Open Veterinary Journal, (2024), Vol. 14(12): 3189-3202
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Review Article
DOI: *10.5455/OVJ.2024.v14.i12.4*

Submitted: 01/11/2024 Accepted: 29/11/2024 Published: 31/12/2024

A review of myostatin gene mutations: Enhancing meat production and potential in livestock genetic selection

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ABSTRACT
Myostatin (MSTN) is an essential gene that regulates muscle growth and development in livestock animals, influencing meat production and carcass quality. This review looks at MSTN genetic diversity in a variety of animals, including cattle, sheep, goats, and poultry, focusing on correlations between particular mutations and phenotypic parameters such as muscle mass and growth rate. Mutations in the MSTN gene have been linked to desirable features such as greater body weight and higher meat quality, making it an ideal candidate for genetic selection in breeding programs. Animals carrying this mutation frequently transform feed into muscle more efficiently, resulting in faster growth with less feed input. This is advantageous for growers seeking to cut feed costs while raising yields. MSTN-mutated animals convert feed into muscle more efficiently, lowering production costs over time and making them more appealing for commercial breeding operations. Advances in molecular genetics, especially CRISPR-Cas9 technology, have made it possible to precisely manipulate the MSTN gene, allowing for the development of calves with enhanced muscle mass. This article also examines the effects of MSTN variation on meat production efficiency, as well as current issues in animal genetics. This research synthesis emphasizes the significance of MSTN in cattle breeding, specifically its potential as a genetic marker to improve production and meat quality in commercial farming.
Keywords: Growth, Poultry, Productivity, Muscle, Myostatin.

Introduction

Myostatin (MSTN), also known as growth and differentiation factor 8 (GDF8), is a key component of the TGF-β superfamily that regulates muscle development (Liu *et al*., 2021a; Malila *et al*., 2022). MSTN gene mutations can increase muscle mass up to threefold, making it an important possibility for increasing muscle growth in livestock (Malila *et al*., 2022). These mutations have been found across numerous animals, including poultry (Kim et al., 2020a; Gaina and Amalo, 2022), sheep (Kim *et al*., 2022), cattle (Csürhés *et al*., 2023), and pigs (Ryan *et al*., 2023), with only a rare incidence in humans (Saif *et al*., 2024). MSTN mutations in animals have been linked to double-muscled breeds, such as cattle (Bhattacharya *et al*., 2022; Ceccobelli *et al*., 2022).

The MSTN gene, also known as MSTN, encodes a protein that is a negative regulator of muscle growth. Mutations or alterations in this gene lead to muscle hypertrophy, where muscle mass increases significantly. However, MSTN is involved in several aspects of metabolism in addition to muscle formation

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(Kumaravel *et al*., 2023; Zhao *et al*., 2023). Although it is largely expressed in skeletal muscle, MSTN is also discovered in other tissues, such as the liver, heart, brain, and mammary glands, indicating its participation in larger physiological processes (Swanson *et al*., 2022; Garba *et al*., 2024). Understanding MSTN's molecular genetics and tissue-specific bioactivity is critical for improving livestock output (Shoyombo *et al*., 2022; Thepa and Tyasi, 2024).

MSTN is a 376-amino acid propeptide that undergoes proteolytic processing to form an active protein (Yan *et al*., 2021). Similar to other TGF-β family members, it has a cysteine knot pattern in its C-terminal region (Nejad *et al*., 2024). Interestingly, the active C-terminal region is retained across several species, including humans, chickens, pigs, and mice (Baig *et al*., 2022; Sonali *et al*., 2022). MSTN is expressed early in embryonic muscle development, with levels extending until adulthood, notably in muscle-dominant tissues (Mohammadabadi *et al*., 2021; Alnajm *et al*., 2024). However, changes in its expression between slow- and fast-twitch muscle fibers have been reported across species, with differing outcomes in humans, mice, and chickens (Iroanya *et al*., 2021; Paek *et al*., 2021).

Recent investigations have further revealed the role of MSTN in controlling muscle growth, specifically by inhibiting myoblast proliferation and differentiation. It interferes with the cell cycle via altering cyclin-dependent kinases and their inhibitors, which are required for cell cycle transitions (Lu *et al*., 2011; Smołucha *et al*., 2019). In vitro tests show that recombinant MSTN suppresses myoblast growth in mice and cattle, but these effects are reversible (Dementeva *et al*., 2019; Bruno *et al*., 2022). Interestingly, while MSTN does not normally promote apoptosis, certain studies have revealed that its overexpression may prevent apoptosis in specific cells (Tanjung *et al*., 2019).

Advances in molecular genetics have found several variants in the MSTN gene, and these polymorphisms are connected with quantitative trait loci for growth-related phenotypes in various species, including humans, livestock, and research animals (Tian *et al*., 2021; Maeta *et al*., 2023). These polymorphisms are effective markers for enhancing meat quality and yield through selective breeding (Kalds *et al*., 2023). Furthermore, MSTN mutations can improve genetic features in livestock and can be passed down through generations, presenting intriguing prospects for breeding programs (Du *et al*., 2022; Kruszewski and Aksenov, 2022). This review will highlight the current understanding of the role of MSTN in muscle development, metabolism, and productivity, with a focus on its uses in cattle breeding. This review aims to investigate the physiological mechanisms, tissue-specific roles, and genetic variants of MSTN in order to emphasize its potential to improve cattle performance.

MSTN as a regulator of muscle development

MSTN or GDF8 is expressed in several tissues, with the most notable expression occurring in skeletal muscle (Zhao *et al*., 2022). The MSTN gene, renowned for its involvement in muscle control, has actually been preserved throughout evolution. It has a structural organization that includes three exons and two introns (Vinet *et al*., 2021; Bendig *et al*., 2024). MSTN polypeptide processing begins with intracellular homodimerization via disulfide bond formation, followed by cleavage into the N-terminal propeptide and the C-terminal mature portion. This maturation phase is crucial for MSTN's function as a negative regulator of muscle development (Muramatsu *et al*., 2021; Paez *et al*., 2021). MSTN's mature C-terminal portion, which weighs 12 kDa, is essential for muscle control because it initiates intracellular signaling cascades (Osman *et al*., 2021). The biologically active form of MSTN is the 12-kDa mature C-terminal segment formed by cleaving the pro-MSTN polypeptide. This fragment contains the functional area required for MSTN action in muscle cells (Meyermans *et al*., 2022; Zakka *et al*., 2024). The mature MSTN fragment interacts with the activin type II receptors on the surface of target cells, especially in skeletal muscle. TGF-β receptors play a crucial role in modulating MSTN's actions (Al-Zayadi and Hamid, 2022).

Muscle activity and metabolism are the primary regulators of muscle fiber expansion, often known as hypertrophy (Zhang *et al*., 2018). During embryogenesis, muscle precursors differentiate into myoblasts using a highly regulated sequential sequence (Rodgers and Ward, 2022). MSTN governs muscle development at critical moments throughout prenatal stages, such as muscle precursor proliferation and myoblast proliferation and differentiation (Sousa-Junior *et al*., 2022; Waller *et al*., 2023). Ectopic expression of MSTN in muscle reduces critical indicators of proliferating muscle precursors (Li *et al*., 2021). In contrast, MSTN upregulates proteins that suppress the proliferation of MyoD-expressing myoblasts (Giri *et al*., 2022; Isık *et al*., 2022). During myogenesis, there is a substantial correlation between MyoD activity and MSTN expression, with MyoD playing an important role in regulating MSTN expression (Azhar *et al*., 2020). The E-box motif in the MSTN promoter region plays an important role because it serves as a binding site for basic helix-loop-helix transcription factors (MRFs) (Jakaria *et al*., 2021). MyoD and MSTN cooperate to regulate muscle differentiation and control muscle cell development. By regulating the number of myoblast precursor pools and committed myoblasts, MSTN serves as a critical regulatory element that inhibits unregulated differentiation caused by MyoD. Downregulating MSTN expression would increase both of these populations (Esposito *et al*., 2022).

Muscular mass development is a complex process that involves the differentiation and fusing of myoblasts

to generate muscular fibers. This process is separated into two discrete phases: primary and secondary fiber creation (Lin *et al*., 2019; O'Hara *et al*., 2021). An increase in the myoblast pool prior to fiber production encourages the creation of more primary and secondary myofibers (Zegyer and Mutlag, 2022). MSTN reduces myoblast growth during muscle development. MSTN mutations decrease or eliminate the gene's inhibitory signal, allowing myoblasts to proliferate faster than usual. This increases the amount of myoblasts, which eventually merge to generate additional muscle fibers, resulting in hyperplasia (Nasr *et al*., 2021). To produce considerable fiber hypertrophy or a rise in the size of muscle fibers, specific circumstances must be maintained not just during prenatal development but also throughout postnatal life (Kim *et al*., 2020b). MSTN levels in ruminant animals are relatively low between embryonic days 15 and 29, with increasing expression found after day 31 (Wu *et al*., 2022). Increase in the number of muscle fibers, and the majority of this process is finished during fetal development. In many animals, the total number of muscle fibers generated in the fetal period is generally fixed at birth, and therefore, postnatal muscle growth is predominantly based on hypertrophy (fiber enlargement) (Bi *et al*., 2020). During development, mesodermal progenitor cells differentiate into primary myoblasts. These cells are the precursors to muscle fibers and are vital for forming the early muscular structure. Growth factors, hormones, and the animal's genetic background all have an effect on the quantity of primary myoblasts (Hu *et al*., 2015).

MSTN gene in poultry

MSTN's main function is to inhibit muscle growth by controlling the number and size of muscle fibers. It prevents excessive muscle development by decreasing the proliferation, differentiation, and fusion of myoblasts (muscle precursor cells) (Zhang *et al*., 2021). MSTN treatment inhibits the proliferation and development of myoblasts (muscle cells) (Tekerli *et al*., 2022). Research on the impacts of MSTN has gone beyond muscle mass to study its involvement in poultry, notably egg production (Hai *et al*., 2023). MSTN's regulatory mechanisms could potentially be modified to boost egg-laying ability, albeit this field is less understood than its implications for muscle growth (Al-Musawi and Al-Ameri, 2022). Further research is required to clarify the role of MSTN in poultry reproductive and egg production. Investigating the particular molecular processes via which MSTN regulates these qualities may open the way for innovative breeding tactics.

Another study discovered a negative link between MSTN marker expression and the growth of the pectoralis major muscle, indicating that MSTN expression could be employed as a marker for selecting improved muscle growth in poultry (Abhijith *et al*., 2021). MSTN is a negative regulator of muscle growth in chicken, as it is in other mammals. By limiting myoblast proliferation and differentiation, MSTN guarantees that muscle fiber growth does not exceed the limitations that the body can sustain (Chen and Lee, 2016). MSTN is extensively expressed in poultry's skeletal muscles, notably in fast-twitch muscles that are crucial for movement and flight. The expression of the MSTN gene in chicken species can vary depending on parameters such as age, breed, and the specific muscle under examination (Lin *et al*., 2019; Esposito *et al*., 2022). A study on hens found that mutations in the regulatory area of the MSTN gene were related to abdomen fat percentage, birth weight, breast meat production, and body weight (O'Hara *et al*., 2021; Zegyer and Mutlag, 2022).

The chicken MSTN gene consists of three exons and two introns, which span around 6693 base pairs. This structure is conserved across numerous species, showing the essential importance of MSTN in muscle biology, and it encodes roughly 375 latent amino acids (Kim *et al*., 2020b; Nasr *et al*., 2021; Wu *et al*., 2022). Variations in this region may influence the effectiveness of MSTN production, secretion, or its first processing, changing its inhibitory impact on muscle growth. These discrepancies are likely related to disparities in muscle mass and growth rates in broiler chickens (Chen *et al*., 2021; Prihandini *et al*., 2021). Polymorphisms that diminish MSTN's inhibitory activity are often related to greater muscle growth and improved growth rates in broiler chickens. These birds usually have larger breast muscle mass and improved feed conversion efficiency, making them more suitable for meat production (Zhao *et al*., 2019). Notably, a nonidentical single nucleotide polymorphism (SNP) (T4842G) was associated with amino acid alterations in MSTN, influencing body weight (Lassiter *et al*., 2019). In China's Bian chicken breed, which is dual-purpose (meat and eggs), a mutation at 234G>A was identified in exon 1 of the MSTN gene (Ju *et al*., 2021). Polymorphisms in chicken MSTN have been connected with differences in breast muscle mass, birth weight, belly fat percentage, and adult body weight (Konovalova *et al*., 2021; Dushyanth *et al*., 2022). In addition to the previously revealed polymorphisms in the coding and intronic sections of the MSTN gene, researchers discovered three SNPs in the 5' regulatory area and two SNPs in the 3' region of the MSTN gene in broilers. These areas serve critical roles in the control of gene expression, regulating the amount and when MSTN is produced during development (Aiello *et al*., 2018). These SNPs are particularly important in commercial broilers because they may influence essential qualities such as growth rate, feed efficiency, and meat output (Rybalka *et al*., 2020). SNPs in this region may promote or inhibit the binding of these transcription factors, therefore regulating the level of MSTN mRNA generated. This control is critical for

determining the balance of muscle growth and other metabolic activities (Kim *et al*., 2021a).

Research on MSTN polymorphisms in ducks has emphasized its potential impact on crucial slaughter features, specifically leg muscle mass and breast muscle percentage (Duan *et al*., 2016). Studies have suggested that ducks with specific MSTN alleles may exhibit improved muscular features, leading to greater leg muscle mass and higher breast muscle percentages than others lacking similar alleles (Tao *et al*., 2015). Marker-assisted selection can be used in breeding programs to boost certain traits related to MSTN variants, resulting in improved growth rates and meat quality (Huang *et al*., 2011). Including MSTN polymorphism data in breeding techniques provides for more informed decision-making. Breeders can target specific genetic markers to improve slaughter qualities, resulting in increased meat quality and market value (Chen and Lee, 2016).

The link between MSTN mRNA expression, body weight, muscle mass, and growth rate in chickens is still under active research. Although MSTN is a known negative regulator of muscle growth, its precise effects and mechanisms in poultry, particularly about growth metrics, are not yet well understood (Yuan *et al*., 2024). The study on MSTN expression in Ross × Ross hens sheds light on its impact on muscle development and growth, particularly during critical growth phases (Bigford *et al*., 2021). Cobb × Cobb embryos did not show similar expression patterns (Kour *et al*., 2024). Although the explanation for the discrepancy remains unknown, the early expression of MSTN in Ross × Ross embryos before the creation of the myogenic lineage implies its crucial function in early embryonic development (Rodgers and Ward, 2022).

The ontogeny of MSTN expression in the pectoralis muscle of chicken embryos includes investigating its involvement in muscle fiber creation and development. MSTN, a member of the TGF-β superfamily, is largely recognized for suppressing muscle growth (Sukhija *et al*., 2023). During this period, MSTN expression may decrease, allowing muscle fiber populations to grow. Regulating MSTN levels is crucial to promote the growth in muscle mass associated with secondary fiber development (Al Amaz *et al*., 2024). MSTN works with other growth hormones and signaling pathways (like IGF-1) to enhance muscle growth. The balance between MSTN and these anabolic signals is necessary for optimal muscular development (Liu *et al*., 2021b). MSTN expression starts early in embryogenesis. In chickens, MSTN is usually identified at the commencement of muscle differentiation, which corresponds to the onset of primary myofiber production (Chen *et al*., 2023). Several studies have looked into distinct MSTN polymorphisms across poultry breeds. Researchers found polymorphisms in the Daheng Broiler, Jinghai, and Bian breeds (Zhang *et al*., 2019; He *et al*., 2020; Jang *et al*., 2021). Investigations on MSTN polymorphisms in Sansui, Gaoyou, and Pekin ducks demonstrate the amount of work conducted on MSTN in poultry from varied climates (Wang *et al*., 2021). Studies on MSTN expression and its involvement in muscle growth show its enormous potential for application in breeding strategies within the poultry industry (Lee *et al*., 2023). Breeding programs that focus on lowering MSTN expression could result in faster growth rates and increased muscle yield. This can lead to increased meat production efficiency and cheaper production costs (Hou *et al*., 2021). Selective breeding for decreased MSTN activity can increase muscle growth and enhance feed conversion ratios. Birds that gain weight effectively can minimize the amount of feed required per kilogram of weight growth, which is economically beneficial to poultry farmers (Koohgivi *et al*., 2019).

Mutations in the MSTN gene in ruminants

In ruminants, such as cattle, goats, and sheep, MSTN plays an important function in preventing skeletal muscle development (Sheng *et al*., 2021). This protein functions as a natural regulator of excessive muscle growth, maintaining a balance between muscular development and the animal's metabolism. MSTN inhibits the growth and development of muscle cells (myocytes) (Ryan and Li, 2021). When MSTN activity is reduced, muscle development becomes greater and faster. Therefore, regulating or changing the expression of MSTN using genetic engineering or biotechnology can produce advantages in meat production while not affecting animal health (Lou *et al*., 2021). Overall, MSTN is a key regulator of muscle growth in ruminants, and understanding its involvement can help improve livestock production efficiency. However, the influence on animal welfare should also be considered (Han *et al*., 2021).

The discovery of around 20 different types of genetic polymorphisms inside the MSTN gene in ruminants, including deletions, insertions, and nucleotide substitutions (SNPs) emphasizes the gene's role in muscle development and growth control (Basha *et al*., 2022; Sakar and Zülkadir, 2022). Allele variations and inactive MSTN are associated with growth rate and desired carcass features, which has substantial consequences for beef cattle production (Priyadharsini *et al*., 2021; Cheng-Li *et al*., 2023). From the perspective of high-quality meat production, the features linked with MSTN variations in beef cattle are very amazing (Pira *et al*., 2021). The higher meat quality attributes reported in cattle with particular MSTN variations can be linked to many major physiological changes that improve the overall quality and consumer appeal of the meat (Dilger *et al*., 2022). Variations in the expression levels of MSTN in skeletal muscle are certainly of great interest in the field of animal breeding, notably in cattle production. MSTN's significance as a fundamental regulator of muscle growth and development shows its potential as a target for genetic selection and breeding

strategies to enhance the desired features (Bruno *et al*., 2022). The understanding of null alleles and polymorphisms in the MSTN gene has proved useful in improving selection techniques for beef cattle and sheep. Understanding the genetic basis of features regulated by MSTN allows breeders to make better decisions to increase cattle quality and production efficiency (Cao *et al*., 2024).

Role of MSTN in muscle regulation

MSTN's genetic mechanisms can direct selective breeding programs in livestock and poultry to improve desirable qualities such as muscle mass and growth rates (Zhou *et al*., 2022a). By altering MSTN expression, breeders can generate hybrids with increased muscle growth and feed efficiency, maximizing productivity in many animal species (Wu *et al*., 2021). Advances in genomic technologies allow for the discovery of specific MSTN gene variations associated with improved muscle characteristics, enabling more targeted breeding tactics (Cheng-Li *et al*., 2023). MSTN inhibitors have generated interest due to their potential to improve muscle function and recuperation in athletes. Research on MSTN may lead to new training regimens or treatment therapies that optimize muscle development (Liu *et al*., 2021b). Advances in gene therapy targeting MSTN may provide viable techniques for restoring muscular function in people suffering from inherited muscle diseases (Abhijith *et al*., 2021).

Mutations in the MSTN gene (GDF8) are actually linked to muscular hypertrophy. This gene is highly conserved across species, which means that MSTN's sequence and function are very similar in a variety of creatures, from mice to humans to sheep. MSTN regulates skeletal muscle growth during embryogenesis and into adulthood (Wu *et al*., 2021; Zhou *et al*., 2022a). Researchers can gain insights into the genetic regulation of muscle development in cattle by studying the location and function of the MSTN gene on chromosome 2, which is vital for understanding growth patterns and productivity. Mapping the gene also allows association studies to identify correlations between specific MSTN gene variants and phenotypic traits, such as muscle size and carcass quality (Zhou *et al*., 2022b). This can inform breeding decisions and enhance production efficiency (Lee *et al*., 2021), including pigs (AY208121) (Hua *et al*., 2023), buffalo (AH013313) (Zhu *et al*., 2018), zebrafish (AY323521) (Mohamedien *et al*., 2023), gilthead freshwater fish (AF258447) (Zhang *et al*., 2023), chickens (AF346599) (Gu *et al*., 2022), and mice (AY204900) (Kim *et al*., 2021b). MSTN is a dimeric protein that is generated from a precursor. It undergoes proteolytic cleavage to generate the active dimer, which consists of two identical subunits connected by disulfide links. This structure is necessary for its role as a signaling molecule, a wide group of cytokines involved in the regulation of diverse cellular processes, including growth, differentiation, and development in many tissues. MSTN shares structural similarities with other members of this superfamily, but it is unique enough to not be easily classified into well-known TGF-β subfamilies such as bone morphogenetic proteins (BMPs) or inhibins (Li *et al*., 2021). MSTN actively suppresses skeletal muscle growth, and when a mutant mh allele (MSTN gene mutation) is present in a homozygous loss-of-function state, it causes muscle hypertrophy, which is an abnormal increase in muscle size (Li *et al*., 2022). However, mutations in the MSTN gene (MSTN gene) do not have to be present in a homozygous situation for the effects of muscle hypertrophy to be noticed. Even those with a heterozygous situation (having one mutant allele and one normal allele) can display some degree of enhanced muscle growth, though the effects tend to be more evident in persons or animals with homozygous loss-of-function mutations. Six mutations in the MSTN sequence have been identified as causing muscle hypertrophy via inactivating the gene (Liu *et al*., 2021a). The six discovered mutations within the MSTN gene produce muscle hypertrophy by deactivating or drastically lowering the action of MSTN. Mutations might be nonsense, missense, frameshift, or deletion, and they vary across animals and individuals (Ren *et al*., 2023).

These mutations include insertions, deletions, and nucleotide transitions that alter the gene's capacity to create a functioning MSTN protein (Wang *et al*., 2021; Ren *et al*., 2023). MSTN normally functions as a negative regulator of muscle growth, reducing the proliferation and differentiation of myoblasts (muscle precursor cells) into mature muscle fibers. Mutations that inactivate MSTN lose its capacity to suppress muscle growth, resulting in increased muscle fiber deposition and muscle hypertrophy (Zhu *et al*., 2018). Aside from the loss-of-function mutations in the coding area of the MSTN gene, some other mechanisms and mutations can impact MSTN activity and lead to muscle hypertrophy. These include mutations or alterations in noncoding areas, as well as changes in the regulatory elements that affect MSTN expression. Furthermore, factors influencing the processing and secretion of the MSTN protein can change its activity (Hou *et al*., 2021). In the examination of 35 double-muscled calves from ten European breeds, numerous mutations were detected in both the intronic and coding regions of the MSTN gene, contributing to the muscle hypertrophy observed in these breeds (Dilger *et al*., 2022).

Despite the fact that Limousin cattle are known for their excellent muscle growth and high carcass production, no loss-of-function mutation in the MSTN gene has been detected (Tanjung *et al*., 2019). According to studies, unlike these breeds, Limousin cattle do not have an obvious loss-of-function mutation in the MSTN gene that would explain their muscular phenotype (Cao *et al*., 2024). There are still undiscovered variables that may trigger muscle hypertrophy, presumably situated

beyond the coding areas of MSTN (Maeta *et al*., 2023). Because of its substantial function in regulating muscle growth, MSTN inhibition is a possible target for treating diseases linked with muscle weakness or muscular dystrophy, such as Duchenne muscular dystrophy (Kalds *et al*., 2023). MSTN not only regulates muscle growth but also maintains muscular homeostasis during periods of inactivity (such as illness or disuse) (Zhang *et al*., 2019). In instances such as muscular atrophy (loss of muscle mass), MSTN levels tend to rise, speeding up the process of muscle mass decrease (Sakar and Zülkadir, 2022).

Phylogenetics of the MSTN gene

Systematic investigation of protein-coding genes under positive selective pressure frequently yields crucial insights into the evolutionary adaption of certain features. The MSTN gene family is a famous example of a gene that has been subject to positive selection, especially in species such as *Artiodactyls* (even-toed ungulates, such as deer, cattle, and pigs) and teleost fish. Positive selection in these species has been found by measuring nucleotide substitution rates, a key sign of how specific mutations may be favored in evolution due to their favorable impact on the organism (Wang *et al*., 2021). Understanding evolutionary processes and potential changes in protein function requires identifying periods when the average selected pressure for substitutions in a gene surpasses the average selective pressure for conserving ancestral sequences. This study frequently focuses on the balance of positive selection (favoring beneficial mutations) and purifying selection (favoring the preservation of existing functional sequences) (Cheng-Li *et al*., 2023). The sequencing of MSTN in goats (Zhao *et al*., 2022), cattle lineages (Dilger *et al*., 2022), and Evidence indicates that MSTN has experienced recent selective pressure during the domestication process of various species, particularly in livestock (Bendig *et al*., 2024). Examining substitution patterns between cattle and sheep, particularly in the context of BMP2 structure, indicates significant amino acid alterations that may have significance for the functional differences between these two species. BMP2, a member of the TGF-β superfamily, is essential for bone and cartilage formation, as well as muscle growth (Ryan and Li, 2021). Understanding the functional dynamics of BMP2, particularly in relation to MSTN, requires understanding the remaining alterations around the α-helix. To completely appreciate how these alterations affect protein function, it is crucial to investigate the sequence–structure–function relationship of MSTN and how it parallels or diverges from BMP2 (Zhou *et al*., 2022a). Current phylogenetic data demonstrating selective forces on MSTN substitutions create difficulty in determining the exact date of these evolutionary events. Understanding when these selection pressures arose is critical for evaluating the physiological consequences of certain mutations and their contributions to muscle development in different animals (Osman *et al*., 2021). The divergence of MSTN sequences, molecular functional connections, and resultant variances in muscle and body size have all been significantly influenced by differential adaptation to climatic change and southern migration in bovine lineages (Basha *et al*., 2022).

MSTN phylogenetics studies the evolutionary links and genetic variation of the MSTN gene across different species. MSTN regulates muscle growth, and its function has been retained throughout animals (Ren *et al*., 2023). This conservation emphasizes its importance in biological mechanisms, including muscle mass regulation. Phylogenetic investigation of the MSTN gene usually entails comparing MSTN gene sequences across taxa (Kumaravel *et al*., 2023). These gene sequences stay mostly constant across species, showing a strong evolutionary pressure to keep this function (Wang *et al*., 2021). By comparing sequences, scientists may track how different species have generated these genetic variants over time, discovering species with mutations that may result in greater muscle growth or changed control (Cheng-Li *et al*., 2023). MSTN has been extensively investigated in mammals, including cattle, sheep, and humans. For example, many cow breeds (e.g., Belgian Blue) have natural mutations in the MSTN gene, which causes a trait known as double muscling (Pira *et al*., 2021).

Phylogenetic investigation of MSTN in livestock indicates genetic variants that also influence muscle growth, but they may differ significantly due to environmental adaptations (Waller *et al*., 2023). Comparative investigations reveal that MSTN function has been conserved in wild populations. Species with spontaneous or intentionally induced mutations in the MSTN gene frequently show accelerated muscle growth (Sheng *et al*., 2021). Selective breeding has been used to produce desirable features by targeting the MSTN gene (Kim *et al*., 2021b). Overall, MSTN is highly conserved, making it a useful target for investigating muscle development, genetics, and evolutionary biology across species (Zhou *et al*., 2022a).

Functional consequences of MSTN mutations

Mutations in the MSTN gene can dramatically influence muscle growth, resulting in increased muscular mass and altered body composition. This assessment focuses on the nature of these mutations, their functional ramifications, and the implications for muscle growth regulation (Lee *et al*., 2021). The sequencing of the MSTN gene across many species, including cattle, sheep, chickens, goats, and pigs, provides substantial insights into its structure and evolutionary conservation. Here is a full explanation of the gene's architecture, its consequences for muscle regulation, and the significance of comparative sequencing across these species (He *et al*., 2020). Several mutations in the coding area of GDF-8 have been found, with nine mutations described thus far, resulting in a spectrum of

functional alterations in the protein (Basha *et al*., 2022). The finding of mutations in the MSTN gene (GDF-8) that cause premature stop codons has substantial consequences for muscle growth regulation. Here is a review of these mutations, their ramifications, and their significance to the double-muscle phenotype observed in specific livestock breeds (Jang *et al*., 2021). The MSTN gene in cattle, located at the end of chromosome 2, has received substantial attention due to the multiple mutations found within its sequence. These mutations lead to the creation of different genotypes and phenotypes, especially those associated with muscle growth and development (Priyadharsini *et al*., 2021). The sequencing of the GDF-8 gene (MSTN) in Belgian Blue cattle has shown a major mutation that impairs muscle growth regulation.

In cattle, a mutation in exon 3 changes a nucleotide from G to A, converting a cysteine codon to tyrosine, which may impair the action of the MSTN gene (Basha *et al*., 2022). The MSTN gene is located on chromosome 2 in sheep, similar to other livestock species, including cattle (Jang *et al*., 2021). The Belgian Texel breed is distinguished by its unusual muscle hypertrophy, which has garnered attention in the study of genetic variables influencing muscle growth (Esposito *et al*., 2022). A SNP g+6223G>A in the 3' untranslated region (3' UTR) of the MSTN gene (GDF-8) has been discovered as a significant marker related to the muscular phenotype exhibited in Belgian Texel sheep (Zhang *et al*., 2019). The SNP g+6223G>A in the 3' UTR of the MSTN gene (GDF-8) has shown significant value outside the Belgian Texel breed (Pira *et al*., 2021). The SNP g+6223G>A in the MSTN gene (GDF-8) has been demonstrated to have a substantial connection with birth weight in sheep, as well as muscle growth and carcass features.

MSTN, primarily known for its role in controlling muscle growth, has a larger expression profile that extends beyond skeletal muscle. MSTN expression is not limited to skeletal muscle but is also present in many nonskeletal tissues, such as the brain, heart, mammary glands, and particular muscle types. However, the particular functions of MSTN in these tissues are mostly unclear, necessitating additional research (Dilger *et al*., 2022). Research findings on muscle cells in chickens differ dramatically from those in other species, with no significant variation in MSTN mRNA levels in slow and rapid chicken muscles (Sheng *et al*., 2021). Furthermore, MSTN levels remain steady during myoblast growth, with changes in satellite cell mRNA content from mature cells (Nasr *et al*., 2021).

Mutations in the MSTN gene in poultry have yet to be observed. Studies on several chicken breeds have shown distinct mutations in the MSTN gene, elucidating its association with fat metabolism and body weight features (O'Hara *et al*., 2021). The MSTN gene (GDF-8) shows a high degree of commonality in its amino acid sequences across different sheep breeds, showing remarkable conservation of its function within the species (Basha *et al*., 2022). MSTN is expressed not only in skeletal muscle but also in other tissues such as the optic lobes, cerebellum, and hypothalamus (Wu *et al*., 2021). MSTN variants have been demonstrated to promote ovulation in several fish breeds and are expressed in the red muscle of several strains, indicating that MSTN performs a different role in fish than in higher vertebrates (Lee *et al*., 2021). Studies have revealed that MSTN expression varies according to muscle type across different species (Zhou *et al*., 2022a). In mice, MSTN protein levels are higher in rapid muscle fibers than in slow ones (Sheng *et al*., 2021). However, the mRNA and protein levels in mice agree with this finding, while the amounts of mRNA and protein in human muscle fibers are comparable (Han *et al*., 2021; Zhou *et al*., 2022a).

The role of MSTN in meat production

In livestock growth, both the quality and quantity of corpses are essential elements that influence animal performance (Lou *et al*., 2021). Genetics, nutrition, climate, and other factors can all influence the consequences (Sakar and Zülkadir, 2022). Genetic factors are the most significant drivers of meat quality (Han *et al*., 2021). There is an increasing demand for lean beef with low-fat content that meets health standards (Lee *et al*., 2023). Over the last few decades, advances in breeding programs and genetic enhancements in commercial livestock have resulted in increased production levels (Al Amaz *et al*., 2024). In recent years, considerable progress has been made in the disciplines of quantity and molecular genetics through the genomic mapping of commercial animals, creating new potential to increase meat production and meet market needs (Lou *et al*., 2021).

The MSTN gene has a substantial influence on muscle growth and meat qualities. Mutations in MSTN have been linked to increased body weight and enhanced meat characteristics across various species (Dilger *et al*., 2022). Mutated alleles and inactive MSTN are highly associated with growth rates and excellent meat characteristics (Priyadharsini *et al*., 2021). Numerous genes influence growth traits and carcass quality and may serve as candidate genes for the aforementioned features or are closely related to genes that govern these characteristics (Cheng-Li *et al*., 2023). Calpastatin, leptin, growth hormone, prolactin, IGF, and PIT are key genes that directly affect meat quality and quantity, either individually or by their impact on other hormones (Chen and Lee, 2016). These genes are beneficial in meat production, and their polymorphisms are connected with both meat quality and quantitative features, making them useful as genetic markers in breeding programs aiming at increasing precision and responsiveness to selection (Zhou *et al*., 2022b).

The role of the MSTN gene in animal breeding

The MSTN gene is recognized as an essential locus in production characteristics (Lee *et al*., 2021). Several

mutations in this gene cause a twofold muscling phenotype and result in greater muscle mass, which is one of the goals of breeding commercial animals (Zhu *et al*., 2018). For decades, conventional breeding has involved selecting people with spontaneous mutations in the MSTN gene. With the advancement of genetic engineering tools, scientists may now selectively modify this gene in animals using methods such as CRISPR-Cas9 (Mohamedien *et al*., 2023). More precise genetic editing tries to develop animals with desirable traits, such as enhanced muscular mass (Kruszewski and Aksenov, 2022).

The MSTN gene serves an important role in controlling muscle growth in animals. It works by suppressing muscle growth and proliferation, therefore naturally opposing the increase of muscle mass (Abhijith *et al*., 2021). In the context of animal breeding, altering the MSTN gene has gained attention because of its potential to boost meat production in animals (Yan *et al*., 2021). This gene encodes the MSTN protein, which is a natural inhibitor of muscle growth (Yanesari *et al*., 2023). By altering or lowering the expression of the MSTN gene, breeders can develop cattle with increased muscle mass, thus boosting meat production efficiency (Wang *et al*., 2021). Some livestock breeds naturally carry mutations in the MSTN gene that affect MSTN function. For example, Belgian Blue cattle and Texel sheep have mutations in this gene, causing a condition known as double muscling (O'Hara *et al*., 2021). These animals have larger muscles and less fat than other cattle breeds, which results in a higher meat yield per animal (Duan *et al*., 2016).

In breeding programs, breeders can select people with MSTN gene mutations or other genetic polymorphisms that influence MSTN function (Mohammadabadi *et al*., 2021). This genetic selection allows them to generate generations of animals with more muscle mass, which can improve meat production efficiency (Hai *et al*., 2023). Choosing cattle or sheep based on their MSTN features can provide major financial benefits (Paez *et al*., 2021). MSTN-modified animals had a higher muscle-to-fat ratio, which means more muscle and less fat (Koohgivi *et al*., 2019). This results in leaner meat, which is often considered premium quality on the market. As a result, this gene influences not only the quantity but also the quality of meat produced (Hai *et al*., 2023).

Conclusion

The MSTN gene plays a critical function in animal breeding, specifically regarding growth, increased muscle mass, and meat production efficiency. MSTN is largely involved in muscle production and growth, making it a good candidate gene for improving muscle development in animals. Mutations discovered in the MSTN sequence using molecular techniques give effective solutions and assist breeders in collecting essential information for making educated decisions about management and selecting the optimal populations for reproduction. This allows breeders to collect comprehensive data on the genetic state of the MSTN gene in animals. One of the key benefits of the MSTN gene is its significance as a target gene for increasing productivity.

Acknowledgments

The authors would like to acknowledge the Kementerian Pendidikan, Kebudayaan, Riset dan Teknologi, which has funded this research.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

The authors are grateful to the Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia, for the facility supporting this study. This study was supported in part by the Program Penelitian Penelitian Skema Penelitian Dasar Unggulan (PDU) Universitas Airlangga, in the fiscal year 2024, with grant number: 1508/UN3.FKH/PT.01.03/2024.

Author's contributions

SRA, ML, MAA, and SHW drafted the manuscript. WPL, ZNA, and ARK revised and edited the manuscripts. SG, LA, IBM, and MA took part in preparing and critical checking this manuscript. RY, RG and MA edited the references. All authors read and approved the final manuscript.

Data availability

All references are open access, so data can be obtained from the online web.

References

Abhijith, A., Sejian, V., Ruban, W., Krishnan, G., Bagath, M., Pragna, P., Manjunathareddy, G.B. and Bhatta, R. 2021. Summer season induced heat stress associated changes on meat production and quality characteristics, myostatin and HSP70 gene expression patterns in indigenous goat. Small Rumin. Res. 203(1), 106490.

Aiello, D., Patel, K. and Lasagna, E. 2018. The myostatin gene: an overview of mechanisms of action and its relevance to livestock animals. Anim. Genet. 49(6), 505–519.

Al Amaz, S., Shahid, M.A.H., Jha, R. and Mishra, B. 2024. Prehatch thermal manipulation of embryos and posthatch baicalein supplementation increased liver metabolism, and muscle proliferation in broiler chickens. Poult. Sci. 103(11), 104155.

Al-Musawi, H.K. and Al-Ameri, M.M. 2022. The effect of the multiple genotypes of Myostatin gene on reproductive characteristics of the Iraqi brown local chickens. Kufa J. Agric. Sci. 14(1), 1–10.

Alnajm, H.R., Imari, Z.K. and Al-Rubeaye, T.A. 2024. Study of genetic variation of myostatin (MSTN) and calpastatin (CAST) genes in two native Iraqi sheep by PCR-RFLP technique. Iraqi J. Vet. Sci. 38(1), 97–103.

Al-Zayadi, Z.A. and Hamid, A.S. 2022. Genetic morphology of the myostatin gene and its relationship to some carcass traits in Arabian sheep. NeuroQuantology 20(10), 8191.

Azhar, A., Akmal, M., Hambal, M., Sabri, M. and Rosa, T.S. 2020. Effects of polymorphism of myostatin and fatty acid-binding protein 4 genes on the chemical composition of meat in cull female Aceh cattle. Vet. World 13(7), 1334–1343.

Baig, M.H., Ahmad, K., Moon, J.S., Park, S.Y., Lim, J.H., Chun, H.J., Qadri, A.F., Hwang, Y.C., Jan, A.T., Ahmad, S.S., Ali, S., Shaikh, S., Lee, E.J. and Choi, I. 2022. Myostatin and its regulation: a comprehensive review of myostatin inhibiting strategies. Front. Physiol. 13(1), 876078.

Basha, H.A., Abd El Naby, W.S., Ibrahim, S.E. and Elnahas, A.F. 2022. Screening for snps in mstn and igf-2 genes and its relationship with body weight and carcass traits in black bronze turkey. Adv. Anim. Vet. Sci. 10(7), 1559–1566.

Bendig, S., Marín-García, P.J., Lesta, A., Ramos, J.J., Ruvira, G. and Llobat, L. 2024. Myostatin serum levels depends on age and diet in athletic and no athletic dogs. Vet. J. 307(1), 106207.

Bhattacharya, T.K., Reddy, B.R., Chatterjee, R.N. and Ashwini, R. 2022. Myostatin (GDF8) gene and its intriguing role in regulating growth in poultry. Indian J. Anim. Sci. 92(10), 1141–1148.

Bi, Y., Feng, B., Wang, Z., Zhu, H., Qu, L., Lan, X., Pan, C. and Song, X. 2020. Myostatin (MSTN) gene indel variation and its associations with body traits in shaanbei white cashmere goat. Animals 10(1), 168.

Bigford, G.E., Donovan, A., Webster, M.T., Dietrich, W.D. and Nash, M.S. 2021. Selective myostatin inhibition spares sublesional muscle mass and myopenia-related dysfunction after severe spinal cord contusion in mice. J. Neurotrauma 38(24), 3440–3455.

Bruno, S., Landi, V., Senczuk, G., Brooks, S.A., Almathen, F., Faye, B., Gaouar, S.S., Piro, M., Kim, K.S., David, X. and Eggen, A. 2022. Refining the *Camelus dromedarius* myostatin gene polymorphism through worldwide whole-genome sequencing. Animals 12(16), 2068.

Cao, L., Ma, J., Chen, P., Hou, X., Yang, N., Lu, Y. and Huang, H. 2024. Exploring the influence of DNA methylation and single nucleotide polymorphisms of the Myostatin gene on growth traits in the hybrid grouper (*Epinephelus fuscoguttatus* (female) × *Epinephelus polyphekadion* (male)). Front. Genet. 14(1), 1277647.

Ceccobelli, S., Perini, F., Trombetta, M.F., Tavoletti, S., Lasagna, E. and Pasquini, M. 2022. Effect of myostatin gene mutation on slaughtering performance and meat quality in Marchigiana bulls. Animals 12(4), 518.

Chen, M., Lian, D., Li, Y., Zhao, Y., Xu, X., Liu, Z., Zhang, J., Zhang, X., Wu, S., Qi, S., Deng, S., Yu, K. and Lian, Z. 2023. Global long noncoding RNA expression profiling of MSTN and FGF5 double-knockout sheep reveals the key gatekeepers of skeletal muscle development. DNA Cell. Biol. 42(3), 163–175.

Chen, M.M., Zhao, Y.P., Zhao, Y., Deng, S.L. and Yu, K. 2021. Regulation of myostatin on the growth and development of skeletal muscle. Front. Cell. Dev. Biol. 9(1), 785712.

Chen, P.R. and Lee, K. 2016. Invited review: inhibitors of myostatin as methods of enhancing muscle growth and development. J. Anim. Sci. 94(8), 3125–3134.

Cheng-Li, L., Ri-Su, N., Wei-Wei, N., Guang-Xin, E., Yan-Guo, H., Yan, Z., Xiao, W., Shu-Zhu, C., Bai-Gao, Y., Xing-Hai, D. and Ze-Hui, G. 2023. Genetic diversity identification and haplotype distribution of myostatin gene in goats. Indian J. Anim. Res. 57(3), 273–281.

Csürhés, T., Szabó, F., Holló, G., Mikó, E., Török, M. and Bene, S. 2023. Relationship between some myostatin variants and meat production related calving, weaning and muscularity traits in charolais cattle. Animals 13(12), 1895.

Dementeva, N.V., Vakhrameev, A.B., Larkina, T.A. and Mitrofanova, O.V. 2019. Efficiency of using SNP markers in the MSTN gene in the selection of the Pushkin breed chickens. Vavilov. J. Genet. Breed. 23(8), 993–998.

Dilger, A.C., Chen, X., Honegger, L.T., Marron, B.M. and Beever, J.E. 2022. The potential for gene-editing to increase muscle growth in pigs: experiences with editing myostatin. CABI Agric. Biosci. 3(1), 36–45.

Du, C., Zhou, X., Zhang, K., Huang, S., Wang, X., Zhou, S. and Chen, Y. 2022. Inactivation of the MSTN gene expression changes the composition and function of the gut microbiome in sheep. BMC Microbiol. 22(1), 273.

Duan, X., Ji, W., Dong, B., Sun, G. and Bian, Y. 2016. Myostatin in black Muscovy duck (*Cairina moschata*): full-length cDNA cloning and age-dependent mRNA expression compared with IGF-I. Br. Poult. Sci. 57(5), 619–627.

Dushyanth, K., Shukla, R., Chatterjee, R.N. and Bhattacharya, T.K. 2022. Expression and polymorphism of follistatin (FST) gene and its association with growth traits in native and exotic chicken. Anim. Biotechnol. 33(5), 824–834.

Esposito, P., Picciotto, D., Battaglia, Y., Costigliolo, F., Viazzi, F. and Verzola, D. 2022. Myostatin: basic biology to clinical application. Adv. Clin. Chem. 106(1), 181–234.

Gaina, C.D. and Amalo, F.A. 2022. Genetic polymorphism of myostatin gene in Sumba Ongole

(*Bos indicus*) cattle and its association with growth traits. J. Adv. Vet. Anim. Res. 9(4), 565–572.

Garba, A.M., Mancha, Y.P., Bello, K.M., Panshak, L.N. and Zakka, T.E. 2024. Molecular genetic variation (polymorphisms) using in-silico methods in determining effect of GDF8 gene in cattle, sheep and goats. Niger. J. Agric. Agric. Technol. 4(3), 129–141.

Giri, S.K., Nayan, V., Legha, R.A., Pal, Y. and Bhardwaj, A. 2022. Characterization of partial sequence of myostatin gene Exon 2 along with SNP detection in Indian horse breeds (*Equus caballus*). J. Equine Vet. Sci. 116(1), 104047.

Gu, S., Wen, C., Li, J., Liu, H., Huang, Q., Zheng, J., Sun, C. and Yang, N. 2022. Temporal expression of myogenic regulatory genes in different chicken breeds during embryonic development. Int. J. Mol. Sci. 23(17), 10115.

Hai, C., Bai, C., Yang, L., Wei, Z., Wang, H., Ma, H., Ma, H., Zhao, Y., Su, G. and Li, G. 2023. Effects of different generations and sex on physiological, biochemical, and growth parameters of crossbred beef cattle by myostatin gene-edited luxi bulls and simmental cows. Animals 13(20), 3216.

Han, S.Z., Li, Z.Y., Paek, H.J., Choe, H.M., Yin, X.J. and Quan, B.H. 2021. Reproduction traits of heterozygous myostatin knockout sows crossbred with homozygous myostatin knockout boars. Reprod. Domest. Anim. 56(1), 26–33.

He, M., Wu, P., Chen, F., Zhang, B., Chen, L., Zhang, T., Zhang, L., Li, P., Wang, J. and Zhang, G. 2020. Transcriptome analysis of leg muscles in fast and slow growth Bian chickens. Anim. Biotechnol. 31(4), 295–305.

Hou, H., Wang, X., Yang, C., Cai, X., Lv, W., Tu, Y., Bao, A., Wu, Q., Zhao, W., Yao, J. and Ding, W. 2021. Comparative genome and transcriptome integration studies reveal the mechanism of pectoral muscle development and function in pigeons. Front. Genet. 12(1), 735795.

Hu, Y., Liu, H., Shan, Y., Ji, G., Xu, W., Shu, J. and Li, H. 2015. The relative expression levels of insulin-like growth factor 1 and myostatin mRNA in the asynchronous development of skeletal muscle in ducks during early development. Gene 567(2), 235–243.

Hua, Z., Xu, K., Xiao, W., Shu, C., Li, N., Li, K., Gu, H., Zhu, Z., Zhang, L., Ren, H., Zeng, Q., Yin, Y. and Bi, Y. 2023. Dual single guide RNAs-mediating deletion of mature myostatin peptide results in concomitant muscle fibre hyperplasia and adipocyte hypotrophy in pigs. Biochem. Biophys. Res. Commun. 673(1), 145–152.

Huang, K.L., Wang, J.W., Han, C.C., Liu, H.H., Li, L., Dai, F., Pan, Z., Xu, F., He, H. and Xu, H. 2011. Developmental expression and alternative splicing of the duck myostatin gene. Comp. Biochem. Physiol. Part D Genomics Proteomics 6(3), 238–243.

Iroanya, G., Osaiyuwu, O., Emmanuel, H. and Fijabi, O. 2021. Genetic polymorphism of myostatin (MSTN) in Nigerian sheep breeds. J. Anim. Vet. Sci. 6(2), 64–73.

Isık, R., Ozdil, F. and Meral, S. 2022. Evaluation of variation on Myostatin (MSTN) gene of Turkish donkey populations in Thrace Region of Turkey. J. Tekirdag Agric. Facul. 19(2), 426–434.

Jakaria, J., Aliyya, W.L.N., Ismail, R., Siswanti, S.Y., Ulum, M.F. and Priyanto, R. 2021. Discovery of SNPs and indel 11-bp of the myostatin gene and its association with the double-muscled phenotype in Belgian blue crossbred cattle. Gene 784(1), 145598.

Jang, J., Park, S., Kim, Y., Jung, J., Lee, J., Chang, Y., Lee, S.P., Park, B.C., Wolfe, R.R., Choi, C.S. and Kim, I.Y. 2021. Myostatin inhibition-induced increase in muscle mass and strength was amplified by resistance exercise training, and dietary essential amino acids improved muscle quality in mice. Nutrients 13(5), 1508.

Ju, X., Liu, Y., Shan, Y., Ji, G., Zhang, M., Tu, Y., Zou, J., Chen, X., Geng, Z. and Shu, J. 2021. Analysis of potential regulatory LncRNAs and CircRNAs in the oxidative myofiber and glycolytic myofiber of chickens. Sci. Rep. 11(1), 20861.

Kalds, P., Zhou, S., Huang, S., Gao, Y., Wang, X. and Chen, Y. 2023. When less is more: targeting the myostatin gene in livestock for augmenting meat production. J. Agric. Food Chem. 71(10), 4216–4227.

Kim, D.H., Choi, Y.M., Lee, J., Shin, S., Kim, S., Suh, Y. and Lee, K. 2022. Differential Expression of MSTN isoforms in muscle between broiler and layer chickens. Animals 12(5), 539.

Kim, D.H., Choi, Y.M., Suh, Y., Shin, S., Lee, J., Hwang, S. and Lee, K. 2020a. Research note: association of temporal expression of myostatin with hypertrophic muscle growth in different Japanese quail lines. Poult. Sci. 99(6), 2926–2930.

Kim, D.H., Choi, Y.M., Suh, Y., Shin, S., Lee, J., Hwang, S., Lee, S.S. and Lee, K. 2021a. Research note: increased myostatin expression and decreased expression of myogenic regulatory factors in embryonic ages in a quail line with muscle hypoplasia. Poult. Sci. 100(4), 100978.

Kim, G.D., Lee, J.H., Song, S., Kim, S.W., Han, J.S., Shin, S.P., Park, B.C. and Park, T.S. 2020b. Generation of myostatin-knockout chickens mediated by D10A-Cas9 nickase. FASEB J. 34(4), 5688–5696.

Kim, J.H., Kim, J.H., Jang, J.P., Jang, J.H., Jin, D.H., Kim, Y.S. and Jin, H.J. 2021b. Identification of molecules from coffee silverskin that suppresses myostatin activity and improves muscle mass and strength in mice. Molecules 26(9), 2676.

Konovalova, E., Romanenkova, O., Zimina, A., Volkova, V. and Sermyagin, A. 2021. Genetic variations and haplotypic diversity in the myostatin gene of different cattle breeds in Russia. Animals 11(10), 2810.

Koohgivi, K., Rooshanfekr, H.A., Nazari, M. and Tatar, A. 2019. Effect of DL-methionine replacement with L-methionine and different dietary protein levels on myostatin gene expression in Japanese quails. Agric. Biotechnol. J. 11(2), 23–36.

Kour, A., Haunshi, S., Rajaravindra, K.S., Prince, L.L. and Rajkumar, U. 2024. Poultry breeding under public sector in India–Achievements and future perspectives: a comprehensive review. Indian J. Anim. Sci. 94(3), 191–202.

Kruszewski, M. and Aksenov, M.O. 2022. Association of myostatin gene polymorphisms with strength and muscle mass in athletes: a systematic review and meta-analysis of the MSTN Rs1805086 mutation. Genes 13(11), 2055.

Kumaravel, V., Mohan, B., Natarajan, A., Murali, N., Selvaraj, P. and Vasanthakumar, P. 2023. Effect on growth performance, carcass traits, and myostatin gene expression in Aseel chicken fed varied levels of dietary protein in isocaloric energy diets. Trop. Anim. Health Prod. 55(2), 82.

Lassiter, K., Kong, B.C., Piekarski-Welsher, A., Dridi, S. and Bottje, W.G. 2019. Gene expression essential for myostatin signaling and skeletal muscle development is associated with divergent feed efficiency in pedigree male broilers. Front. Physiol. 10(1), 126.

Lee, J., Kim, D.H., Brower, A.M., Schlachter, I. and Lee, K. 2021. Effects of myostatin mutation on onset of laying, egg production, fertility, and hatchability. Animals 11(7), 1935.

Lee, J., Tompkins, Y., Kim, D.H., Kim, W.K. and Lee, K. 2023. The effects of myostatin mutation on the tibia bone quality in female Japanese quail before and after sexual maturation. Poult. Sci. 102(7), 102734.

Li, X., Zhang, M., Feng, J. and Zhou, Y. 2021. Myostatin and related factors are involved in skeletal muscle protein breakdown in growing broilers exposed to constant heat stress. Animals 11(5), 1467.

Li, Y.D., Xue, B.A., Xin, L.I., Wang, W.J., Li, Z.W., Ning, W.A., Fan, X.I., Gao, H.H., Guo, H.S., Hui, L.I. and Wang, S.Z. 2022. Integration of genome-wide association study and selection signatures reveals genetic determinants for skeletal muscle production traits in an F2 chicken population. J. Integr. Agric. 21(7), 2065–2075

Lin, Y.S., Lin, F.Y. and Hsiao, Y.H. 2019. Myostatin is associated with cognitive decline in an animal model of Alzheimer's disease. Mol. Neurobiol. 56(3), 1984–1991.

Liu, J., Zhou, Y., Hu, X., Yang, J., Lei, Q., Liu, W., Han, H., Li, F. and Cao, D. 2021b. Transcriptome analysis reveals the profile of long non-coding RNAs during chicken muscle development. Front. Physiol. 12(1), 660370.

Liu, Y., Xu, C., Asiamah, C.A., Ye, R., Pan, Y., Lu, L.L., Zhao, Z., Jiang, P. and Su, Y. 2021a. Decorin regulates myostatin and enhances proliferation and differentiation of embryonic myoblasts in Leizhou black duck. Gene 804(1), 145884.

Lou, Z., Zhao, Y., Zhang, Y., Zheng, B., Feng, H., Hosain, M.A. and Xue, L. 2021. MiR-2014-5p and miR-1231-5p regulate muscle growth of *Larimichthys crocea* by targeting MSTN gene. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 252(1), 110535.

Lu, J., Hou, S., Huang, W., Yu, J. and Wang, W. 2011. Polymorphisms in the myostatin gene and their association with growth and carcass traits in duck. Afr. J. Biotechnol. 10(54), 11309–11312.

Maeta, K., Farea, M., Nishio, H. and Matsuo, M. 2023. A novel splice variant of the human MSTN gene encodes a myostatin-specific myostatin inhibitor. J. Cachexia Sarcopenia Muscle 14(5), 2289–2300.

Malila, Y., Thanatsang, K.V., Sanpinit, P., Arayamethakorn, S., Soglia, F., Zappaterra, M., Bordini, M., Sirri, F., Rungrassamee, W., Davoli, R. and Petracci, M. 2022. Differential expression patterns of genes associated with metabolisms, muscle growth and repair in Pectoralis major muscles of fast-and medium-growing chickens. PLoS One 17(10), e0275160.

Meyermans, R., Janssens, S., Coussé, A., Gorssen, W., Hubin, X., Mayeres, P., Veulemans, W., Claerebout, E., Charlier, C. and Buys, N. 2022. Myostatin mutation causing double muscling could affect increased psoroptic mange sensitivity in dual purpose Belgian Blue cattle. Animal 16(3), 100460.

Mohamedien, D., Mokhtar, D.M., Abdellah, N., Awad, M., Albano, M. and Sayed, R.K.A. 2023. Ovary of zebrafish during spawning season: ultrastructure and immunohistochemical profiles of Sox9 and myostatin. Animals 13(21), 3362.

Mohammadabadi, M., Bordbar, F., Jensen, J., Du, M. and Guo, W. 2021. Key genes regulating skeletal muscle development and growth in farm animals. Animals 11(3), 835.

Muramatsu, H., Kuramochi, T., Katada, H., Ueyama, A., Ruike, Y., Ohmine, K., Shida-Kawazoe, M., Miyano-Nishizawa, R., Shimizu, Y., Okuda, M., Hori, Y., Hayashi, M., Haraya, K., Ban, N., Nonaka, T., Honda, M., Kitamura, H., Hattori, K., Kitazawa, T., Igawa, T., Kawabe, Y. and Nezu, J. 2021. Novel myostatin-specific antibody enhances muscle strength in muscle disease models. Sci. Rep. 11(1), 2160.

Nasr, M.A.F., Reda, R.M., Ismail, T.A. and Moustafa, A. 2021. Growth, hemato-biochemical parameters, body composition, and myostatin gene expression

of clarias gariepinus fed by replacing fishmeal with plant protein. Animals 11(3), 889.

Nejad, F.M., Mohammadabadi, M., Roudbari, Z., Gorji, A.E. and Sadkowski, T. 2024. Network visualization of genes involved in skeletal muscle myogenesis in livestock animals. BMC Genomics 25(1), 294.

O'Hara, V., Cowan, A., Riddell, D., Massey, C., Martin, J. and Piercy, R.J. 2021. A highly prevalent SINE mutation in the myostatin (MSTN) gene promoter is associated with low circulating myostatin concentration in Thoroughbred racehorses. Sci. Rep. 11(1), 7916.

Osman, N.M., Shafey, H.I., Abdelhafez, M.A., Sallam, A.M. and Mahrous, K.F. 2021. Genetic variations in the myostatin gene affecting growth traits in sheep. Vet. World 14(2), 475–482.

Paek, H.J., Luo, Z.B., Choe, H.M., Quan, B.H., Gao, K., Han, S.Z., Li, Z.Y., Kang, J.D. and Yin, X.J. 2021. Association of myostatin deficiency with collagen related disease-umbilical hernia and tippy toe standing in pigs. Transgenic Res. 30(5), 663–674.

Paez, M.A., Tovar, N.J., MendozaSánchez, G., Muñoz, M.F., Molina, B.J., Torres, A.F., Gutíerrez, J.A. and Buitrago, J.D. 2021. Sequencing and analysis of the myostatin gene (GDF-8) in *Bubalus bubalis* young animals to determine the existence of possible mutations expressed in double musculature phenotype. J. Buffalo Sci. 10(1), 1–5.

Pira, E., Vacca, G.M., Dettori, M.L., Piras, G., Moro, M., Paschino, P. and Pazzola, M. 2021. Polymorphisms at myostatin gene (MSTN) and the associations with sport performances in Anglo-Arabian racehorses. Animals 11(4), 964.

Prihandini, P.W., Primasari, A., Aryogi, A., Efendy, J., Luthfi, M., Pamungkas, D. and Hariyono, D.N.H. 2021. Genetic variation in the first intron and exon of the myostatin gene in several Indonesian cattle populations. Vet. World 14(5), 1197–1201.

Priyadharsini, R., Gopinathan, A., Karthickeyan, S.M. and Jagatheesan, P.N. 2021. Association of myostatin gene with on-field sporting traits of indigenous cattle breeds (*Bos indicus*) of Tamil Nadu. Indian J. Vet. Sci. Biotechnol. 17(3), 6–10.

Ren, P., Chen, M., Li, J., Lin, Z., Yang, C., Yu, C., Zhang, D. and Liu, Y. 2023. MYH1F promotes the proliferation and differentiation of chicken skeletal muscle satellite cells into myotubes. Anim. Biotechnol. 34(7), 3074–3084.

Rodgers, B.D. and Ward, C.W. 2022. Myostatin/activin receptor ligands in muscle and the development status of attenuating drugs. Endocr. Rev. 43(2), 329–365.

Ryan, A.S. and Li, G. 2021. Skeletal muscle myostatin gene expression and sarcopenia in overweight and obese middle-aged and older adults. JCSM Clin. Rep. 6(4), 137–142.

Ryan, C.A., Purfield, D.C., Naderi, S. and Berry, D.P. 2023. Associations between polymorphisms in the myostatin gene with calving difficulty and carcass merit in cattle. J. Anim. Sci. 101(1), skad371.

Rybalka, E., Timpani, C.A., Debruin, D.A., Bagaric, R.M., Campelj, D.G. and Hayes, A. 2020. The failed clinical story of myostatin inhibitors against duchenne muscular dystrophy: exploring the biology behind the battle. Cells 9(12), 2657.

Saif, R., Raza, M.H., Zafar, M.O., Tariq, W., Danish, M. and Wasim, M. 2024. Genetic Association of MSTN gene variant (18: 66493737t> c) with track performance & muscle development in Pakistani horses: genetic association of MSTN gene variant in horses. Futuristic Biotechnol. 4(1), 43–47.

Sakar, Ç.M. and Zülkadir, U. 2022. Determination of the relationship between Anatolian black cattle growth properties and myostatin, GHR and Pit-1 gene. Anim. Biotechnol. 33(3), 536–545.

Sheng, H., Guo, Y., Zhang, L., Zhang, J., Miao, M., Tan, H., Hu, D., Li, X., Ding, X., Li, G. and Guo, H. 2021. Proteomic studies on the mechanism of myostatin regulating cattle skeletal muscle development. Front. Genet. 12(1), 752129.

Shoyombo, A.J., Abdulmojeed, Y., Alabi, O.O., Popoola, M.A., Okon, E.M. and Arije, D.O. 2022. Applications of myostatin in poultry and aquaculture-a review. Open Agric. J. 16(1), 1–9.

Smołucha, G., Kozubska-Sobocińska, A., Koseniuk, A., Żukowski, K., Lisowski, M. and Grajewski, B. 2019. Polymorphism of the myostatin (MSTN) gene in Landes and Kielecka geese breeds. Animals 10(1), 10.

Sonali, Giri, S.K., Unnati, Nayan, V., Legha, R.A., Pal, Y. and Bhardwaj, A. 2022. Characterization of partial sequence of myostatin gene exon 2 along with SNP detection in Indian horse breeds (*Equus caballus*). J. Equine Vet. Sci. 116(1), 104047.

Sousa-Junior, L.P., Meira, A.N., Azevedo, H.C., Muniz, E.N., Coutinho, L.L., Mourão, G.B., Leão, A.G., Pedrosa, V.B. and Pinto, L.F. 2022. Variants in myostatin and MyoD family genes are associated with meat quality traits in Santa Inês sheep. Anim. Biotechnol. 33(2), 201–213.

Sukhija, N., Kanaka, K.K., Goli, R.C., Kapoor, P., Sivalingam, J., Verma, A., Sharma, R., Tripathi, S.B. and Malik, A.A. 2023. The flight of chicken genomics and allied omics-a mini review. Ecol. Genet. Genom. 29(1), 100201.

Swanson, D.L., Zhang, Y. and Jimenez, A.G. 2022. Skeletal muscle and metabolic flexibility in response to changing energy demands in wild birds. Front. Physiol. 13(1), 961392.

Tanjung, A., Saragih, H.T.S.S.G., Trijoko, Soenarwan, H.P., Widianto, S., Mahardhika, I.W.S. and Daryono, B.S. 2019. Polymorphism of myostatin gene and its association with body weight traits in a hybrid of

GAMA chicken (*Gallus gallus domesticus* Linn. 1758). Biodiversitas 20(11), 3207–3212.

Tao, Z., Zhu, C., Song, C., Song, W., Ji, G., Shan, Y., Xu, W. and Li, H. 2015. Lentivirus-mediated RNA interference of myostatin gene affects MyoD and Myf5 gene expression in duck embryonic myoblasts. Br. Poult. Sci. 56(5), 551–558.

Tekerli, M., Erdoğan, M., Koçak, S., Çelikeloğlu, K., Yazıcı, E., Hacan, Ö., Bozkurt, Z., Demirtaş, M. and Çinkaya, S. 2022. The comparative results of myostatin introgression from donor Texel to recipient Ramlıç sheep with the aspects of growth, pre-, and post-slaughter carcass traits in the second backcross generation. Arch. Anim. Breed 65(2), 231–238.

Thepa, T.L. and Tyasi, T.L. 2024. A systematic review of myostatin gene variations and their association with growth traits in sheep. Adv. Anim. Vet. Sci. 12(6), 1199–1205.

Tian, W., Wang, Z., Wang, D., Zhi, Y., Dong, J., Jiang, R., Han, R., Li, Z., Kang, X., Li, H. and Liu, X. 2021. Chromatin interaction responds to breast muscle development and intramuscular fat deposition between Chinese indigenous chicken and fast-growing broiler. Front. Cell Dev. Biol. 9(1), 782268.

Vinet, A., Bouyer, C., Forestier, L., Oulmouden, A., Blanquet, V., Picard, B., Cassar-Malek, I., Bonnet, M., Rocha, D. and Renand, G. 2021. The Blonde d'Aquitaine T3811> G3811 mutation in the myostatin gene: association with growth, carcass, and muscle phenotypes in veal calves. J. Anim. Sci. 99(2), skab039.

Waller, B.E., Garcia, S.R., Fuerniss, L.K., Johnson, B.J., Woerner, D.R. and Wulf, D.M. 2023. Effects of the F94L myostatin gene mutation in beef× dairy crossed cattle on muscle fiber type, live performance, carcass characteristics, and boxed beef and retail cut yields. J. Anim. Sci. 101(1), skad324.

Wang, J., Li, J., Ge, Q. and Li, J. 2021. A potential negative regulation of myostatin in muscle growth during the intermolt stage in *Exopalaemon carinicauda*. Gen. Comp. Endocrinol. 314(1), 113902.

Wu, D., Gu, M., Wei, Z., Bai, C., Su, G., Liu, X., Zhao, Y., Yang, L. and Li, G. 2022. Myostatin knockout regulates bile acid metabolism by promoting bile acid synthesis in cattle. Animals 12(2), 205.

Wu, P., Zhou, K., Zhang, L., Li, P., He, M., Zhang, X., Ye, H., Zhang, Q., Wei, Q. and Zhang, G. 2021. High-throughput sequencing reveals crucial miRNAs in skeletal muscle development of Bian chicken. Br. Poult. Sci. 62(5), 658–665.

Yan, Z., Yan, Z., Liu, S., Yin, Y., Yang, T. and Chen, Q. 2021. Regulative mechanism of guanidinoacetic acid on skeletal muscle development and its application prospects in animal husbandry: a review. Front. Nutr. 8(1), 714567.

Yanesari, F.R., Hassani, S. and Najafi, M. 2023. Identification of different allelic forms of myostatin gene and investigation of body weight and carcass biometric traits measured by ultrasound in Kurdi sheep. J. Rumin. Res. 11(1), 37–54.

Yuan, Y., Duan, W., Yang, N., Sun, C., Nie, Q., Li, J. and Lian, L. 2024. Transcriptome analysis of long non-coding RNA associated with embryonic muscle development in chickens. Br. Poult. Sci. 65(4), 394–402.

Zakka, T.E., Mancha, Y.P., Bello, K.M., Garba, A.M. and Luka, P.N. 2024. Effects of mutations of myostatin (MSTN) gene in breeds of sheep in Nigeria. Niger. J. Agric. Agric. Technol. 4(3), 187–193.

Zegyer, E.A.K. and Mutlag, A.M. 2022. Histological and chronological study of myostatin (MSTN) role on muscles growth in domestic broiler chicken. Int. J. Health Sci. 6(S3), 2415–2432.

Zhang, G., He, M., Wu, P., Zhang, X., Zhou, K., Li, T., Zhang, T., Xie, K., Dai, G. and Wang, J. 2021. MicroRNA-27b-3p targets the myostatin gene to regulate myoblast proliferation and is involved in myoblast differentiation. Cells 10(2), 423.

Zhang, L., Liu, L., Chen, H., Wang, R. and Yue, B. 2018. Single nucleotide polymorphism screening and bioinformatics analysis of myostatin gene in Jingning chicken. J. Zhejiang Univ. Agric. Life Sci. 44(5), 629–637.

Zhang, X., Wang, F., Ou, M., Liu, H., Luo, Q., Fei, S., Zhao, J., Chen, K., Zhao, Q. and Li, K. 2023. Effects of myostatin B knockout on offspring body length and skeleton in yellow catfish (*Pelteobagrus fulvidraco*). Biology 12(10), 1331.

Zhang, X.X., Ran, J.S., Lian, T., Li, Z.Q., Yang, C.W., Jiang, X.S., Du, H.R., Cui, Z.F. and Liu, Y.P. 2019. The single nucleotide polymorphisms of myostatin gene and their associations with growth and carcass traits in Daheng broiler. Braz. J. Poult. Sci. 21(1), eRBCA-2018

Zhao, X., Wang, G., Han, H., Zhou, Y., Feng, J. and Zhang, M. 2023. Effects of atmospheric ammonia on skeletal muscle growth in broilers. Animals 13(12), 1926.

Zhao, X.H., Li, M.Y., Xu, S.S., Sun, J.Y. and Liu, G.J. 2019. Expression of myostatin (MSTN) and myogenin (MYOG) genes in Zi and Rhine goose and their correlation with carcass traits. Braz. J. Poult. Sci. 21(1), eRBCA-2019.

Zhao, Y., Yang, L., Su, G., Wei, Z., Liu, X., Song, L., Hai, C., Wu, D., Hao, Z., Wu, Y. and Zhang, L. 2022. Growth traits and sperm proteomics analyses of myostatin gene-edited Chinese yellow cattle. Life 12(5), 627.

Zhou, K.Z., Wu, P.F., Zhang, X.C., Ling, X.Z., Zhang, J., Zhang, L., Li, P.F., Zhang, T., Wei, Q.Y. and Zhang, G.X. 2022b. Comparative analysis of

miRNA expression profiles in skeletal muscle of bian chickens at different embryonic ages. Animals 12(8), 1003.

Zhou, T., Wu, Y., Bi, Y., Bai, H., Jiang, Y., Chen, G., Chang, G. and Wang, Z. 2022a. MYOZ1 gene promotes muscle growth and development in meat ducks. Genes 13(9), 1574.

Zhu, P., Li, H., Huang, G., Cui, J., Zhang, R., Cui, K., Yang, S. and Shi, D. 2018. Molecular cloning, identification, and expression patterns of myostatin gene in water buffalo (*Bubalus bubalis*). Anim. Biotechnol. 29(1), 26–33.