutinho; Rosário; Jorge, 2010; Rosa, 2015; Santos *et al.* 2024).

Dentro do universo das galinhas caipiras, a Canela-Preta se destaca como uma das raças mais representativas nas regiões Norte e Nordeste do Brasil. Ela é apreciada não só pela qualidade da carne, mas também pela postura de ovos. Estudos indicam que as galinhas Canela-Preta apresentam uma média de 180 a 200 ovos por ano, dependendo das condições de manejo e da nutrição ofertada (Araújo De Carvalho *et al.* 2020;Santos *et al.* 2024). Conhecida por sua rusticidade e adaptação a condições adversas, esta raça é altamente valorizada tanto pela sua contribuição econômica em sistemas de agricultura familiar quanto por seu papel na segurança alimentar em áreas rurais. Sua produção envolve sistemas de criação extensivos, onde a ave é criada em liberdade e com alimentação baseada em recursos naturais, o que contribui para a sustentabilidade da produção. Estudos recentes têm explorado o potencial genético dessa raça para melhoramento, visando a ampliação da sua produção sem comprometer as características tradicionais que a tornam valorizada no mercado (Araújo de Carvalho *et al.* 2020).

A galinha Canela-Preta possui características morfológicas distintas que a diferenciam de outras raças caipiras. A plumagem é predominantemente negra, com penas brilhantes e firmes, enquanto as pernas são de cor preta ou escura, características que lhe conferem o nome. A conformação corporal é de porte médio, com um corpo robusto, pescoço alongado e cauda de tamanho médio (Figura 1).

Figura 1. Exemplares da Galinha da Raça Canela-Preta. Autoria: Claudiane Morais.

Geneticamente, ela é um importante reservatório de variabilidade genética. Estudos apontam que essa variabilidade é fundamental para a resistência da raça a doenças e para sua capacidade de adaptação a diferentes condições ambientais, incluindo climas áridos e semiáridos. Pesquisas conduzidas pela Embrapa revelaram que a diversidade genética dessa raça permite que ela seja utilizada em programas de melhoramento genético visando à conservação de raças locais e ao aumento da produção sem perder suas características adaptativas (Otto *et al.* 2024)

A análise genética da Canela-Preta sugere que ela possui uma alta heterogeneidade genética, o que é um indicativo de sua adaptabilidade e resiliência em ambientes hostis. Essa diversidade genética também é crucial para a manutenção de características fenotípicas importantes, como a resistência a parasitas (Luca; Leal, 2022), e a capacidade de forrageamento, que são essenciais para a sobrevivência em sistemas de criação extensiva.

Em termos de produção, a galinha Canela-Preta é conhecida por sua dupla aptidão: produção de carne e ovos. Embora a produção de ovos seja inferior à das raças comerciais, e tenha dificuldades relacionadas ao alto índice de choco, a Canela-Preta produz ovos de alta qualidade, com casca resistente e conteúdo nutricional adequado, o que a torna adequada para sistemas de produção que valorizam a qualidade em detrimento da quantidade. Além disso, a carne da Canela-Preta é apreciada por sua textura e sabor diferenciados, sendo especialmente valorizada em mercados locais e feiras de produtores (Luca; Leal, 2022).

Apesar de suas qualidades, a galinha Canela-Preta, até pouco tempo atrás, enfrentava desafios significativos em termos de conservação devido à pressão por raças comerciais mais produtivas e a falta de políticas públicas específicas para a preservação de raças autóctones ameaçavam a continuidade da Canela-Preta. Porém, em 2021, a galinha caipira Canela-Preta foi

oficialmente reconhecida como patrimônio histórico, cultural e genético do estado do Piauí, conforme estabelecido pela Lei nº 7.615/2021. Esse reconhecimento sublinha a importância da raça para a identidade regional. A cidade de Queimada Nova – PI, onde foram realizadas as primeiras pesquisas sobre a Canela-Preta, foi designada como a capital dessa raça, consolidando-se como o epicentro dos estudos e da preservação desses animais (PIAUÍ, 2021).

Os programas de conservação são essenciais para garantir que a galinha Canela-Preta continue a ser uma opção viável e sustentável para a produção avícola, especialmente em regiões onde a adaptabilidade e a rusticidade são características cruciais para a sobrevivência e a produção das aves.

A galinha Canela-Preta representa um recurso genético valioso para a avicultura brasileira, especialmente em regiões semiáridas como o Piauí. Suas características morfológicas, genéticas e zootécnicas fazem dela uma raça única, adaptada a condições adversas e com um grande potencial para sistemas de produção sustentáveis. A continuidade dos esforços de conservação é vital para preservar essa raça e maximizar seu potencial produtivo, garantindo, assim, a segurança alimentar e a diversidade genética nas regiões onde ela é criada.

No estado do Piauí, a criação de galinhas Canela-Preta possui grande importância socioeconômica, sendo uma prática comum entre pequenos produtores. A rusticidade dessa raça é particularmente vantajosa nas condições semiáridas do Piauí, onde a disponibilidade de recursos é limitada e a resiliência das aves é crucial para a viabilidade da produção. Pesquisas conduzidas na região têm demonstrado o impacto positivo da criação de Canela-Preta na renda das famílias rurais, além de destacar a importância de programas de conservação genética para preservar as características únicas dessa raça (Carvalho *et al.* 2017). A valorização da Canela-Preta no Piauí também está alinhada com as políticas de desenvolvimento sustentável, que buscam promover a produção animal integrada aos recursos naturais disponíveis na região.

2.2 INVESTIGAÇÃO E GENOTIPAGEM DE SNP’s EM GALINHAS CAIPIRAS

A maioria das características de interesse econômico segue um padrão de herança poligênica, sendo controlada por diversos genes, que podem ter efeitos grandes ou pequenos, e que são fortemente influenciadas por fatores ambientais (Coutinho; Rosário; Jorge, 2010). O uso de marcadores moleculares no processo de caracterização genética tem sido amplamente adotado devido a várias características desejáveis.

Um marcador molecular é uma variação na sequência de DNA que está situada perto ou associada a um gene responsável pela expressão de uma característica específica. Essa variação pode ou não ter sua posição exata conhecida dentro do genoma (Amiteye, 2021). Um marcador molecular ideal deve ter alto polimorfismo, codominância, presença frequente no genoma,

ausência de influência ambiental, acessibilidade, facilidade de ensaio, detecção, automação e também de ter uma boa reprodutibilidade (Carrer; Barbosa; Ramiro, 2010).

Em galinhas caipiras, os marcadores moleculares podem evidenciar áreas gênicas que estão ligadas a características de interesse econômico como: carcaça, qualidade da carne, produção de ovos, resistência a doenças, crescimento, adaptabilidade e reprodução (Silva Júnior *et al.* 2021). Nesse aspecto os marcadores genéticos são examinados com base nos polimorfismos presentes nas variações alélicas, permitindo, dessa forma, a diferenciação entre os indivíduos, possibilitando uma seleção mais precisa e eficaz. Esses polimorfismos podem ser classificados em inserção/deleção (Indel), duplicação, translocação, como também mutações pontuais como transições e transversões (Bhattacharya *et al.* 2011).

Os projetos de sequenciamento genético permitiram a identificação de polimorfismos de nucleotídeo único, popularmente denominados de SNPs (Single Nucleotyde Polymorphisms). Eles ocorrem quando um nucleotídeo é substituído por outro ou quando um ou mais nucleotídeos são adicionados ou excluídos (Barros Polido *et al.* 2012). Para que uma variação seja classificada como SNP, ela deve estar presente em pelo menos 1% da população (Veruska; Da Silva, 2012). Os SNPs são encontrados em grande quantidade no genoma, e também podem ser localizados em regiões codificadoras de proteínas, tendo assim a possibilidade de influenciar algumas variações e características importantes nos indivíduos estudados (Yang *et al.* 2013). Este tipo de polimorfismo é interessante para os estudos que envolvem a necessidade de genotipagem, visto que são geneticamente estáveis e de fácil acesso, são bi-alélicos, tem baixa taxa de mutação e podem ser amplificados em técnica de reação em cadeia de polimerase (PCR) convencional (Medrano; de Oliveira, 2014).

Com o avanço das tecnologias moleculares para genotipagem de SNPs foi possível detectar diferenças no arcabouço genético para características de interesse econômico, tais como a identificação de *loci de característica quantitativa* (QTL) para o mapeamento dos fatores genéticos que afetam o valor de traços quantitativos. Foram detectados QTLs para galinhas nativas poedeiras e de corte, sendo esses *loci* associados a crescimento e alta produção de ovos, validando a expressão de oito genes (GHR, GHRHR, IGF2BP1, OVALX, ELF2, MGARP, NOCT, SLC25A15). Essas informações ajudam na identificação de caracteres controlados por um ou mais genes, sua localização e se existe interação com outros mecanismos moleculares (Wu *et al.* 2024).

Dentre as técnicas utilizadas para detecção dos SNPs, está a abordagem do gene candidato que consiste na investigação de genes específicos que são suspeitos de estarem associados a uma determinada condição ou característica. Essa abordagem pode servir para seleção de genes, estudos de associação e validação de marcadores (Bello *et al.* 2023)

A estratégia é bastante eficaz, pois facilita a coleta de dados e informações, sendo amplamente utilizada em estudos de associação genômica para a identificação de SNPs (Wang *et*

al. 2021). Inicialmente a detecção de SNP's estava restrita ao uso de técnicas como PCR-RFLP, que envolve a utilização de PCR seguida da aplicação enzimas de restrição para a identificação dos polimorfismos genéticos. No entanto esta técnica apresenta algumas desvantagens, como a demora no processo de análise, a necessidade de sítios de restrição específicos no local do polimorfismo e a adaptação dos protocolos das enzimas para garantir a precisão dos resultados. Além disso, a necessidade de enzimologia detalhada e de etapas adicionais tornam o processo relativamente complexo e dispendioso. Opcionalmente, estes polimorfismos também podem ser detectados com o uso de sequenciamento direto, uma abordagem mais moderna que, embora eficaz, é onerosa e exige equipamentos de alta capacidade, o que a torna menos acessível para laboratórios com orçamento mais restrito. Mais recentemente técnicas como a Tetra-*primer* ARMS PCR (*Amplification Refractory Mutation System* PCR) emergiram como solução. A técnica de Tetra-*primer* ARMS-PCR é uma metodologia desenvolvida para a detecção de mutações específicas, como polimorfismos de nucleotídeo único (SNPs), onde quatro *primers* (dois internos e dois externos) são utilizados simultaneamente em uma única PCR (Medrano; de Oliveira, 2014; Newton *et al.* 1989).

A técnica de Tetra-*primers* ARMS-PCR é uma metodologia desenvolvida para a detecção de mutações específicas, como polimorfismos de nucleotídeo único (SNPs) onde quatro *primers* (dois internos e dois externos) são utilizados simultaneamente em uma PCR única (Collins; Ke, 2012). Os *primers* externos são usados para amplificar a região do DNA ao redor da mutação, gerando amplicons de controle. Ao mesmo tempo, os *primers* internos, projetados para se ligar a alelos específicos e orientados de forma oposta aos *primers* externos, permitem a amplificação simultânea dos dois alelos distintos. Os produtos da amplificação geram fragmentos de tamanhos variados que podem ser facilmente diferenciados por eletroforese em gel de agarose, permitindo a distinção entre homozigotos e heterozigotos com base no tamanho dos fragmentos (Fatima *et al.* 2022).

Uma das principais vantagens da técnica de Tetra-*primers* ARMS-PCR é o seu baixo custo operacional em comparação com outros métodos de genotipagem. Por não demandar o uso de equipamentos sofisticados, como sequenciadores automáticos, e por permitir a discriminação dos alelos diretamente após a amplificação, essa técnica se torna acessível e viável para laboratórios de menor porte e para pesquisas em larga escala. Segundo Mendes *et al* (2020), o custo reduzido de reagentes e a simplicidade no design dos *primers* tornam essa metodologia particularmente atraente em contextos em que o financiamento é limitado. Além disso, é um método rápido, já que a reação ocorre em uma única etapa, com resultados facilmente visualizáveis por eletroforese, oferecendo precisão e simplicidade na identificação de SNPs (Wolc *et al.* 2014).

A técnica também apresenta algumas limitações. Entre elas, podemos citar a necessidade de uma otimização criteriosa das condições de PCR, como a concentração de $MgCl_2$ e a temperatura de anelamento, para garantir que não haja amplificação cruzada entre os *primers*

internos e externos. Além disso, a especificidade dos *primers* é um fator crítico, uma vez que uma má escolha dos *primers* pode resultar em amplificações não específicas, comprometendo a análise dos resultados (Dou *et al.* 2022). O desenho de *primers* requer cuidado extra, principalmente quando há alta similaridade entre os alelos a serem discriminados. Esses desafios, embora possam ser contornados, exigem experiência no design experimental e otimização contínua.

A técnica de Tetra-*primers* ARMS-PCR foi utilizada para amplificar os SNPs nos genes PRL e VIPR1 no presente trabalho, genes estes envolvidos em características produtivas e reprodutivas de galinhas caipiras. Para desenvolver essa técnica, é preciso primeiro localizar as sequências disponíveis em bancos de dados genéticos, como o GenBank. Um exemplo é verificar as sequências ID AB011438.2 para o gene PRL e ID NC_052533.1 para o gene VIPR1. Em seguida, é necessário localizar os SNPs nas sequências escolhidas. Após essa etapa, é utilizado uma interface específica, como a *PRIMER1: primer design for tetra-primer ARMS-PCR* (https://*primer*1.soton.ac.uk/*primer*1.html), para projetar os tetra*primers*, garantindo uma especificidade adequada para os SNPs de interesse. Essa metodologia permite a identificação dos diferentes genótipos através da análise dos amplicons por eletroforese em gel, facilitando a discriminação de características como a produção de ovos e a qualidade do ovo (Li *et al.* 2011).

2.3 GENES CANDIDATOS E POLIMORFISMOS ASSOCIADOS A PRODUÇÃO DE OVOS

A produção de ovos é a característica econômica mais importante para galinhas caipiras. Em 2023, a produção global de ovos de galinha foi de aproximadamente 88,68 milhões de toneladas, respondendo por cerca de 93% da produção de ovos de aves (FAO, 2023).

O ovo é uma excelente fonte de proteína de alto valor biológico, rico em vitaminas e minerais, e um alimento acessível a diversos estratos sociais. O baixo custo desse alimento tem do ponto de vista social pode ajudar países subdesenvolvidos a combater a fome (Figueiredo, 2017), além disso a criação de aves soltas e com pequeno custo de investimento é mais acessível as camadas mais pobres.

A produção de ovos é uma métrica fundamental para a avaliação da eficiência reprodutiva e produtiva em sistemas avícolas. Tradicionalmente, este parâmetro é calculado através da seguinte fórmula:

$$Índice de Postura = \frac{Total de ovos}{Número de Galinhas}$$

Este índice representa a quantidade média de ovos produzidos por cada ave em um determinado período, geralmente expresso em ovos por ave por dia (OAD). A produção de ovos é influenciada por diversos fatores, incluindo genética, manejo alimentar, condições ambientais,

e saúde das aves, sendo um indicador essencial para a otimização dos processos de produção no setor avícola (Rakonjac *et al.* 2021). A eficiência produtiva, representada por este índice, é um dos principais objetivos na seleção genética e nas práticas de manejo, visto que impacta diretamente na rentabilidade da produção.

O processo de seleção artificial da indústria de postura levou produção das galinhas poedeiras, que podem produzir mais de 400 ovos em um ciclo de 52 semanas (Rusnadi; Dewi; Sriyani, 2022). Embora tenha ocorrido mudanças genéticas significativas ao decorrer das práticas de seleção e reprodução, as relações genótipo e fenótipo ainda não são bem caracterizados (Goto; Tsudzuki, 2017).

Pesquisas focadas na genética da produção de ovos identificaram 31 genes candidatos fortemente ligados a essa característica. Além disso, foram descobertos 64 novos genes e 108 SNPs relacionados ao desempenho na postura através de análises de sequenciamento do genoma. (Du *et al.* 2020b). Foram relatados mais de 440 *loci* de características quantitativas (QTL) no cromossomo de *Gallus gallus* (GGA) que estão ligados à quantidade de ovos produzidos por galinhas (Hu; Park; Reecy, 2016).

Com os avanços no sequenciamento de nova geração e no aumento da identificação de SNPs, os estudos de associação genômica ampla (GWAS) e as análises de varredura de seleção surgiram como ferramentas úteis para identificar *loci* de característica quantitativa (QTL) em várias raças de galinhas (Li *et al.* 2021). Essas novas tecnologias permitem com maior precisão investigar polimorfismos associados a características de interesse econômico, e com isso aumentar a produção e eficiência na avicultura rural, tendo em vista que o uso de tecnologias emergentes deve beneficiar toda gama de estrato social, desde da indústria até o pequeno produtor.

Na produção de ovos, a idade do primeiro ovo (IPO), o número de ovos (NO), peso do ovo (PO), taxa de postura (TP), qualidade do ovo (QO), abertura dos ossos pélvicos (APELVE), temperatura cloacal (TCLOAC), peso da casca do ovo (PCO), espessura da casca (EC), cor da casca (CC), resistência da casca (RC), índice de forma do ovo (IFO), peso da gema (PG) e a unidade de Haugh (UH) são parâmetros importantes na produção avícola (DU *et al* 2020a). Outros parâmetros como índice de choco (IC) e frequência de choco (CHOCO) também são bastante utilizados em programas de seleção de aves com melhor desempenho na produção de ovos.

Esses parâmetros são regulados principalmente por fatores genéticos e endócrinos e são padrões valiosos para se obter uma boa análise do desempenho de postura (Sharp; Dawson; Lea, 1998; Wolc *et al.* 2014; Wu *et al.* 2024). A característica genética para postura de ovos é classificada como de baixa ou média herdabilidade variando de 0,16 a 0,64 (Li *et al.* 2011; Luo; Yang; Yang, 2007).

Muitos avicultores tendem a utilizar técnicas de melhoramento tradicional, selecionando fenotipicamente aves com melhor aptidão para postura, porém, isso torna a seleção muito difícil e imprecisa visto que a característica analisada é grandemente influenciada pelo ambiente, com as ferramentas moleculares emergentes é possível identificar e obter uma seleção mais precisa e eficiente, poupando recursos nutricionais e logísticos (Dunn *et al.* 2004; Qin *et al.* 2015a, 2015b).

Essa característica também é classificada como poligênica, ou seja, é controlada por vários genes o que torna mais complexo e desafiante o estudo genético da postura (Andersson; Georges, 2004). Estudos indicam que existem variações nesses genes que afetam características quantitativas, classificando-os em micro efetores e genes principais (Shrimpton; Robertson, 1988).

Para compreender melhor o desempenho na produção de ovos em galinhas, é essencial identificar mais genes candidatos e polimorfismos associados a essa característica. Inicialmente pensava-se que a postura de ovos era relacionada somente a genes ligados a características endócrinas, como o eixo hipotálamo-hipófise-gonadal (HHG), que regula a função reprodutiva em aves. Esse eixo é responsável pela produção e liberação de hormônios gonadotróficos (LH e FSH), essenciais para o desenvolvimento folicular e ovulação. Com os avanços das pesquisas, descobriu-se que alguns genes fora desse eixo também influenciam significativamente a postura de ovos em galinhas, como o gene do nanismo ligado ao sexo (DW) e o fator de crescimento semelhante à insulina 1 (IGF-1) (Nagaraja *et al.* 2000; Sadjadi *et al.* 1983).

A formação do ovo é um processo complexo e multifatorial, controlado pela interação de hormônios do eixo HHG e de genes regulatórios. A prolactina (PRL), secretada pela hipófise anterior, desempenha papel essencial na regulação da incubação, influenciando negativamente a oviposição ao inibir a secreção de gonadotrofinas. A PRL é ativada em resposta a estímulos sensoriais do ambiente (como o comportamento de nidificação) e atua diretamente no tecido gonadal e no controle da formação folicular.

O gene VIPR1 codifica o receptor do peptídeo intestinal vasoativo (VIP), que é expresso no hipotálamo e desempenha uma função crucial na estimulação da liberação de prolactina. O VIP interage com vias intracelulares de sinalização, como a ativação da adenilato ciclase e o aumento de AMP cíclico, resultando na expressão aumentada de PRL. Essa interação é mediada por mecanismos autócrinos e parácrinos no hipotálamo e na hipófise, modulando a homeostase hormonal necessária para a produção do ovo.

Os hormônios PRL e VIP participam de vias metabólicas interligadas que regulam a síntese e o transporte de nutrientes para o oócito em desenvolvimento. A prolactina influencia a mobilização de cálcio, essencial para a calcificação da casca, ao atuar nos tecidos-alvo, como o osso medular e o oviduto. Essa via envolve o aumento da expressão de transportadores de cálcio (como o NCX1) e a ativação da enzima cálcio-ATPase, crucial para a mineralização do ovo.

O VIP, por sua vez, regula a ativação do eixo HHG e promove a síntese de lipoproteínas na gema, aumentando a biodisponibilidade de nutrientes essenciais, como lipídeos e proteínas, para o crescimento do folículo. Além disso, o VIP potencializa a expressão de receptores hormonais, como os receptores de estrógeno (ESR), amplificando o efeito dos esteroides gonadais na maturação folicular e na ovulação.

O ciclo bioquímico da formação do ovo envolve processos coordenados de síntese de proteínas, lipídeos e deposição de cálcio. Nesse contexto, a PRL e o VIP modulam a atividade das células da granulosa e da teca nos folículos ovarianos. A prolactina regula a diferenciação celular e a liberação de hormônios esteroides, enquanto o VIP mantém a integridade do eixo HHG, favorecendo a produção cíclica de LH e FSH, necessários para o recrutamento e maturação dos folículos. Portanto, o estudo desses genes e suas vias metabólicas não só amplia a compreensão dos mecanismos endócrinos da postura de ovos, mas também possibilita a aplicação de ferramentas moleculares para melhorar o desempenho reprodutivo das galinhas.

Vários genes candidatos como o GnRH-I (hormônio liberador de gonadotrofina I), NPY (neuropeptídeo Y), GNRHR (receptor de hormônio liberador de gonadotrofina), GnIH (hormônio inibidor de gonadotrofina), FSHR (receptor do hormônio folículo estimulante), PRL (prolactina), PRLR (receptor de prolactina), ESR (receptor de estrogênio) e VIP (peptídeo intestinal vasoativo) foram identificados por estudos de associação. Atualmente os genes mais explorados que estão significativamente associados ao desempenho na postura de ovos (Quadro 1).

Quadro 1. Genes associados a produção de ovos

Gene	Característica primária associada	Referência / NCBI Gene ID
IGF1	Crescimento e desenvolvimento	395900
PRL	Reprodução e comportamento de incubação	396568
GH	Crescimento corporal e produção de carne	374108
LEP	Regulação do apetite e metabolismo	396048
TSHR	Comportamento reprodutivo	395315
VIP	Regulação da reprodução e crescimento	395676
GHRHR	Crescimento e desenvolvimento	396353

Desses, genes os mais destacados são o PRL (*Prolactin*) que está relacionado à produção do hormônio prolactina, que desempenha um papel crucial na incubação e comportamento de choco em galinhas. Estudos mostram que a expressão do gene PRL pode ser modulada por diferentes doses de antiprolactina (Bana; Barlian; Ridwan, 2021). O gene IGF1

está associado ao crescimento e desenvolvimento das galinhas. Polimorfismos neste gene podem influenciar o desempenho reprodutivo e a produção das galinhas (Ogunpaimo *et al.* 2021). E o gene VIP está envolvido na regulação da produção de ovos e comportamento de choco em galinhas. Polimorfismos neste gene podem afetar a produção de ovos e a duração do comportamento de choco (Zhou *et al.* 2010).

Esses genes estão localizados principalmente nos cromossomos GGA1, 2, 3, 9, 13, e Z (Du *et al.* 2020a). Tais estudos permitem uma base para elucidar com maior precisão os mecanismos genéticos moleculares que influenciam a postura de ovos em galinhas, o desenvolvimento de marcadores moleculares promoverá ainda mais a criação e seleção de raças locais e pode estabelecer núcleos de conservação e desenvolvimento avícola sustentável.

Com o avanço dos estudos de associação genômica ampla (GWAS) foram identificados vários genes e QTLs (*Quantitative Trait Loci*) que influenciam no desempenho de postura, e o crescente interesse do uso de marcadores que possam estudar essas variações pode desempenhar um papel crucial na genômica. Por exemplo, os estudos de associação genômica ampla (GWAS) foi usado para identificar variações de a idade do primeiro ovo (IPO), e número de ovos (NO) em galinhas poedeiras Leghorn (Li *et al*. 2011). A maioria dos *loci* significativamente associados ao desempenho na postura de ovos através do GWAS foi encontrada nos cromossomos GGA1 (36 SNPs), GGA2 (4 SNPs), GGA3 (10 SNPs), GGA 4 (7 SNPs), GGA5 (27 SNPs) e GGA21 (5 SNPs).

Foi relatado um SNP (GGA092322) no cromossomo 13 de galinhas (*Gallus gallus*) no íntron 2, sendo esse significativamente associado a idade do primeiro ovo (IPO) (Li *et al.* 2011), outros artigos demostraram que essa variação no íntron 2 desse SNP também foi expresso no cérebro, na retina e no sistema óptico das galinhas (Kenzelmann *et al.* 2008).

Um SNP relacionado ao número de ovos (NO) foi identificado em 21,67 Mb de GGA7, localizado no íntron 12 do gene GRB14, esse gene em mamíferos é expresso nos ovários e interage com o receptor de insulina (IR) e o receptor do fator de crescimento semelhante a insulina (IGFR) (Daly *et al*. 1996). Sendo esses *IGF* e *o IGFR* que regulam a função do ovário e o desenvolvimento folicular em galinhas (Kim; Seo; Ko, 2004).

Identificaram que quatro SNPs nos cromossomos GGA1, GGA2 e GGA11 apresentaram correlação significativa com número de ovos (NO) na faixa etária de 300 a 462 dias. Especificamente, um SNP (rs13905010) foi localizado em GGA1, outro SNP (rs15938574) foi encontrado em GGA2, e dois SNPs foram detectados na região de 2,33-2,35 Mb do GGA11 (Fan *et al.* 2017).

Um *locus* significativo em todo o genoma (ss1985401199) foi identificado no cromossomo sexual Z, associado à produção de ovos na faixa etária de 25-45 semanas. Foram

encontrados também 23 SNPs nos cromossomos GGA1, GGA5 e GGA23, significativamente ligados à produção de ovos, com destaque para os 21 SNPs localizados na região de 40,1-43,16 Mb do GGA5. Adicionalmente, descobriu-se que a região do QTL (38,62-38,69 Mb) associada à produção de ovos aos 300 dias de idade no GGA5 está a 1,41 Mb de distância dessa região (40,1-43,16 Mb), sugerindo que essa área pode ser um importante QTL relacionado ao desempenho na postura de ovos (Shen *et al.* 2012; Yuan *et al.* 2015).

Em relação ao peso do ovo (PO), foi relatado que um SNP (rs13588750A), localizado no GGA5, está significativamente associado ao PO aos 300 dias de idade, sendo o gene candidato o FBLN5 (Zhang *et al.* 2015).

Há também estudos que associam a abertura dos ossos pélvicos (APELVE) com os números de ovos, e que essa característica está estreitamente relacionada a produção de ovos. Nas galinhas durante a fase de produção máxima, os órgãos abdominais expandem a distância entre a extremidade da quilha e os ossos pélvicos (Torres; Graner, 1951). O mesmo autor sugere que há correlação positiva entre a dispersão dos ossos pélvicos e a produção de ovos (r = + 0,23 e pr = + 0,16).

A temperatura cloacal (TCLOAC) é vista como uma medida representativa da temperatura corporal, servindo como um bom indicador das condições de conforto ou estresse térmico dos animais, o que pode influenciar seus índices de produção (T. M. Brown-Brandl *et al.* 2003).

Na análise sobre o peso da casca do ovo (PCO), foram revelados dois SNPs significativamente associados: um localizado no GGA2 e outro no GGA3. Os genes promissores identificados foram GALNT1 e BLK, respectivamente (Li *et al.* 2011).

Um SNP localizado em 184,63 Mb no GGA1 apresentou uma associação significativa com o peso da gema (PG) em poedeiras de 40 semanas de idade, sendo o gene candidato correspondente o ATM, além disso, foram identificados dois SNPs no GGA1, situados em 8,1 Mb (171,2-179,3 Mb), que demonstraram uma correlação significativa com a espessura da casca do ovo (EST) em galinhas poedeiras com 40 semanas de idade. Os genes candidatos associados foram LOC418918 e ENOX1 (Li *et al.* 2011).

O desempenho na produção de ovos é crucial para o rendimento econômico das galinhas poedeiras, tornando esses estudos essenciais para esclarecer os mecanismos genéticos associados à produção de ovos. Apesar de um grande número de genes relacionados ao desempenho na postura de ovos ter sido identificado tanto no eixo hipotálamo-hipófise-gonadal (HHG) quanto fora. O conhecimento e aplicação de marcadores moleculares polimórficos pode promover o aprimoramento de raças locais, bem como sua conservação e utilização como recurso eficiente e sustentável.

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Article 1. A Global Survey of SNP Marker Studies in Chickens (*Gallus gallus domesticus*): A Bibliometric Approach

Global survey of studies with SNP markers in chickens (*Gallus gallus domesticus*): A bibliometric approach

ABSTRACT: This research aimed to review the scientific literature on the application of single nucleotide polymorphism (SNP) markers in chickens, using data from the Web of Science (WoS) Core Collection and Scopus. The "bibliometrix" package in the R platform was used to analyze data exported in BibTeX format. The research focused on articles on SNPs in chickens (*Gallus gallus domesticus*), covering the period from 2008 to 2024. A total of 1668 articles were analyzed, with a significant growth in publications since 2008. The year 2024 stood out as the most productive, with 137 articles, and the annual average was 87 publications. The countries with the greatest scientific contribution were China, the United States, and South Korea, with China leading with 723 articles. The predominance of developing countries, such as China and Brazil, reinforces the potential for global collaboration in the advancement of poultry genetics. The most frequently used keywords were “growth” and “egg production”, mainly associated with production and reproduction traits. The most explored genes include GH, PRL, MC1R, MHC, IGF1 and VIP, which are linked to egg production, growth and disease resistance. Therefore, the results indicated potential genes for application in breeding studies. The research identified that more than 40% of the publications are linked to state institutions in China, with emphasis on the Ministry of Agriculture and the China Agricultural University. The review highlights the growth of research with SNP markers in chickens, reflecting the interest in applying these technologies to improve production and sustainability in poultry farming.

Key words: Poultry Genetics; Bibliometrix; Poultry Production; Egg Production.

Levantamento global de estudos com marcadores SNPs em galinhas (*Gallus gallus domesticus*): Uma abordagem bibliométrica

RESUMO: A presente pesquisa foi desenvolvida objetivando-se revisar a literatura científica sobre a aplicação de marcadores de polimorfismos de nucleotídeo único (SNPs) em galinhas, utilizando dados da Coleção Principal da Web of Science (WoS) e da Scopus. Utilizou-se o pacote "bibliometrix" na plataforma R para analisar dados exportados no formato BibTeX. A pesquisa focou em artigos sobre SNPs em galinhas (*Gallus gallus domesticus*), cobrindo o período de 2008 a 2024. Foram analisados 1668 artigos com um crescimento significativo nas publicações desde 2008. O ano de 2024 destacou-se como o mais produtivo, com 137 artigos, e a média anual foi de 87 publicações. Os países com maior contribuição científica foram China, Estados Unidos, e Coreia do Sul, com a China liderando com 723 artigos. A predominância de países em desenvolvimento, como China e Brasil, reforça o potencial de colaboração global no avanço da genética avícola. As palavras-chave mais recorrentes foram "crescimento" e "produção de ovos", associadas principalmente a características de produção e reprodução. Entre os genes mais explorados estão GH, PRL, MC1R, MHC, IGF1 e VIP, ligados à produção de ovos, crescimento e resistência a doenças. Portanto, os resultados apontaram genes potenciais para aplicação em estudos de melhoramento. A pesquisa identificou que mais de 40% das publicações estão vinculadas a instituições estatais da China, com destaque para o Ministério da Agricultura e a Universidade de Agricultura da China. A revisão evidencia o crescimento da pesquisa com marcadores SNPs em galinhas, refletindo o interesse na aplicação dessas tecnologias para melhorar a produção e sustentabilidade na avicultura.

Palavras-chave: Genética avícola, bibliometrix, produção avícola, produção de ovos.

1. INTRODUCTION

Chickens are distributed worldwide and comprise approximately 70 genera with over 250 species. The domestic subspecies (*Gallus gallus domesticus*) is the predominant one, with a worldwide flock estimated at 24 billion FAO (2023); Bruce Campbell (2013). Chickens, which are members of the Phasianidae family and the order Galliformes, were domesticated thousands of years ago and have since become essential members of human societies. In addition to being the world's main source of protein, they are also a major source of revenue for small and medium-sized farmers. (Takahashi *et al.*, 2006). From a socioeconomic point of view, poultry farming is mostly one of the biggest pillars of agriculture, especially when we're talking about small and medium producers who really depend on it. It's not just numbers on a paper, it's food on the table and money circulating in rural areas. In 2023, for instance, chicken meat production hit something like 131 million metric tons, and egg production. Over 1.2 trillion units, which is kind of insane if you stop to think about it. Altogether, this whole chain keeps moving economies and people, with the industry estimated to support around 4.3 billion jobs, directly and indirectly, all over the world (FAO, 2023).

The demand for chicken meat and eggs just keeps growing, and that's been pushing the industry to chase more and more efficiency and productivity. It's kind of wild to realize that poultry has at heart taken the lead and is now considered the world's main protein source (Ledur, 2007; ABPA, 2023). So, in this whole scenario, genetics ends up being a big deal it's what helps pick out the traits we want, like faster growth, better meat, stronger resistance to diseases, and even things tied to animal welfare. But then, there's the tricky part: producing more while still adapting chickens to keep up with global food demand. That's no small task, especially with population numbers climbing, the planet heating up, and all the socioeconomic struggles piling on (FAO, 2023). Genotype-by-environment interaction is a real headache, with stuff like scarce resources, tropical diseases, local weather, and how the farms are run all messing with things. Picking birds that can handle this and still perform well is super important if you wanna get the most out of your flock, make the resources count, and boost production, since animals respond to whatever's around them. That's why studies using genetic markers tied to production traits have been getting way more attention lately (Barros Polido *et al.*, 2012; Amiteye, 2021).

SNPs, or Single Nucleotide Polymorphisms, have taken over modern genetics. They're basically tiny differences in DNA between individuals, and now we can check out tons of them at once using microarrays packed with thousands of markers. Such approaches make it possible to map genes connected to economically important traits and apply this knowledge to increase production and sustainability in poultry farming (Burt, 2005; Salvian *et al.*, 2023), while also contributing to animal welfare (Blokhuis *et al.*, 2003). The number of studies using SNPs in

chickens has increased considerably in recent years. Several countries, including the United States, China, Brazil, and the United Kingdom, are investing in biotechnological tools to identify genes that influence traits of interest to the poultry industry.

Based on this background, the present bibliometric analysis used data extracted from the Web of Science Core Collection (Clarivate Analytics) and Scopus to examine scientific production on SNP markers in *Gallus gallus domesticus*. The study also evaluates the main genes cited in the literature and discusses their potential applications in marker-assisted selection projects.

2. MATERIALS AND METHODS

Para a obtenção dos dados, foram utilizadas as plataformas Web of Science (WoS) e Scopus, buscando artigos relacionados à aplicação de Single Nucleotide Polymorphisms (SNPs) na espécie *Gallus gallus domesticus*. Data were obtained from the Web of Science (WoS) and Scopus platforms, focusing on articles that addressed the use of Single Nucleotide Polymorphisms (SNPs) in *Gallus gallus domesticus*. The analysis period spanned from 2008 to 2024, starting from 2008 to capture a significant phase in SNP research in chickens, characterized by relevant discoveries and shifts in research practices in this field (Groenen *et al*. 2009; Souza, 2011). Boolean search descriptors were adapted to encompass the broadest possible range of publications and were defined as follows:

- Web of Science: ((TI=(snp)) OR TI=(snps)) AND TI=(chicken)) or AB=(snp)) OR AB=(snps)) AND AB=(chicken)) or AK=(snp)) OR AK=(snps)) AND AK=(chicken)
- Scopus: snp OR snps AND chicken

Results were extracted in BibTeX format, containing complete records with information on authors, article title, abstract, country, publication year, affiliation, citations, and keywords. After data extraction, the records from both databases were consolidated into a single dataset. Duplicate entries were identified and removed using the bibliometrix package in R (Aria & Cuccurullo, 2017), with matching criteria based on Digital Object Identifier (DOI) and article title. The search and screening process targeted only research articles, using information from titles, abstracts, and keywords.

The bibliometrix package was also used for the core bibliometric analyses, such as evaluating publication output by country and keyword frequency. The country attributed to each publication was determined based on the correspondence address of the first author. For enhanced visual network analysis, co-occurrence maps and network graphs were generated using VOSviewer software (van Eck & Waltman, 2010).

The following analytical procedures were applied for data presentation:

- Temporal publication trend analysis: The histPlot function was used to generate a graph displaying the annual number of publications combined with citation counts.
- Country-level production analysis: A mapping function based on bibliographic data was used for country analysis in VOSviewer. Publication output by country was also assessed using bibliometrix.
- Keyword frequency analysis: Keyword co-occurrence maps were created using VOSviewer, supplemented by network relationship analysis.
- Institutional analysis: We compiled a list of educational and research institutions based on the affiliation addresses of both domestic and international authors. This approach helps reveal where scientific capacity is concentrated and how collaborations are distributed across countries. In my view, however, it is also important to note that such lists tend to highlight well-funded institutions, while smaller universities or research centers in developing regions — including Brazil — often remain underrepresented, despite their significant contributions to poultry science under tropical conditions.
- Gene prominence analysis: We used AI (OpenAI, 2024) just to help us sort through the big pile of data to identify the genes that showed up the most and linked them to the traits people talk about. When you've got too much info, AI is useful, but you still have to use common sense. In other words, the fact that a gene is highly cited in international papers doesn't always mean it's the one that really matters. When it comes to tropical poultry, sometimes what makes the difference is whether the bird can handle the heat, survive local bugs, or fit into small free-range or family farms - which doesn't always show up as a "top priority" in research reports.

We made sure all the data followed the standard format set out by Moura *et al.* (2017).

Todos os dados foram padronizados de acordo com a metodologia bibliográfica proposta por (Moura; Dawson; Nogueira, 2017).

3. RESULTS

The first search across the Web of Science (WoS) and Scopus databases gave 2,055 articles about poultry science. This focused on studies about "SNPs in *Gallus gallus domesticus*", as the search rules stated. After removing 387 copies, 1,668 separate publications stayed for the next bibliometric analysis. (Table 1).

Table 1. The total number of publications retrieved from Web of Science but also Scopus databases, the number of duplicate articles, and the final article count

DATA DESCRIPTION	COUNT
SCOPUS	1473
WEB OF SCIENCE	582
SUBTOTAL	2055
DUPLICATES	387
TOTAL	1668

When we looked at how many papers came out over the years, it was pretty clear the numbers just kept going up. Back in 2008 there were like 50 of them, and then every year it kind of climbed a bit more. By 2024 it hit the highest point, 137 papers that year (Figure 1). On average it ended up being about 87 a year, but the main thing is you can see this steady growth if you follow the timeline.

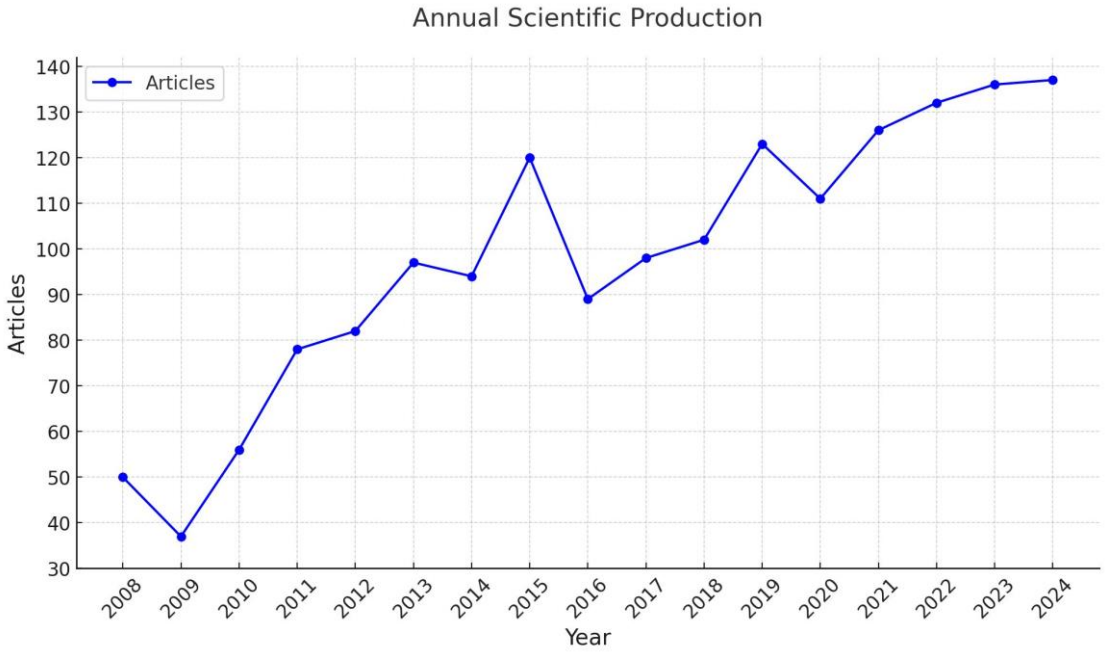

Figure 1. Annual number of scientific publications based on the use of SNP markers for research in *Gallus gallus domesticus* by publication year.

Articles from 67 countries exploring the use of SNPs in chickens were recorded. China led scientific output with 723 publications, followed by the United States (133 publications) and South Korea (51 publications). Other notable contributing countries included the United Kingdom (47 publications), Japan (49 publications), and Iran (41 publications) (Figure 2).

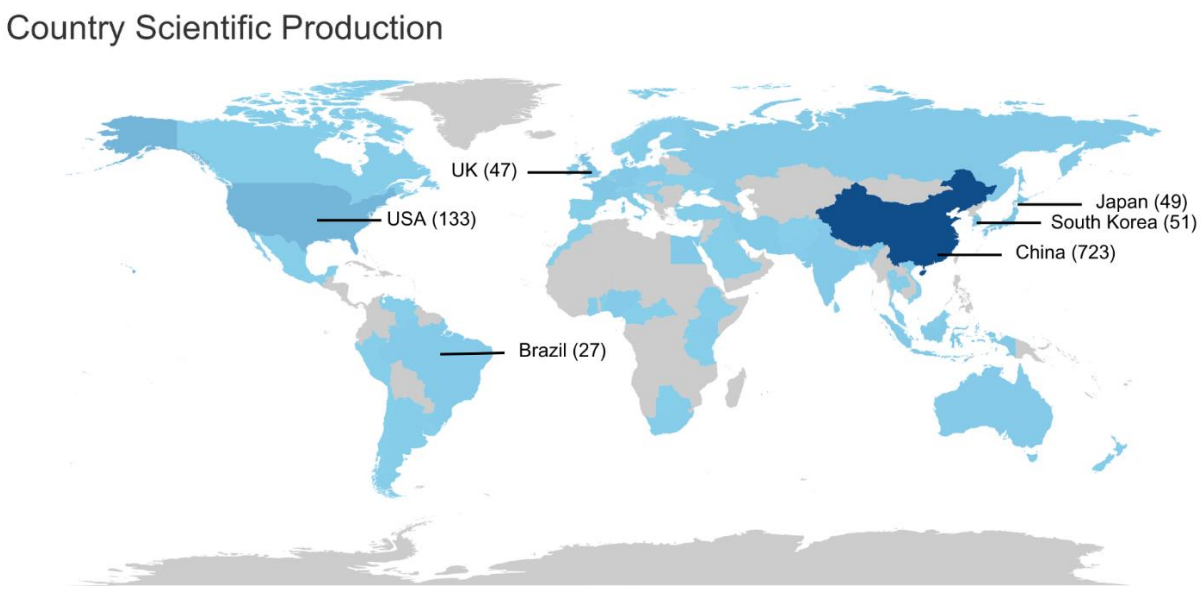

Figure 2. Geographic distribution of scientific publications on SNPs in *Gallus gallus domesticus* by country. Darker blue shades indicate higher publication output, while lighter shades correspond to lower output. Countries shaded in gray had no publications in the analyzed databases.

So, Brazil shows up in 10th place, with 27 papers on the subject if you put together Scopus and Web of Science. Out of the top 20, four countries are still considered "developing" China, Brazil, India, and Iran and three of those are in BRICS. Some others worth pointing out: Indonesia had 58, India and the Netherlands both hit 56, Russia came in with 54, and Egypt had 52. If you check (Figure 3) you'll see how the publication numbers tie into collaborations China's big red bubble and the US's purple one dominates the map, which makes sense given how much they publish and how many people they work with.

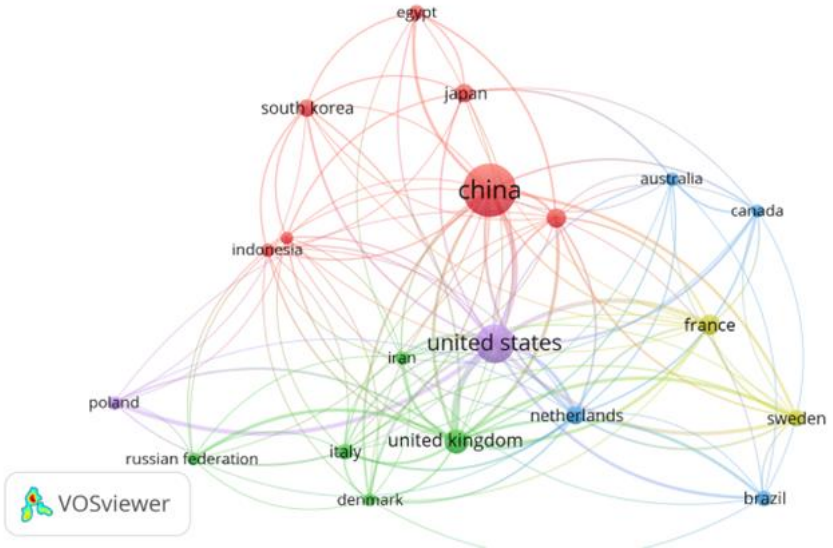

Figure 3. Worldwide scientific publications on SNP markers in *Gallus gallus domesticus* by country of corresponding author and collaborative networks. Node size corresponds to publication volume, and connecting lines represent collaborative linkages between countries.

A total of 6,868 keywords were identified. Using keywords from publications on *Gallus gallus domesticus* SNPs, the biggest research areas are directly related to production and zootechnical traits. There were lots of keywords relating to chicken, *SNPs, GWASs (Genome-Wide Association Studies), egg production, growth, associations, traits, reproductive traits, candidate genes, and egg quality*. (Figure 4).

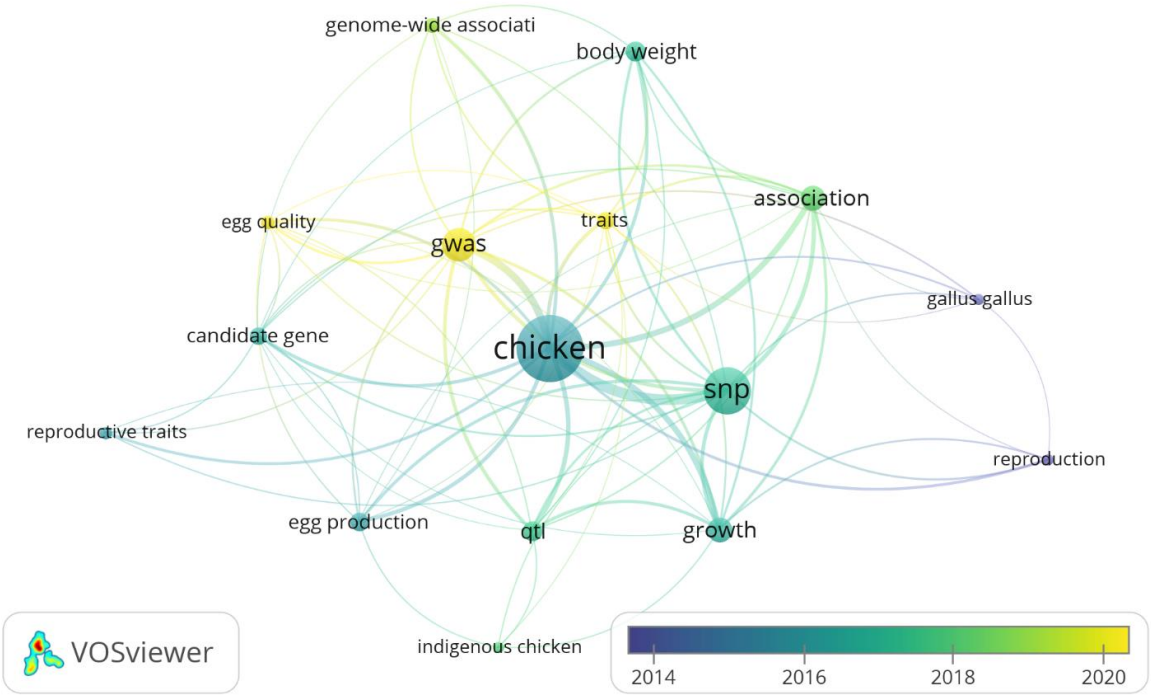

Figure 4. Co-occurrence network analysis of keywords in publications on SNP markers in *Gallus gallus domesticus*. The bigger the node, the more times that keyword showed up, and the lines just show how the terms are kind of connected in meaning.

The publication records show author affiliations with state institutions in the People's Republic of China for more than 40 % of the entries - these institutions really contribute to scientific research on single nucleotide polymorphism (SNP) markers and their application in chickens.

Among the institutions that published articles in the Web of Science (WoS) and Scopus databases, China Agricultural University ranked first with 131 publications, followed by other Chinese institutions, including Yangzhou University (127 publications) and South China Agricultural University (100 publications). All ten of the most prolific institutions were based in China (Table 2).

Table 2. Publication output by institution: Article count and affiliation records from Web of Science and Scopus. All the listed institutions are from China.

Affiliation	Articles	Percentage	Country
CHINA AGR UNIV	131	2.36%	China
YANGZHOU UNIV	127	2.33%	China
SOUTH CHINA AGR UNIV	100	1.81%	China
SICHUAN AGR UNIV	91	1.64%	China
CHINA AGRICULTURAL UNIVERSITY	79	1.43%	China
INST ANIM SCI	79	1.43%	China
HENAN AGR UNIV	67	1.21%	China
SOUTH CHINA AGRICULTURAL UNIVERSITY	63	1.14%	China
YANGZHOU UNIVERSITY	63	1.14%	China
CHUNGNAM NATL UNIV	52	0.94%	China

The most frequently studied genes in this species include Growth Hormone (GH), Insulin-like Growth Factor (IGF), Major Histocompatibility Complex (MHC), Corticotropin (CORT), Prolactin (PRL), Melanocortin 1 Receptor (MC1R), and Vasoactive Intestinal Peptide (VIP). These genes are predominantly connected to economically important traits such as egg production, disease resistance, and growth performance (Figure 5).

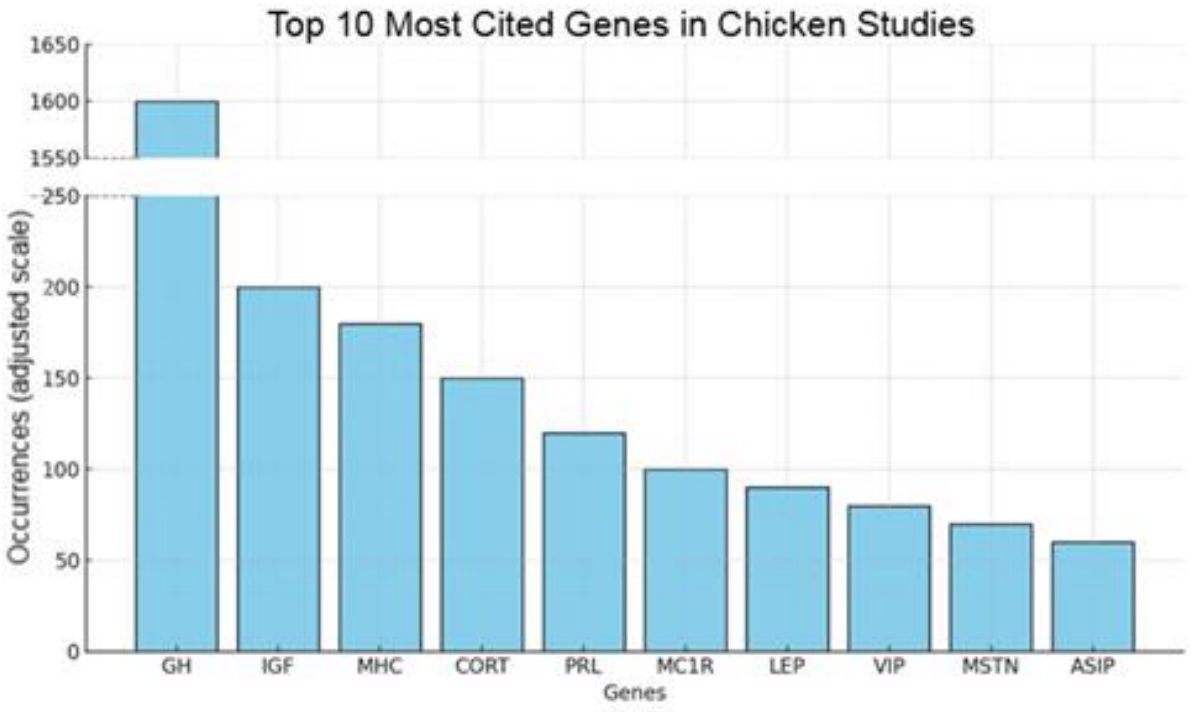

Figure 5. Frequency of the most studied genes and their associated traits in *Gallus gallus domesticus* research. GH: Growth Hormone IGF: Insulin-like Growth Factor, MHC: Major Histocompatibility Complex, CORT: Corticosterone, PRL: Prolactin, MC1R: Melanocortin 1 Receptor, LEP: Leptin, VIP: Vasoactive Intestinal Peptide, MSTN: Myostatin, ASIP: Agouti Signaling Protein.

4. DISCUSSION

A considerable rise in scientific publications about poultry farming but also SNP markers in chickens (*Gallus gallus domesticus*) shows a bigger interest in this research area - it also shows a better understanding of how genetic markers help production. The regular growth in publications from 2008 points to a slow acceptance of how important those markers are in poultry science. (Ledur, 2007; Souza, 2011). The timeframe we picked lines up with when researchers first started spotting SNPs across full genomes, like the chicken genome (Groenen *et al.*, 2009). Funny enough, this also covers the COVID-19 years (2020–2023), and even with all the lockdowns and chaos, research and papers kept coming.

Rising worldwide l demand for chicken meat and eggs has driven the need for improvements in poultry production efficiency. Studies emphasize the importance of SNP markers in identifying genes connected to economically valuable traits in birds (Xu *et al.*, 2011; Liu *et al.*, 2018).

Basically, the research coming out of countries like China, the US, South Korea, and Japan really shows they're leading the field — it just highlights how important poultry science is for food and the economy. And yeah, studies with SNP markers prove that it makes a difference everywhere, no matter the country or context (Wolc *et al.*, 2014; Cheng *et al.*, 2023;). Analysis of the worldwide research landscape highlights significant findings regarding geographic distribution and contributions from various countries.

Looking at research all over the world, you kinda see some clear stuff about where it's coming from and which countries are putting in the work. China's ahead for a bunch of reasons massive poultry industry, tons of people eating chicken, and they're pumping a lot of money into genetic R&D. Studies like Dou *et al.* (2022) and Fang *et al.* (2023) really show that. Overall, China plays a huge role in poultry genetics, especially when it comes to using SNP markers to boost production and improve bird healt traits in chickens. Their research shows how these tools can be applied to enhance important characteristics across flocks.

The United States also holds a very notable place here. This is backed up by its top research centers and universities, along with a long track record of investing in poultry genetics. Studies like Wolc *et al.* (2014) and Fulton (2012) point out how the U.S. has contributed to identifying genetic markers tied to traits that really matter for the poultry industry.

South Korea's contribution is smaller when you compare it with China or the U.S., but it still matters and actually shows how broad SNP marker research has become in poultry. The work of Drobik-Czwarno *et al.* (2018) and Kim *et al.* (2023) reflects how studies in different countries are pushing forward improvements in poultry genetics and production.

The papers a country publishes doesn't always mean its poultry industry is stronger or more successful. Take Brazil, for example — the country produces a huge amount of chicken, which already speaks to a high level of skill and know-how in poultry production. (ABPA, 2023), yet it has relatively low scientific output in this domain. One possible explanation is the difference in research capacity. Having proper infrastructure, like well-equipped labs and advanced tech, makes a big difference in what can be done. And in many cases the poultry industry itself tends to put efficiency and productivity first, leaving research and development in second place. In Brazil, industry often focuses on productive efficiency, while research is primarily conducted by universities or specialized institutions.

Notably, research was not limited to developed countries. Contributions also came from developing nations (e.g., China, Brazil, India, Indonesia, Iran, Egypt, South Africa, Thailand, Argentina, Chile, Ecuador, Malaysia, and Vietnam) and countries with low human development indices and GDP per capita (e.g., Nigeria, Pakistan, Rwanda, Tanzania, and Kenya), as classified by the International Monetary Fund (IMF) and the United Nations (UN). These countries also employed advanced techniques in SNP studies.

Regarding institutions, China's predominance in SNP marker research reflects not only substantial government investment in R&D but also the country's emerging leadership in agricultural. China's National Bureau of Statistics reports that R&D spending has gone up, which already points to new possibilities in areas like agriculture. With initiatives such as *Made in China 2025*, focused on pushing high-tech sectors like agriculture and biotechnology, the level of investment in agricultural research has risen a lot (ISPD, 2018).

Having big names like the University of Edinburgh in the UK and Iowa State University in the U.S. just shows how global and relevant genetic marker research in poultry really is. These institutions are key for building international collaboration and spreading scientific knowledge in poultry genetics. The use of SNPs in chickens also depends on the sociocultural context (East/West) of the institution, suggesting a diversity of approaches (production, resistance, aesthetics) and perspectives (cultural, sociological, religious).

In Brazil the amount of publications connected to institutions is pretty limited, EMBRAPA for example shows up with just two references. That kinda shows the gap when it comes to modern research approaches for agricultural development, especially for the real challenges of tropical farming. Still, EMBRAPA does work on poultry genetics and markers, trying to develop varieties better suited for tropical conditions and to push sustainability in Brazilian poultry production (EMBRAPA, 2007). EMBRAPA teamed up with the Federal University of Viçosa and came up with a free-range chicken line that really fits the Brazilian climate and the way people manage their flocks, called Embrapa 041 Colonial Broiler. This line

could really help family farmers keep their chickens healthy and productive while making their farms work better for them (Figueiredo, 2017). However, in 2019, EMBRAPA faced a budget cut of nearly half, which severely impact its research capacity.

Research on poultry genetics in Brazil has a lot of problems, like not enough money, crappy infrastructure, and too few skilled people, according to stuff from CAPES and CNPq (Costa & Costa, 2023). Fixing all this means throwing more money at science and tech, and getting the government, private companies, and research labs to actually work together to push innovation and science forward.

Looking at the keywords from the searches, it's clear people are kinda into checking out gene expression and spotting genetic markers tied to stuff like growth rate and egg production. Makes sense, it's pretty much what everyone's doing in poultry molecular genetics right now. Studies such as those by Muir *et al.* (2008) and Negash *et al.* (2021) highlight the importance of these themes in understanding the genetic basis of production traits and developing breeding strategies.

Most of the articles relied on next-gen sequencing (NGS) and GWAS using microarrays or chips. These tools made it a lot easier to find and study genetic markers like SNPs, giving us a much clearer picture of chicken genetics and the traits that really matter for raising birds (Khanzadeh, 2010; Pan *et al.*, 2024). Chickens have such a wild mix of traits that you can't just focus on genetics you also have to consider the environment and how they're raised. Studies like Tixier-Boichard *et al.* (2011) and Negash *et al.* (2021) really show how crucial the whole genotype-environment interaction is for how these traits actually appear in chickens.

The gene that showed up the most in the survey was GH (Growth Hormone), and it's absolutely central for chicken growth and development. The gist is, it helps them build more muscle, bone, and other tissues. Here's how it works: the pituitary gland releases GH, which then signals the liver to produce IGF-1, and that kicks cell growth and tissue repair into motion. Which also means, recent studies have found that SNPs in the GH gene can tweak both how much GH is made and how well it functions, which in turn has a direct impact on growth and meat production in chickens (Wang *et al.*, 2021).

IGF-1 (Insulin-like Growth Factor 1) is primarily the main guy behind GH, doing a ton of work for cell growth, bones, and muscles. SNPs in the IGF-1 gene can mess with how much of it gets made, which can affect meat growth and even egg production. In chickens, it's also tied to reproduction because it kicks the ovaries into gear and gets egg laying rolling. And on top of that, it helps them fight off diseases by boosting the immune system and helping cells regenerate (Kim *et al.*, 2004; Ogunpaimo *et al.*, 2021).

It produces proteins that act like flags, helping the body spot harmful pathogens and foreign cells. The MHC (Major Histocompatibility Complex) is a really important part of the chicken genome that keeps their immune system running. Variations in the MHC gene, like certain SNPs, can change how well a chicken can fight off diseases such as Marek's disease or infectious bronchitis. Some versions of this gene actually give birds a stronger defense, giving them a real advantage against viruses and bacteria. In short, the MHC is essential for adaptive immunity because it shows antigens to T cells, and it plays a major role in whether a chicken will easily resist or be more susceptible to certain infections. (Kaufman, 2022).

CORT, or Corticotropin, is in simple terms the hormone that tells chickens how to deal with stress. When things get rough—like the environment changes too fast or management isn't ideal—CORT levels shoot up, which can throw their metabolism and appetite off balance. Some variations in the CORT gene, like certain SNPs, seem to help chickens manage stress better. Birds with these "better" versions can handle tough situations more smoothly, keep laying eggs regularly, and make the most out of their feed (Zhang *et al.*, 2015; Lou *et al.*, 2019).

In chickens, prolactin (PRL) isn't about milk at all—it's all about reproduction and how hens take care of their eggs. This hormone helps them with incubation and plays a big part in overall reproductive performance, influencing not just egg laying but also how well hens look after their chicks (Bole-Feysot *et al.*, 1998). SNPs in the PRL gene can shake up ovulation and incubation, affecting how many eggs get fertilized and how many actually hatch. Some of these SNPs are even tied to parental behavior, influencing how attentive hens are with their chicks. In short, PRL is a major player when it comes to incubation and brooding in chickens (Bana *et al.*, 2021).

The MC1R gene is at heart the mastermind behind chicken feather color—it controls melanin in the cells, which is why birds end up with all those shades. But here's the thing: it's not just about looking good. Different versions of MC1R can actually mess with how chickens deal with stress and how fast they grow. Some SNPs even change feather colors, which might seem trivial, but in commercial breeds it can seriously affect sales people do notice. And then there's the bonus: certain versions help chickens grow better and handle heat like champs, which is a lifesaver in hotter climates. So yeah, MC1R isn't just about feathers it's juggling color, growth, stress, and even survival, all at once (Qi *et al.*, 2023).

The VIP (Vasoactive Intestinal Peptide) gene helps control egg production and brooding behavior in chickens. (Zhou *et al.*, 2010). These genes have shown SNPs that could be used as markers to boost productivity. VIP is a neuropeptide that helps control digestion, immunity, and the body's internal clock. SNPs in the VIP gene can affect feed efficiency and gut health in chickens, helping them absorb nutrients better and fight off intestinal infections.

Additionally, VIP plays an important role in modulating stress response, which can impact egg production and bird growth (Bana *et al.*, 2021).

Among these, the most prominent genes are PRL (Prolactin), IGF1 and VIP aside, PRL is all about controlling prolactin, the hormone that helps hens with incubation and brooding. Interestingly, studies show that how much the PRL gene gets expressed can change depending on the dose of antiprolactin, meaning its activity isn't fixed—it can actually be adjusted (Bana *et al.*, 2021). Putting IGF1 and VIP aside for a moment, PRL is basically in charge of prolactin, the hormone that gets hens through incubation and brooding. The cool thing is, studies show that the PRL gene doesn't always act the same—it can be dialed up or down depending on how much antiprolactin is around. So its activity isn't set in stone; it's kind of flexible (Ogunpaimo *et al.*, 2021). The VIP gene helps chickens manage egg laying and their brooding habits. Variations in this gene, like certain SNPs, can change not just how many eggs a hen lays but also how long she spends brooding over them (Zhou *et al.*, 2010).

These genes are located in the on chromosomes 1, 2, 3, 9, 13, and Z (Du *et al.*, 2020a). Such studies provide a basis for more precisely elucidating the molecular genetic mechanisms influencing egg laying in chickens. The development of molecular markers will further promote the breeding and selection of local breeds and may establish centers for conservation and sustainable poultry development. These genes stand out because they have a big say in how chickens reproduce and produce. On top of that, the bird genome is kind of messy—genes get duplicated, repeated, and sometimes you find copies or other regulatory bits scattered across different chromosomes. It's not neat, but that's part of what makes chicken genetics so interesting (Du *et al.*, 2020a). For example, prolactin (PRL) and the stuff that controls it can be affected by regions on different chromosomes.

With advances in genome-wide association studies (GWAS), several genes and QTLs (Quantitative Trait Loci) that influence laying performance have been identified. The growing interest in using markers to study these variations can play a crucial role in genomics. For example, GWAS has been used to identify variations in age at first egg (AFE) and egg number (EN) in Leghorn laying hens (Li *et al.*, 2011). Most loci significantly connected to egg-laying performance through GWAS were found on chromosomes GGA1 (36 SNPs), GGA2 (4 SNPs), GGA3 (10 SNPs), GGA4 (7 SNPs), GGA5 (27 SNPs), and GGA21 (5 SNPs).

One SNP (GGA092322) on chromosome 13 of chickens (*Gallus gallus*) in intron 2 was connected to age at first egg (AFE) (Li *et al.*, 2011). Other articles demonstrated that this variation in intron 2 of this SNP was also expressed in the brain, retina, and optical system of chickens (Kenzelmann *et al.*, 2008).

There’s a single nucleotide polymorphism linked to egg number EN, it's at 21. 67 Mb on GGA7, smack-dab in the 12th intron of the GRB14 gene. Active in the ovaries of mammals, the gene has relationships with the insulin receptor IR and the IGF receptor IGFR. These pathways influence ovarian function and follicle development in chickens, subsequently affecting how many eggs a hen lays. (Daly *et al.*, 1996). Right, so with these IGF and IGFR elements, the main takeaway centers around governing ovarian function. It's also related to follicle development in chickens (Kim & Seo, 2004).

Right away, Researchers, they discovered four SNPs, sprinkled across GGA1, GGA2, and GGA11. These, they reckon, might mess with the number of eggs laid between days 300 and 462. Specifically, rs13905010 chills on GGA1. Also, rs15938574 is situated on GGA2. Finally, the last two snuck in, around a wee stretch (Fan *et al.*, 2017).

They discovered a fascinating spot ss1985401199 located on the Z sex chromosome, and it appears to be strongly connected to egg production taking place from week 25 up until week 45. Plus, a impressive twenty three SNPs on GGA1, GGA5, plus GGA23 demonstrate a clear connection with egg production, and among them, 21 are tightly packed in a 40. 1–43. 16 Mb area on GGA5. Also, there exists a QTL area spanning from 38. 62–38. 69 Mb on GGA5 associated with egg production, at 300 days, just a measly 1. 41 Mb away from that aforementioned massive region. That specific region just might be a crucial QTL for how well the hens lay eggs, looks that way (Shen *et al.*, 2012; Yuan *et al.*, 2015).

Regarding egg weight (EW), one SNP (rs13588750A) on GGA5 was significantly connected to EW at 300 days of age, with FBLN5 identified as the candidate gene (Zhang *et al.*, 2015). Some studies link pelvic bone spread (PBS) to egg number, suggesting that this trait is strongly linked to egg production. During peak egg production, the abdominal organs in chickens increase the distance between the pelvic bones and the keel tip (Torres & Graner, 1951). The same author proposes a positive correlation between pelvic bone width and egg production. (r = +0.23 and pr = +0.16).

Cloacal temperature (CT) is considered a representative measure of body temperature, serving as a good indicator of comfort or thermal stress conditions, which can influence production indices (Brown-Brandl *et al.*, 2003).

In the analysis of eggshell weight (ESW), two significantly associated SNPs were revealed: one located on GGA2 and another on GGA3. The promising genes identified were GALNT1 and BLK, respectively (Li *et al.*, 2011).

One SNP located at 184.63 Mb on GGA1 showed a significant association with yolk weight (YW) in 40-week-old laying hens, with ATM as the corresponding candidate gene. Additionally, two SNPs on GGA1, situated at 8.1 Mb (171.2–179.3 Mb), demonstrated a

significant correlation with eggshell thickness (EST) in 40-week-old laying hens. The candidate genes associated were LOC418918 and ENOX1 (Li *et al.*, 2011).

Data extraction from the studies included the genes used in each study, and based on the methodology of each article, the genes and their frequency across all publications were analysed. These genes play crucial roles in chicken productivity and health, and SNPs within them hold great potential for use in genetic improvement programs. Identifying SNPs connected to traits such as growth, egg production, and disease resistance can help optimize poultry production, enabling more effective control over these factors. Combining these SNPs with other phenotypic and genetic traits can enhance production efficiency and resilience in chickens, with significant economic benefits for the poultry industry.

CONCLUSION

A comprehensive analysis of studies on single nucleotide polymorphism (SNP) genetic markers in poultry reveals the current state of scientific production, along with growing worldwide interest and working together in this area. China, the United States, and South Korea are examples of countries that are leaders in scientific output. The words "growth" and "egg production" show that poultry breeding and production traits are very important. The large number of Chinese, Korean, and Japanese institutions in this area shows that Asia is a major center for scientific research in this field. The main genes of interest identified in SNP-based research include GH (Growth Hormone), IGF (Insulin-like Growth Factor), MHC (Major Histocompatibility Complex), CORT (Corticotropin), PRL (Prolactin), MC1R (Melanocortin 1 Receptor), and VIP (Vasoactive Intestinal Peptide). These findings provide valuable insights to guide future research and development strategies in poultry genetics, such as linking explored genes to zootechnical production outcomes.

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Article 2. Pleiotropic PRL and VIPR-1 Genes Modulate Egg Production Traits in Native Canela-Preta Chickens

Pleiotropic PRL and VIPR-1 Genes Modulate Egg Production Traits in Native Canela-Preta Chickens

ABSTRACT: The conservation and genetic improvement of native poultry breeds are crucial for sustainable animal production. This study investigated the association of PRL and VIPR-1 gene polymorphisms with egg production-related traits – pelvic bone opening (PBO), cloacal temperature (CT), and broodiness behavior frequency (BBF) – in the Brazilian Canela-Preta free-range chicken. We genotyped 40 hens for five polymorphisms and evaluated phenotypic traits over four 28-day cycles. Population genetics analysis revealed high fixation indices (up to $F = 0.846$ for PRL1) and significant deviation from Hardy-Weinberg equilibrium, indicating a history of inbreeding and non-random mating, likely due to traditional management with limited breeder rotation. Despite this, the population maintained considerable diversity (mean effective alleles = 1.97). While PBO and CT showed no significant genetic associations, being primarily influenced by random and temporal factors, broodiness (BBF) demonstrated strong genetic regulation. The PRL2-TC and PRL3-CG genotypes were significantly associated with reduced broodiness ($p < 0.05$), with the PRL3 C allele showing a suggestive additive effect for increased broodiness. Models for BBF showed high explanatory power (conditional R^2 up to 0.779). Our findings points to PRL2 and PRL3 as promising markers for marker-assisted selection. We propose a practical breeding strategy that selects against unfavorable homozygotes rather than targeting unstable heterozygotes, integrated with a reproductive management plan to enhance genetic diversity. This approach offers a viable path for improving productivity while ensuring the long-term conservation of the Canela-Preta breed.

Keywords: Marker-assisted selection; Native chickens; Broodiness; Genetic conservation; Reproductive traits

Genes pleiotrópicos PRL e VIPR-1 associados a características de produção de ovos em galinhas nativas Canela-Preta

RESUMO: Objetivou-se com este estudo investigar a associação de polimorfismos nos genes da prolactina (PRL) e do receptor de peptídeo intestinal vasoativo 1 (VIPR1) com características produtivas em galinhas da raça Canela-Preta, incluindo a abertura dos ossos pélvicos (APELVE), temperatura cloacal (TCLOAC) e comportamento de choco (CHOCO). Foram analisados 40 indivíduos, com DNA extraído via kit Quick-DNA/RNA™ MagBead e genotipagem realizada por PCR convencional (PRL1) e tetraprimer ARMS-PCR (PRL2, PRL3, VIPR1A e VIPR1B). As frequências alélicas e genotípicas foram calculadas, e o equilíbrio de Hardy-Weinberg foi avaliado pelo software GenAlEx. Os dados fenotípicos foram associados aos genótipos por meio de Modelos Lineares Generalizados (GLMs), com avaliação de efeitos categóricos e aditivos. A heterozigose observada foi baixa (Ho média = 0,287), com destaque para o *locus* PRL1, que apresentou a menor heterozigose (Ho = 0,077) e elevado índice de fixação (F = 0,846), indicando alta endogamia. A análise GLM revelou que o genótipo PRL3-CG teve efeito negativo significativo sobre APELVE (p = 0,0491), enquanto PRL2-TC, VIPR1A-CT e VIPR1B-CT mostraram efeitos sugestivos. Para CHOCO, os genótipos PRL3-CG e PRL3-GG apresentaram efeitos negativos significativos, e o alelo C (PRL3) foi positivamente associado ao aumento da frequência de choco (p = 0,042). Não foi identificada associação significativa entre os genótipos e a temperatura cloacal, embora o tempo (semana 32) tenha influenciado essa variável. Os achados reforçam o potencial de uso dos marcadores PRL e VIPR1 em programas de seleção genética voltados à produtividade e comportamento reprodutivo em galinhas Canela-Preta.

Palavras-chave: Seleção assistida por marcadores; Galinhas nativas; Choco; Conservação genética; Características reprodutivas.

1 INTRODUCTION

Producing and eating free-range chicken and eggs is a long-standing tradition. These foods are not only excellent sources of protein but are also prized for their unique taste and texture (Santos, 2023). Free-range chickens are very different from their industrial counterparts considering diet, healthcare, genetics, and overall management, which often results in significant differences in their growth, productivity, and welfare. This distinction is also reflected in consumer choices between the two main products. Eggs are generally the more popular choice, as they are more affordable than the meat and valued for both their cost-effectiveness and nutritional benefits (Hirvonen, 2021).

In Brazil, most layer birds are derived from foreign commercial strains (Carvalho *et al*., 2020), leading to the gradual displacement of local free-range breeds. This trend places native chicken populations at risk (Santos, 2023). Concurrently, consumer demand for alternative, organic, or less industrially processed poultry products is growing (Blokhuis *et al*., 2003), increasing interest in local genetic resources and conservation. Among Brazil's native chickens, the Canela-Preta breed stands out as an autochthonous variety thought to have arisen from random crosses of domestic chickens (*Gallus gallus*) brought by Portuguese colonizers (Carvalho *et al*., 2017). Canela-Preta chickens possess considerable genetic diversity and thus considerable potential for selective improvement (Carvalho *et al*., 2017).

Egg production in poultry is a polygenic trait with low to moderate heritability (Luo *et al*., 2007), making it difficult to evaluate and improve through genetic selection. This challenge is even greater in Canela-Preta free-range chickens due to their strong tendency to become broody (Carvalho *et al*., 2017; Santos, 2023). Broodiness sets in after a laying period and causes the hen to stop laying eggs. Females will instead focus on nesting, become more vocal and defensive, and experience a rise in body temperature while eating and drinking less (Sharp, Dawson & Lea, 1998). This behavior is common in native breeds like the Canela-Preta and significantly disrupts continuous egg production (Santos, 2023).

Another key trait linked to egg production is the pelvic bone opening, which has been correlated with both egg number and overall laying capacity (Rosa *et al*., 2007). Today, modern molecular technologies offer more efficient ways to select for these traits in free-range chicken lines (Dunn *et al*., 2004; Qin *et al*., 2015a).

Researchers have already identified several candidate genes in chickens that influence important economic and reproductive characteristics. Notable among these are the prolactin gene (PRL) and the vasoactive intestinal peptide receptor 1 gene (VIPR-1) (Li *et al*., 2013; Zhou *et al*., 2010). The PRL gene has pleiotropic effects, including mediation of ovarian follicle

development and the induction of physiological changes associated with incubation and parental care (Bole-Feysot *et al*., 1998; Schindler & Darnell, 1995). VIPR-1 influences gastrointestinal motility and has been linked to reproductive traits and egg production, in part because digestive efficiency and nutrient absorption are important for reproductive output (El-Halawani *et al*., 2000).

Single Nucleotide Polymorphisms (SNPs) have been identified in these two genes as promising molecular markers for faster genetic selection. This is a significant advantage over the traditional method, which required waiting until birds were sexually mature to identify superior egg-layers. With SNPs, evaluation can occur early, even at the embryonic stage. Furthermore, this technology enables breeders to assess the genetic potential of male birds for traits that are only expressed in females, such as egg production itself (Burt, 2005; Salvian *et al*., 2023). As a result, a major focus in animal reproduction has been to find SNPs that are significantly associated with productive traits (Zhou *et al*., 2008; Li *et al*., 2013). The relationship in Canela-Preta chickens, however, remains a gap in our knowledge—specifically, how polymorphisms in these genes influence measurable phenotypes like cloacal temperature, pelvic bone opening, and broodiness.

We aimed to determine whether polymorphisms in the Prolactin (PRL) and Vasoactive Intestinal Peptide Receptor 1 (VIPR-1) genes could serve as reliable markers for selecting improved egg production in Canela-Preta free-range chickens. We analyzed their association with cloacal temperature, pelvic bone opening, and broodiness behavior to assess their potential for use in a breeding program that also prioritizes breed conservation and animal welfare.

2 MATERIAL E METHODS

2.1 *Animal Parameters, Management, and Identification*

A total of 120 twenty-week-old female Canela-Preta chickens were obtained from a producer in the Teresina-PI region of Brazil. The birds were housed in an experimental shed (13.0 m × 8.0 m) with a concrete floor and allocated to 20 identical pens, each measuring 2.75 m × 0.90 m, resulting in a stocking density of 2.42 birds/m² (6 birds per pen).

The chickens also had access to a pasture area consisting of 20 paddocks, each measuring 1.0 m × 9.0 m, with a density of 1.5 birds/m². The paddocks were planted with Bahiagrass (*Paspalum notatum*) and irrigated, allowing the birds free daytime access. This configuration characterizes the rearing system as semi-intensive, following ABNT guidelines (2015).

The field experiment was conducted at the Department of Animal Science (DZO) within the Center for Agricultural Sciences (CCA) of the Federal University of Piauí (UFPI) in Teresina-PI, Brazil (geographic coordinates: 5º02'31.07" S, 42º46'57.85" W). The study was

approved by the university's Animal Ethics Committee (CEUA/UFPI) under protocol No. 669/2021. Molecular analyses were performed at the Molecular Biology Laboratory of the Department of Animal Science (DZO) and the Molecular Biology Laboratory of the Department of Biology (DBio) at UFPI in Teresina.

Each pen was equipped with pendulum-type automatic drinkers and tubular feeders for adult birds with a 10 kg capacity. Wooden nests (30 cm wide × 60 cm long × 30 cm deep) were lined with rice straw to prevent egg cracking. Nest substrate was replaced and renewed every 30 days or as needed.

A continuous lighting program provided 17 hours of light per day (a combination of natural and artificial light from 4:00 AM to 10:00 PM), controlled by an automatic timer, following ABNT recommendations (2016). The birds were identified with leg bands and underwent an adaptation period. Nutritional levels followed EMBRAPA manual recommendations (2017) and met ABNT criteria (2016), with 100g of feed provided per bird daily and water available ad libitum.

The experiment was structured into four distinct phases, following an initial 30-day period for bird adaptation and quarantine. The subsequent experimental phases, each lasting 28 days, corresponded to bird ages of 28, 32, 36, and 42 weeks. Data collection and evaluation of the following parameters were conducted during these phases:

- **Pelvic Bone Opening (PBO):** This parameter was assessed at 28-day intervals via manual palpation. The opening width was converted to a centimeter scale based on finger-width equivalents, ranging from 1.5 cm (one finger) to 6.0 cm (four fingers).
- **Cloacal Temperature (CT):** Recorded at 28-day intervals using a clinical thermometer, with measurements reported in degrees Celsius (°C).
- **Broodiness Behavior Frequency (BBF):** Broody behavior was monitored daily in all experimental units throughout the intervals between the 28-day phases. The frequency was recorded as the total count of occurrences for each bird during each interval (28, 32, 36, and 42 weeks).

2.2 *DNA Isolation*

Approximately 1 mL of blood was collected from the wing vein of two randomly selected individuals per pen (experimental unit), for a total of 40 samples. Genomic DNA was extracted using the Quick-DNA/RNA™ MagBead Kit (Zymo Research, R2141-E) following the manufacturer’s protocol.

2.3 *Selection of Markers and Genomic DNA Amplification by PCR*

The genes listed in Table 1, previously reported to be associated with traits of interest in poultry, were analyzed to detect polymorphisms within genomic regions described in earlier studies. Specific primers were designed for these regions to amplify the target fragments.

Table 1. Genetic polymorphisms associated with specific traits and their corresponding genes, based on literature reports. PRL (Prolactin; ID: AB011438.2) and VIPR1 (Vasoactive Intestinal Peptide Receptor; ID: NC_052533.1); EP: Egg production (weeks), AFE: Age at first egg and/or sexual maturity, BB: Broodiness behavior. (all polymorphisms were identified on Chromosome 2 in chickens).

GENE	**ID**	**Polymorphism**	**Production data**	**Reference**
PRL	PRL1	24-bp indel	EP 17-42, 17-72	Cui *et al* 2006
	PRL2	SNP 8052TC	AFE	Li *et al* 2013
	PRL3	SNP 8113GC	AFE	Li *et al* 2013
VIPR1	VIPR1A	SNP C+598T (intron)	BB	Zhou *et al* 2008
	VIPR1B	SNP C+53327T	BB	Zhou *et al* 2008

Genotyping of SNPs was performed using the tetra-primer ARMS-PCR technique (Fatima *et al*., 2022), except for the polymorphic 24-bp indel marker, in which the polymorphism was detected directly by the size of the generated amplicon, as it represents a deletion rather than a SNP. The primers used for amplification of these regions were designed based on the sequences of the PRL (GenBank ID: AB011438.2) and VIPR1 (GenBank ID: NC_052533.1) genes, using the GeneRunner software, version 6.3.0.3 (Hastings Software, Inc., NY; http://www.generunner.com), and the tetra-primer ARMS-PCR interface (https://primer1.soton.ac.uk/primer1.html) for primer design (Table 2).

Table 2. Primers used to flank the polymorphic regions of the studied genes, expected fragment size of each allele, and annealing temperature (AT). (F – Forward, R – Reverse, FI – Forward Inner, FO – Forward Outer, RI – Reverse Inner, RO – Reverse Outer; D – deletion, I – insertion; C – cytosine, T – thymine, G – guanine.)

Locus	***Primers* (5'→ 3')**	**Allele sizes (pb)**	**T ºC**
PRL1	F - TTTAATATTGGTGGGTGAAGAGACA R - ATGCCACTGATCCTCGAAAACTC	130 D / 154 I	54
PRL2	FI - AAATCGACAACTATCTTAAAGTTTTGACGC RI - CAATTGCTATCATGGATTAGGCGTCA FO - GATGAGGACTCCAGACTCTTTGCTTTTTA RO - AAGTTACTGATGATCCTGGTGCTGTACA	181 C / 119 T	66
PRL3	FI - AATCCATGATAGCAATTGCTAAGTACGTG RI - GGTTTCAGTGAGTAATGCAGCCGAG FO - TTTGCTTTTTATAACCTGCTGCATTGC RO -TAAATGGCACTAATAGTTGCAGCCAGAA	191 G / 136 C	65
VIPR1A	FI - GACCCTCAGGCTCACRTCCCTGTCT RI - GAGAAATGGCACAGATGAAGCAGATGTTAG FO - GGATGCATATCCCCATGGTACATTGA RO - CTGTGGGTGATCATCATCAAGCTATTCA	200 T / 300 C	68
VIPR1B	FI - GTAAGCCCATGGACTCACTTTTCTGTC RI - CTTGCACGTATCCTTGGGTAGC FO - TCCTACCTGGGATGTTTCCAATCTAAATC RO - GATGTGTTTATGAGGTTTCTGCTGTGCT	161 C / 289 T	67

F – Foward, R – Reverse, FI – Foward Inner, FO – Foward Outer, RI – Reverse Inner, RO – Reverse Outer.

The PCR method for the PRL1 marker was based on the protocol described by Cui *et al*. (2006), using the following reagents: 1× PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTP mix, 0.4 µM of each primer, 1.0 U Taq DNA polymerase, and approximately 10 ng of genomic DNA, in a final volume of 20 µL. The amplification protocol consisted of an initial denaturation and enzyme activation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 5 min.

The remaining markers had primers designed in this study and were individually optimized. For the SNP markers PRL2 and PRL3, the PCR mixture consisted of the following reagents: 1× PCR buffer, 3 mM $MgCl_2$, 0.8 mM dNTP mix, 0.5 µM of each inner primer, 0.15 µM of each outer primer, 1.0 U Taq DNA polymerase, and genomic DNA, in a final volume of 20 µL. The amplification protocol included an initial denaturation and enzyme activation step at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 66°C (PRL2) or 65°C (PRL3) for 1 min, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min.

For the SNP markers VIPR1A and VIPR1B, the PCR reaction contained the following reagents: 1× PCR buffer, 2 mM $MgCl_2$, 0.8 mM dNTP mix, 0.5 µM of each inner primer, 0.15 µM of each outer primer, 1.0 U Taq DNA polymerase, and genomic DNA, in a final volume of 20 µL. The amplification protocol consisted of an initial denaturation and enzyme activation step at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58.2°C for 45 s, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min.

Negative controls were included in all PCR reactions to verify the reliability of the results. DNA amplification was carried out in an Applied Biosystems™ 2720 thermal cycler, and all PCR products were subjected to electrophoresis on 3% agarose gels in 0.5× TBE buffer (Tris–borate–EDTA). A 100 bp DNA ladder (Kasvi) was used as a molecular weight marker. Electrophoresis was performed at 120 V, 0.30 mA, and 21 W for 2 h. Gels were subsequently stained with ethidium bromide (~0.5 µg/mL in 0.5× TBE buffer) for 40 min, and DNA bands were visualized under UV light using an L-PIX photodocumentation system (*Loccus Biotecnology*).

2.4 *Genotyping and Statistical Analyses*

After detecting the amplicons corresponding to each allele, allelic and genotypic frequencies of the genotyped SNPs were calculated, and the existence of Hardy–Weinberg equilibrium (HWE) in the sampled population was assessed using GenAlEx version 6.5. The inbreeding coefficient

(F) for each locus, as well as the effective number of alleles (N_e), were also estimated based on observed and expected heterozygosity values provided by GenAlEx.

To evaluate the effect of genotype on the traits, a linear mixed model was fitted using the *glmmTMB* package in R. The model included the five genotypic markers (PRL1, PRL2, PRL3, VIPR1A, and VIPR1B) and repeated measurements of phenotypic traits over different weeks as fixed effects, and individuals as random effects. The significance of the effects was assessed via type II analysis of variance (ANOVA) for mixed models, complemented by inspection of 95% confidence intervals for the estimated parameters.

The linear model was tested under two scenarios for the genetic information. In the first scenario, genotypes were treated as independent categorical variables, and in the second, the additive effect of allelic variation was estimated. For the categorical genotypes, the statistical model tested can be described as:

$$Y_{ij} = \beta_0 + \sum_{k=1}^{5} \beta_k \, G_{ki} + \sum_{s=2}^{S} \gamma_s \, S_{sij} + u_i + \varepsilon_{ij}$$

where Y_{ij} is the phenotypic value of the trait measured for the *i-th* individual in week *j*. *b0* represents the model intercept, b_k represents the fixed effect of genotype G_{ki} of the *i-th* individual, and g_s represents the fixed effect of the week of measurement S_{ij} for individual *i*. e_{ij} denotes the residual error.

To evaluate the additive genetic contribution, genotypes were numerically coded according to the number of reference alleles (0 = reference homozygote, 1 = heterozygote, 2 = alternative homozygote), assuming a linear model in which each allele contributes a fixed effect to the phenotype. This approach, which simplifies the analysis by using a single coefficient per gene, is suitable for estimating additive allelic effects. The statistical model tested can be described as:

$$Y_{ij} = \beta_0 + \sum_{k=1}^{5} \alpha_k \, A_{ki} + \sum_{s=2}^{S} \gamma_s \, S_{sij} + u_i + \varepsilon_{ij}$$

where the variables follow the same definitions described previously, with a_k representing the additive fixed effect (0, 1, or 2) of allele A_{ki} of individual *i*.

All analyses accounted for the hierarchical structure of the data with repeated measurements per individual, evaluating four covariance structures. The first model assumed compound symmetry (CS) with homogeneous variance, the second incorporated heterogeneous variance across weeks (CSH), the third included a first-order autoregressive correlation structure (AR1), and the fourth combined AR1 with heterogeneous variance across weeks (AR1H). All models included fixed effects for genotypes and weeks, and random intercepts for individuals.

Comparing these covariance structures allowed assessment of the robustness of the results under different assumptions about the variability of the longitudinal data.

Models were applied to the response variables PBO (pelvic bone opening), TC (cloacal temperature), and BBF (broodiness behavior frequency) for both categorical and additive genotypes. Model evaluation included the Akaike Information Criterion (AIC), ANOVA, and regression coefficients for each genotype and factor. Analyses proceeded using linear models when normality and homoscedasticity assumptions were met, as assessed by the Shapiro–Wilk test. Given the nature of the data, when normality assumptions were not satisfied, analyses were adjusted using Generalized Linear Models (GLMs).

Alternative distributions tested included Gamma, Log-Normal, Poisson, and Negative Binomial, depending on the type of response variable. For the continuous variables PBO and TC, GLMs were fitted using Log-Normal and Gamma distributions, while for the discrete quantitative variable BBF, models were fitted with Poisson, Zero-Inflated Poisson, and Negative Binomial distributions. Model selection was based on Akaike (AIC) and Bayesian (BIC) information criteria.

Following model fitting, adjusted means were compared for the levels of PRL1, PRL2, PRL3, VIPR1A, and "week" using the *emmeans* function from the R emmeans package, with Tukey adjustment for multiple comparisons.

Considering the potentially multifactorial nature of the traits, gene effects were evaluated at the conventional 5% significance level ($p < 0.05$) for significant effects, and additionally at a more lenient 10% level ($p < 0.10$) to detect suggestive effects.

Model fit was further assessed by calculating the coefficient of determination (R^2) using the *r2* function from the R performance package (Lüdecke *et al.*, 2021), obtaining both the marginal R^2 (R^2m), representing the variance explained solely by fixed effects, and the conditional R^2 (R^2c), representing variance explained jointly by fixed and random effects in mixed models. This approach allows quantification of model robustness in explaining the data and enables comparisons between different model structures.

3 RESULTS

3.1. *Genotypic Patterns of the PRL and VIPR1 Genes*

The PRL1 indel polymorphism (24-bp indel) was successfully amplified in 39 of the 40 specimens. Genotypes were identified as II (insertion homozygote), DD (deletion homozygote), and ID (heterozygote carrying both insertion and deletion alleles). The resulting patterns for the PRL1 polymorphism were consistent with previous reports, with 130 base pairs (bp) for the D allele and 154 bp for the I allele, this locus being located in the promoter region of the gene.

For the SNPs PRL2, PRL3, and VIPR1A, genotypes were successfully determined for all individuals, except for VIPR1B, for which one of the 40 samples failed genotyping. The SNPs PRL2 (TT, TC, and CC), PRL3 (CC, CG, and GG), and VIPR1A and VIPR1B (TT, TC, and CC) displayed electrophoretic patterns consistent with the tetra-primer ARMS-PCR technique, showing two or three bands. For example, SNP PRL2 produced a 119-bp band corresponding to the T allele (genotype TT) or a 181-bp band corresponding to the C allele (genotype CC). The simultaneous presence of both bands indicates the heterozygous genotype TC. An additional 244-bp band served as a control for the technique (amplification of the outer primers) and was not used for genotyping (Figure 1).

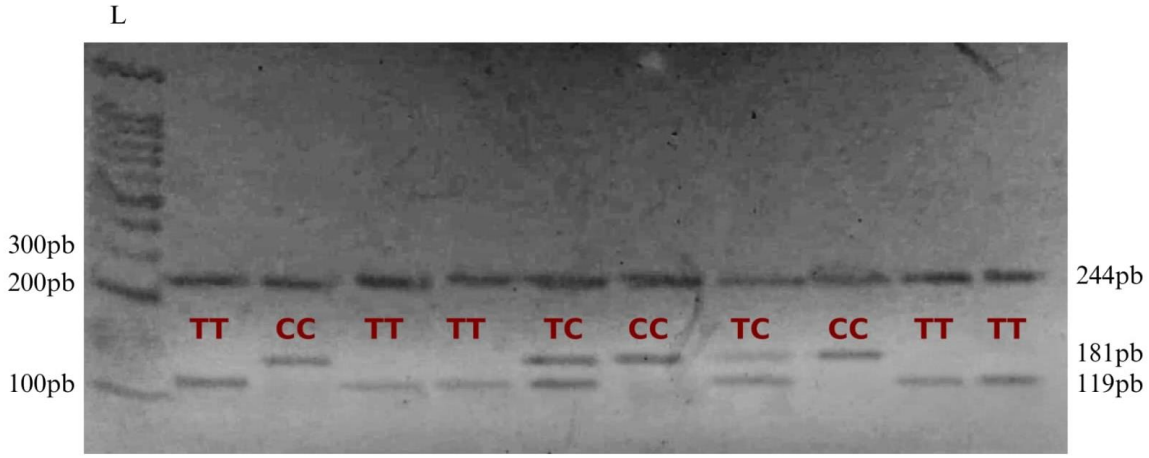

Figure 1. Electrophoretic pattern of amplified fragments for the PRL2 SNP (genotypes TT, TC, and CC) on an agarose gel (inverted image). L: molecular weight ladder; TT and CC: homozygous genotypes; TC: heterozygous genotype.

For SNP PRL3, the amplicon sizes were 136 bp (C allele) and 191 bp (G allele), with the outer primer amplification product measuring 273 bp. For SNP VIPR1A, the amplicon sizes were 200 bp (T allele) and 300 bp (C allele), with the outer primer product of 445 bp. Finally, for SNP VIPR1B, the amplicon sizes were 161 bp (C allele) and 289 bp (T allele), with the amplification product of both outer primers being 398 bp. All obtained amplicons corresponded to the expected sizes based on the designed primer references.

The five selected markers exhibited an average heterozygosity of 0.287 (observed, Ho) and 0.492 (expected, He) (Table 3).

Table 3. Genetic parameter estimates in Canela-Preta chickens. (N = number of individuals, Ho = observed heterozygosity, He = expected heterozygosity, F = fixation index, P-value = chi-square P-values for Hardy–Weinberg equilibrium assessment, N_e = effective number of alleles.)

Locus	N	Ho	He	F	P-valor	N_e
PRL1	39	0,077	0,505	0,846	<0,001	1,99
PRL2	40	0,325	0,503	0,346	0,028	1,99
PRL3	40	0,350	0,501	0,293	0,064*	1,99

VIPR1A	40	0,325	0,481	0,315	0,046	1,90
VIPR1B	39	0,359	0,498	0,270	0,092*	1.96

N = number of individuals, Ho = observed heterozygosity, He = expected heterozygosity, F = Fixation index [1 - (Ho/He)], P-value = P-values referring to X2 in the Hardy-Weinberg Equilibrium assessment, N_e = Effective number of alleles.

The PRL1 marker exhibited the lowest observed heterozygosity (Ho = 0.077) and a large difference compared to the expected heterozygosity (He = 0.505). The fixation index (F = 0.846) was very high, suggesting a high degree of inbreeding at this locus. The P-value was highly significant (P < 0.001) for the Hardy–Weinberg test, indicating a significant deviation between observed and expected genotype frequencies.

At the PRL2 locus, observed heterozygosity (Ho = 0.325) also differed considerably from expected heterozygosity (He = 0.503), with a significant deviation from Hardy–Weinberg equilibrium (P = 0.028). The fixation index (F = 0.346) was moderate, indicating an intermediate level of inbreeding.

The PRL3 locus showed differences between Ho (0.350) and He (0.503), with a slightly lower fixation index (F = 0.293) than PRL2, suggesting a modest reduction in inbreeding. However, the P-value for the Hardy–Weinberg test was marginal and not significant (P = 0.064).

For VIPR1A, Ho was 0.325 and He was 0.481, showing a moderate difference between observed and expected values. The fixation index (F = 0.315) was also moderate, and the Hardy–Weinberg test indicated a significant deviation (P = 0.046).

VIPR1B exhibited the highest observed heterozygosity (Ho = 0.359), which was closest to the expected value (He = 0.492). The fixation index (F = 0.270) was the lowest among the analyzed loci, suggesting a lower degree of inbreeding, although the value cannot be considered very low. The P-value (P = 0.092) indicates that the difference between observed and expected genotype frequencies was not statistically significant. The effective number of alleles (Ne), calculated based on expected heterozygosity (He), averaged 1.97.

3.2. *Analysis of Genotype Effects on Response Variables Using Generalized Linear Models (GLMs)*

For the analysis of Pelvic Bone Opening (PBO), two mixed-effects models were fitted to evaluate the influence of genetic polymorphisms (PRL1, PRL2, PRL3, VIPR1A, and VIPR1B) and the effect of time (“weeks”) on the response variable. The first model treated genotypes as categorical variables, whereas the second assessed the additive contribution of allele substitution. In both cases, models assuming compound symmetry (CS), heterogeneous compound symmetry (CSH), first-order autoregressive correlation (AR1), and heterogeneous first-order autoregressive correlation (AR1H) were tested.

For none of these scenarios were the assumptions of residual normality met, as indicated by the Shapiro–Wilk test (p-values ranged from 6.00e−17 to 8.00e−10 across models). A log-normal distribution was also evaluated, but normality remained unfulfilled (p-values between 2.71e−17 and 3.83e−13). Given these results and the nature of the response variable, the analyses proceeded using a Gamma distribution.

Model comparisons using the Akaike Information Criterion (AIC) indicated that the Gamma distribution provided the best overall fit. Under the normal distribution, AIC values ranged from 381.9 (CS) to 389.0 (AR1H); for the log-normal, from 371.2 (AR1) to 373.3 (CSH); and for the Gamma distribution, from 358.4 (CS) to 366.0 (AR1H). Therefore, subsequent analyses were conducted using the Gamma distribution, which better captured data variability.

Under both CS and AR1 covariance structures, a suggestive effect was observed for the VIPR1B locus (CT genotype, p-value = 0.0778), with conditional and marginal R^2 values of 0.348 and 0.134, respectively, indicating moderate explanatory power of the fixed and random effects (Table 4).

For the additive models, residual normality was again not achieved under either the normal (p-values between 6.65e−17 and 1.03e−11) or log-normal (p-values between 2.71e−17 and 8.07e−15) distributions. Consequently, analyses also proceeded under the Gamma distribution, which yielded the lowest AIC values among the tested alternatives. The AIC under the normal distribution ranged from 384.9 (CS) to 390.9 (AR1H); for the log-normal, from 373.6 (AR1) to 378.6 (CSH); and for the Gamma distribution, from 361.3 (CS) to 373.6 (AR1H). Despite providing a better fit, the marginal R^2 value was low (0.028), and no significant or suggestive additive effects of alleles on PBO were detected (Table 4).

Table 4. Comparison of categorical and additive mixed models for the variables Pelvic Bone Opening (PBO), Cloacal Temperature (CT), and Broodiness Behavior Frequency (BBF), considering the distributions that provided the best data fit according to the Akaike Information Criterion (AIC). R^2c and R^2m: conditional and marginal coefficients of determination, respectively.

Trait	Genetic model	Best model/ Distribution	AIC	R^2c	R^2m	Significant variables	Contribution	P-value
PBO	Categorical	CS/Gamma	358.4	0.348	0.136	**VIPR1B-CT**	-0.148	0.0778
	Additive	CS/Gamma	361.3	0.351	0.028	-	-	-
CT	Categorical	CS/Gamma	195.2	0.113	0.113	32 weeks	-0.006	0.0149
	Additive	CS/Gamma	190.8	0.085	0.085	32 weeks	-0.006	0.0170
BBF	Categorical	CS/Poisson	168.9	0.779	0.568	**PRL2-TC**	-2.664	0.0302
						PRL3-CG	-2.654	0.0121
						36 weeks	1.425	0.0131

					42 weeks	2.051	0.0002
Additive	CS/Poisson	172.1	0.617	0.201	**PRL3-C**	0.787	0.0904
					36 weeks	1.432	0.0133
					42 weeks	2.069	0.0002

For the variable Cloacal Temperature (CT), the influence of genotypes treated as categorical information was tested under the CS, CSH, AR1, and AR1H models. Once again, the assumptions of normality for the residuals were not met (p-values ranging from 0.014 to 0.013), and the same pattern was observed for the Log-Normal distribution (p-values ranging from 0.013 to 0.017). The analyses therefore proceeded using the Gamma distribution, which also proved to be more informative for this phenotypic variable. Among the models tested, the CS + Gamma configuration presented the lowest AIC value (195.2). For the CSH + Gamma model, the AIC was 198.8. Unsatisfactory results were obtained with the AR1 and AR1H models, which exhibited convergence issues. This condition prevented reliable AIC estimation and accurate parameter inference.

Considering the additive influence of the tested genes on CT, the normality assumptions for residuals were met, although with p-values close to the significance threshold (p = 0.058 for all models tested). In this context, the effects of allelic substitution were also evaluated under Log-Normal and Gamma distributions to identify the most explanatory model. For the Log-Normal distribution, normality assumptions remained valid (p-values ranging from 0.058 to 0.084). Regarding AIC indices, the CS + Gamma configuration again provided the best fit (AIC = 190.8) compared with other models. However, the AIC values were relatively close among the tested configurations, with results of 191.0 for CS + Log-Normal, 191.2 for CS + Normal, 193.1 for CSH + Normal, 193.5 for CSH + Log-Normal, and 193.6 for CSH + Gamma. Once again, AR1 and AR1H models failed to converge for this variable.

Across all models applied to CT, no significant effects of the tested genotypes were detected. Only the “32 weeks” variable showed a significant effect in the analyses (Table 4).

Finally, the effect of genetic polymorphisms (genotypes) on broodiness (BBF) was evaluated. Given that BBF was measured as a count of observed behavior events, the data were expected to violate normality assumptions, which was confirmed by Shapiro-Wilk tests (p-values = 7.44e-06 for all models).

Given the count nature of the data, Poisson, Zero-Inflated Poisson, and Negative Binomial distributions were tested. Model comparisons using the Akaike Information Criterion (AIC) indicated that Poisson distributions provided the best fit for most scenarios. The lowest AIC values were similar for the CS+Poisson and CSH+Poisson configurations (AIC = 168.9), which demonstrated the best model fit. Intermediate AIC values were obtained with Zero-

Inflated Poisson and Negative Binomial distributions (range 170.9 to 172.9), while the highest AIC values resulted from using a Normal distribution (217.2 to 340.2).

Among all tested models, only CS+Poisson showed significant contributions of categorical genetic variables to BBF (Table 4). Significant effects were observed for the PRL2 (p-value = 0.0302) and PRL3 (p-value = 0.0121) loci. The conditional and marginal R^2 values for this model were 0.779 and 0.568, respectively. Additionally, the 36- and 42-week intervals also showed significant effects (Table 4).

Allelic substitution effects (additive effects) for BBF were evaluated using Poisson distributions, as none of the models met normality assumptions (p-values = 2.93e-07 in Shapiro-Wilk tests). AIC comparisons again favored Poisson distributions in most scenarios, with the lowest values for CS+Poisson and CSH+Poisson configurations (AIC = 172.1). Intermediate AIC values were obtained with Zero-Inflated Poisson and Negative Binomial distributions (range 174.1 to 209.8), while Normal distribution yielded the highest AIC values (226.4 to 364.5).

As with the categorical genotype models, among all additive allelic models tested, only CS+Poisson showed significant genetic contributions to broodiness (Table 4). The PRL3 locus showed a suggestive effect (p-value = 0.0904), while the 36- and 42-week time points again demonstrated significant effects. The conditional and marginal R^2 values were 0.773 and 0.253, respectively.

The contribution of significant variables for each model were evaluated to estimate their contribution on phenotypic traits. For the Pelvic Bone Opening (PBO) trait, only the categorical genotype model showed suggestive effects for the VIPR1B gene (CT genotype; p-value = 0.0778), with a negative estimate (-0.148 cm), indicating a reduction in the phenotypic value associated with this genotype (Table 4).

For Cloacal Temperature (CT), no significant effects of the genetic variables were detected (in either the categorical or additive approach). Only the "32-week" time point showed a significant effect in both models (p = 0.0149), with a small negative estimate of -0.006 cm (Table 4).

Regarding Broodiness Behavior Frequency (BBF), the CS/Poisson model applied to the categorical data revealed significant effects of the PRL2 and PRL3 genetic variables. For the PRL2 locus, the TC genotype was associated with a reduction of -2.66 in the predicted count of the behavior (p-value = 0.0302) (Table 4). For the PRL3 locus, the CG genotype was associated with an estimated reduction of -2.6543 in the predicted values (p-value = 0.0122). An effect of this locus was also observed in the additive model, with the PRL3-C allele showing a suggestive

effect (p-value = 0.0904) and a positive estimate (+0.7871), indicating that the presence of this allele was associated with an increase in the behavior.

Considering only PBO and BBF, where significant or suggestive genetic associations were found, the analysis of least-squares means (LSMEANS) revealed distinct patterns among the different genotypes. For PBO, the contributions of each genotype for the VIPR1B locus were estimated, with the heterozygous CT genotype showing the smallest contribution to the variable (Figure 2A). However, despite these variations, no statistically significant differences were found among the three detected genotypes (p-values for genotype comparisons after post-hoc Tukey tests: CC/CT = 0.1820; CC/TT = 0.6735; CT/TT = 0.6689).

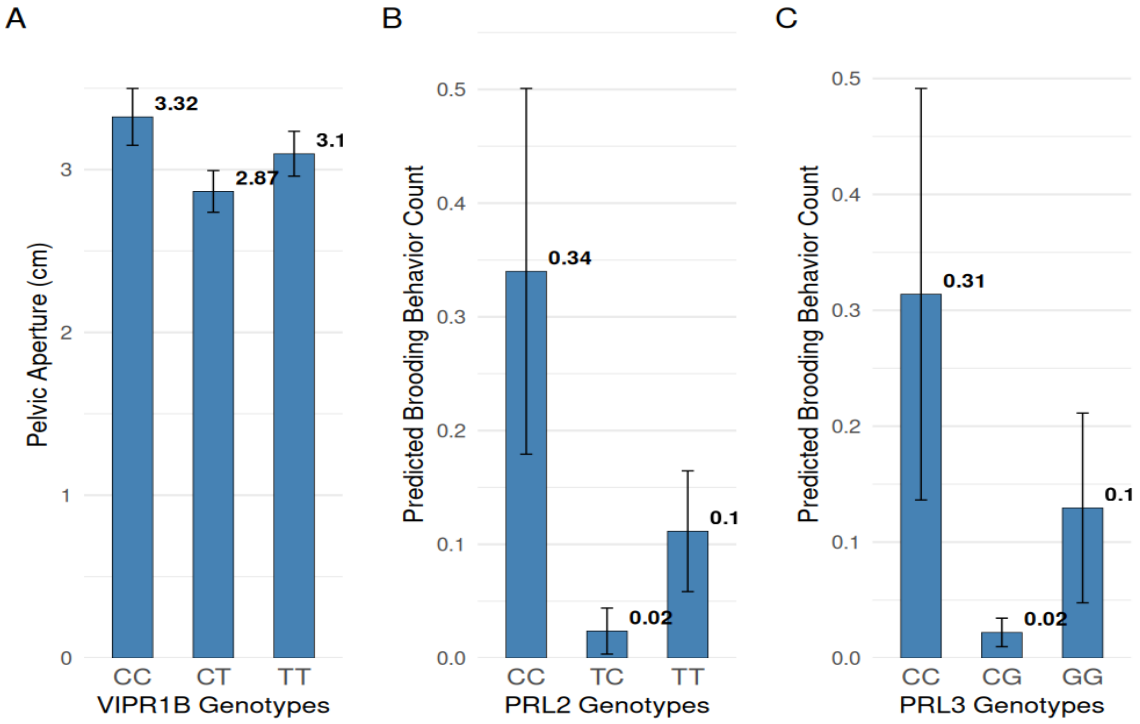

Figure 2. Adjusted means (± standard error) for the pelvic opening (APELVE) and brooding behavior (CHOCO) phenotypes as a function of the significant genotypes found. (A) Pelvic opening values for the VIPR1-B locus genotypes. (B and C) Predicted brooding count considering the PRL2 and PRL3 loci genotypes.

For the BBF phenotype, modeled with a Poisson distribution and CS covariance structure, suggestive and significant genetic effects were identified. For the PRL2 locus, the heterozygous TC genotype showed the lowest estimated contribution to the behavior (Figure 2B). The LSMEANS analysis showed a suggestive variation only in the TC/CC genotype comparison (p-value = 0.0770). Comparisons between other genotypes were not significant (CC/TT = 0.2580; TC/TT = 0.4734). The PRL3 locus also showed a lower contribution to BBF for the heterozygous CG genotype (Figure 2C). The LSMEANS analysis showed a significant

variation only in the CG/CC genotype comparison (p-value = 0.0327). Comparisons between other genotypes were not significant (CC/GG = 0.3984; CG/GG = 0.2611).

The BBF phenotype also showed a suggestive association under the additive effect model for the PRL3 locus. The substitution effect of the C allele was evaluated by counting the number of allele copies per individual: 2 copies (CC), 1 copy (CG), or 0 copies (GG). The presence of a greater number of C alleles was associated with a higher predicted count of broody behavior (Figure 3). The model yielded predicted values of 0.0296 (95% CI: 0.00634–0.139) for 0 copies, 0.0651 (95% CI: 0.02299–0.185) for 1 copy, and 0.1431 (95% CI: 0.04297–0.477) for 2 copies. Although the linear model indicated a suggestive effect for this trait (p-value = 0.0904), post-hoc Tukey tests did not detect statistically significant differences among the three genotype groups (p-value = 0.2077 for all comparisons).

4 DISCUSSION

Genetic analysis of the selected Canela-Preta population, based on marker data, revealed high fixation indices (F) – with an extreme value of 0.846 for the PRL1 locus – indicating the presence of inbreeding or selection for specific alleles, possibly favored by dominant breeding males. This pattern is corroborated by observed heterozygosity values that were significantly lower than expected, suggesting a strong influence of non-random mating. Only the PRL3 and VIPR1B loci were in Hardy-Weinberg equilibrium, which may reflect targeted matings for traits like egg production, representing an indirect selection for these genes.

These findings can be explained by the breed's historical and management context. The Canela-Preta originated in Quilombola and Indigenous communities in the Queimada Nova region of Piauí, Brazil, suggesting a restricted founder population with a potential founder effect (Carvalho *et al.*, 2016). This situation is compounded by reproductive management practices where a limited number of males are often maintained for extended periods (up to five years), coupled with the dominance of specific males. This reduces random mating and results in the selection of particular alleles and increased inbreeding (Favati *et al.*, 2014). This phenomenon of sexual selection, where few males dominate reproduction, is consistent with observations by other authors (Bruce Campbell, 2013; Moura, Dawson & Nogueira, 2017; Sharp, Dawson & Lea, 1998). Consequently, the absence of proper breeding management intensifies this process, leading to the high fixation indices observed in this study.

Despite these conditions, the effective number of alleles (N_e) showed a mean of 1.97. These values indicate a relatively high level of diversity for the markers evaluated, especially given the biallelic inheritance of SNPs. This suggests the population retains a reservoir of

genetic diversity, though it is structured in a distinctive pattern, as evidenced by the high inbreeding coefficients.

To increase the breed's genetic variability and reduce the risk of homozygous allele accumulation, a reproductive management strategy incorporating a larger number of breeding males and more frequent male rotation would be necessary. Furthermore, introducing new individuals from other populations could increase heterozygosity and reduce fixation indices. Even with more polymorphic, multi-allelic markers like microsatellites, where expected heterozygosity rates are generally higher, observed rates in chicken typically remain lower. For example, Brazilian creole chickens laying blue eggs showed a mean observed heterozygosity (Ho) of 0.491 and expected heterozygosity (He) of 0.756 for microsatellites (Fonteque *et al*., 2014). Native Turkish chickens (Kaya and Yıldız, 2008) exhibited Ho of 0.380 and He of 0.475, while Indigenous chicken breeds (Chatterjee *et al*., 2015) showed Ho of 0.50 and He of 0.60.

The egg production traits evaluated in this study – pelvic bone opening (PBO), cloacal temperature (CT), and broodiness behavior frequency (BBF) – are genetically complex. They are considered polygenic and can be influenced by various environmental factors (Luo *et al*., 2007). The PRL gene, associated with egg production in several studies, is responsible for various functions, including ovarian follicle development. During the reproductive period, prolactin induces a suite of physiological changes that prepare the hen for incubation and broodiness (Bole-Feysot *et al*., 1998; Schindler & Darnell, 1995). The VIPR-1 gene, in contrast, is involved in regulating gastrointestinal activity and intestinal motility. Furthermore, VIPR-1 is linked to reproductive processes and egg production, as gastrointestinal function is directly tied to nutrient absorption and reproductive efficiency in poultry. The vasodilatory function of this gene further aids in regulating blood flow and intestinal motility (El-Halawani *et al*., 2000). The correlation of these genes with the response variables explored in this study could facilitate the selection of more productive chickens (Carvalho *et al*., 2017; Santos, 2023), an advantage afforded by their pleiotropic nature.

While most research on egg production in chickens uses direct egg count as a parameter in breeding programs, this study focused on traits that are indirectly associated with production but are nonetheless important for selection purposes. The association analyses between the genetic markers and response variables, conducted via linear models, revealed distinct patterns for each case examined.

For the analysis of pelvic bone opening, a Gamma distribution was adopted due to the variable's asymmetric and potentially heteroscedastic nature. In the categorical genotype models, the CT genotype at the VIPR1B locus showed a suggestive effect with an inverse relationship to pelvic opening (p-value = 0.0778), indicating a potential functional role. Literature suggests an

important relationship between pelvic bone opening and laying capacity (Rosa *et al*., 2007). Selecting layers with a narrower pelvic opening could negatively impact their egg-laying potential, as a smaller aperture may physically obstruct egg passage. This trait also affects the hen's musculoskeletal system, where anatomy and behavior can be linked to the artificial selection for traits like egg production (Paxton *et al*., 2010). Some studies suggest that hens with wider pelvic openings appear to have longer laying periods (Corr *et al*., 2003).

As early as 1951, Torres and Graner reported a larger pelvic opening during peak egg production. Subsequent work by Oliveira *et al*. (1997) and Rosa *et al*. (2007) has also identified pelvic bone distance as a fundamental characteristic for evaluating laying hens.

However, the analysis of the coefficients of determination indicated that only 13.6% of the variability in PBO was attributable to the model's fixed effects. This relatively low marginal R^2 suggests that the fixed variables, including the genotypic markers, have limited explanatory power on their own. This is reinforced by the absence of significant differences among the VIPR1B genotypes (CC vs. CT, CC vs. TT, and CT vs. TT), indicating that the individual contribution of this locus to pelvic opening is limited. In contrast, the conditional R^2 of 34.8% highlights the substantial role of random effects – such as intrinsic animal characteristics, environmental conditions, or management factors – in determining this morphometric trait.

Cloacal temperature, in turn, is recognized as a representative measure of core body temperature and a reliable indicator of an animal's thermal comfort or stress, which can influence production performance (Brown *et al*., 2003). Prolactin, regulated by the PRL gene, may also have indirect impacts on thermoregulation during reproductive cycles (Bole-Feysot *et al*., 1998). In this study, the models applied to this trait also indicated violations of normality assumptions. The Gamma distribution again showed the best performance, with the CS+Gamma configuration achieving the lowest AIC (195.8). However, none of the evaluated genotypes showed a significant or suggestive effect on this variable. Only the time factor (32-week mark) was significant, indicating that cloacal temperature was influenced by temporal variation in the sampled population, potentially related to physiological maturation or environmental adaptation within the experimental design.

Even when considering additive genetic models, the results remained consistent, showing no significant genetic effects. This supports the conclusion that cloacal temperature, while influenced by the bird's physiological state, is not significantly modulated by the specific genetic polymorphisms analyzed in this study.

Finally, broodiness, or incubating behavior, is strongly regulated by prolactin. The PRL gene plays a central role in this behavior, as the hormone is directly responsible for inducing maternal behavior in birds. The significant associations demonstrated between SNPs in the PRL

and VIPR-1 genes and broodiness frequency suggest a genetic modulation of this reproductive behavior. VIPR-1 is also involved in neurohormonal signaling, which may further influence this trait (Jiang *et al*., 2005; Zhou *et al*., 2010). Given the discrete, count-based nature of this variable, non-Gaussian distributions were assumed for the models. The Poisson distribution provided the best fit (AIC = 173.9 for categorical genotypic data and 165.8 for additive allelic data). The CS+Poisson models were the only ones to capture significant genetic effects.

For the categorical genotypic data, the TC genotype (PRL2 locus) and the CG genotype (PRL3 locus) showed statistically significant effects ($p < 0.05$). It is possible that different genotypes at these loci are associated with hormonal patterns that influence natural incubating behavior, as a lower frequency of broodiness was observed in heterozygous individuals. Furthermore, the analysis of genotypic contrasts revealed a suggestive difference between the TC and CC genotypes of the PRL2 locus (p-value = 0.0770) and a significant difference between the CG and CC genotypes of the PRL3 locus (p-value = 0.0327). The additive genetic model reinforced the findings for the PRL3 locus. Despite a suggestive, marginal significance (p-value = 0.0904), the C allele was associated with a higher incidence of broody behavior.

The high conditional R^2 values (0.779 and 0.617 for the categorical and additive data, respectively) indicate strong explanatory power for the models, particularly when random time effects are considered. The marginal R^2 values (0.568 and 0.201 for the categorical and additive data, respectively) further confirm the importance of the fixed genetic effects.

Few studies have investigated the association between the PRL gene and broodiness, underscoring the significance of our findings on its regulatory role in this behavior (Dou *et al*., 2022; Jiang *et al*., 2005; Zhou *et al*., 2010). Our results align with Li *et al*. (2013), who found that the H2H3 diplotype (equivalent to the heterozygous CT/CG combination at the PRL2 and PRL3 loci) was associated with significantly higher egg production. In the present study, these same loci were associated with reduced broodiness incidence, supporting the hypothesis that this genotypic combination confers an advantage by shortening the intervals between laying cycles. This suggests a pleiotropic genetic mechanism, where the same polymorphisms that positively influence egg production also suppress incubating behavior.

The strong association of the CG genotype (PRL3) with reduced broodiness, coupled with the high conditional R^2 values of the models, indicates that variation in the PRL gene is a key hereditary factor modulating this complex behavior. Therefore, selecting for the favorable genotypes identified, particularly the heterozygous CG at the PRL3 locus, could be an effective strategy in breeding programs aimed at simultaneously improving productivity in chickens.

A significant drawback, however, is that selecting for specific heterozygous genotypes, while genetically advantageous in the short term, presents considerable practical challenges.

Maintaining heterozygosis is inherently unstable, as mating between individuals with the advantageous genotype will inevitably produce segregating offspring, including homozygotes – a classic outcome in models of heterozygote advantage or overdominance (Mitton, 2002; Mitton & Hedrick, 2015). This requires continuous genotypic monitoring of the flock, making the breeding program more complex, costly, and dependent on genotyping tools (Wiggans *et al*., 2017; Burrow *et al*., 2021). Maintaining and crossing animals specifically for a heterozygous genotype requires a far more elaborate mating system than mass selection. Genomic mating strategies, as demonstrated by Liu *et al*. (2016), involve careful planning to minimize coancestry, control inbreeding, and preserve genetic diversity, all of which increase operational, logistical, and data management costs.

Consequently, while the genetic results are promising, practical implementation faces sustainability and cost-benefit hurdles. These considerations are particularly relevant for breeds targeted for use in family farming, where financial resources, infrastructure, and technical capacity are often limited (Meuwissen *et al*., 2009; Zhao *et al*., 2020).

Further research on the PRL gene could reveal alternative strategies, such as selecting against unfavorable homozygotes. Although evidence for the additive effect was only suggestive, our findings show that birds can be selected for reduced broodiness and general robustness simply by avoiding the CC genotypes at PRL2 and PRL3 loci.

In summary, this study demonstrates that polymorphisms in the PRL and VIPR-1 genes significantly modulate complex reproductive traits in Canela-Preta chickens, most notably broodiness. The link between specific heterozygous genotypes (TC at PRL2 and CG at PRL3) and reduced broodiness, which in turn may boost egg production, confirms the pleiotropic nature of these loci and establishes them as promising molecular markers.

However, the population's high fixation indices, a legacy of historical management practices, pose a major challenge. While selecting directly for these advantageous heterozygous genotypes may improve short-term phenotypes, it must be approached with caution due to Mendelian segregation. A more practical approach is to use these markers for selecting against the unfavorable homozygous genotypes (e.g., CC at both loci). This strategy should be part of a comprehensive breeding program focused on increasing the effective number of breeders and rotating males to restore genetic diversity and ensure the breed's long-term viability.

CONCLUSION

Our findings reveal that the Canela-Preta chicken population has a history of inbreeding and non-random mating (considering the evaluated loci), evidenced by high fixation indices and low observed heterozygosity. This genetic structure likely stems from traditional practices using few breeding males over extended periods. Nevertheless, the population retains diversity at the tested loci, as shown by the effective number of alleles. Pelvic bone opening and cloacal temperature were not significantly affected by the PRL and VIPR-1 genotypes, underscoring the polygenic and environmental nature of these traits. In contrast, we identified a strong association between specific genotypes (TC at PRL2 and CG at PRL3) and reduced broodiness, marking them as favorable for productivity. The PRL3 C allele was also suggestively linked to increased broodiness considering additive allele effect. These polymorphisms are thus valuable for marker-assisted selection. This approach should be coupled with a reproductive management plan that enhances genetic diversity through breeder rotation, successfully balancing productivity with the conservation of the Canela-Preta breed.

FINAL CONSIDERATIONS

Research on genetic polymorphisms, especially SNPs (Single Nucleotide Polymorphisms), has proven to be a promising and innovative field in the study of poultry genetics, with direct implications for improving egg production in chickens (*Gallus gallus domesticus*). Both articles under review provide a detailed analysis of the relationship between genetic polymorphisms and poultry production traits, revealing not only the genetic underpinnings involved but also the modern tools and methodologies being applied to achieve a more precise and effective understanding of these mechanisms. From these studies, it is clear that the use of specific genetic markers can represent a significant advance for the poultry industry, contributing to the selection of birds with desirable traits, such as high laying rates. The bibliometric data collected in this research showed that the most studied genes (GH, PRL, MC1R, MHC, IGF1, and VIP) are associated with traits such as growth, egg production, and disease resistance.

The results point to the potential use of these genes in genetic improvement programs. The second article presents an innovative approach by exploring the impact of population-specific genetic markers of the Canela-Preta chicken breed, revealing important aspects related to population structure, genetic variability, and the influence of PRL and VIPR1 markers on reproductive traits. The combination of historical data, management practices, and molecular information shows that the population under study exhibits signs of inbreeding, likely resulting from a founder effect and reproductive practices that favor homozygosity. High fixation rates at some loci and Hardy-Weinberg disequilibrium reinforce this scenario.

The identification of associations between SNPs in the PRL and VIPR1 genes and traits such as pelvic opening (APELVE) and broody behavior (CHOCO) highlights the potential of these markers as auxiliary tools in genetic improvement programs. The selection of appropriate statistical models, such as the Gamma distribution for asymmetric variables, allowed for more accurate capture of data variability and identification of significant effects, especially in the categorical model.

Despite the absence of significant genotypic effects for cloacal temperature (TCLOAC), the role of time as a relevant factor indicates that this variable can be modulated by physiological and environmental processes over time. The complexity of the traits evaluated, many of them polygenic in nature, reinforces the need for further studies with a larger number of individuals and additional markers. Finally, the conservation and proper management of the Canela-Preta breed are essential to ensure the maintenance of genetic variability and the exploitation of its productive potential. Applying the genetic knowledge obtained in this study can contribute to

more efficient selection strategies, promoting a balance between productivity and genetic conservation.

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APPENDIX

Características fenotípicas analisadas das 40 aves da raça Canela-Preta, contendo as estatísticas para as variáveis Abertura da Pelve (APELVE), Temperatura Cloacal (TCLOAC) e choco (CHOCO).

Variável	Média	Desvio Padrão	Mínimo	Máximo	CV (%)
APELVE (cm)	3.02	1.17	1.5	6	38.74
TCLOAC (°C)	40.78	1.58	30.8	42	3.87
CHOCO (Z)	0.50	1.14	0	5	228.00

*A alta CV para CHOCO sugere que esta tem uma distribuição altamente variável, com muitos valores zero e alguns valores altos, o que é típico para variáveis que indicam um evento raro ou esporádico como o choco.