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Review Article

A Systematic Review of Single Nucleotide Polymorphisms and its Association with Eggshell Thickness in Chickens

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ABSTRACT

Eggshell prevents microbial contamination of the egg's internal content, and its quality determines the commercial acceptance or rejection of eggs. Broken eggs amount to about 6 to 8% of total egg production, resulting in economic loss to the egg industry. The task of animal genomics is the development of accurate genetic methods to identify genes that play a role in the manifestation of commercial traits. The current report aimed to systematically review literature on single nucleotide polymorphism and its association with chicken eggshell thickness. Literature used for the current study was obtained from the Google Scholar, PubMed, ScienceDirect, and Web of Science databases using single nucleotide polymorphisms, eggshell thickness, and chickens as key words combined in various ways. From the primary search, 76 articles were obtained, and 15 qualified to be used in the study after further screening for eligibility. These 15 studies were published from 2008 to 2023, and most of them originated from China (6), Japan (2) and Nigeria (2). *Ovocleidin-116* (20%), *Ovocalyxin-32* (13%) and *ChEST985k21*(13%) were mostly investigated in different chicken breeds. In conclusion, they are considered to be the best candidate genes playing a role in the development of eggshell thickness, as there was association between genotypes and eggshell thickness.

INTRODUCTION

The egg industry around the world has a significant impact on the economy (Liu *et al.,* 2011). The quality of chicken egg shells is one of its economically important traits, being affected by many factors such as environment, nutrition, diseases and genetic makeup of the animal (Jiang *et al.,* 2010). Liu *et al*. (2011) further reports that eggshell quality determines whether eggs are accepted or rejected during marketing. In addition, Jiang *et al*. (2010) reported that the quality of the eggshell is important in chicken breeding, as it prevents microbial contamination, loss of water and plays an important role in embryonic development through the provision of calcium. However, broken eggs amount to about 6 to 8% of total egg production, resulting in economic loss to the egg industry (Dunn *et al.,* 2008). Barkova *et al*. (2010) suggests that the task of animal genomics is to develop accurate genetic methods of identifying genes playing a role in the manifestation of commercial traits.

Common genes used to improve chicken eggshell thickness include *Low-density Lipoprotein Receptor* (*LRP2*) in the Pureline dwarf layers breed, *Ovocalyxin-32* (*OCX-32*) in the White Leghorn (WL) and Rhode Island Red (RIR) breeds, *Low-Density Lipoprotein Receptor-*

Related Protein 8 (*LRP8*) in dwarf chickens breeds, *Ovocleidin-116* (*OC-116*) in the Nigerian Heavy Local Chicken Ecotype, Rhode Island Red hens and Pureline Rhode Island White layers breeds, *Osteopontin* (*SPP1*), *Ovocalyxin-32*, *Ovotransferrin* (*LTF*), *Ovalbumin and Ovocalyxin-36*, *Estrogen Receptor* (*ESR1*) and *Carbonic Anhydrase II* (*CAII*) in the Rhode Island Red hens breed, *ChEST985k21* in Rhode Island layers, Polish breed Green and Rhode Island Red breed, *Non-SMC Condensin I Complex, Subunit G* (*NCAPG*) in Island chicken breed, *Insulin-like Growth Factor-1* (*IGF-1*) in the Normal feather, Naked neck, Frizzle, Kuroiler and Sasso breeds, *Subunit Beta 2* (*PSMB2*) in the LingKun Chickens breed, *Parathyroid hormone* gene in the Houdan hens breed, *calmodulin1* (*CALM1*) in the Pureline Rhode Island White layers breed, *GRB14* and *GALNT1* in White Leghorn and Brown-Egg Dwarf Layers, *Ovoinhibitor* (*OIH*) in White Leghorn breed and *Phospholipase C zeta* (*PLCZ1*) in the Rhode Island Red breed.

To the authors' knowledge, there is no literature on single nucleotide polymorphism and its association with chicken eggshell thickness. Hence the objective of this study was to systematically review literature on single nucleotide polymorphism and its association with chicken eggshell thickness. This could assist to address the identified gaps and provide guidance to chicken breeders on selecting genes to improve eggshell thickness.

MATERIALS AND METHODS

Eligibility criteria

In the current systematic review, the identification of Population, Exposure, and Outcomes (PEO) components of the research question was performed. Chickens were defined as a population of the review, eggshell thickness was defined as the exposure, and single nucleotide polymorphism of candidate genes associated with chicken eggshell thickness were the outcomes. Before the start of the study, a search of PEO elements on Google Scholar, ScienceDirect, PubMed, and Web of Science was conducted.

Search Strategy

Systematic review of the articles was performed by Kagisho Madikadike Molabe and Thobela Louis Tyasi on Google Scholar, PubMed, ScienceDirect and Web of Science databases using key combination (combined in various ways) of single nucleotide polymorphisms, eggshell thickness, and chickens.

Inclusion criteria

Systematic review included articles that were published in English investigating candidate genes associated with eggshell thickness of chickens. Articles on either genome-wide or single nucleotide polymorphism were included.

Exclusion criteria

Studies using other species than the species of interest, duplicate records, and studies not meeting the requirement (e.g. not including the key combination and using another genetic polymorphism) were excluded.

Data extraction

Kagisho Madikadike Molabe and Thobela Louis Tyasi independently selected and extracted studies (articles with authors, year of publication and type of model) of the systematic review based on the above-mentioned exclusion and inclusion criteria and agreed regarding all the materials.

Ethical considerations

To ensure the quality of the report, data manipulation, misconduct, plagiarism, and informed concern were considered ethically by all authors when conducting the systematic review.

RESULTS

Searched articles

A flowchart of the identification and selection of articles for the systematic review with reasons for exclusion of articles are shown in Figure 1. A total number of 76 studies were selected from the primary search on Google Scholar, PubMed, ScienceDirect and Web of Science using the key combination (Single nucleotide polymorphisms, eggshell thickness, and chickens). A total of 56 studies remained after the extraction of duplicates, and 32 studies were excluded after screening of the title and abstract. Further screening for eligibility resulted in 15 studies being included in the current systematic review.

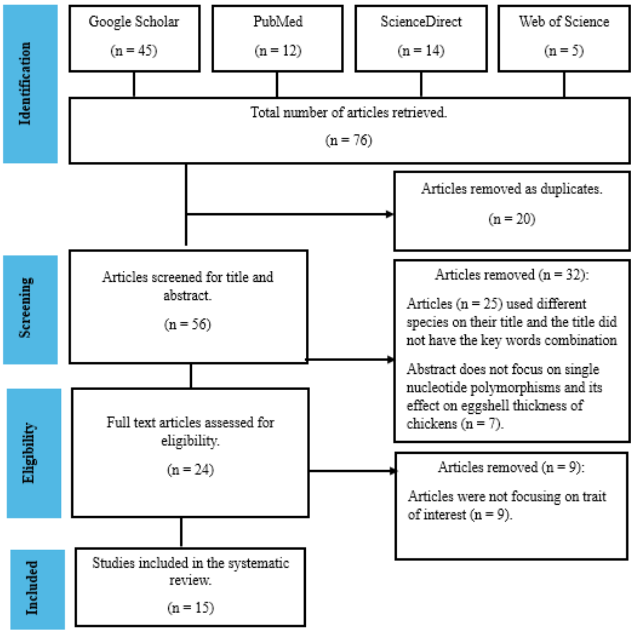

Figure 1 – Flowchart of identification and selection of studies for systematic review.

Characteristics of the included studies

The current systematic review used 15 articles that investigate the potential association between single nucleotide polymorphisms and chicken eggshell thickness, as shown in Table 1. Amongst the articles, Barkova published 3 articles in different years (Barkova *et al.*, 2011; 2021; 2015). White Leghorn was used by 5 articles as the experimental animal, while other articles (n = 4) used Rhodes Island Red. About 20% (3/15) of the included articles investigated the *ovocalyxin-32* gene. The results also showed that Dunn *et al*. (2008), Wang *et al*. (2016), and Ikeh *et al*. (2020) investigated the association of the same gene (*Ovocleidin-116*) with chicken eggshell thickness. About 60% (9/15) of the included articles focused on one gene of interest, whereas the others (40%) focused on more than one gene (6/15).

Table 1 – Characteristics of included studies.

Author	Year	Country	Chicken	N	Nº of genes	Gene name
Zhang *et al.*	2011	China	Pureline dwarf layers	568	1	*Low-density lipoprotein receptor (LRP2)*
Uemoto *et al.*	2009	Nigeria	White Leghorn (WL) males and Rhode Island Red (RIR)	272	1	*Ovocalyxin-32 (OCX-32)*
Yao *et al.*	2010	China	dwarf chickens	747	1	*Low-density lipoprotein receptor-related protein 8 (LRP8)*
Ikeh *et al.*	2020	Nigeria	Nigerian Heavy Local Chicken Ecotype	240	1	*Ovocleidin-116 (OC-116)*
Dunn *et al.*	2008	Germany	Rhode Island Red hens	2000	6	*Ovocleidin-116, osteopontin (SPP1), ovocalyxin-32, ovotransferrin (LTF), ovocalyxin-36 (OCX-36), estrogen receptor (ESR1) and carbonic anhydrase II (CAII).*
Barkova *et al.*	2013	Poland	Rhode Island layers	46	1	*ChEST985k21*
Barkova & Smaragdov	2015	Russia	Island chicken breed	241	1	*Non-SMC Condensin I Complex, Subunit G (NCAPG)*
Barkova *et al.*	2010	Iran	Polish breed Green and Rhode Island Red breed	136	1	*ChEST985k21*
Whetto *et al.*	2023	Singapore	(50 Normal feather, 50 Naked neck and 50 Frizzle feather), 50 Kuroiler and 50 Sasso	250	1	*Insulin-like Growth Factor-1 (IGF-1)*
Gao *et al.*	2022	China	LingKun Chickens	266	1	*Subunit Beta 2 (PSMB2)*
Jiang *et al.*	2010	China	Houdan hens	300	1	*Parathyroid hormone (PHT)*
Wang *et al.*	2016	China	Pureline Rhode Island White layers	384	2	*Ovocleidin-116 (OC-116)* *calmodulin1 (CALM1)*
Liu *et al.*	2011	China	White Leghorn and Brown-Egg Dwarf Layers	746	2	*GRB14 and GALNT1*
Duan *et al.*	2016	Japan	White Leghorn	230	1	*Ovoinhibitor (OIH)*
Duan *et al.*	2015	Japan	Rhode Island Red	320	1	*Phospholipase C zeta (PLCZ1)*

N: *Population size*

Publication by year

Figure 2 shows the number of articles published per year. The years 2008 (Dunn *et al.*, 2008), 2009 (Uemoto *et al.*, 2009), 2013 (Barkova *et al.*, 2013), 2020 (Ikeh *et al.*, 2020) and 2023 (Whetto *et al.*, 2013) had the lowest number of publications, each with one article (n=1). On the other hand, 2010 (Yao *et al.*, 2010) had the highest number of publications (20%, 3/15), while 2011 (Liu *et al.*, 2011) and 2016 (Wang *et al.*, 2016) had an equal number of publications (n=2) (13%, 2/15).

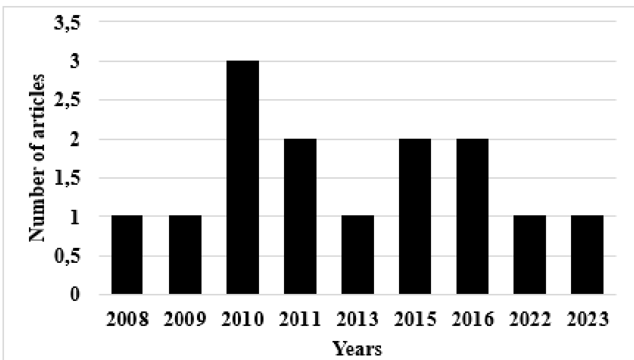

Figure 2 – Publications by year.

Publication by country

The number of articles published per country are represented in Figure 3. The results indicated that the included articles had the following countries of origin: Russia (Barkova & Smaragdov, 2015), Singapore (Whetto *et al.,* 2023), Iran (Barkova *et al.,* 2010), Poland (Barkova *et al.,* 2010). Furthermore, Russia (Barkova & Smaragdov, 2015) had the lowest number of articles (n = 1), while China had the highest number of publications 40% (6/15).

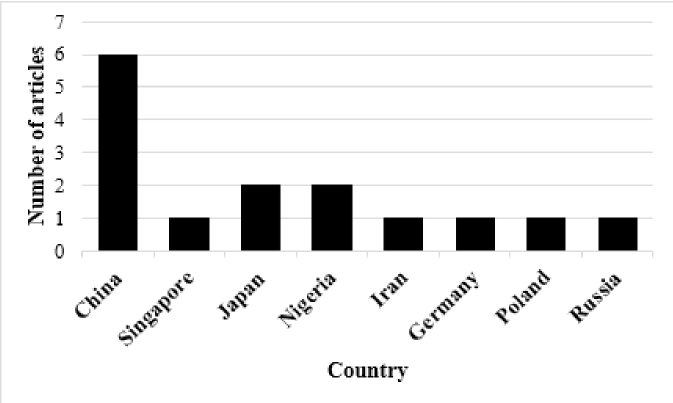

Figure 3 – Publication by country.

Publication by population size

Figure 4 shows the population size of the chickens used in the studies. The results indicated that all the included articles informed the population size used. About 40% (6/15) of the included articles used population sizes between 100 and 500 chickens, and the largest population size was noted in the articles by Dunn *et al*. (2008) and Yao *et al*. (2010).

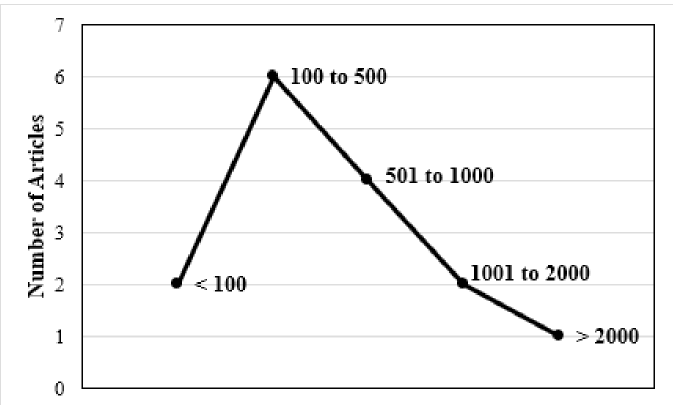

Figure 4 – Publication by population size.

Distribution of the included articles by genes

Results on Figure 5 show the number of genes per article. About 20% (3/15) of the articles investigated the same gene [*Ovocleidin-116* (*OC-116*)] (Dunn *et al.,* 2008; Wang *et al.,* 2016; Ikeh *et al.,* 2020). *Ovocalyxin-32* was investigated by 2 articles (Dunn *et al.,* 2008; Uemoto *et al.,* 2009), and *ChEST985k21* was also investigated by the same author in different years (Barkova *et al.,* 2010 and 2013).

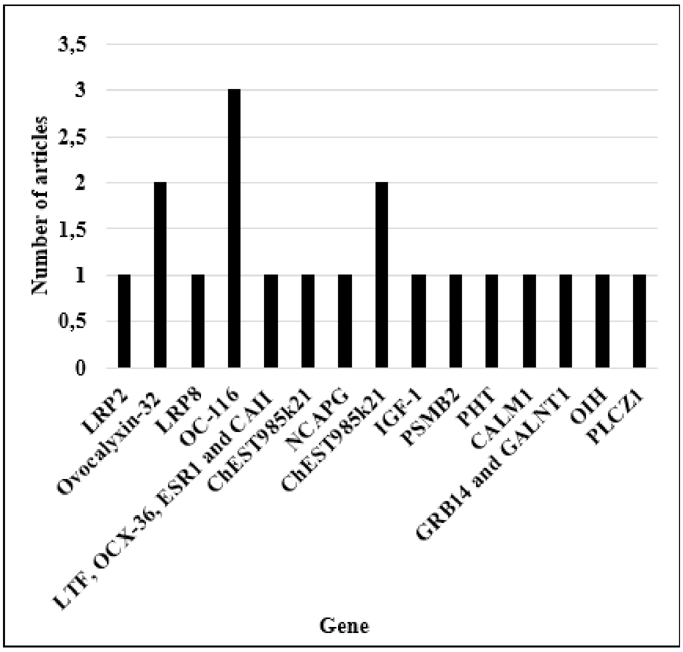

Figure 5 – Distribution by gene.

Identified SNPs and their regions

The identified SNPs and their positions from the included articles are presented in Table 2. The results indicated that about 73% (11/15) of the included articles identified 1 SNP. Twenty seven percent (4/15) of included articles identified 2 (rs13968878, rs13978498; Liu *et al.,* 2011), 3 (T267G, A494C, G381C; Uemoto *et al.,* 2009), 4 (rs15841856, rs14986134, s16544657,ss538155652; Duan *et al.,* 2015) or 6 (S2_1 (GATCAGYAGTAAA), S3_1 (CATTTTRCTCTCA), S3_2 (GTTCAGYGTGGTC), S3_3 (TGTAAAYGCAGCA), S3_4 (CAGAATYGCACAG), S6_1 (AGTTTTYGGGTGC; Barkova *et al.,* 2013) SNPs. The results of the current study also revealed that 47% (7/15) of the included articles showed the SNPs positions, while 53% (8/15) articles did not show the SNP positions. It was recognised that amongst the 47% (7/15) of the articles that identified SNP positions, 86% (6/7) of the SNPs were noted on exons, whereases 4% (1/7) identified

SNP on intron (Retnosari & Daryon 2022). The results further indicated that about 67% (4/6) of articles identified 4 SNPs on exon 4. Amongst the investigated breeds, 27% (4/15) of included articles used the Rhode Island Red chicken breed.

Table 2 – Identified SNPs and their regions.

Author	Breed	SNP	Region
Duan *et al*. 2016	White Leghorn	GGA	-
Duan *et al*. 2015	Rhode Island Red	rs15841856 rs14986134 s16544657 ss538155652	Exon 7 Exon 4 Exon 5 Exon 6
Zhang *et al*. 2011	Pureline dwarf layers	T14347C	-
Uemoto *et al*. 2009	White Leghorn (WL) males and Rhode Island Red (RIR)	T267G A494C G381C	Exon 2 Exon 4 Exon 3
Liu *et al*. 2011	White Leghorn (WL) and Dongxiang (DX) chickens	rs13968878 rs13978498	-
Wang *et al*. 2016	Pureline Rhode Island White layers	rs16030727	-
Jiang *et al*. 2010	Houdan hens	A2205G	Exon 3
Barkova *et al*. 2013	Rhode Island layers	S2_1 (GATCAGYAGTAAA) S3_1 (CATTTTRCTCTCA) S3_2 (GTTCAGYGTGGTC) S3_3 (TGTAAAYGCAGCA) S3_4 (CAGAATYGCACAG) S6_1 (AGTTTTYGGGTGC)	-
Barkova *et al*. 2015	Island chicken breed	rs14491030	Exon 8
Barkova *et al*. 2010	Polish breed Green and Rhode Island Red breed	ST2_1GATCAGYAGTAAA	-
Gao *et al*. 2022	LingKun Chickens	C4415750T	Exon 3
Yao *et al*. 2010	Dwarf chickens	C1623T	-
Ikeh *et al*. 2020	Nigerian Heavy Local Chicken Ecotype	C1110G	-
Dunn *et al*. 2008	Rhode Island Red hens	C00895G	Exon 4
Retnosari & Daryon 2022	Golden Kamper Hybrid chickens	G4363T	Intron 7

Allelic and genotypic frequencies

Allelic and genotypic frequencies are shown in Table 3. Our findings showed that about 53% (8/15) of the included articles did not highlight the gene frequencies, 67% (10/15) did not indicate genotypic frequencies, and 73% (11/15) did not indicate if the populations were under Hardy Weinberg equilibrium or not. In addition,about 33% (5/15) of the included articles indicated both the gene and genotypic frequencies. Out of the 5 included articles that indicated frequencies, 50% (2/5) revealed that the population was under Hardy Weinberg equilibrium (Jiang *et al.,* 2010; Ikeh *et al.,* 2020), while those by Yao *et al*. (2010), and Zhang *et al*. (2011) were not under Hardy Weinberg equilibrium. The gene frequency of the investigated reports ranged between 0.09 and 0.91, with genotypic frequency ranging from 0.01 to 51.8.

Table 3 – Gene and genotypic frequencies.

Author	Gene frequency	Genotypic frequency	HWE (Sign)
Duan *et al*. 2016	-	-	-
Duan *et al*. 2015	-	-	-
Zhang *et al*. 2011	C = 0.38 T = 0.62	TT = 0.47 TC = 0.30 CC = 0.23	ns
Uemoto *et al*. 2009	T = 0.62 C = 0.38	T>G GG = 35.5 TG = 38.7 TT = 30.9 G>C GG = 22.4 CG = 51.8 CC = 25.7 A>C GC = 33.5 CG = 35.7 CC = 30.9	-
Liu *et al*. 2011	-	-	-
Wang *et al*. 2016	-	-	-
Jiang *et al*. 2010	A = 0.67 G = 0.33	AA = 0.43 AG = 0.49 GG = 0.08	*
Barkova *et al*. 2013	S2_1 (GATCAGYAGTAAA) C = 0.35 T = 0.65 S3_1 (CATTTTRCTCTCA) A = 0.21 G = 0.77 S3_2 (GTTCAGYGTGGTC) C = 0.60 T = 0.38 S3_3 (TGTAAAYGCAGCA) C = 39 T = 0.45 S3_4 (CAGAATYGCACAG) C = 0.41 T = 0.45 S6_1 (AGTTTTYGGGTGC) C = 0.42 T = 0.52	-	-
Barkova *et al*. 2015	-	-	-
Whetto *et al*. 2023	-	-	-
Barkova *et al*. 2010	C = 0.35 T = 0.65	-	-
Gao *et al*. 2022	-	-	-
Yao *et al*. 2010	T = 0.09 C = 0.91	TT = 0.02 CC =0.85 CT =0.13	ns
Ikeh *et al*. 2020	G = 0.75 C = 0.25	GG = 0.6 GC = 0.3 CC = 0.1	*
Dunn *et al*. 2008	-	-	-

HWE: *Hardy-Weinburg Equilibrium,* Sign: *Significant,* ns: *Not significant at p*>0.05, *: *Significant at p*<0.05.

Association of genotypes of the identified SNPs with eggshell thickness and their effect on the protein-coding region

The association of genotypes of the identified SNPs with eggshell thickness and the effect of SNPs on protein-coding region are represented in Table 4.

About 67% (10/15) of included articles showed the association between genotypes of identified SNPs and traits of interest. Analysis of marker-trait association showed that 20% (3/15) of the selected articles did not highlight whether there was a marker-trait association or not (Wang *et al.,* 2016; Gao *et al.,* 2022; Wheto *et al.,* 2023). SNP effect on the protein-coding region was highlighted by 13% (2/15), showing synonymous SNPs (Jiang *et al.,* 2010; Zhang *et al.,* 2011), while 7% (1/15) showed non-synonymous SNP (Barkova *et al.,* 2015), and 80% (12/15) articles did not indicate the type of SNP.

Table 4 – Association of genotypes of the identified SNPs with eggshell thickness and the genetic variation contribution.

Author	Gene	Genotypes	SNP	Association (YES/NO)	Effect on protein-coding region
Duan *et al*. 2016	*PLCZ1*	AA= 12.5 AB= 17.2	GGA	YES	-
Duan *et al*. 2015	*CALM1*	TT= 26.12 AT= 63.40	rs15841856 rs14986134 s16544657 ss538155652	YES	-
Zhang *et al*. 2011	*Low-density lipoprotein receptor (LRP2)*	AA= 16.00 AB= 0.88	T14347C	YES	Synonymous
Uemoto *et al*. 2009	*OCX-32*	TC= 0.86 GC= 3.76 AC= 0.33	T267G A494C G381C	NO	-
Liu *et al*. 2011	*GRB14 and GALNT1*	TC= 35.20 TT= 18.26	rs13968878 rs13978498	YES	-
Wang *et al*. 2016	*Ovocleidin-116 (OC-116)* *calmodulin1 (CALM1)*	AA= 304.55 AG= 302.19 GG= 305.35	rs16030727	-	-
Jiang *et al*. 2010	*Parathyroid hormone gene*	AA= 3.28 AG= 3.71 GG= 3.69	A2205G	YES	Synonymous
Barkova *et al*. 2013	*ChEST985k21*	CC= 389.00 CT= 340.00 TT= 351.00	S2_1 (GATCAGYAGTAAA) S3_1 (CATTTTRCTCTCA) S3_2 (GTTCAGYGTGGTC) S3_3 (TGTAAAYGCAGCA) S3_4 (CAGAATYGCACAG) S6_1 (AGTTTTYGGGTGC)	YES	-
Barkova *et al*. 2015	*NCAPG*	GG= 341.00 AA= 348.00 AG= 352.00	rs14491030	YES	Non - synonymous
Whetto *et al*. 2023	*Insulin-like Growth Factor-1*	AA= 0.40 AC= 0.41 CC= 0.41	-	YES	-
Gao *et al*. 2022	*Subunit Beta 2 (PSMB2)*	CT= 32.04 TC= 13.55 GC= 13.05	-	-	-
Yao *et al*. 2010	*Low-density lipoprotein receptor-related protein (LRP8)*	CC= 342.90 CT= 347.20 TT= 355.40	C1623T	YES	-
Ikeh *et al*. 2020	*Ovocleidin-116 (OC-116)*	CG= .031 GG= 0.31 CC= 0.33	C1110>G	YES	-
Dunn *et al*. 2008	*Ovocleidin-116, osteopontin (SPP1), ovocalyxin-32 (RARRES1), ovotransferrin (LTF), ovalbumin and ovocalyxin-36, [estrogen receptor (ESR1) and carbonic anhydrase II (CAII)].*	GG= 19.18 CG= 19.52 CC= 18.85	C00895G	YES	-
Barkova *et al*. 2010	*ChEST985k21*	GT= 5.30 CC= 4.58	ST2_1 GATCAGYAGTAAA	YES	-

DISCUSSION

The current report systematically reviews single nucleotide polymorphism and its effects on chicken eggshell thickness, which supports marker-trait selection to enhance eggshell thickness. The review comprised 15 articles, with China (40%, 6/15 articles) being the leading country, with different sample sizes (100 and 500). From the investigated articles, *Ovocleidin-116* (20%), *Ovocalyxin-32* (13%) and *ChEST985k21*(13%) were the most studied candidate genes across different chicken breeds for their potential influence on eggshell thickness, an influence that was effectively observed.

Reports by Ikeh *et al*. (2010) on Nigerian Heavy Local chicken ecotype, Dunn *et al*. (2008) on Rhode Island Red hens, and Wang *et al*. (2016) on Pureline Rhode Island White layers agree that Ovocleiding-116 is the best candidate gene for marker trait association to improve eggshell thickness. A study conducted by Dunn *et al*. (2008) on Rhode Island Red hens reveals that *Ovocalyxin-32* is the best gene for improving eggshell thickness. Barkova *et al*. (2010) studied the Polish breed Green and Rhode Island Red breed, and also conducted another study on Rhode Island layers, and indicated the *CHEST985K21* as the gene playing a role in eggshell thickness. rs16030727 in the Ovodeidin-116, rs14491030 in *CHEST985K21* on Exon 8, S2_1 (GATCAGYAGTAAA), S3_1 (CATTTTRCTCTCA), S3_2 (GTTCAGYGTGGTC), S3_3 (TGTAAAYGCAGCA), S3_4 (CAGAATYGCACAG), S6_1 (AGTTTTYG GGTGC) in *CHEST985K21* and C00895G in *Ovocalyxin-32*, C1110G and C00895G in *Ovocleidin-116* could be used as a genetic markers during animal breeding to improve eggshell thickness.

To our knowledge, the current review is the first to report on the use of single nucleotide polymorphism and its association with eggshell thickness in different chicken breeds, with no other studies it could be compared with. Current reports reveal that marker-traits association is a successful molecular approach to improve traits of economic importance such as eggshell thickness to enhance egg production.

CONCLUSION

The review suggests that Ovocleiding-116, *CHEST985K21* and *Ovocalyxin-32* are candidate genes playing a role in chicken eggshell thickness. The current systematic review is supported by the fact that there are no similar studies on single nucleotide polymorphism and its association with chicken eggshell thickness. This review also suggests that rs16030727, C1110G and C00895G in the *Ovocleidin-116*, rs14491030 in *CHEST985K21* on Exon 8, S2_1 (GATCAGYAGTAAA), S3_1 (CATTTTRCTCTCA), S3_2 (GTTCAGYGTGGTC), S3_3 (TGTAAAYGCAGCA), S3_4 (CAGAATYGCACAG), S6_1 (AGTTTTYG GGTGC) in *CHEST985K21* and C00895G in *Ovocalyxin-32* could be used as genetic markers during animal breeding to improve eggshell thickness. However, more studies need to be conducted to increase information of the current review. In conclusion, the marker-trait association may be used to improve eggshell thickness.

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AUTHOR CONTRIBUTIONS

Conceptualization, KMM, TLT and VGM; methodology, KMM; software, KMM and TLT; validation, KMM, TLT and VGM; formal analysis, KMM, TLT; investigation, KMM; resources, KMM; data curation, TLT and VGM; writing—original draft preparation, KMM, TLT; writing—review and editing, KMM, TLT and VGM; visualization, TLT and VGM; supervision, TLT and VGM; project administration, KMM; funding acquisition, KMM. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data is available upon request to the corresponding author.

CONFLICT OF INTEREST

No conflicts of interest.

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