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Original Research Article

APPLICATION OF CRISPR TO INCREASE MEAT YIELD IN CHICKEN

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Abstract *Meat production is one of the major purposes of the poultry industry. Meat is full of protein and accomplishes the protein requirement of the diet. This study was carried out to attempt to use CRISPR-mediated genome editing of chicken to increase meat yield. For this purpose, CRISPR was used to edit the gene sequence and then it was allowed to infect the PGC's (Primordial germ cells). The successfully edited PGC's having blocked exon 3 of MSTN gene were then injected into the blood bloodstream of 2.6 days of fertilized egg cells. The eggs were incubated up to hatch. The wild type was taken as control and the Mutated ones as T1. The CRD was used with three replications to find the significance of variation in To and T1. The weight of chicks for T0 (wild type) and T1 (Mutated) was significantly different. The average weight of three replications of each treatment was compared through a bar graph. it was found that the CRISPR-mediated edited chicks were more weighted than that of their wild types. The CRISP-mediated inhibition of MSTN has a significant effect on muscle growth in chickens. The more such genes may be targeted to get more meat yield.*

Keywords*:* *CRISPR Cas9; Poultry industry; Chicken production; Meat yield; PGS's MSTN gene; Chicken*

Introduction

Poultry chicken is the most important source of protein worldwide. The demand for chicken is increasing day by day along with population (Mottet et al., 2017). According to FAO global chicken meat production should reach over 70% by 2050 to meet the increasing population's demand. So, it is necessary to work on food security following an increase in high meat yield in production (Gerber et al., 2013). In chickens, the muscle growth is dependent upon many genetic factors. One of the important genes involved in muscle development in chicken is the myostatin (MSTN) gene (Yang et al., 2013). The expression of this gene has a negative role in muscle development. It inhibits the proliferation of myoblasts, the precursors of muscle tissues. In many species (mice, rats, cattle, and chicken) the MSTN gene expression is inhibited through mutations, and this increases the muscle mass of chicken. The MSTN inhibition is a target in the poultry industry for increased chicken meat production (Li et al., 2018). The CRISPR Cas9 system, along with other recent gene editing techniques, revolutionized the field of genetic engineering. It has an edge over other techniques in that it can target the gene sequence to make desirable gene modifications very precisely. It can also control the expression of a gene (Doudna et al., 2014). In poultry, it can be used for inhibition of MSTN gene for increment in muscle mass of chicken. The knockout of MSTN gene can inhibit the expression of MSTN gene. The production of myostatin protein which hurts muscle growth can stopped. As a result, more muscle growth can increase yield in the chicken (Kald et al., 2023).

Materials and Methods

The selected gene (MSTN) sequence which is present on chromosome number 7 of the chicken genome, was obtained from the Ensembl genome database. The exon-3 of MSTN gene is usually considered for myostatin function. So, it was selected as a target site for CRISPR-mediated mutagenesis. MIT was used to

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design sgRNA to be attached to the target site. Meanwhile, the fertilized chicken eggs were incubated for two days (germinal crescent stage) at 37.5° C and 50% humidity. After two days primordial germ cells were separated from egg cells with the help of a fine needle. After this, it was cultured in DMEM (Dulbecco's Modified Eagle Medium) strengthened with 10% FBS *(fetal bovine serum FBS)*, penicillin-streptomycin, and 2 mM L-glutamine under standard conditions in a CO2 incubator. The Cas9 gene and the sgRNA (CRISPR Cas9 Plasmid) were then transfected into the PGCs in an electroporator. These PGCs were then cultured in DMEM supplemented with 10 % FBS for 48 hours to give time for CRISPR/Cas9 components to express. The screening of transfected cells was done through neomycin (300 µg/mL) for 7-10 days. After screening the surviving cells were selected and their DNA was extracted. PCR was performed using primers flanking the targeted *MSTN* locus. The PCR products were analyzed by Sanger sequencing to confirm the presence of indels at the target site. The confirmed correctly edited PGC as an outcome of Sanger sequencing, was injected into blood bloodstream of 2.6 days-old embryos of chicken. The embryos were incubated under standard conditions to hatch chicks. The wild types were taken as control and CRISPR-mediated mutated chicks as T1. Three replications of each treatment were maintained. The weight of chicks was measured with digital weight balance after 21 days and 35 days. The average weight of all three replications was analyzed through analysis of variance by using Staitstix 8.1.

Reagents and Chemicals used in the whole process

1. Plasmid vectors: pX330-U6-Chimeric_BB-CBh-hSpCas9
2. Single guide RNA (sgRNA)
3. CRISPR Design Tool (MIT)
4. DNA oligonucleotides
5. Neomycin for selection
6. Dulbecco's Modified Eagle Medium (DMEM)
7. Fetal bovine serum (FBS)
8. PCR reagents: Taq DNA polymerase, dNTPs, MgCl2, and primers
9. Sanger sequencing reagents: BigDye Terminator v3.1 Cycle Sequencing Kit

Equipment Used

1. CO2 incubator
2. Biosafety cabinet
3. Electroporator (Bio-Rad Gene Pulser X-cell)
4. Thermocycler:
5. Gel electrophoresis apparatus
6. Fluorescent microscope
7. Histology equipment: Microtome, tissue processor, and staining setup
8. Real-time PCR machine
9. Biological Materials
10. Fertilized chicken eggs
11. Chicken primordial germ cells (PGCs)
12. Wild-type hens and roosters

Results and discussion

The average weight of three replications of chicken for wild type and mutated type is given in Fig 1. It can be seen that in control (wild type) the weight at 21 days was 1.28 kg and the weight after 35th was 1.43 kg. In mutated chicks in which MSTN gene was knocked out by using CRISPR, the average weight after 21 days was 1.25 kg, and the average weight after 35 days was 1.54 kg. In control, the chicks gained a weight of 0.18 kg in 14 days, and mutated chicks gained 0.29 kg in 14 days.

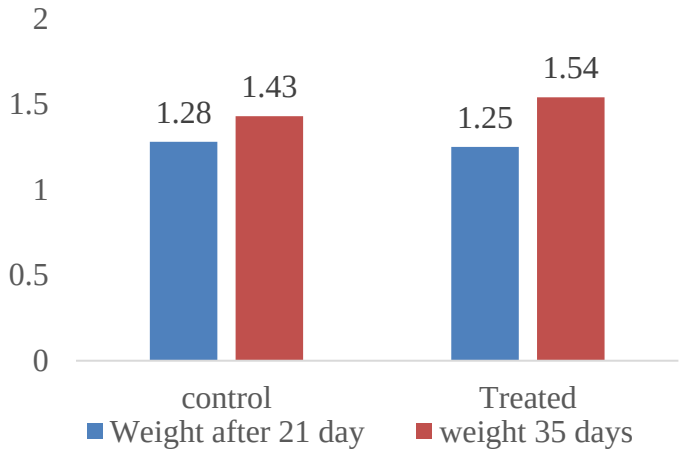

Fig 1 The variation in chicken weight in wild type and mutated type in at 21st day and 35th Day

Table 1 shows the analysis of variance in wild type and mutant types. It suggests high significant variation in the wild and mutant. It means that the wild type and mutated chicks were very different from each other in weight in leg muscle weight.

Table 1 ANOVA table for chicken weight in wild type and mutated type through CRISPR Cas9 system in at 21st day and 35th Day

Source	DF	SS	MS	F	P
Treatment	1	0.14963	0.14963	58.8**	0.002
Error	10	0.02543	0.00254		
Total	11	0.17507			

The coding of MSTN gene (Wild type)

ATGAAGGACGGCATTGACAGCTGTACACATC
CGAGGCCAGCTTCAAGGTGTTGCAGGTGGCT

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CCTGCTGAGATACACGGGGTACAGGAAGGA
GCGGAGCCTGCCGTGGTGCTGACCAAGGGG
ACGGCCTTCCCTGTTGACTCTGCTCGCTGGTG
CAGCCTTGACTCCAACTTCTACAACTCCAGA
CTCCTGGGTCCTACCTCAGCACCACCACCTT
CTGGATACACACATGAGGCCTCGGCTCCTGG
CACCTGTTGGAGCCTGGAAGAGTGGATGAA
GGTGGAAGATGATGACGACATCTCCATGGTC
CACCTGAGCCTACGGGCAGGAAGCCCGCGA
CGCCTTCCAGCCCTCCATCAACGACGACGAG
AGCCTGGCCCGAGTGGTGGCGTCTTGATCTT
CAAGAAGAGCCCTGACCGTGCCAGACATCGT
GGAGGAGTACATCGTCTGGCAGATGTCTGAG
ACAGCAGCGGGTGGAATCTGCAGACTGCAC
GTGACCCAGGTCCTGAGGAGCGTGTGGGTGG
CTGAAGATGGCCTCTGCTCTCCCTGAGCTTTC
TGATGAGTGGGTGGCCGAGTGGGAAGTGTG
AGGTGCGCAGGGTGGGGCCAGGCAGCTGCA
GTGTTGTGAGCCGGAAGCTGAGGAGGAGCG
TGCTGTGAGCCTGCTGGCCTTCCGTCAGGCG
GTTGTGTAGCAGTGCCTGCAGCGTGTGCCGG
TGTGAGAGCCACAGTGTTGTGAGCGGGAAG
CCATCCGAGGCGGTTGCGGGGAGCCGTGGA
ACAGCCGGGTCTGA

Guide RNA for MSTN gene (promotor region)

5'-CACACATCCGAGGCCAGC-3'

Wang et al., (2015) reported high efficiency (85%) of CRISPR in knocking out the MSTN gene and hitting the target precisely). The use of electroporation and antibiotic selection ensured that only successfully edited cells were propagated, enhancing the overall effectiveness of the gene editing process. The phenotypic changes (muscle growth) in pigs were observed by Qian et al., (2015) in G_o and subsequent generations due to inhibition of MSTN gene. The same type of increased muscle growth was observed in cattle by Grobet et al., (1997). Previous studies in cattle have shown that while *MSTN* knockout improves muscle mass, it can also lead to changes in muscle fiber composition and meat tenderness (Fiems, 2012). The effective transmission of the edited gene across generations without significant loss of function suggests that the MSTN knockout can be stably maintained in a breeding program, potentially leading to the establishment of a new line of high-yield poultry (Hi et al., 2014). Van Eenennaam and Young (2018). got the same results as our research. They found increased meat yield in chickens by knocking out the MSTN gene with the help of CRISPR. Moreover, they suggested assessing the long-term effects of *MSTN* knockout on chicken physiology and welfare. The potential impacts on reproductive fitness, health, and behavior are critical considerations before commercial application. Additionally, ethical concerns surrounding genetic modifications in animals must be addressed, particularly in the context of food production. The use of CRISPR/Cas9 in livestock raises questions about the balance between technological advances and the principles of animal welfare and sustainability (Van Eenennaam & Young, 2018).

Conclusion

It is concluded that inhibition of the expression of MSTN gene has a significant effect on muscle growth and development. There was found significant increase in meat production in wild and mutated types. CRISPR Cas9 proved to be a very useful and powerful tool for gene editing in chickens to increase muscle mass.

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Declaration

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

The study was approved by authors.

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Authors' Contribution

All authors contributed equally.

Conflict of interest

There is no conflict of interest among the authors of the manuscript.