Research Note: Improved feed efficiency in quail with targeted genome editing in the myostatin gene

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ABSTRACT Increased growth rate and decreased cost of feed are main focuses to increase revenue of poultry farms. Myostatin (**MSTN**) is a negative regulator of muscle growth and mutation on MSTN results in increased muscle growth. Due to the antimyogenic function of MSTN, MSTN gains high attention as a potential target and genetic selection marker to increase meat yield in the livestock industry. In addition, MSTN can affect feed efficiencies and, thus decrease total feed requirement as shown in increased feed efficiencies in pigs and cattle with MSTN mutations. Although MSTN mutation in various animal species has been previously studied, MSTN mutation in avian species has only recently been generated to characterize its biological function. However, beneficial effects of MSTN mutation on poultry production need to be further investigated. In this study, using the MSTN mutant quail, feed efficiency related to interplay of changes in body weight gain (**WG**), feed intake (**FI**), and fat accretion were investigated. WG of mutant quail were significantly higher ($P< 0.001$) than those of wild-type (**WT**) from all time periods, 10-d interval from post-hatching day (**D**)10 to 40. Feed intake of mutant quail were significantly higher than those of WT from D 10 to 20 ($P< 0.01$) and D 20 to 30 ($P< 0.001$), but not from D 30 to 40, resulting in a significantly lower feed conversion ratio (**FCR**) of mutant quail compared to WT quail only from D 30 to 40 ($P< 0.001$). From those results, overall (D 10 to 40) FCR was significantly lower in mutant quail ($P< 0.001$) indicating improved feed efficiency by MSTN mutation. In addition, percentages of leg or abdominal fat compared to body weight in mutant quail at 8 wk were significantly lower than WT ($P< 0.05$). In combination to greater WG, less fat accretion might partially contribute to improved feed efficiency in MSTN mutant quail. As there is a current preference of meat with lower fat as a healthy food, MSTN can be used for the potential selection marker for not only bigger and leaner poultry, but also better feed efficiency that can satisfy both producers and consumers.

Key words: myostatin, feed conversion ratio, weight gain, fat content, quail

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INTRODUCTION

Myostatin (**MSTN**) is known to negatively regulate muscle growth and MSTN mutations result in increasing body weight and muscle mass, along with decreasing fat deposition, in cattle, mice, lamb, fishes, and pigs. (McPherron & Lee 1997; McPherron et al., 1997; Boman et al., 2010; Ohama et al., 2020; Ren et al., 2020). Such characteristics are important traits in the livestock industry to increase carcass yield and thus, MSTN gained attention as a potential selection marker (Aiello et al., 2018). For these reasons, animals with natural and genetically induced mutations in the *MSTN* gene are actively investigated to understand growth performance and carcass characteristics (Boman et al., 2010; Fiems 2012; Ohama et al., 2020; Ren et al., 2020). However, studies about MSTN function in avian species have been limited until the recent generation of MSTN mutant quail and chickens (Kim et al., 2020; Lee et al., 2020).

In our previous study (Lee et al., 2020), MSTN mutant quail with the deletion of three base pairs causing C42del in the propeptide region was generated by an injection of adenoviral CRISPR/Cas9 system into the blastoderm and showed a significantly higher body weight and muscle mass compared to wild-type quail. Increased body weight and muscle mass were also found in MSTN knockout chickens (Kim et al., 2020). However, other important traits for growth performance still need to be further investigated in MSTN mutant birds.

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Indeed, better growth performances, such as greater weight gain and efficient feed conversion ratio, from MSTN knockout cattle, fishes, and pigs compared to wild-type have been reported (Fiems 2012; Ohama et al., 2020; Ren et al., 2020). As feed accounts for the main production cost, analysis of feed efficiency of MSTN mutant quail can give a potential application for reducing production cost in the poultry industry. The objective of the current study was to investigate interplay of body weight gain (**WG**), feed intake (**FI**), and fatness on feed conversion ratio (**FCR**) of MSTN mutant quail.

MATERIALS AND METHODS

Animal Care

Japanese quail (*Coturnix coturnix japonica*) used in this study were maintained at The Ohio State University Poultry Facilities in Columbus, OH, USA. All animal care protocols and procedures were approved by the Institutional Care and Use Committee at The Ohio State University (Protocol 2019A00000024). WT quail were obtained from a random bred control line and mutant quail were obtained from a MSTN homozygous mutant line with the deletion of three base pairs causing C42del in the propeptide region generated from our previous study (Lee et al., 2020). Quail were fed ad libitum and euthanized at 8 wk of age via CO_2 inhalation.

Experimental Design and Data Collection

After hatching, WT and mutant quail were raised in the same brooder cages and transferred to individual cages at 7-day-old to give 3 days of adaptation period before conducting the experiment. At post-hatching day (**D**) 10, feed was replaced with new feed after measuring the weight of new feed. A cover with holes was placed on the feeder to prevent any spills by quail. The weight of leftover feed was measured at D 20 and replaced to new feed after measuring the weight. The cycle was repeated two more times from D 20 to 30 and from D 30 to 40. Body weight of quail was measured at D 10, 20, 30, and 40 right before changing feed and transfer to the brooder cages after finishing the feed intake experiment at D 40. Final body weight, along with leg and abdominal fat pad weight were measured after euthanization at wk 8.

Statistical Analysis

The number of quail analyzed in this study was 10 and 12 for WT and MSTN mutant males, respectively and 9 for both WT and MSTN mutant females. Analysis of variance were performed using GraphPad Prism software, version 6.02, and all data were expressed as means ± SEM. Student t tests were used to analyze measurement of body weight at each time point (D 10, 20, 30, and 40) and fat percentage. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$. Also, multiple means were compared by two-way ANOVA for the analysis of WG, FI, and FCR followed by a Tukey's multiple comparison test ($P < 0.05$).

RESULTS AND DISCUSSION

As a negative regulator of muscle growth, MSTN has been considered as a future selection marker to increase meat yield in the livestock industry (Aiello et al., 2018). Although better growth performance with bigger muscle is a desired trait in the poultry industry, function of MSTN in avian species has been revealed lately using genome edited quail and chickens carrying MSTN mutation (Kim et al., 2020; Lee et al., 2020). In agreement with other MSTN mutant mammals (McPherron & Lee 1997; McPherron et al., 1997; Boman et al., 2010; Ren et al., 2020), MSTN mutant quail in this study showed significantly higher body weight (Figure 1A and B). Especially, higher body weights of mutant quail than WT quail were attributed by differences in WG between mutant and WT quail from D 10 to 30; approximately 9% and 12% of increased WG in male (mutant, 61.95 g vs. WT, 56.7 g) and female (mutant, 66.83 g vs. WT, 59.45 g), respectively, and from D 30 to 40; approximately 26% of increased WG in both male (mutant, 19.52 g vs. WT, 15.48 g) and female (mutant, 25.77 g vs. WT, 20.52 g). After D 40, the growth curve of both WT and mutant males reached plateaus, but the body weights of mutant quail in both sexes were significantly heavier than those of WT quail (Figure 1A and B). These data suggested that MSTN mutation greatly affected WG at the later stage (D 30 to 40) of the 56 d post-hatch growth period.

In the early growth phase from D 10 to 30, mutant quail grew faster and ate more, having no significant difference in FCR compared to WT quail. In the late growth phase from D 30 to 40, there was significantly higher WG in mutant quail compared to WT quail, but no significant difference in FI. Analysis of WG and FI revealed a significantly lower FCR in mutant quail compared to WT quail only from D 30 to 40, which leads to significantly lower FCR in overall period (D 10 to 40) (Table 1).

As shown in several MSTN mutant animals, low body fat is one of the characteristics of MSTN mutation (Boman et al., 2010; Fiems 2012; Ren et al., 2020). Likewise, MSTN mutant quail showed a significantly lower percent of leg and abdominal fat compared to WT in both males and females (Figure 1C and D). Generally, lower fat deposition contributes to better feed efficiency by distributing energy intake toward muscle growth instead of storing fat, because a gram of fat accretion requires more calories than protein and adipose tissue contains about 80% fat and 15% water, whereas muscle tissue contains about 20% protein and 75% water (Whittemore and Kyriazakis, 2006). Therefore, in addition to greater WG, improved feed efficiency might be partially

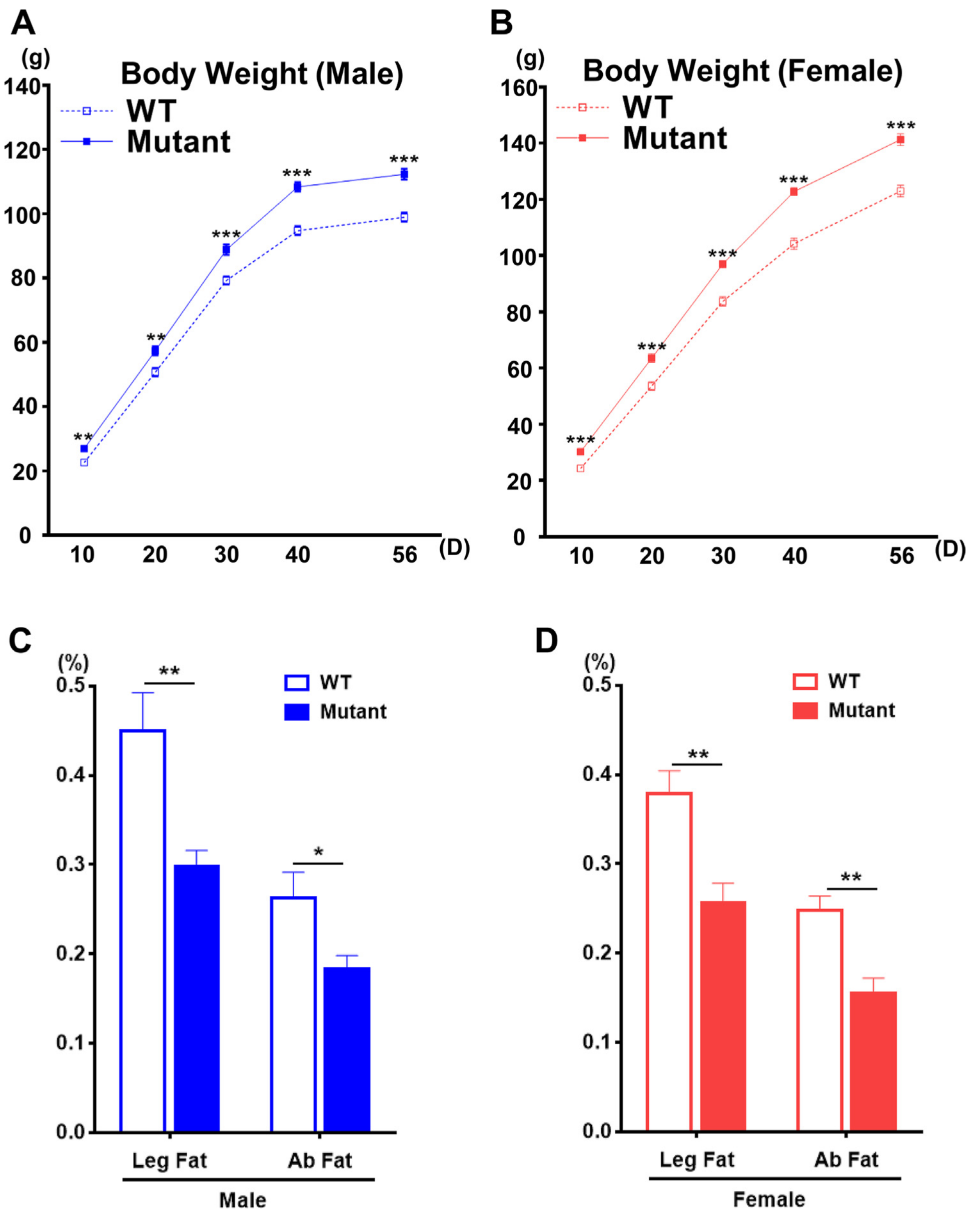

Figure 1. Comparison of body weights and fat percentages during growth. Body weights were measured weekly from post-hatching day (D) 10 to D 56 in male (A) and female (B). Values were compared for statistical analysis at each of the measuring days (D 10, D 20, D 30, D 40, and D 56). Opened circle with a dotted line represents wild-type (WT) quail. Closed circle with a solid line represents mutant quail. Leg and Abdominal (Ab) fat percentages compared to body weight were measured at D 56 in male (C) and female (D). Opened box represents WT and closed box represents mutant. Blue color represents male and red color represents female. Values present means ± SEM. n = 10 WT males, 12 mutant males, 9 WT females, and 9 mutant females. Student *t* tests were used for statistical analysis by the GraphPad PRISM 6.02 program. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

contributed by low body fat in mutant quail as shown in the current and previous studies (Lee et al., 2020).

Interestingly, bigger body weight is one of the characteristics in female quail (Murali Krishnan, 2019), unlike other poultry species, such as the chicken and turkey, showing heavier body weight in males compared to females (Gous et al., 1999; Isgüzar, 2003). Indeed, statistical comparison of body weight at D 40 between females and males showed a heavier body weight in females in both WT (female, 104.28 g vs. male, 94.77 g, $P < 0.01$) and mutant (female, 122.83 g vs. male, 108.39 g, $P < 0.001$) quail. In the present study, WG of the female was the greatest from D 30 to 40 compared to the male ($P < 0.001$), being accompanied with higher FI in the female than male ($P < 0.001$) (Table 1). However, there was a significantly lower FCR in female quail compared to male quail regardless of genotypes from D 30 to 40 and overall period than males ($P < 0.01$), suggesting better feed efficiency in female than male quail. Opposite to quail, body weight of the male chicken is greater than the female chicken and feed efficiency of the male is better than the female as well (Benyi et al., 2015), indicating growth rate might be one of the important factors affecting feed efficiency.

In summary, a leaner and bigger body with better feed efficiency by MSTN mutation can benefit the poultry producers and satisfy the customers' preference for

Table 1. Comparisons of feed conversion ratio between WT and mutant quail during growth.

		Male		Female		Comparison between groups		
	Age	WT	Mutant	WT	Mutant	Sex	Genotype	Interaction
WG (g)	D 10−20	28.13 ± 0.51	30.48 ± 0.91	29.34 ± 0.84	33.35 ± 1.10	<0.05	<0.001	NS
	D 20−30	28.57 ± 0.69	31.47 ± 1.08	30.11 ± 0.68	33.48 ± 0.67	<0.05	<0.001	NS
	D 30−40	15.48 ± 0.84	19.52 ± 0.66	20.52 ± 1.30	25.77 ± 1.23	<0.001	<0.001	NS
	Overall (D 10−40)	72.19 ± 1.37	81.48 ± 1.18	79.97 ± 1.86	92.60 ± 0.99	<0.001	<0.001	NS
FI (g)	D 10−20	82.62 ± 2.27	89.49 ± 2.36	84.17 ± 3.09	93.69 ± 1.40	NS	<0.01	NS
	D 20−30	112.62 ± 1.99	117.54 ± 1.61	111.37 ± 2.60	122.32 ± 0.85	NS	<0.001	NS
	D 30−40	133.35 ± 3.81	132.05 ± 2.97	143.97 ± 3.51	152.38 ± 4.03	<0.001	NS	NS
	Overall (D 10−40)	328.59 ± 6.71	339.03 ± 6.22	339.85 ± 7.06	368.40 ± 4.75	<0.01	<0.01	NS
FCR	D 10−20	2.94 ± 0.06	2.96 ± 0.11	2.88 ± 0.10	2.83 ± 0.08	NS	NS	NS
	D 20−30	3.97 ± 0.13	3.77 ± 0.10	3.72 ± 0.10	3.66 ± 0.06	NS	NS ($P = 0.1$)	NS
	D 30−40	8.85 ± 0.56	6.84 ± 0.26	7.24 ± 0.45	5.97 ± 0.17	<0.01	<0.001	NS
	Overall (D 10−40)	4.56 ± 0.11	4.17 ± 0.08	4.26 ± 0.07	3.98 ± 0.04	<0.01	<0.001	NS

All data were presented as means ± SEM.
Significant differences were determined using Two-Way ANOVA followed by Tukey's multiple comparison ($P < 0.05$).
Abbreviations: FI, feed intake; FCR, feed conversion ratio; NS, no significance; WG, weight gain.
n = 10 WT males, 12 mutant males, 9 WT females, and 9 mutant females.

poultry meat with low fat. Although further studies to understand MSTN function in commercial broiler poultry breeds are needed, the current study provides important information on improved feed efficiency by MSTN mutation in quail that can be potentially applied to the broiler industry.

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DISCLOSURES

The authors declare that they have no competing interests.

REFERENCES

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

Benyi, K., T. S. Tshilate, A. J. Netshipale, and K. T. Mahlako. 2015. Effects of genotype and sex on the growth performance and carcass characteristics of broiler chickens. Trop. Anim. Health Prod. 47:1225–1231.

Boman, I. A., G. Klemetsdal, O. Nafstad, T. Blichfeldt, and D. I. Våge. 2010. Impact of two myostatin (MSTN) mutations on weight gain and lamb carcass classification in Norwegian White Sheep (Ovis aries). Genet. Sel. Evol. 42:4.

Fiems, L. O. 2012. Double muscling in cattle: genes, husbandry, carcasses and meat. Animals (Basel) 2:472–506.

Gous, R. M., E. T. Moran, H. R. Stilborn, G. D. Bradford, and G. C. Emmans. 1999. Evaluation of the parameters needed to describe the overall growth, the chemical growth, and the growth of feathers and breast muscles of broilers. Poult. Sci. 78:812–821.

Isgüzar, E. 2003. Growth, carcass traits and meat quality of Bronze and White turkeys in Isparta province of Turkey. Arch. Anim. Breed. 46:471–481.

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

Lee, J., D. H. Kim, and K. Lee. 2020. Muscle hyperplasia in Japanese Quail by single amino acid deletion in MSTN propeptide. Int. J. Mol. Sci. 21:1504.

McPherron, A. C., and S. J. Lee. 1997. Double muscling in cattle due to mutations in the myostatin gene. Proc. Natl. Acad. Sci. USA. 94:12457–12461.

McPherron, A. C., A. M. Lawler, and S. J. Lee. 1997. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature 387:83–90.

Murali Krishnan, L. 2019. A comparative study on carcass yield in male and female Japanese quail (Coturnix coturnix japonica). Plant Arch 19:2099–2100.

Ohama, M., Y. Washio, K. Kishimoto, M. Kinoshita, and K. Keitaro. 2020. Growth performance of myostatin knockout red sea bream *Pagrus major* juveniles produced by genome editing with CRISPR/Cas9. Aquaculture 529:735672.

Ren, H., W. Xiao, X. Qin, G. Cai, H. Chen, Z. Hua, C. Cheng, X. Li, W. Hua, H. Xiao, L. Zhang, J. Dai, X. Zheng, Z. Zhu, C. Qian, J. Yao, and Y. Bi. 2020. Myostatin regulates fatty acid desaturation and fat deposition through MEF2C/miR222/SCD5 cascade in pigs. Commun. Biol. 3:612.

Whittemore, C., and I. Kyriazakis. 2006. Growth and body composition changes in pigs. Pages 65−103 in Whittemore's Science and Practice of Pig Production. I. Kyriazakis, C. T. Whittemore, ed. Blackwell Publishing, Oxford, UK.