Engineering xxx (xxxx) xxx

Contents lists available at ScienceDirect

Engineering

journal homepage: www.elsevier.com/locate/eng

Research
Agricultural Engineering—Article

Evidence that Genome Editing is Preferable to Transgenesis for Enhancing Animal Traits

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ARTICLE INFO

Article history:
Received 24 January 2025
Revised 3 November 2025
Accepted 19 November 2025
Available online xxxx

Keywords:
Genetic engineering
Trait enhancement
Livestock
Meta-analysis

ABSTRACT

Production traits such as growth, disease resistance, and fatty acid content in engineered animals are anticipated to be enhanced via transgenesis (TG) or genome editing (GE). It is, however, unclear whether this expectation is upheld when making global comparisons across taxa. In this study, we performed a meta-analysis of 154 research papers covering 72 species and 55 genes, with the aim of quantifying and comparing the effects of TG and GE on animal production traits through overexpressing or disrupting key genes. Although TG is more commonly used for trait enhancement, GE has more pronounced and widespread effects, particularly on growth and disease resistance traits. This is reflected in larger effect sizes and broader impacts across trait responses. Yet, we observe differences in patterns of trait enhancement that are specific to taxon and parameter. For instance, TG reduces pathogen load in chickens and cattle, but not in pigs; conversely, GE lowers virus RNA levels in pigs, but is less successful in chickens and cattle. In contrast, both TG and GE significantly increase growth rates in ray-finned fish. It is notable that, although transgenes or edited genes remain highly expressed or repressed in Filial 1 (F_1) offspring, the magnitude of trait improvement is diminished compared to the founder generations. This study provides evidence-based insights to assist researchers in refining their methods and directing future investigations into trait enhancement in genetically engineered animals, while also informing policymaking.

1. Introduction

Modern genetic modification techniques fall into two broad categories: transgenesis (TG) and genome editing (GE). TG, which was developed in the 1980s, involves randomly inserting foreign DNA (e.g., growth hormone (GH) genes) into a host genome. In contrast, GE—enabled by tools such as clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) since 2012—allows precise modifications to be made, such as knockouts (disrupting a gene) or knock-ins (inserting a sequence at a specific location). While TG remains useful for certain applications, GE is more precise and has become the dominant approach for optimizing traits in livestock and aquaculture. Techniques such as CRISPR/Cas9 [1], transcription activator-like effector nucleases (TALENs) [2], and zinc finger nucleases (ZFNs) [3] have been widely adopted due to their ability to target specific genes associated with desirable animal traits. These technologies have been applied to a wide range of species, including laboratory animals, livestock, poultry, and fish. The aim is to enhance desirable traits in animals, such as increased growth rates [4,5], improved disease resistance [6–8], and optimized fatty acid composition [9–11]. Moreover, these technologies play a key role in biomedical research, helping to generate animal models of human diseases [12,13], and in conservation biology, supporting the protection of endangered species [14,15].

Since the 1980s, the potential of transgenic farm animals as bioreactors capable of producing recombinant proteins has been investigated [16,17]. These studies have led to the introduction of various GH and growth factor genes via TG to promote growth in livestock species, such as pigs [18,19] and sheep [20,21], as well

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https://doi.org/10.1016/j.eng.2025.11.032

as in fish, including Atlantic salmon (*Salmo salar*) [22,23], common carp (*Cyprinus carpio*) [24], Nile tilapia (*Oreochromis niloticus*) [25], and channel catfish (*Ictalurus punctatus*) [26]. Additionally, transgenic farm animals with enhanced disease tolerance and altered fatty acid profiles have been developed, encompassing both livestock and aquatic species [27,28]. Recent advancements in GE technologies, particularly CRISPR-based tools, have increasingly facilitated genetic modifications in farm animals to a greater extent. Nevertheless, to date, only GH-transgenic AquAdvantage salmon [29,30] and transgenic GalSafe pigs [31] have been approved for human consumption. Stringent regulatory oversight makes it challenging for genetically modified organisms to enter the food supply. In contrast, GE can result in changes that mimic natural mutations and may therefore face fewer regulatory obstacles than TG in some countries [32,33]. Several gene-edited animals, including myostain (*mstn*)-edited red sea bream (*Pagrosomus major*) and leptin receptor-deficient tiger puffer (*Takifugu rubripes*), have been approved by Japanese government [34]. Given the advancements over recent decades, there remains a critical need to communicate to policymakers the potential benefits of genetic engineering in animal applications. While both methods can effectively improve desired traits, there have been no comprehensive studies that have systematically compared the relative efficacy of TG and GE in achieving this, apart from their distinct regulatory challenges.

Current evidence for up-scaling favourable traits via TG or GE remains inconclusive, with independent studies yielding varied and, in some cases, conflicting conclusions. The effect of TG or GE on trait improvement may vary according to different parameters and animal taxa. For example, Pursel et al. [19] reported that insulin-like growth factor I (*IGF-I*) transgenic pigs did not exhibit improved body weight or specific growth rate (SGR) compared to non-TG individuals. However, a subsequent study by Bee et al. [35] suggested that, although *IGF-I* transgenic pigs had lower carcass dressing percentage, their longissimus dorsi muscle was larger than that of sibling controls. Several studies have shown that disrupting the *mstn* gene improves growth by increasing myofiber numbers in pigs [36,37], sheep [5], and fish [38], without changing myofiber size. Conversely, other studies have reported increases in both myofiber size and number in *mstn*-mutant pigs [39], chickens [40], and fish [41–43]. Additionally, transgenic channel catfish expressing lysozyme exhibited enhanced lysozyme activity following bacterial infection, compared to non-transgenic controls [44]. However, no significant differences in serum lysozyme levels were observed between lactoferrin-transgenic and control grass carp (*Ctenopharyngodon idellus*) following a bacterial challenge [45]. Investigations have shown that cluster of differentiation 163 (*CD163*)-edited pigs have enhanced resistance to porcine reproductive and respiratory syndrome virus (PRRSV) [46–49], but not African swine fever virus [50]. Similarly, fatty acid-related traits have demonstrated inconsistent outcomes. For example, transgenic pigs and cattle expressing the humanized *Caenorhabditis elegans* gene 1 (*fat1*) transgene did not show significant alterations in the content of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), or *n*-6 polyunsaturated fatty acid (ω-6) [51,52], whereas other studies have reported significant increases in EPA and DHA, as well as reductions in ω-6 levels of *fat1*-transgenic pigs [53,54]. Furthermore, studies on fatty acid elongase 2 (*elovl2*)-transgenic channel catfish have reported both significant and non-significant changes in DHA, *n*-3 polyunsaturated fatty acid (ω-3), and ω-6 content depending on the study [11,55].

The variability in the reported effects of TG and GE on trait enhancement may be due to various biological and methodological factors influencing the outcomes of these interventions. A meta-analytical approach is essential to understand the influence of these 'moderators' and explore general effects on trait performance. Consequently, the observed heterogeneity in trait performance enhancements reflects the specificity of these factors, which must be considered when evaluating the effectiveness of TG or GE in improving traits. However, no study has approached this systematically in relation to animals. Closing this knowledge gap requires the comprehensive integration of all available data and a re-quantitative analysis.

The aim of this study was to investigate the impact of potential determinants on trait performance improvement using multiple moderator analyses based on a meta-data matrix. Our primary objective was to quantitatively integrate empirical data on the application of TG and GE to enhance traits in farm animals. Specifically, we sought to ① identify consistencies across studies involving TG or GE in different animal taxa to evaluate the performance of transgenic and gene-edited animals in terms of gene/protein expression, growth, disease resistance, and fatty acid profiles based on various parameters; ② compare the effects of TG and GE on trait enhancement by different parameters to determine whether GE offers superior results compared to traditional gene transfer; ③ assess whether the impact of TG and GE on trait improvement varies with the age of the engineered animals; and ④ determine promising candidate transgenes or innate genes capable of consistently improving traits across taxonomic classes. Herein, through a cross-taxon meta-analysis and a global synthesis of published data, we provide herein a comprehensive understanding of how to optimize transgene or innate gene manipulation to enhance trait performance in farm animals using genetic engineering approaches.

2. Materials and methods

2.1. Literature search

A literature search of the PubMed, Web of Science, and SCOPUS databases using the search strings (Note 1 in Appendix A) yielded over 2000 potentially eligible published articles from each database. The literature search and collection were carried out based on the statement of Preferred Reporting Items for Systematic Reviews and Meta-Analyses for Ecology and Evolutionary Biology (PRISMA-EcoEvo) [56]. We also conducted a forward search using ten relevant review papers related to the topic of our meta-analysis [7,27,57–64]. The last retrieval date for these online databases was 17 September 2024. Initially, 10299 peer-reviewed scientific articles were retrieved using the aforementioned search strings (Fig. S1 in Appendix A). After thoroughly reviewing the abstracts and full texts, we deemed 154 publications, comprising a total of 1145 data entries, to create our meta-dataset (Appendix B). We did not request the unpublished datasets from colleagues due to the risk of biasing the estimates of effect size [65]. We ensured that the screening process was highly reproducible (Note 2 in Appendix A).

2.2. Selection criteria

To ensure the relevance and rigor of the meta-analysis, the following selection criteria were applied: ① the article must be a study-based research (not a meta-analysis, a review, or case study) involving non-human animals and written in English; ② peer-reviewed studies involving controlled experiments with genetically engineered or edited animals were included, while observational or theoretical studies were excluded; ③ recruited articles must have investigated quantitative data on at least one trait performance metric *in vivo*, such as growth, disease resistance, or fatty acid content in wild-type (WT, non-transgenic or non-edited) and treated (transgenic or gene-edited) groups, in order to calculate effect sizes.

2.3. Information extraction and moderators

To determine gene/protein expression and quantify the evidence of improved trait performance (growth, disease resistance, or fatty acid) through TG or GE in animals, specific data were extracted from selected studies. This process included the following details: author information (first author and year of publication), article title, taxon, species/breed within each taxon, method used (TG or GE), target gene, and trait of interest, as well as sample size, means and standard deviation (SD) or standard error (SE) of the outcome data in WT and treated groups (Appendix B). Where figures were the only source of data, we employed ImageJ.JS to derive the mean and SD or SE.

To investigate the influence of methodological moderators on trait performance (Note 3 in Appendix A), we initially categorized the dataset into two groups based on the method applied (TG or GE) using a method-moderator analysis. Moderator analyses were then conducted separately within each group. To understand how biological moderators affect trait enhancement, we compiled data on various biological variables from the 154 studies included in our meta-analysis. First, we evaluated the overall effects of TG and GE on target gene or protein expression. For each study, we captured information on the taxon, species or breed, target gene, trait, and metrics for gene or protein expression. As is well-established, TG typically involves integrating transgenes into the host genome, often under the control of specific promoters to drive overexpression [7]. Furthermore, gene or protein expression often displays tissue-specific patterns, as described in individual studies [64]. Consequently, we conducted moderator analyses stratified by taxon, species, gene, trait, promoter, and tissue, in order to identify significant variations in gene or protein expression. We conducted parallel analyses for the GE group, excluding promoter-moderator analyses, to evaluate the effects of these moderators on gene/protein expression.

With respect to growth traits, various measured parameters were considered, including body weight (e.g., mean weight, average weight, carcass weight, wet weight, mean mass, live weight, and dressing percentage), condition factor (e.g., condition score), feed conversion efficiency (FCE), myofiber number (e.g., fiber cell number, myofiber nuclei number, total number of fibers, percentage of fibers, and myofiber density), and myofiber size (e.g., mean muscle fiber cross-sectional area, muscle fiber area, area of fiber cells, and mean/total area of fibers). Additional measurements included plasma GH (e.g., concentration of GH), SGR (e.g., average growth speed, standard growth rate, and percentage of body weight increase), and weight gain (e.g., percentage weight gain and daily weight gain). The disease resistance traits were measured using various parameters, including antibody response, cumulative survival rate (CSR), lysozyme activity, pathogen load (e.g., number of virus-positive cells, colony-forming units, viral growth, number of surviving bacteria, and viral particle production), phagocytic activity, sign score (e.g., clinical and pathological sign scores), virus RNA (e.g., relative virus RNA expression, viral nucleic acid, viral RNA fold change, viral RNA load level, and viral RNA copies), and virus titer (Log_{10}(tissue culture infectious dose 50% ($TCID_{50}$)), mean viral shedding titer and number of plaques). Similarly, fatty acid traits were captured by measuring multiple parameters, including the content of DHA, EPA, ω-3, ω-6, and the ratio of ω-6 to ω-3 (ω-6/ω-3) (Note 4 in Appendix A).

2.4. Effect-size calculations

The goal of the study was twofold: first, to determine trait enhancement in transgenic and gene-edited animals relative to WT individuals; and second, to compare the effects of TG and GE on trait enhancement across various parameters in animals. All outcome parameters were continuous variables, and sample sizes were not identical between WT and transgenic or gene-edited groups. We employed random-effect models to calculate the effect size for the meta-analysis using the heteroscedastic standardized mean difference (SMD) [66] (Note 5 in Appendix A). We estimated the overall mean effect sizes with 95% confidence intervals (CIs) and 95% prediction intervals (PIs) using the rma.mv function from the metafor package [67]. This enabled comparison of WT versus transgenic or gene-edited groups across parameters for each trait performance. Mean effect size estimates and their 95% CIs were reported, unless otherwise specified. Furthermore, the SMD was interpreted using a rule of thumb similar to Cohen's *d*, but with some modifications: $0 < |SMD| \leq 0.5$: small effect; $0.5 < |SMD| \leq 0.8$: medium effect; $|SMD| > 0.8$: large effect [68].

2.5. Moderator analysis

First, we created a null model to determine the overall effect of TG or GE on enhancing animal traits. We included the effect size in the null model, as well as the random effects of effect size ID, study ID, and species name, in order to control the non-independence of effect sizes. We used the *I*-squared statistic (I^2) [69] to calculate the percentage of variance due to inconsistencies in the population effect across studies. $I^2 > 50\%$ indicated significant between-study heterogeneity. We also quantified the partial heterogeneity explained by each potential moderator using the i2_ml function from the orchard package [70]. The full model included the following moderators as random effects: taxon, parameter, method, species, gene, promoter, tissue, pathogen, generation, and phylogenetic relatedness. Additionally, we constructed a variance–covariance (VCV) matrix of species-phylogenetic relatedness to control for non-independent variation from the same study individuals in each dataset. We first conducted meta-regressions (full models) to determine the effect of moderators on trait enhancement (growth, disease resistance, and fatty acid content) led by TG and GE. We then established several meta-regressions to investigate the effect of each moderator (Note 6 in Appendix A). We calculated the Cochran's *Q* (Q_M) and the proportion of the total heterogeneity explained by the moderators (marginal R^2), using the r2_ml function in the orchard package [70].

Specifically, we initially performed a method-moderator analysis to compare the effects of different methods (TG vs GE) on trait enhancement in animals. This analysis incorporated all parameters for each trait performance. Subsequent moderator analyses were then conducted based on specific traits. For gene/protein expression, we assessed the overall effects of TG and GE using a global model based on a method-moderator test (all data). We also conducted a taxon-moderator analysis to assess expression profiles across taxa for both TG and GE. Differences in gene/protein expression were examined across traits using trait-specific moderator analyses. In addition, gene- and tissue-moderator analyses were performed on both the TG and GE datasets to investigate whether the expression of messenger RNA (mRNA) and protein was gene- and tissue-specific. Likewise, several moderators were analyzed in the growth trait, where a method-moderator analysis was used to compare the effects of TG and GE on enhancing growth trait across different parameters. This was followed by taxon- and gene-moderator analyses to determine the effects across taxonomic classes and genes. Gene- and promoter-moderator analyses identified the most promising candidate transgenes (coupled with promoters) and innate growth-regulating genes.

In terms of disease resistance and fatty acid traits, the positive and negative effect sizes jointly led to a null effect result when we combined all parameter datasets from TG and GE. To address this, we first conducted a parameter-moderator analysis to compare the effects of TG and GE on individual traits across

parameters. We then conducted a taxon-moderator analysis for disease resistance, assessing the impact of TG or GE on CSR, virus RNA, pathogen load, and virus titer across taxonomic classes. Gene- and promoter-moderator analyses were also performed to confirm the valid candidate transgenes (coupled with promoters) and innate genes for disease resistance enhancement. Furthermore, we evaluated the effects of TG and GE on improving disease resistance through pathogen-moderator analysis. For the fatty acid traits, the available dataset of GE was limited to a single study by Park et al. [71]. All moderator analyses were carried out using the TG dataset without a method-moderator analysis being conducted. Taxon-moderator analyses were performed across parameters, such as ω-3, ω-6, and the ratio of ω-6/ω-3, as well as DHA and EPA, to evaluate the effect of TG on various fatty acid traits. Based on gene-moderator analyses, the most effective candidate transgenes for fatty acid enhancement were proposed. Finally, generation-moderator analyses were performed for growth, disease resistance, and fatty acid traits, investigating whether improved traits caused by TG or GE were similarly effective in the offspring. Given the high degree of partial heterogeneity observed in these two traits, meta-regressions were generated using the metafor package to investigate how age influenced the effects of TG and GE on growth and fatty acid traits.

2.6. Publication bias

To assess publication bias, funnel plots were generated, and all estimates were subjected to a classic Egger's regression test (weighted regression with multiplicative dispersion) to check for funnel plot asymmetry [72]. A *P*-value of less than 0.05 implies that the funnel plot is asymmetric and that publication bias may be present. In this case, the "trim and fill" approach [73] was used to determine whether additional studies needed to be generated at random to reduce potential publication bias. Finally, we tested for a time-lag bias in each trait to determine whether effect sizes decline in more recent publications [72].

2.7. Sensitivity analysis

We identified potential outliers and influential observations of the estimated measures across studies using sensitivity analyses to assess the stability and reliability of our meta-analysis. We performed a "leave-one-out" analysis for each trait, consecutively removing one study from the dataset each time and recalculating the global meta-analytic mean and 95% CIs [74].

3. Results

3.1. Data overview

The current meta-dataset comprises 171 figures extracted from 154 studies (86 on TG and 68 on GE), which provide relevant data on the impact of genetic engineering on trait performance. In total, 1145 effect sizes (SMD) were obtained across ten taxonomic classes and 72 species/breeds. The majority of studies were conducted on livestock and ray-finned fish (77 and 68 studies, respectively) (Figs. 1(a) and (b)). Of the 55 genes linked to enhanced target traits, 23 were associated with improved growth traits (15 for TG and eight for GE), 27 with enhanced disease resistance (18 for TG and nine for GE), and five with altered fatty acid content (four for TG and one for GE) (Fig. 1(c)).

3.2. Effects of TG/GE on gene/protein expression

First, we assessed the expression profiles (both mRNA and protein) of target transgenes and innate genes at a global level by combining all the data. Our multivariate meta-analysis model showed a moderate effect of TG/GE on gene/protein expression (mean [95% CI]: 0.376 [−0.536 to 1.289], t_{98} = 0.819, P = 0.4149). The dataset exhibited high heterogeneity (I^2 = 100%), with 38% attributed to differences between studies, and 17.2% to differences in effect sizes. Notably, most of the effect sizes were derived from fish and pig species. Phylogenetic relatedness (Fig. 2(a)) explained 44.8% of the heterogeneity, which suggests that there is a phylogenetic effect on gene/protein expression.

Obviously, TG and GE were found to significantly induce (mean [95% CI]: 4.876 [3.590 to 6.163], P < 0.0001) and reduce (mean [95% CI]: −3.990 [−5.789 to −2.192], P < 0.0001) mRNA/protein expression, respectively (Fig. 2(b)). When effects were averaged across all taxa, taxonomic class explained a large proportion of heterogeneity (R^2 = 0.723, Q_M = 4.763, P = 0.9421, df = 11). Specifically, TG was found to significantly increase mRNA levels and protein expression of the transgenes across different taxa (pig, mean [95% CI]: 5.653 [3.541 to 7.764], P < 0.0001 > sheep, mean [95% CI]: 4.953 [−0.542 to 10.447], P = 0.0773 > fish, mean [95% CI]: 3.336 [0.686 to 5.985], P = 0.0136) (Fig. 2(c), Fig. S2(a) in Appendix A). This was observed in all three traits, including growth (mean [95% CI]: 4.701 [0.812 to 8.590], P = 0.0178), disease resistance (mean [95% CI]: 5.387 [2.951 to 7.823], P < 0.0001), and fatty acid content (mean [95% CI]: 4.072 [1.007 to 7.137], P = 0.0092) (Fig. 2(d)). The expression of transgenes and proteins was found to be gene- (R^2 = 0.472, Q_M = 61.667, P < 0.0001, df = 10), promoter- (R^2 = 0.265, Q_M = 22.140, P = 0.0011, df = 6), and tissue-specific (R^2 = 0.263, Q_M = 25.210, P = 0.1537, df = 19) (Figs. S2(b)–(d) in Appendix A). Compared to other promoters, the H1 promoter exhibited the greatest induction of gene expression (mean [95% CI]: 12.618 [2.662 to 22.574], P = 0.0130), followed by the β-actin and U6 promoters (mean [95% CI]: 7.612 [4.258 to 10.967], P < 0.0001 for β-actin; mean [95% CI]: 4.562 [−4.114 to 13.239], P = 0.0863 for U6), while cytomegalovirus (CMV) and ubiquitin (UBI) promoters showed similar effects on gene expression (Fig. S2(e) in Appendix A). At the global level, these transgenes showed consistent expression in both the Parental 1 (P1) and Filial 1 (F_1) generations (Fig. S2(f) in Appendix A).

GE had a comparable effect on gene/protein expression to TG, as indicated by the similar absolute values of the overall effect size (3.91 vs 4.60) (Fig. 2(b)). GE effectively disrupted gene/protein expression (Fig. S3(a) in Appendix A), with significant downregulation of target innate genes/proteins observed in both fish and pigs (mean [95% CI]: −2.834 [−5.238 to −0.430], P = 0.0209 for fish; mean [95% CI]: −3.692 [−5.922 to −1.462], P = 0.0012 for pigs) (Fig. 2(c)). Similar to TG, moderator analysis showed that expression of the edited innate genes was gene- (R^2 = 0.007), species- (R^2 = 0.519), and tissue-specific (R^2 = 0.821) (Figs. S3(b)–(d) in Appendix A). Although 18 genes were edited across 42 animal species/breeds, the expression data primarily related to growth and disease resistance traits (Fig. 2(d)), particularly for *mstn*, insulin-like growth factor 2 (*IGF2*), leptin receptor (*LepR*), and *CD163* (Figs. S3(c) and (e) in Appendix A), while expression data for other genes were rarely recorded.

3.3. Effects of TG/GE on growth

According to our meta-analysis, altered gene/protein expression resulting from TG or GE led to enhanced trait performance, though these improvements were not consistent across traits and taxa. With respect to growth, our full model, which included all moderators and parameters, revealed significant effects of TG/GE, (mean [95% CI]: 0.337 [0.230 to 0.444], t_{499} = 6.213, P < 0.0001). Heterogeneity in this dataset was high (I^2 = 99.8%), with 16.8% attributed to true differences between studies, and 48.2% to differences in

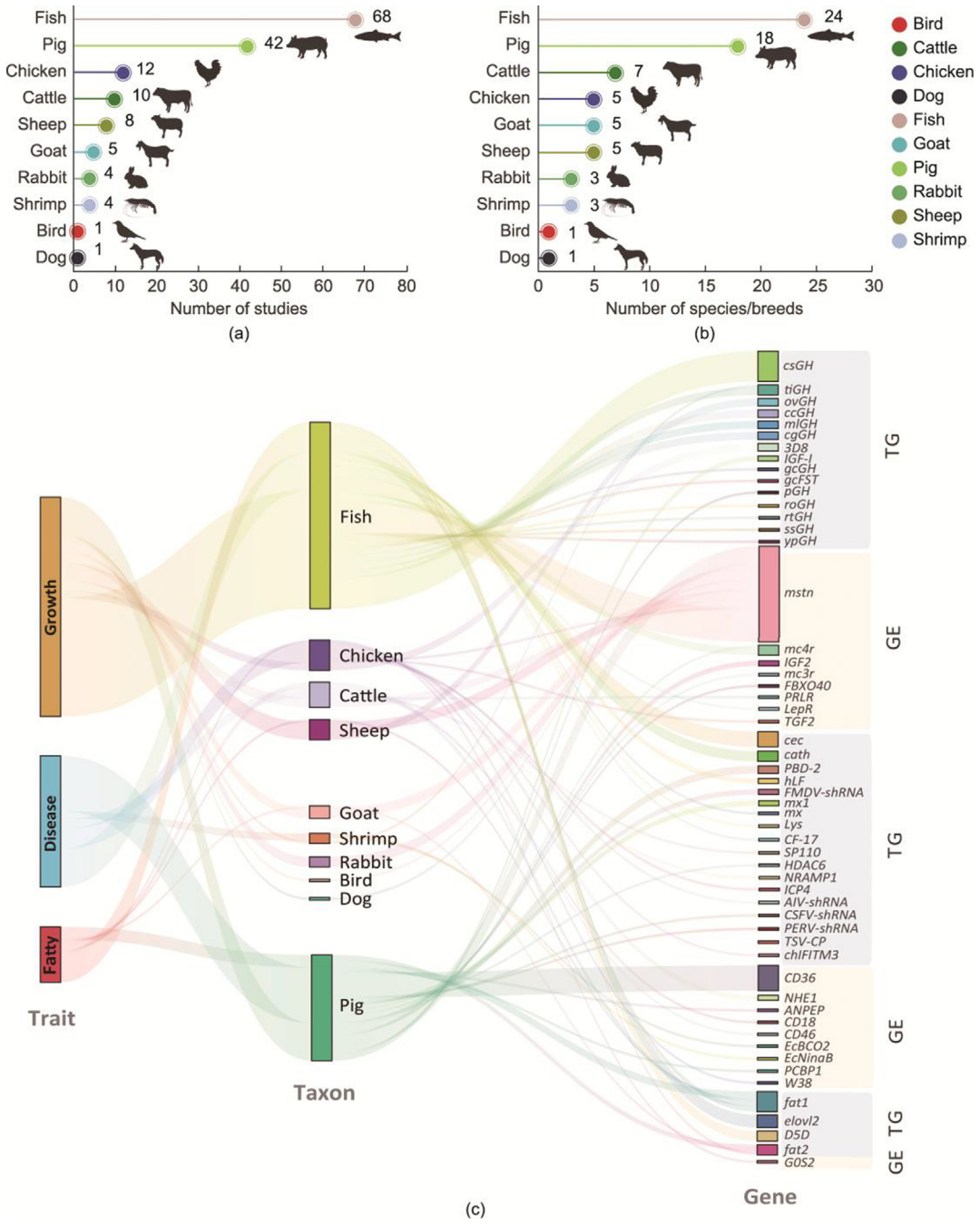

Fig. 1. A summary of the current applications of TG and GE for trait enhancement in farmed animals. (a) The number of studies focuses on trait enhancement (growth, disease resistance, and fatty acids) across taxa using TG and GE. (b) The number of species/breeds involved in each taxonomic class. (c) The Sankey diagram illustrates the proportion of studies focusing on different traits per taxon, and the target transgenes or innate genes are listed in the right column. *csGH*: coho salmon growth hormone; *tiGH*: tilapia growth hormone; *ovGH*: ovine growth hormone; *ccGH*: channel catfish growth hormone; *mlGH*: mud loach growth hormone; *cgGH*: *Clarias gariepinus* growth hormone; *gcGH*: grass carp growth hormone; *gcFST*: grass carp follistatin; *pGH*: porcine growth hormone; *roGH*: rohu growth hormone; *rtGH*: rainbow trout growth hormone; *ssGH*: Atlantic salmon growth hormone; *mc4r*: melanocortin-4 receptor; *IGF2*: insulin-like growth factor 2; *mc3r*: melanocortin-3 receptor; *FBXO40*: fox box protein 40; *PRLR*: prolactin receptor; *LepR*, leptin receptor; *TGFβ2*: growth factor-beta 2; *cec*: cecropin; *cath*: cathelicidin; *PBD-2*: porcine beta-defensin 2; *hLF*: human lactoferrin; *FMDV-shRNA*: small hairpin RNA targeting foot-and-mouth disease virus; *mx*: myxovirus; *Lys*: lysozyme; *CF-17*: synthetic cecropin B analog; *SP110*: SP110 nuclear body protein; *HDAC6*: histone deacetylase 6; *NRAMP1*: natural resistance-associated macrophage protein-1; *ICP4*: infected-cell polypeptide-4; *AIV-shRNA*: small hairpin RNA targeting avian influenza virus; *CSFV-shRNA*: small hairpin RNA targeting classical swine fever virus; *PERV-shRNA*: small hairpin RNA targeting porcine endogenous retrovirus; *TSV-CP*: taura syndrome virus coat protein; *chIFITM3*: chicken interferon-induced transmembrane 3; *NHE1*: chicken ALV-J receptor Na^+/H^+ exchanger type 1; *ANPEP*: amino peptidase N; *EcoBCO2*: β-carotene 9'/10'-oxygenase; *EcNinaB*: carotenoid isomerooxygenase; *PCBP1*: poly-binding protein 1; *W38*: amino acid tryptophan 38; *D5D*: delta5-desaturase; *fat2*: humanized *Caenorhabditis elegans* gene 2; *G0S2*: G0/G1 switch gene 2.

effect sizes. Phylogenetic relatedness explained a further 34.9% of the heterogeneity (Fig. 3(a)).

Significant positive effects on growth enhancement were observed with both TG and GE, either through the integration of foreign genes or the disruption of innate growth-regulating genes (mean [95% CI]: 1.626 [0.679 to 2.572], k = 172, P = 0.0008 for TG; mean [95% CI]: 2.009 [1.141 to 2.877], k = 343, P < 0.0001 for GE) (Fig. S4(a) in Appendix A). This improvement in growth traits

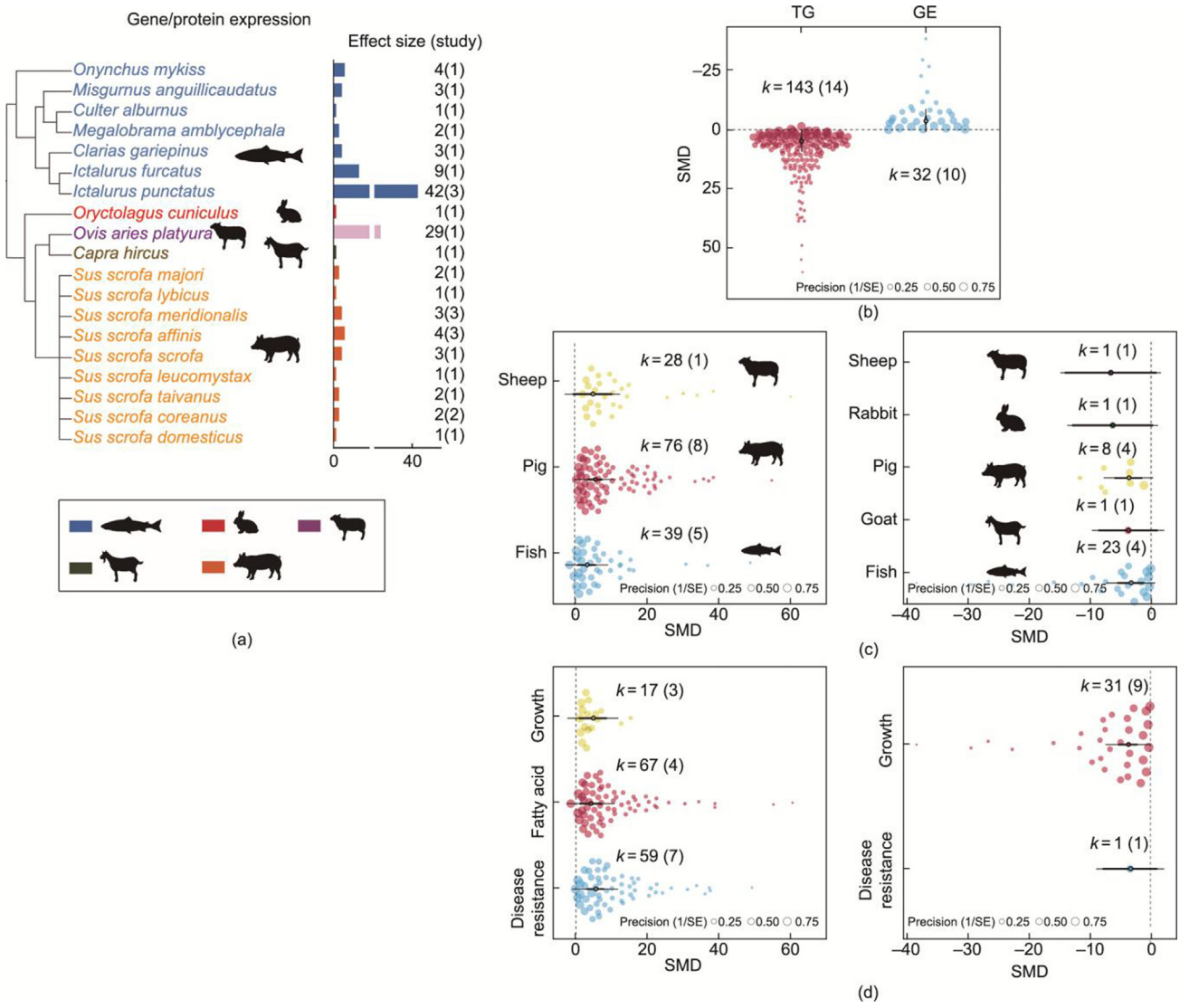

Fig. 2. Significant induction of transgenes and inhibition of innate genes were observed across taxonomic classes and traits. (a) Phylogenetic tree shows all species including in the gene/protein expression meta-dataset. Colors represent taxonomic class. The bars and the numbers in the first column represent the number of effect sizes, followed by the number of studies in brackets. (b) Overall effects of TG and GE on gene/protein expression in the global model, based on a method-moderator analysis. (c) Effect of TG and GE on gene/protein expression for each taxon. (d) Effect of TG and GE on gene/protein expression for each trait. The *x*-axis shows the values of effect sizes as a heteroscedastic SMD, and the *y*-axis represents the density distribution of the effect sizes. The size of the dots represents the precision of each effect size (1/SE). Thick black whisker lines and thin black lines represent 95% CIs and 95% PIs, respectively. A bold error bar (95% CI) indicates whether the overall effect size is significantly ($P < 0.05$) different from zero (i.e., not overlapping zero). *k* is the number of effect sizes and the number of studies (the number in brackets).

was confirmed by changes in several parameters, including increased body weight, weight gain, SGR, plasma GH, and decreased FCE (Fig. 3(b)). Although large, significant effects were detected in the P1 generation, the F1 generation inherited these effects with smaller effect sizes than their parents (mean [95% CI]: 4.465 [2.706 to 6.225], k = 48, $P < 0.0001$ for P1; mean [95% CI]: 1.205 [−0.507 to 2.918], k = 124, $P = 0.1677$ for F1) (Fig. S4 (b) in Appendix A). Taxon explained only a small proportion of the heterogeneity ($R^2 = 0.021$, $Q_M = 1.528$, $P = 0.6757$, $df = 3$), and significant positive effects of TG on growth were primarily observed in fish (mean [95% CI]: 1.982 [0.207 to 3.756], k = 172, $P = 0.0287$). Although small and large effects were noted in TG pigs and sheep, respectively, these differences were not statistically significant compared to the WT group (mean [95% CI]: −0.254 [−6.099 to 5.591], k = 9, $P = 0.9320$ for pigs; mean [95% CI]: 3.998 [−1.947 to 9.942], k = 23, $P = 0.1875$ for sheep) (Fig. S4(c) in Appendix A). In addition, nine (*mlGH*, *cgGH*, *ovGH*, *roGH*, *csGH*, *ssGH*, *tiGH*, *gcFST*, and *rtGH*) out of 15 GH transgenes showed large positive effects on growth enhancement, with *mlGH*, *cgGH*, and *ovGH* being the most impactful (Fig. S4(d) in Appendix A).

Compared to TG, GE had a greater impact on growth enhancement, as reflected by a larger effect size (2.01 vs 1.63) (Fig. S4(a) in Appendix A). GE significantly improved growth traits with large effects in five of ten taxonomic classes, including cattle, fish, goats, pigs, and sheep (mean [95% CI]: 2.007 [0.386 to 3.628], k = 14, $P = 0.0152$ for cattle; mean [95% CI]: 1.898 [1.221 to 2.575], k = 113, $P < 0.0001$ for fish; mean [95% CI]: 2.073 [1.204 to 2.943], k = 25, $P < 0.0001$ for goats; mean [95% CI]: 1.865 [1.054 to 2.676], k = 56, $P < 0.0001$ for pigs; mean [95% CI]: 3.023 [1.525 to 4.521], k = 15, $P < 0.0001$ for sheep). Inexplicably, GE showed an insignificant effect on chicken growth (mean [95% CI]: 0.051 [−1.932 to 2.033], $P = 0.9601$), despite a large sample size ($k = 46 > 30$) being collected (Fig. S4(e) in Appendix A). Compared to TG, GE had a broader impact on growth traits, significantly improving a wider range of growth parameters. These included condition factor (mean [95% CI]: 3.098 [2.527 to 3.670], k = 13,

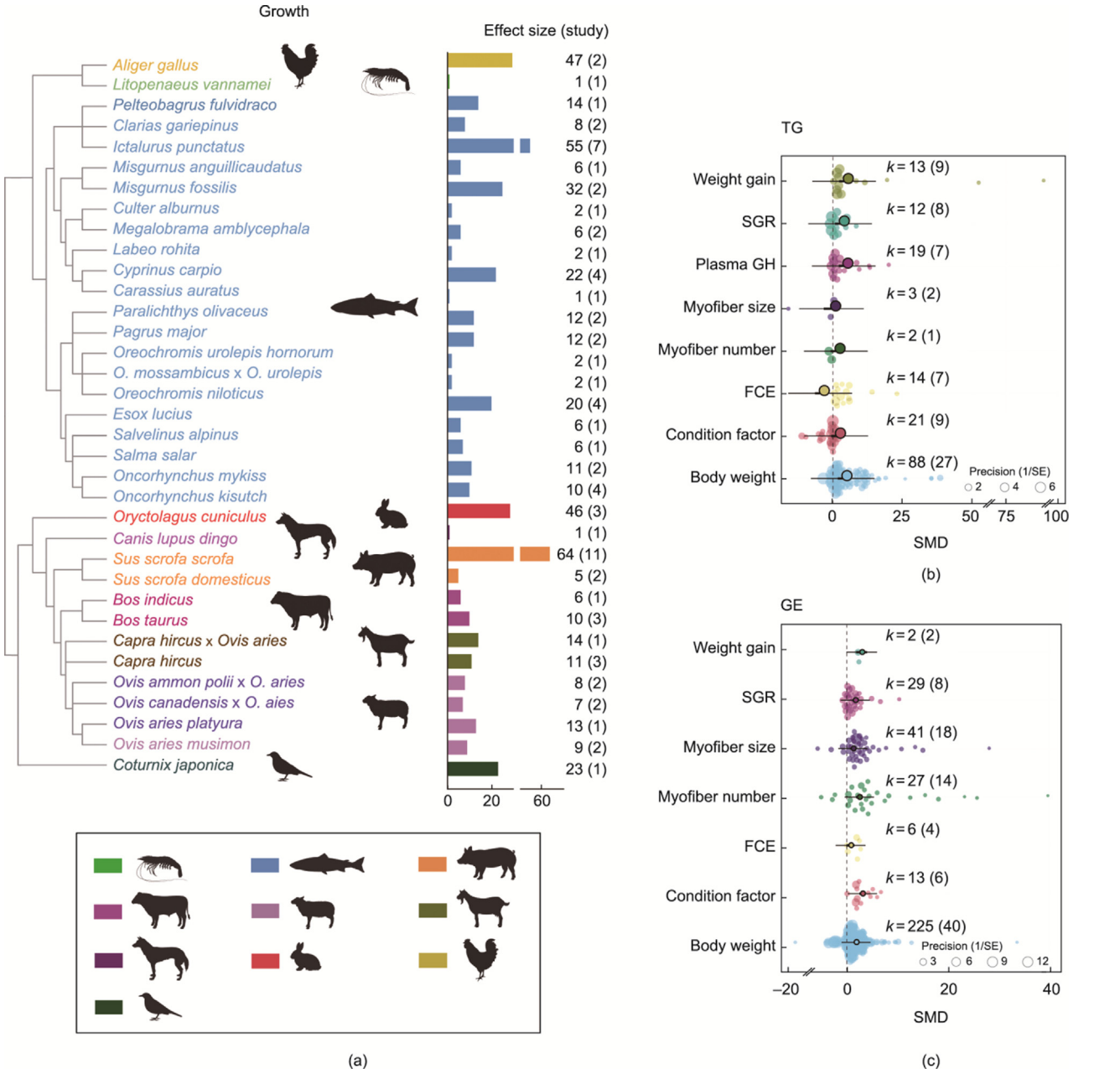

Fig. 3. Comparison of the effects of TG and GE on growth in farmed animals across various parameters. (a) Phylogenetic tree shows all species including in the growth meta-dataset. Colors represent taxonomic class. The bars and the numbers in the first column represent the number of effect sizes, followed by the number of studies in brackets. (b) The effect of TG on individual growth traits in 19 species/breeds in the meta-dataset. (c) The effect of GE on individual growth traits in 38 species/breeds. The *x*-axis shows the values of effect sizes as a heteroscedastic SMD, and the *y*-axis represents the density distribution of the effect sizes. The size of the dots represents the precision of each effect size (1/SE). Thick black whisker lines and thin black lines represent 95% CIs and 95% PIs, respectively. A bold error bar (95% CI) indicates whether the overall effect size is significantly ($P < 0.05$) different from zero (i.e., not overlapping zero). *k* is the number of effect sizes and the number of studies (the number in brackets).

$P < 0.0001$), myofiber number (mean [95% CI]: 2.493 [1.869 to 3.118], $k = 27$, $P < 0.0001$), and myofiber size (mean [95% CI]: 1.247 [0.685 to 1.808], $k = 41$, $P < 0.0001$), in addition to body weight, weight gain, and SGR. However, the meta-analysis indicated that GE only had a moderate, non-significant effect on FCE (mean [95% CI]: 0.759 [−0.042 to 1.561], $k = 6$, $P = 0.0634$) (Fig. 3(c)). Although a total of eight growth-regulating genes were targeted by GE tools to increase growth in farmed animals, the effects varied across studies. Encouragingly, disruption of *mstn*, melanocortin 4 receptor (*mc4r*), or *IGF2* showed a large, significant positive effect on growth, followed by prolactin receptor (*PRLR*), *LepR*, and melanocortin 3 receptor (*mc3r*). There were no significant general effects on animal growth when the growth factor-beta 2 (*TGFβ2*) or fox box protein 40 (*FBXO40*) was edited (Fig. S4(f) in Appendix A).

3.4. Effects of TG/GE on disease resistance

We did not find a significant general effect of TG/GE on disease resistance using a full model (including all moderators and parameters; mean [95% CI]: −0.373 [−0.887 to 0.140], $t_{235} = -1.433$, $P = 0.1531$). Heterogeneity in this dataset remained still high ($I^2 = 100\%$), with 41.3% attributed to true differences between studies, and 15.8% to variations in effect sizes. Phylogenetic relatedness explained an additional 42.8% of the heterogeneity (Fig. 4(a)).

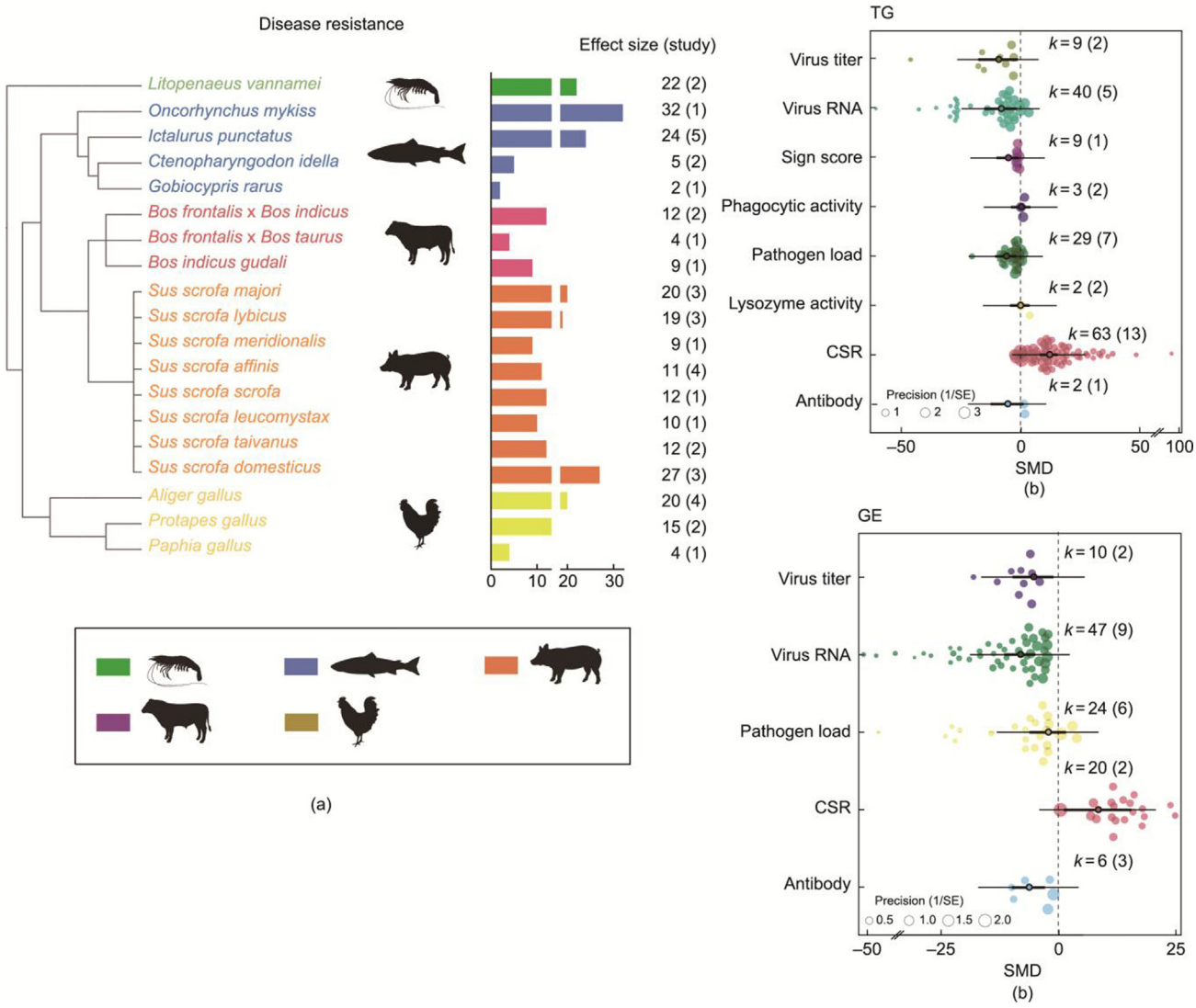

Fig. 4. Comparison of the effects of TG and GE on disease resistance in farmed animals across various parameters. (a) Phylogenetic tree shows all species including in the disease resistance meta-dataset. Colors represent taxonomic class. The bars and the numbers in the first column represent the number of effect sizes, followed by the number of studies in brackets. (b) The effect of TG on disease resistance traits in 16 species/breeds. (c) The effect of GE on disease resistance traits in seven species/breeds. The *x*-axis shows the values of effect sizes as a heteroscedastic SMD, and the *y*-axis represents the density distribution of the effect sizes. The size of the dots represents the precision of each effect size (1/SE). Thick black whisker lines and thin black lines represent 95% CIs and 95% PIs, respectively. A bold error bar (95% CI) indicates whether the overall effect size is significantly ($P < 0.05$) different from zero (i.e., not overlapping zero). *k* is the number of effect sizes and the number of studies (the number in brackets).

A total of eight parameters (Appendix B) related to disease resistance trait were collected in our dataset. Parameters explained a large proportion of the significant heterogeneity (R^2 = 0.564, Q_M = 52.164, $P < 0.0001$, df = 7). Parameter-moderator analysis revealed that TG exerted various effects (both positive and negative) on these parameters, resulting in a non-significant pooled effect size (k = 157, P = 0.0981). In this case, we first determined the different effects of TG on various parameters, including significant positive effects on CSR (mean [95% CI]: 11.928 [8.216 to 15.640], k = 63, $P < 0.0001$), and negative effects on pathogen load (mean [95% CI]: –6.191 [–10.671 to –1.710], k = 29, P = 0.0068), sign score (mean [95% CI]: –5.425 [–10.051 to –0.780], k = 9, P = 0.0215), virus RNA (mean [95% CI]: –8.398 [–15.212 to –1.584], k = 40, P = 0.0157), and virus titer (mean [95% CI]: –9.483 [–17.806 to –1.160], k = 9, P = = 0.0255). However, TG is less likely to affect the antibody response (mean [95% CI]: – 5.650 [–12.568 to 1.268], k = 2, P = 0.1094), lysozyme activity (mean [95% CI]: – 0.299 [–4.380 to 4.152], k = 2, P = 0.8859), and phagocytic activity (mean [95% CI]: 0.0641 [–4.024 to 4.152], k = 3, P = 0.0001) (Fig. 4(b)).

Improvements in disease resistance through TG varied depending on the specific parameters assessed and the taxonomic class. Specifically, we observed a significant increase in CSR in fish (Fig. S5(a) in Appendix A), a substantial reduction in virus RNA levels in pigs (Fig. S5(b) in Appendix A), and a significant decrease in pathogen load in both chickens and cattle (Fig. S5(c) in Appendix A). Regarding virus titers, our dataset reported a total of nine effect sizes from chicken and pig, showing no general reduction in virus titers for either species (Fig. S5(d) in Appendix A). Our findings suggest that different transgenes, in combination with various promoters, have diverse effects on enhancing disease resistance traits. The top five genes [synthetic cecropin B analog (*CF-17*), myxovirus (*mx*), cathelicidin (*cath*), cecropin (*cec*), and natural resistance-associated macrophage protein-1 (*NRAMP1*)] (Figs. S5

(e) and (f) in Appendix A) driven by the most appropriate promoters (UBI, β-actin, CMV, or U6) had the greatest effect on improving disease resistance (Figs. S5(g) and (h) in Appendix A).

GE appeared to have a greater impact on disease resistance trait than TG at a global level, as evidenced by a larger absolute effect size (mean [95% CI]: 1.268 [−2.061 to 4.597], k = 157, P = 0.4554 for TG; mean [95% CI]: −3.912 [−8.080 to 0.257], k = 107, P = 0.0659 for GE) (Fig. S6(a) in Appendix A). Not only did GE significantly enhance CSR post-pathogen infection (mean [95% CI]: 8.355 [1.154 to 15.556], k = 20, P = 0.0230), it also reduced virus RNA or titer levels (mean [95% CI]: − 8.234 [−11.557 to −4.911], k = 47, P < 0.0001 for viral RNA; mean [95% CI]: −5.414 [−9.790 to −1.039], k = 10, P = 0.0153 for viral titer), but it also significantly inhibited antibody responses (mean [95% CI]: − 6.400 [−9.923 to −2.878], k = 6, P = 0.0004). This result was not present in the TG dataset (Fig. 4(c)). Furthermore, the effects of GE on enhancing disease resistance varied across taxa (R^2 = 0.678, Q_M = 38.560, P < 0.0001, df = 3). For instance, a significant reduction in viral RNA levels was observed in pigs, but not in chickens or cattle (Fig. S6(b) in Appendix A), with the poly-binding protein 1 (*PCBP1*) and *CD163* genes being found to contribute to these effects (Fig. S6 (c) in Appendix A). Notably, both TG and GE showed variability in their effects across different pathogens (Figs. S6(d) and (e) in Appendix A), with the greatest inhibitory effects being observed for bacteria, followed by viruses and parasites.

3.5. Effects of TG/GE on fatty acid

The effects of TG or GE on fatty acid composition showed no significant overall impact when using a full model that accounted for all moderators and parameters (mean [95% CI]: −0.09 [−0.392 to 0.218], t_{140} = −0.565, P = 0.5732). However, a high level of heterogeneity (I^2 = 99.8%) was detected, with 12.6% attributed to true differences between studies and 87.2% to differences between genes. We did not find any phylogenetic relatedness (Fig. 5(a)), which indicates that the phylogenetic structure does not contribute to the observed variation in effect sizes.

A large proportion of heterogeneity was observed in both taxon and gene when the effects were averaged across all parameters (R^2_{taxon} = 0.591, R^2_{gene} = 0.575). We performed moderator analyses for each parameter, both for taxa and genes. Although previous studies have reported significant improvements in ω-3, DHA, EPA, and reductions in ω-6 and the ω-6/ω-3 ratio, our meta-analytic findings showed significant effects on ω-3, DHA, and ω-6/ω-3 (mean [95% CI]: 0.584 [−0.009 to 1.177], k = 26, P = 0.0537 for ω-3; mean [95% CI]: 0.792 [0.201 to 1.383], k = 29, P = 0.0086 for DHA; mean [95% CI]: −2.073 [−2.909 to −1.237], k = 32, P < 0.0001 for ω-6/ω-3), with no significant effect observed for EPA or ω-6 (mean [95% CI]: 0.342 [−0.246 to 0.930], k = 30, P = 0.2545 for EPA; mean [95% CI]: −0.471 [−1.059 to 0.117], k = 24, P = 0.1165 for ω-6) when all data were combined globally (Fig. 5(b)). These effects were taxon-specific across different parameters. The largest positive effect of TG on ω-3 was observed in cattle, followed by pigs and fish (mean [95% CI]: 4.671 [0.767 to 8.576], k = 4, P = 0.0190 for cattle; mean [95% CI]: 4.065 [0.657 to 7.474], k = 6, P = 0.0194 for pigs; mean [95% CI]: 1.186 [−0.611 to 2.984], k = 14, P = 0.1959 for fish) (Fig. S7(a) in Appendix A). Similarly, the largest effects on ω-6 and ω-6/ω-3 were found in cattle, while the largest effects on DHA and EPA were found in pigs. Nevertheless, we found no significant effect of TG on the levels of ω-3 in goats (mean = 0.099, k = 1, P = 0.9696), ω-6/ω-3 in fish (mean = 0.797, k = 2, P = 0.8845), or DHA in cattle (mean = 1.571, k = 3, P = 0.2560) (Figs. S7(b)–(e) in Appendix A). Different transgenes appear to play distinct roles in enhancing fatty acid traits. Integration of the *fat1* transgene significantly improved levels of ω-3, DHA, and EPA (Figs. S8(a)–(c) in Appendix A), while decreasing ω-6 and ω-6/ω-3 (Figs. S8(d) and (e) in Appendix A). In contrast, the transfer of the humanized *Caenorhabditis elegans* gene 2 (*fat2*) transgene did not significantly increase ω-3 level or reduce ω-6 level, but it dramatically lowered the ω-6/ω-3 ratio. However, the *elovl2* (k = 6 for ω-3, k = 5 for ω-6, k = 15 for DHA, k = 15 for EPA, all P > 0.05) and delta5-desaturase (*D5D*) transgenes (k = 6 for ω-3, k = 6 for ω-6, k = 6 for DHA, k = 6 for EPA, all P > 0.05) were less likely to alter fatty acid traits across all taxonomic classes (Fig. S8 in Appendix A).

To date, only one gene-editing study involving one gene G0/G1 switch gene 2 (*G0S2*) with two effect sizes has been reported for the enhancement of fatty acid in chickens [71]. Although the study reported significant improvements in ω-3 and ω-6 levels, we found

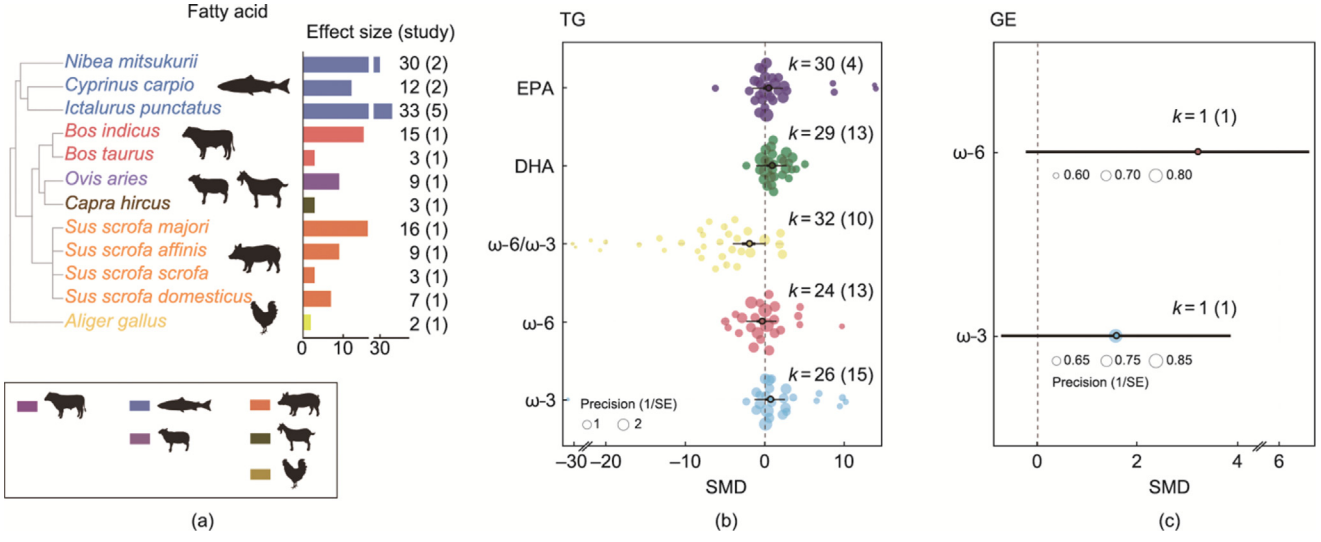

Fig. 5. Comparison of the effects of TG and GE on fatty acid in farmed animals across various parameters. (a) Phylogenetic tree shows all species including in the fatty acid meta-dataset. Colors represent taxonomic class. The bars and the numbers in the first column represent the number of effect sizes, followed by the number of studies in brackets. (b) The effect of TG on fatty acid traits in ten species/breeds. (c) The effect of GE on fatty acid traits in the White Leghorn chicken. The *x*-axis shows the values of effect sizes as a heteroscedastic SMD, and the *y*-axis represents the density distribution of the effect sizes. The size of the dots represents the precision of each effect size (1/SE). Thick black whisker lines and thin black lines represent 95% CIs and 95% PIs, respectively. A bold error bar (95% CI) indicates whether the overall effect size is significantly (P < 0.05) different from zero (i.e., not overlapping zero). k is the number of effect sizes and the number of studies (the number in brackets).

no significant effects on fatty acid alteration in *G0S2*-KO chickens compared to WT individuals (k = 1, P = 0.1813 for ω-3; k = 1, P = 0.1681 for ω-6) (Fig. 5(c)). Furthermore, we found evidence of an age-dependent effect of TG and GE on growth traits. The effect of transgenes or edited genes on growth enhancement decreased with age. This result was primarily evident in SGR and myofiber (number and size), but not body weight (Fig. 6(a)). In addition, the effects of TG and GE on fatty acids diminished with advancing age, as demonstrated by reduced effects on DHA+EPA, ω-3, ω-6, and ω-3/ω-6 (Fig. 6(b)).

3.6. Publication bias

Based on our results, we found little evidence of publication bias for gene/protein expression (t_{173} = 0.582, P = 0.5613) and fatty acid traits (t_{133} = 0.243, P = 0.8083). However, the funnel plots and Egger's regression test indicated potential publication bias for the effect sizes of growth (t_{470} = 5.274, P < 0.0001) and disease resistance (t_{264} = 5.179, P < 0.0001). In these cases, we used the trim-and-fill method to estimate the number of missing effect sizes required for the current meta-analysis. The results revealed that 141 and 73 additional effect sizes were needed, respectively, to compensate for the potential publication bias (Fig. S9 in Appendix A). Nevertheless, TG and GE still had significant effects on growth and disease resistance traits (adjusted mean [95% CI]: −1.249 [−1.516 to −0.982], k = 613, P < 0.0001 for growth; 2.953 [2.142 to 3.764], k = 339, P < 0.0001 for disease resistance) after incorporating these randomly generated effect sizes. We also did not detect a decline in effect size magnitude in more recent years (Fig. S10 in Appendix A).

3.7. Sensitivity analysis

The influence analysis indicated that no individual study carried a significant deviation of pooled effect size from the overall level when we removed one by one (Fig. S11 in Appendix A). Examining

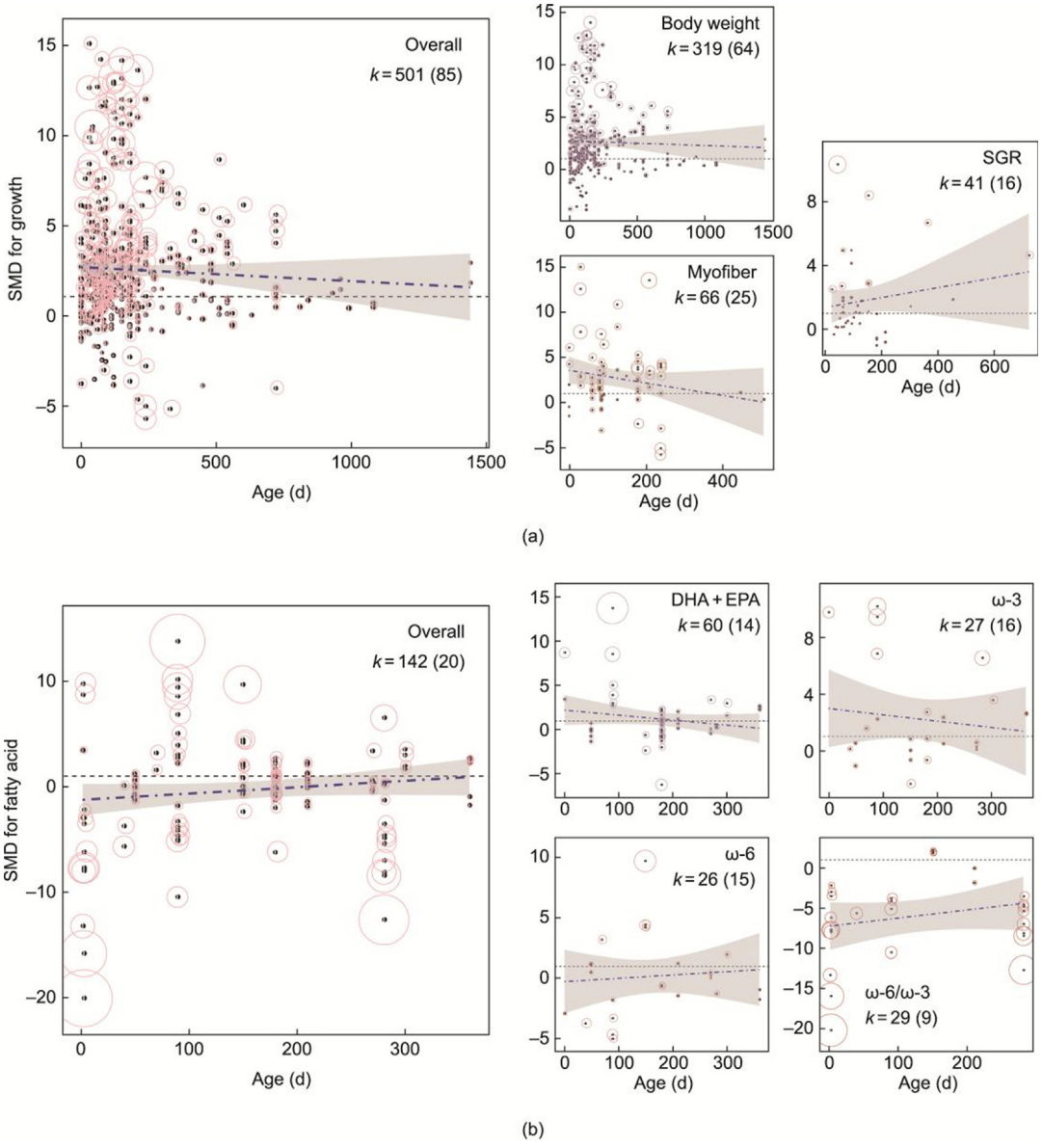

Fig. 6. The impact of transgene integration or gene knockout on traits decreases progressively with advancing age. (a) The effects of age (x-axis) on the effect size (SMD) (y-axis) of growth traits across the entire dataset, and disaggregated for body weight, myofiber and SGR. (b) The effects of age (x-axis) on the effect size (SMD) (y-axis) of fatty acid traits across the entire dataset, and broken down for DHA+EPA, ω-6, ω-3, and ω-6/ω-3. The center of each circle indicates the mean effect size. The diameter of the circles represents the 95% CI of the effect size. The dark line with shaded bars shows the overall effect of age on effect sizes and its 95% CI, respectively, and the blue dotted line shows the mean regression line. k is the number of effect sizes and the number of studies (the number in brackets).

the leave-one-out results confirmed that no single study dominated the results. Notably, the results from the full model were consistent regardless of whether the VCV matrix was included (gene/protein expression, disease resistance, and fatty acid), so the model is presented without the VCV matrix throughout the main text.

4. Discussion

Advances in genetic engineering have consistently enhanced trait performance in gene-modified animals across individual studies. In this study, we conducted a comprehensive meta-analysis to quantify the effects of TG and GE on trait performance in various animal species. Our findings challenge the assumption that genetic modifications uniformly result in trait augmentation, demonstrating significant variability in outcomes such as growth, disease resistance, and fatty acid content. This variability is shaped by factors including the specific animal taxa, the traits being targeted, and the transgenes or innate genes involved. In aggregate, this study reveals the effects of genetic modifications on trait enhancement across animals, highlighting key gaps in knowledge that will facilitate a better understanding of the future applications of TG and GE.

Our results indicate that both TG and GE significantly impact key phenotypic traits, albeit to varying degrees. Genetic modifications notably enhanced gene/protein expression, growth, and disease resistance, whereas the effects on fatty acid composition were more inconsistent. Specifically, gene expression was particularly affected by both methods, highlighting the capacity of TG and GE to effectively upregulate or downregulate target genes. TG generally resulted in higher gene expression by introducing new functional genes under robust promoters such as H1 or β-actin. In contrast, GE often downregulates or eliminates specific genes, particularly growth regulators such as *mstn* and *IGF2*. This illustrates the precision with which gene pathways can be manipulated. This modulation of gene expression led to marked improvements in growth traits, including body weight and SGR, with GE showing a larger effect size for growth than TG, likely due to its precise targeting of negative growth regulators with low off-target events [1]. These enhancements were particularly evident in fish, likely due to differences in genetic architecture and growth physiology across taxa [75,76]. In terms of disease resistance, GE had more consistent and robust effects than TG, as evidenced by larger effect sizes and a broader impact across trait parameters. Our analysis revealed that targeted gene knockdown effectively enhances disease resistance in livestock by significantly reducing the incidence of infectious diseases through suppressing viral receptors and immune regulatory genes. However, variability across species and pathogens indicates that further optimization is needed, particularly with regard to TG, where the overall effects on disease resistance were not significant. Additionally, changes in fatty acid composition were less consistent, with no significant overall effect detected. However, improvements in specific parameters, such as the ω-6/ω-3 ratio and DHA levels, were observed in certain species, such as pigs and cattle. The variability in fatty acid traits suggests that, while it is possible to manipulate lipid metabolism genetically, the success of this manipulation may depend on factors such as species, specific transgenes or edits, and their integration into existing metabolic pathways [11,52,53]. These findings highlight the advantages of GE over TG due to its precision and efficiency, as well as its ability to produce natural-like modifications. This suggests that it will be more widely accepted by regulators and consumers, and it will also be valuable for the government when it comes to regulating transgenic and gene-edited animals.

This meta-analysis reveals substantial taxon-specific variation in responses to genetic interventions across parameters, with fish demonstrating the most significant improvements, particularly in terms of growth. This may be due to the focus on introducing a diversity of GH genes in fish [23,77], which are known to enhance growth rate and body size. In contrast, effects in mammalian species such as pigs, sheep, and cattle were more variable, reflecting differences in the genetic regulation of traits [7,57] such as growth and disease resistance. The variability in effect sizes across traits emphasizes the importance of targeting specific genes or pathways. Growth traits improved consistently across taxa due to simpler interventions, whereas disease resistance exhibited greater variability, likely due to the complexity of immune pathways [78,79]. The success of genetic interventions was also influenced by promoter type, with the H1 promoter driving the most effective gene expression, followed by β-actin and U6, emphasizing the need for careful promoter selection to optimize TG.

In our meta-dataset, which included animals of various ages, we observed that the effects of TG and GE on growth and fatty acid traits varied with age. Specifically, the efficacy of these modifications decreased with age, suggesting that their impact diminishes over the animal's lifespan. This may be due to reduced cellular activity, a slower metabolism, hormonal changes, and compensatory mechanisms [77,80]. Furthermore, trait improvements were more pronounced in the P1 generation than that in the F1 generation. Despite the consistent expression or repression of transgenes or edited genes in the F1 generation (Fig. S2(f), Fig. S12(a) in Appendix A), the effects on growth (Fig. 6(b), Fig. S12(b) in Appendix A), disease resistance (Figs. S12(c) and (d) in Appendix A), and fatty acids (Fig. S12(e) in Appendix A) were less pronounced in the offspring. This could be due to partial dominance or incomplete penetrance; whereby full expression requires homozygosity. However, the F1 generation is often heterozygous [43,46,49,81,82], and exhibits reduced trait manifestation. Epigenetic modifications or other intergenerational regulatory mechanisms may also play a role [83]. To more fully assess these effects, future analyses should include homozygous animals beyond the F1 generation.

Our meta-analysis identified 18 innate genes involved in trait enhancement, of which only five showed significant global-level improvements. Disruption of the *mstn*, *mc4r*, or *IGF2* genes, for instance, significantly enhances growth in both livestock and fish, while knockouts of the *CD163* or *PCBP1* genes increases viral resistance in livestock by reducing viral RNA. Nevertheless, few genes have been validated for improving traits in farm animals, particularly fish, which poses a significant challenge for genome manipulation aimed at enhancing economically important traits. The limited number of genes identified as being linked to such traits restricts the application of genetic manipulation techniques, including GE [58,84]. Therefore, focused efforts to identify specific gene variants through comprehensive genomic studies, such as genome-wide association studies, quantitative trait locus mapping, and functional genomics, are critical [84]. Once these variants have been identified, they can be introduced or removed through targeted genetic manipulation, improving the predictability and effectiveness of breeding programmes.

It is important to note that the genes and modifications analyzed in TG studies were frequently chosen for their ability to improve production traits. This reflects the longer history of TG technology compared to GE, which has only been widely available since around 2012. Early TG experiments were limited by a lack of genomic sequence data and gene structure information, resulting in a focus on well-characterized genes, such as GH. In contrast, GE studies benefit from advanced genomic resources and can target a broader range of genes with greater precision. Both technologies have evolved over time, becoming more efficient and reducing off-target effects and allowing for more controlled genomic inte-

gration. These improvements can influence phenotypic outcomes. These historical and technical differences between TG and GE should be considered when interpreting the results of studies in animal biotechnology.

As with any study, limitations must be acknowledged, and research gaps identified. In this study, we analyzed and interpreted effect sizes across taxa, traits, parameters, and gene-specific factors. We employed species-moderator analysis rather than phylogenetic meta-analysis due to low variation across species. Our literature screening may have overlooked studies, as our meta-dataset only includes English-language publications. We also excluded studies on model animals (i.e., zebrafish, medaka and mice) [85] and *in-vitro* studies identifying potential causative genes using cell lines. For example, several representative genes from fish, such as rhamnose-binding lectin (*RBL*), signal transducer and activator of transcription 2 (*STAT2*), junctional adhesion molecule-A (*JAM-A*), the repressor of RNA polymerase III transcription of *Paralichthys olivaceus* (*PoMaf1*), NEDD-8 activating enzyme 1 (*nae1*), and GRB2-associated binding protein 3 (*GAB3*) have undergone induced mutations that mimic and alter the immunity of the fish and improve the host's resistance to disease [86,87]. Therefore, our results represent broad trends from the current literature that meet our inclusion criteria, and should be interpreted accordingly.

Future primary studies should aim to cover a broader range of taxonomic groups (i.e., model animals in the laboratory) and underrepresented traits (i.e., reproductive outcomes). It would be interesting to examine the effects of genetic modification on model animals beyond livestock and fish (which were not included in this study) would provide valuable insight into the process of TG and GE in captive animals. Another aim of TG and GE in farm animals is reproduction confinement [7,84], and a growing number of studies have identified candidate reproduction-regulating genes, particularly in fish species [84]. Verifying these promising genes by assessing reproductive outcomes (i.e., gamete quality, sperm motility, fertilization rate, and hatching rate) on a global scale for transgenic or gene-edited animals would also be valuable.

CRediT authorship contribution statement

Jinhai Wang: Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Shinichi Nakagawa:** Supervision, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Jiaqi Wang:** Resources, Formal analysis, Data curation. **Robert Stewart:** Resources, Formal analysis, Data curation. **Alexandra Florea:** Resources, Formal analysis, Data curation. **Rex A. Dunham:** Supervision, Resources, Investigation, Formal analysis, Data curation. **Fei Ling:** Methodology, Formal analysis, Data curation. **Gaoxue Wang:** Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lily Liu:** Writing – original draft, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Diego Robledo:** Validation, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Lily Liu was supported by the Ten Thousand Talent Plans for Young Top-notch Talents Grant (20221116). Gaoxue Wang was supported by the Biological Breeding-National Science and Technology Major Project (2023ZD04065). Diego Robledo was supported by the Horizon Europe Framework Programme Grant (101079467).

Data availability statement

All R codes to this article can be found online at https://github.com/Jinhai1017/GenoTrans.

Appendix A and B. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eng.2025.11.032.

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