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## A meta-analysis of the association between Growth Hormone (*GH*) gene polymorphism (*AluI*) and growth traits in cattle breeds

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**ABSTRACT:** The growth hormone (*GH*) is an important gene that affects the productive and physiological traits in livestock. This study aimed to use a meta-analysis to determine the association of the *GH-AluI* gene polymorphism with growth traits in cattle. Four different genetic models were used: dominant LL + LV versus VV, recessive LL versus LV + VV, over dominant LL+VV vs. LV and co-dominant LL vs. LV, LL vs. VV and LV vs. VV. The random-effect model was used in data analysis based on I<sup>2</sup>. Meta-analysis showed a significant effect of *GH-AluI* genotypes on the BW ( $p < 0.05$ ;  $p < 0.01$ ) under the recessive and over dominant models. However, no significant associations were found with the dominant model ( $p > 0.05$ ). In terms of the co-dominant model, the *GH* polymorphism showed a significant association with birth weight (BW) (SMD = 0.359, 95% CI = 0.119 to 0.599,  $p = 0.003$ ) with the LL vs. LV genotype combination patterns. There was no association between the *GH* polymorphism and BW under the LL vs. VV genotype combination. When comparing the models, the results showed that the LL genotype significantly affected BW traits. Moreover, the findings showed the effect of the *GH* on average daily gain (ADG) under the four genetic models ( $p < 0.05$ ). The current study confirmed the association between the *GH* gene and growth traits in cattle.

**Keywords:** *GH*; Birth weight; Average daily gain; Cattle; Meta-analysis

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## INTRODUCTION

Growth plays an essential role in producing meat from animals and is defined as increasing body weight (Fedota *et al.*, 2018; Pal *et al.*, 2004; Sedykh *et al.*, 2020; Tatsuda *et al.*, 2008; Mohammadabadi *et al.*, 2021). Genes are important factors directly affecting growth and development (Aytekin *et al.*, 2020; Fadhil & Zülkadir 2017; Fadhil & Zülkadir 2021). Several candidate genes are involved in the growth processes, such as growth hormone (*GH*), myoblast determination protein 1 (*MyoD*), myogenic factor 5 (*Myf5*), insulin-like growth factor 1 (*IGF1*), myostatin (*MSTN*) and calpastatin (*CAST*) (Abousoliman *et al.*, 2020; Bayraktar & Shoshin 2021; Eghbalsaid *et al.*, 2016; Gebreselassie *et al.*, 2019). *GH*, also called somatotrophic hormone (STH), is a peptide hormone that stimulates the growth process by stimulating the size (hypertrophy) and the number (hyperplasia) of cells and also activates amino acids and protein synthesis (Fedota *et al.* 2018; Ishida *et al.*, 2010; Lee *et al.*, 2013; Papatungan *et al.*, 2016). The most important biological function of the *GH* gene is to stimulate growth in the body. However, the main target organs of the *GH* gene are bones and muscles. *GH* also affects the synthesis of *IGF-1* and increases the free fatty acids and glucose concentration (Møller & Jørgensen 2009). The bovine *GH* gene is located on chromosome 19q and contains four introns and five exons. *GH* is considered a candidate gene associated with growth, milk and reproduction traits (Curi *et al.*, 2006; Dario *et al.*, 2005; Grochowska *et al.*, 2001; Pereira *et al.*, 2005; Sönmez *et al.*, 2018). Researchers have identified several polymorphisms in the *GH* gene (Amiri *et al.*, 2018; Bordonaro *et al.*, 2020). The best-known polymorphisms are the missense mutation C>G in exon five that changes leucine (L) to valine (V) in position 127, which can be characterized using the *AluI* restriction enzyme (Hradecka *et al.*, 2008). Schlee *et al.*, (1994) showed the effects of L/V substitution on carcass gain, meat value, and classification score in Bavarian Simmental bulls. Several studies confirmed the effects of *GH* gene polymorphism on milk production, milk quality, growth, carcass composition and carcass quality (Akçay *et al.*, 2015; Silveira *et al.*, 2008; Sari *et al.*, 2013; Ünal *et al.*, 2020). Some studies reported an association between *GH* gene polymorphism with growth traits (Pal *et al.* 2004; Çinar *et al.*, 2018; Hartatik *et al.*, 2020; Pal & Chakravarty 2020; Reis *et al.*, 2001; Thomas *et al.*, 2007). In contrast, other studies confirmed no association between *GH* and growth traits (Akçay *et*

*al.* 2015; Arnim *et al.*, 2018; Di Stasio *et al.*, 2002; Hartatik *et al.*, 2012; Soewandi *et al.*, 2021). To verify these contradictory results, meta-analysis can use as a tool to address these contradictions by collecting different data and creating a large dataset to overcome the small samples size used in some studies (Lee 2015; Mahmoudi *et al.*, 2019; Mahmoudi *et al.*, 2020). A meta-analysis is a statistical application used to resolve inconsistencies in genetic association studies by integrating previous studies on the same topic (Bangar & Magotra 2021; Bangar *et al.*, 2021a; Özdemir *et al.*, 2018). Meta-analysis raises statistical ability and accuracy in detecting effects by integrating previous studies' results, therefore getting over the small sample size problems and the unsuitable statistical ability of complex genetic traits studies (Bangar *et al.*, 2021b; Chong *et al.*, 2019; Özdemir & Esenbuğa 2020). This study aimed to apply a meta-analysis to the results of previous studies to find out the effects of *GH* polymorphism on BW and ADG in cattle breeds.

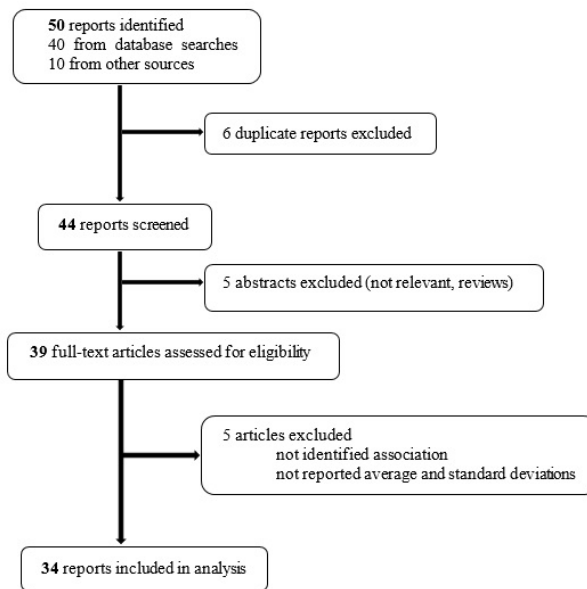
## MATERIALS AND METHODS

### The Search strategy in sources

The Preferred Report Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria selected the required studies to perform the meta-analysis. An accurate and comprehensive search has been carried out about the relationship between the *GH-AluI* gene polymorphisms and growth traits in previous studies that have been published in different languages in different journals and databases. It relied on studies that were published between 2002 to 2021. Studies were searched in Google Scholar, Wiley, Springer, Taylor & Francis, PubMed and Elsevier. Several keywords were used to find the required studies (growth trait, polymorphism, *GH*, association, cattle, body weight BW and average daily gain).

### Criteria of selection and excluding

Specific criteria have been applied when selecting studies, including; (I) effects of *GH* gene polymorphism on growth traits (ADG and BW), (II) animal number per genotypes, (III) least square means were reported for growth traits and (IV) standard deviation/errors and average means of the relevant trait for each genotype (standard errors converted to standard deviations). Studies were excluded if they were as such; (I) publications as a summary, (II) not mentioned the number of animals per genotype, (III) not show standard deviation/errors and average means per genotype and (IV) Studies that have been replicated. A total of



**Figure 1.** PRISMA flow diagrams

50 studies were collected, and 16 were excluded because they did not match the criteria mentioned above, and finally, 34 were used. The 34 studies were from nine countries as Turkey (n=1), Indonesia (n=12), Ukraine (n=6), India (n=6), Russian (n=5), Italy (n=1), Korea (n=1), Iran (n=1), Japan (n=1). Figure 1 shows PRISMA diagrams of the process of selection and exclusion in meta-analysis.

### Data extraction

Relevant data were extracted from the studies using standardized and empirical data extraction models. Data management was implemented by Microsoft Excel 2013. The extracted data included; the names of authors, year of the article, cattle breeds, animal number, genotype number, an average of traits, means with a standard deviation (SE), BW, ADG, significance level and country.

### Statistical analysis

The STATA software performed the meta-analysis. A significance level of less than 0.01 (P-values) was accepted. The database was managed independently for a gene locus and four methods have been followed below;

- The random effect model is used in the meta-analysis when performing differences between means. The model choice depends on the type of study effects (heterogeneous and homogeneous). The fixed model was used with homogeneous data, and the random model

was used with heterogeneous data. The assumption of heterogeneity was calculated based on  $I^2$  (The significance level was identified as 0.01 in the heterogeneity analysis).

- Four genetic model comparisons were used: dominant LL + LV versus VV, recessive LL versus LV + VV, over dominant LL+VV vs. LV and co-dominant LL vs. LV, LL vs. VV and LV vs. VV.
- The standard mean differences (SMDs) and standard deviation were calculated with a 95% CI to estimate the association's ability between genotypes following four genetic models for each trait.
- When calculating SMD, the Hedges method is used when the studies number is less than ten; in contrast, the Cohen method is used when the number of studies is greater than ten. The Cohen method for SMD is appropriate for it tends to assess the effect of size. However, if the number of studies is small, the Hedges method for standardized mean differences is beneficial.

## RESULTS

### Estimation the heterogeneity

In this study, the  $I^2$  test was used to estimate the heterogeneity between the studies. Because of the high heterogeneity so the random effect model was

applied. The  $I^2$  values for dominant, recessive and over dominant genetic models of growth traits were high ( $I^2 > 75\%$ ). The results for BW and ADG traits were as such; dominant model ( $I^2 = 75.4$ ; 98.8, SMD = -0.065; -2.493, 95% CI = -0.337 to 0.207; -4.877 to -0.109,  $p = 0.641$ ; 0.040), recessive model ( $I^2 = 92.9$ ;

98.9, SMD = 0.325; -9.073, 95% CI = -0.095 to 0.556; -11.170 to -6.975,  $p = 0.006$ ; 0.000), over dominant model ( $I^2 = 81.1$ ; 98.9, SMD = 0.232; -9.551, 95% CI = 0.002 to 0.463; -11.549 to -7.553,  $p = 0.048$ ; 0.000) (Table 1, 2; Figure 2, 3, 4 and 5).

**Table 1.** Heterogeneity test of three genetic models dependent on the  $I^2$  statistic

Traits	n	LL+LV vs. VV, Dominant model		LL vs. LV+VV, Recessive model		LL+VV vs. LV, Over dominant model	
		$I^2$	Model	$I^2$	Model	$I^2$	Model
BW	23	75.4	R	92.9	R	81.1	R
ADG	11	98.8	R	98.9	R	98.9	R
Co-dominant model							
		LL vs. LV		LL vs. VV		LV vs. VV	
BW							
		$I^2$		$I^2$		$I^2$	
		71.3		65.8		74.4	
		Model		Model		Model	
		R		R		R	
ADG							
		$I^2$		$I^2$		$I^2$	
		98.7		98.9		97.5	
		Model		Model		Model	
		R		R		R	

n: study numbers; R: Random model

**Table 2.** The results of the Meta-analysis regarding the association between GH and BW and ADG traits under genetic models

Traits	n	SMD	95% CI	p-Value	Model
<b>LL+LV vs. VV, Dominant model</b>					
BW	23	-0.065	-0.337 to 0.207	0.641	R
ADG	11	-2.493	-4.877 to -0.109	<b>0.040**</b>	R
<b>LL vs. LV+VV, Recessive model</b>					
BW	23	0.325	0.095 to 0.556	<b>0.006*</b>	R
ADG	11	-9.073	-11.170 to -6.975	<b>0.000*</b>	R
<b>LL+VV vs. LV, Over dominant model</b>					
BW	23	0.232	0.002 to 0.463	<b>0.048**</b>	R
ADG	11	-9.551	-11.549 to -7.553	<b>0.000*</b>	R
<b>Co-dominant model</b>					
		LL vs. LV		LV vs. VV	
BW					
		SMD		SMD	
		0.359		-0.114	
		95% CI		95% CI	
		0.119 to 0.599		-0.402 to 0.173	
		p-Value		p-Value	
		0.003		0.436	
		Model		Model	
		R		R	
ADG					
		SMD		SMD	
		-7.023		3.713	
		95% CI		95% CI	
		-9.213 to -4.834		-16.368 to -8.273	
		p-Value		p-Value	
		0.000*		0.010**	
		Model		Model	
		R		R	

n: study numbers; SMD: standardized mean difference; CI: Confidence interval; R: Random; \*: ( $p < 0.01$ ); \*\*: ( $p < 0.05$ )

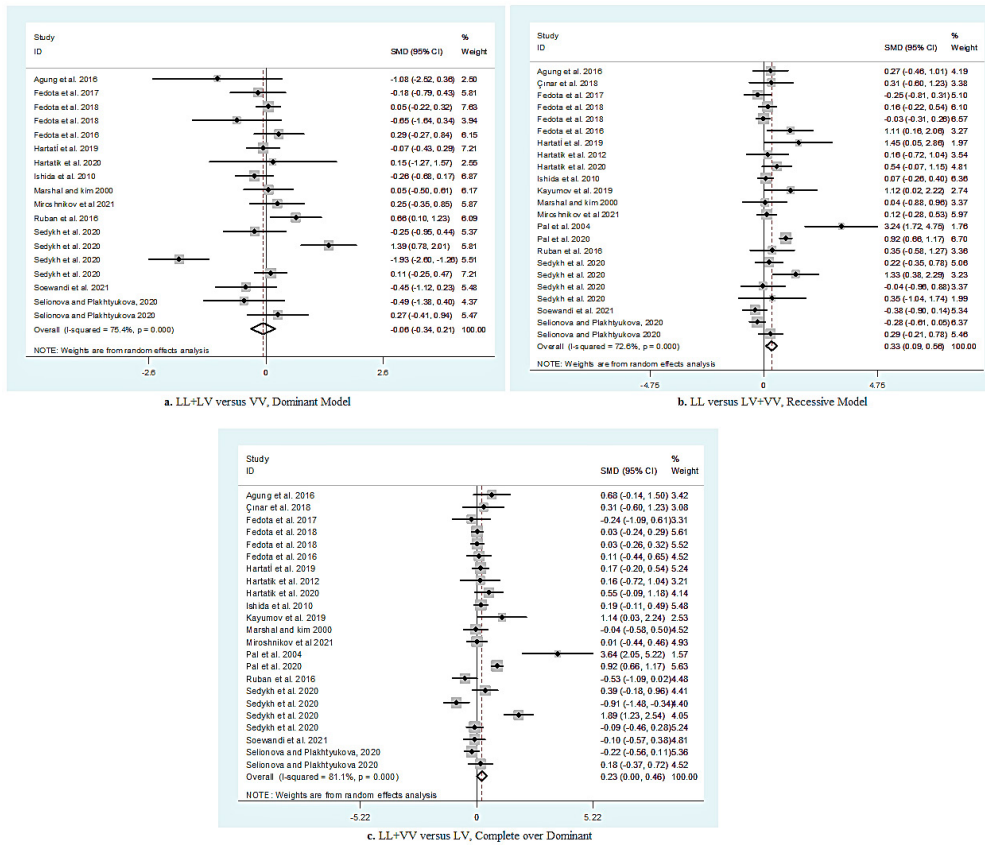


Figure 2. The forest plot of heterogeneity test of three genetic models dependent on the  $I^2$  statistic for birth weight

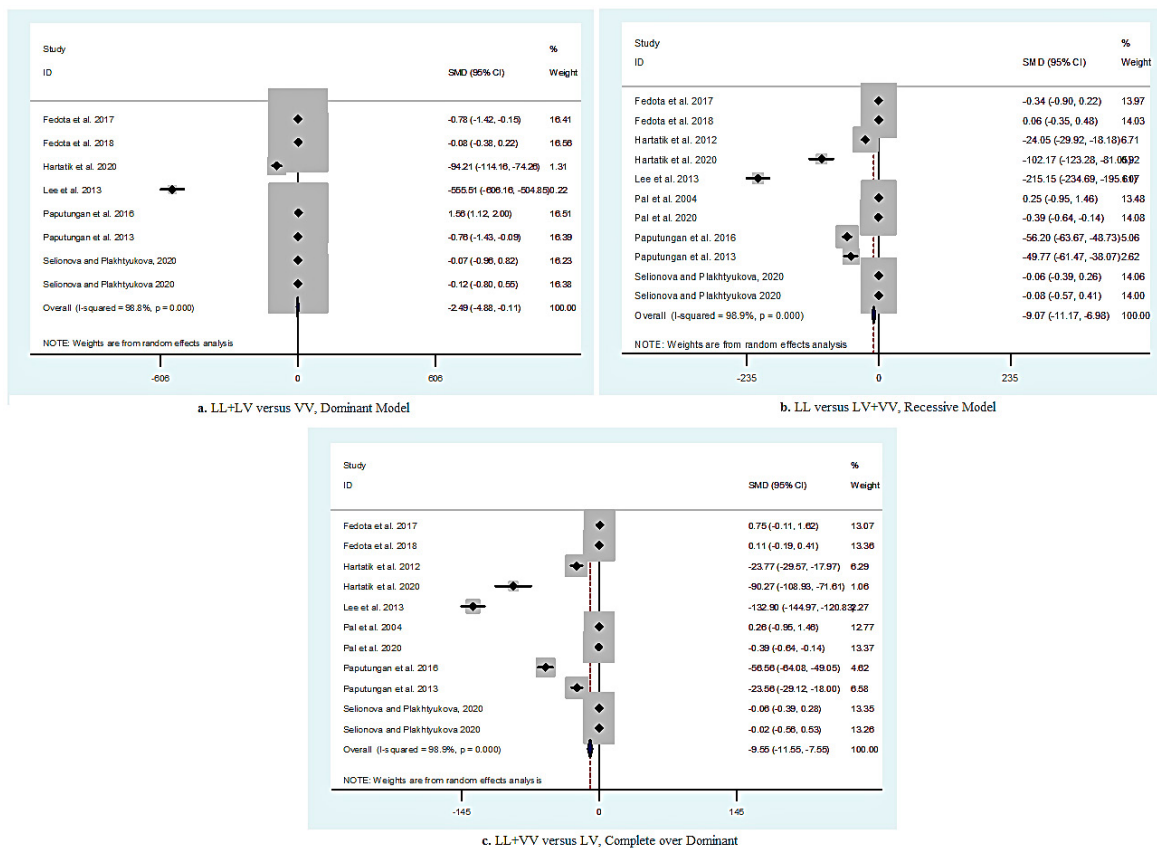


Figure 3. The forest plot of heterogeneity test of three genetic models dependent on the  $I^2$  statistic for average daily gain



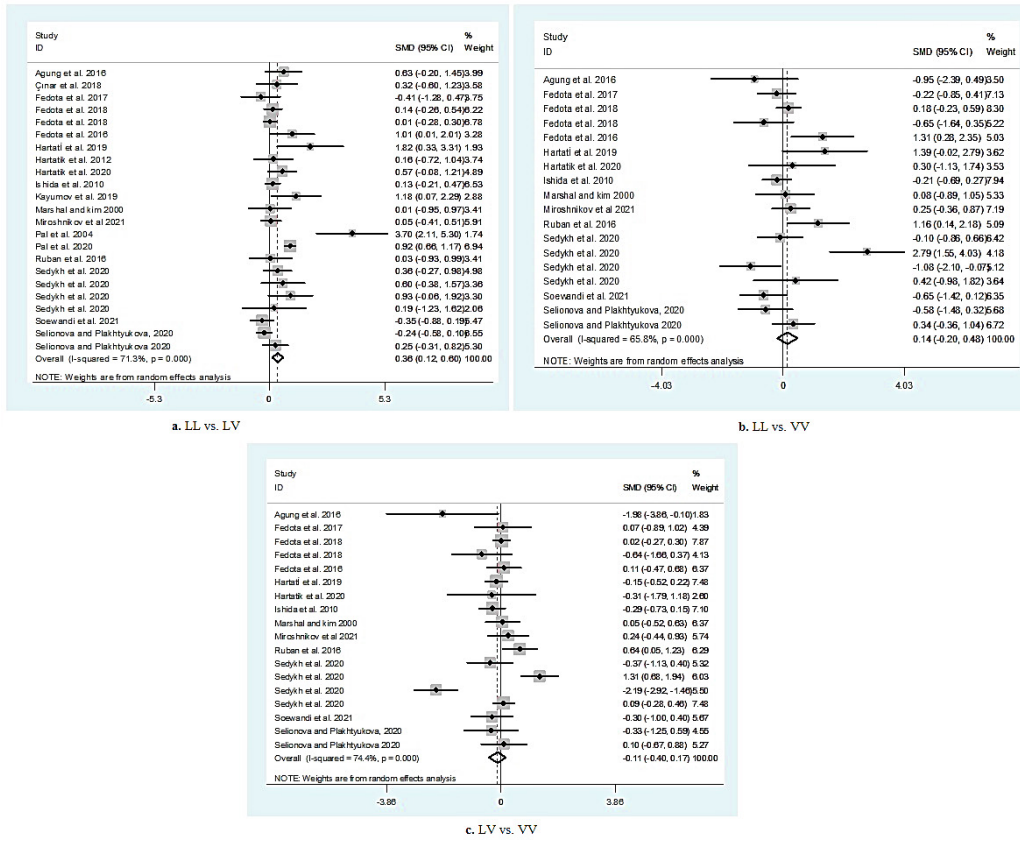


Figure 4. The forest plot of heterogeneity test of birth weight under co-dominant model

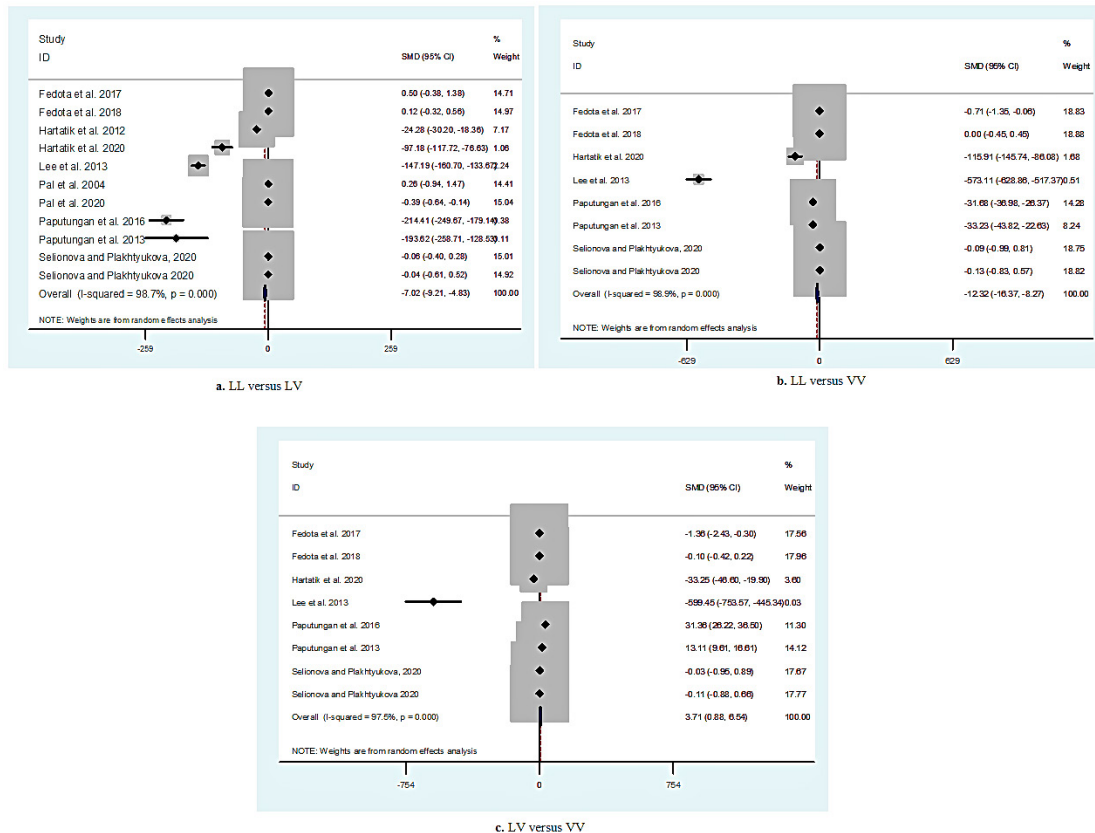
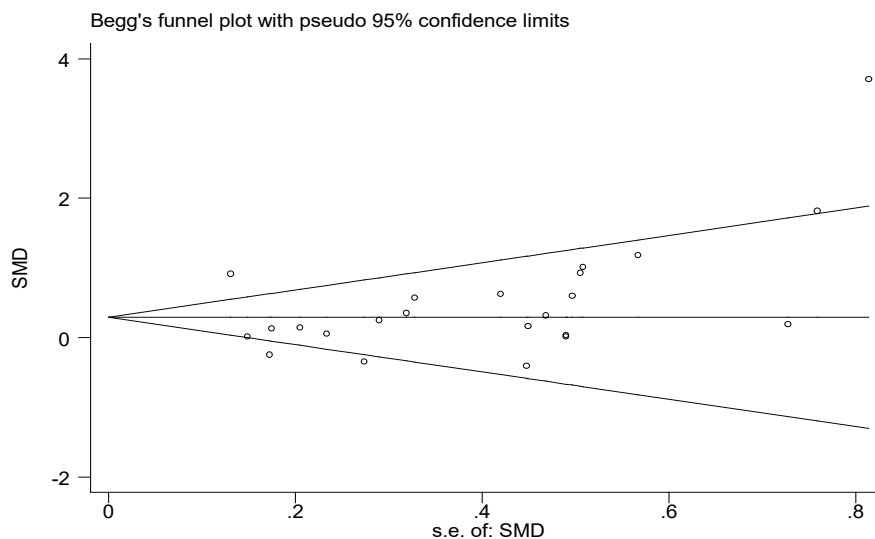


Figure 5. The forest plot of heterogeneity test of average daily gain under co-dominant model

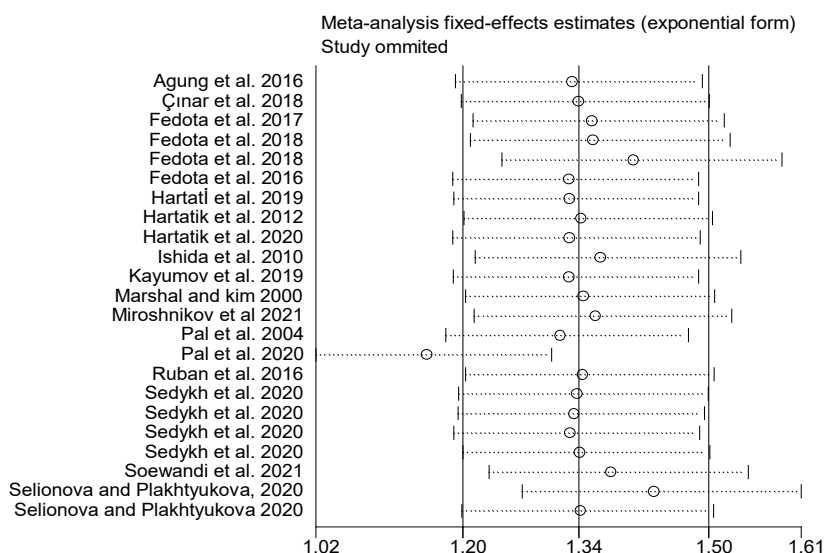
**Publication bias and sensitivity analysis**

In a meta-analysis, a funnel plot is used to assess publication bias. The funnel plot is a scatter graph to estimate the effects of different studies. Egger’s regression test confirmed no publication bias for all genetic models ( $p>0.05$ ) (Figure 6). A sensitivity analysis was performed to assess the effects of all studies

in the meta-analysis on the stability of pooled results by removing one study at a time. After removing individual studies, the sensitivity analysis results showed no differences in pooled SMDs, indicating that none of the single studies was responsible for the overall results (Figure 7).



**Figure 6.** Funnel plot of Begg’s test for publication bias



**Figure 7.** Sensitivity Plot Pooled with Fixed Effects



### Meta-analysis of the association between *GH* polymorphism and growth traits

Four different genetic models, including dominant model (LL+LV versus VV), recessive model (LL versus LV+VV), over the dominant model (LL+VV versus LV) and co-dominant model (LL vs. LV, LL vs. VV and LV vs. VV), were used in the meta-analysis to reveal the association of the *GH* polymorphism with growth traits. The results showed a significant association between *GH* polymorphism and growth traits in cattle ( $p < 0.01$ ;  $p < 0.05$ ). The *GH* polymorphism showed a significant association with ADG (SMD = -2.493, 95% CI = -4.877 to -0.109,  $p = 0.040$ ) under dominant model. However, no association was found between *GH* polymorphism and BW under dominant model. The effects of the *GH* polymorphism on the BW and ADG (SMD = 0.325, 95% CI = 0.095 to 0.556,  $p = 0.006$ ; SMD = -9.073, 95% CI = -11.170 to -6.975,  $p = 0.000$ ) was identified under recessive model (Table 2, Figures 4, 5). No association was found between the *GH* polymorphism and BW under LL vs. VV genotype combination. A significant association was revealed between *GH* polymorphism BW and ADG under over dominant model (SMD = 0.232, 95% CI = 0.002 to 0.463,  $p = 0.048$ ; SMD = -9.551, 95% CI = -11.549 to -7.553,  $p = 0.000$ ). Regarding the co-dominant model, the *GH* polymorphism showed a significant association with BW (SMD = 0.359, 95% CI = 0.119 to 0.599,  $p = 0.003$ ) under LL vs. LV genotype combination patterns. The effects of the *GH* polymorphism on the ADG (SMD = -7.023, 95% CI = -9.213 to -4.834,  $p = 0.000$ ) were detected under LL vs. LV genotype combination patterns (Table 2, Figure 6, 7). No association was found between *GH* polymorphism and BW under LL vs. VV genotype combination. A significant association was revealed between *GH* polymorphism and ADG under LL vs. VV genotype combination (SMD = -12.321, 95% CI = -16.368 to -8.273,  $p = 0.000$ ). There were no effects between *GH* polymorphism and BW Under LV vs. VV genotype combination. In contrast, a significant association was found between *GH* polymorphism and ADG (SMD = 3.713, 95% CI = 0.882 to 6.543,  $p = 0.010$ ).

### DISCUSSION

The candidate gene studies are at the vanguard of genetic association studies by identifying genes associated with a specific trait. This will help breeders select animals early and provide an important economic return. *GH* is an important candidate gene because it

is associated with economic traits, such as growth, reproduction, and milk production. The contradictory results regarding the association of *GH* with growth traits have been reported in previous studies. Researchers have debated whether *GH* polymorphism is associated with growth traits or not. To answer these discrepancies, we performed a meta-analysis of the data obtained from previous studies regarding the association of *GH-AluI* polymorphism with growth traits. Dominant model (LL+LV versus VV), recessive model (LL versus LV+VV), over dominant model (LL+VV versus LV) and co-dominant model (LL vs. LV, LL vs. VV and LV vs. VV) was used to verify the association of *GH* genotypes with growth traits. Meta-analysis showed a significant effect of *GH* gene genotypes on the BW ( $p < 0.05$ ;  $p < 0.01$ ) under the recessive and over dominant models. However, no significant associations were found under the dominant model ( $p > 0.05$ ). Regarding the co-dominant model, the *GH* polymorphism showed a significant association with BW (SMD = 0.359, 95% CI = 0.119 to 0.599,  $p = 0.003$ ) under LL vs. LV genotype combination patterns. There was no association between *GH* polymorphism and BW under LL vs. VV genotype combination. The findings showed that the LL genotype had a significant effect on BW traits from LV and VV genotypes when comparing the models. The findings obtained in the present study are consistent with the previous studies. Pal *et al.* (2004) showed that the LL genotype was significantly higher than LV in birth weight, body weight in three months and average daily gain in Karan Fries Cattle. Hartatik *et al.* (2012) reported that the LL genotype had greater birth weights in Limousin Cross Madura Cattle. Studies have confirmed that the L allele is associated with higher birth weight (Lee *et al.* 2013; Thomas *et al.* 2007; Fedota *et al.*, 2016). Pal & Chakravarty (2020) found that the LL genotype had a relatively superior biological action on the growth, milk and reproduction traits of the LV genotype. Hartatik *et al.* (2020) identified that the LL genotype had the highest birth weight than LV and VV genotypes in crossbred beef cattle. The LL homozygous individuals showed significant weight growth in crossbred cattle (Kayumov *et al.*, 2019). In contrast, some studies reported that the LV genotype had the highest birth weight in Holstein-Friesian (Biswas *et al.*, 2003). Other studies found no significant association between *GH* polymorphism and birth weight (Sedykh *et al.* 2020; Çinar *et al.* 2018; Plakhtukova *et al.*, 2020; Ruban *et al.*, 2016; Selionova & Plakhtyukova 2020). A meta-analysis showed a significant as-

sociation between *GH* polymorphism and ADG under dominant, recessive, over dominant and co-dominant models ( $p < 0.05$ ;  $p < 0.01$ ). Pal *et al.* (2004) and Pal & Chakravarty (2020) indicated that the individuals with the LL genotype had more ADG than the LV genotype in Karan Fries bulls and crossbred cattle. Hartatik *et al.* (2012) reported that LV heterozygotes were significantly higher regarding ADG than LL homozygotes in Karan Fries Cattle. Plakhtukova *et al.* (2020) found that the homozygous VV animals had a significant ADG than LL and LV genotypes. In contrast, no significant effect showed *GH* polymorphism on the ADG in Kazakh white-headed breed and crossbred beef cattle.

## CONCLUSIONS

In conclusion, a meta-analysis is an effective tool for understanding the gene polymorphism association with productive traits in livestock. This study answered the inconsistencies reported in previous studies. The current study confirmed an association between the *GH* gene and the growth traits in cattle. *GH* gene can be used as a genetic marker in cattle improvement programs. The number of studies used in the meta-analysis could be a limitation for this study. However, the use of many numbers of studies in a meta-analysis will be a significant impact on genetic association studies.

## CONFLICT OF INTEREST

None declared by the authors.

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