Research Note: Growth promoting potential in Mstn mutant quail dependent and independent of increased egg size

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ABSTRACT In avian species, positive relationships between egg weight (**EW**) and body weight (**BW**) have been reported. However, the correlation between the body growth rate and different weights of eggs from genetically mutated avian species was not studied yet. Myostatin (**Mstn**), an anti-myogenic factor, mutant quail were recently developed, and it was reported that EW produced from Mstn homozygous mutant quail (**HO**) was heavier compared to those from wild-type quail (**WT**). In the current study, distributions of pre-incubated EW and associations between EW and BW were compared between the Mstn mutant and WT quail lines. Average egg weight for the HO group was significantly heavier than the WT ($P <$ 0.001) and the number of eggs having heavier EW (over 11 g) was higher in the HO compared to the WT ($P <$ 0.01). BWs at wk (**W**) 0, 4, and 6 after hatch were also significantly greater in the HO ($P < 0.001$ in all groups). In addition, linear regression analyses revealed positive relationships between EW and BW from W0 to W6, regardless of sexes and genotypes. Furthermore, Mstn mutant quail were a heavier BW compared to the WT quail originated from eggs with similar weights. These data indicate that increased BW by Mstn mutation is contributed by increased EW and/or growth promoting activity of Mstn mutation independent of increasing egg sizes. These findings provide Mstn as a desirable genetic factor for selection of poultry breeds with superior growth. In addition, the knowledge gained from this study could inspire similar proof-of-concept studies involving standard and commercial lines of poultry.

Key words: myostatin, quail, egg weight, body weight, growth

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INTRODUCTION

In poultry species, body weight (**BW**) of offspring and egg weight (**EW**) are 2 relevant productive traits. It was reported that BW of hens has a positive correlation with EW in diverse avian species including poultry species (Rahn et al., 1975). In addition, heavier hens lay heavier eggs, and heavier chicks are hatched from heavier eggs in the chicken (Bish et al., 1985; Iqbal et al., 2016) and quail (Nestor et al., 1982).

Egg weights from the same breed of chicken are well correlated with chick weights, although this positive correlation decreases with increased age in chickens (Iqbal et al., 2016). Although eggs with similar weights from broiler and layer chicken breeds were incubated, embryo weight at 19-d of incubation is significantly heavier in broilers than layers (Ohta et al., 2004). It is well-known that the size of skeletal muscle mainly makes a difference on BW between broiler and layer chickens. In general, broiler chickens have 5 to 6 times heavier BW with more muscle mass than layers at 5 wk of age (Scheuermann et al., 2004). This suggests that genetic factors significantly affect muscle growth at embryonic and post-hatch ages.

Myostatin (**Mstn**), a well-known negative regulator in muscle development, has been studied as a potential selective marker for meat production in the poultry industry. Mstn mutated poultry species resulted in muscle hypertrophy and hyperplasia in chickens (Kim et al., 2020b) and muscle hyperplasia in quail (Lee et al., 2020). In our previous studies, Mstn mutant quail exhibited delayed egg laying time and produced fewer numbers of eggs during an actively laying period (Lee et al., 2021). In addition, eggs from Mstn mutant quail are larger in size and a heavier egg weight than wild-type (**WT**) eggs (Lee et al., 2022), but have similar fertility and hatchability as wild-type eggs (Lee et al., 2021). However, there is a lack of further investigation focusing

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on the association between egg weights from Mstn mutants and body growth. Therefore, the objectives of this study were to measure the EW, BW, and body growth to understand association of these factors by comparing WT and Mstn mutant (**HO**) quail.

MATERIALS AND METHODS

Animal Care and Experimental Design

Japanese quail (*Coturnix japonica*) used in this study were maintained at The Ohio State University Poultry Facilities in Columbus, OH. 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 Mstn homozygous mutant line generated from our previous study (Lee et al., 2020). All eggs were collected daily from 10 pairs of both WT and HO, and incubated weekly for a total of 11 wk. All quail were raised in the same circumstances such as incubation time and temperature to hatch eggs, room temperature, the size of brooder cages, and the same kind of feed, free access to food and water after hatch.

Comparisons of Egg Weights and Body Weights at Hatch

Weights of all collected eggs including unfertile eggs (n = 609 for WT and 586 for HO) were measured to compared differences in **EWs** at time of pre-incubation between the WT and HO. Percentages of egg weights were analyzed using all collected eggs to compare proportion of collected eggs by weight. To further investigate the relationship between EW and BW at hatch, eggs from the WT and HO were hatched, and then measured BW to compare between the two groups (n = 239 for WT and 137 for HO).

Linear Regression Analyses Between Egg Weights and Body Weights During Growth

Each of the labeled and weighted eggs was hatched, and BWs were measured every 2-wk after hatch (week [**W**] 0, W2, W4, and W6). Using multiple linear regression models, the correlation between EW and BW was analyzed and compared between the two groups, the WT and HO, in male (n = 118 for WT and 57 for HO) or female (n = 120 for WT and 80 for HO) quail. In addition, to further investigate whether Mstn mutation can cause greater body growth compared to the WT quail originated from the same size of eggs. Eggs within the tight range (10.1 g ± 0.2) near average weights were selected so as not to have a significant difference in egg weights between the WT and HO group.

Statistical Analysis

The obtained data were analyzed with the SAS 9.4 software package (SAS Institute, Cary, NC). The paired comparisons of the 2 categorical variables such as between WT and HO was performed with the *t* test. All data were expressed as means ± SEM. *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$. The associations between BW and egg weight of WT and HO in male or female quail, respectively, were explored by applying a multiple linear regression model. The correlation coefficient was calculated every 2 wk after hatch (W 0, 2, 4, and 6). Detailed number of samples used for the experiments were described in each of the Figure Legends.

RESULTS AND DISCUSSION

In this study, to compare EW between WT and HO, 609 eggs from WT quail and 586 eggs from HO quail were collected, and each of the EW was measured. Average egg weight for the HO group was significantly heavier than the WT ([10.03 g ± 0.04 for the HO vs. 9.63 g ± 0.03 for the WT, $P < 0.001$], Figure 1A). As shown in distribution of egg weight (Figure 1B), disruption of the Mstn gene in quail hens resulted in laying fewer numbers of small eggs and larger numbers of large eggs. Specifically, the HO hens produced significantly fewer numbers of eggs in the range of 9 to 10 g compared to the WT ([34.9% ± 2.7 for the HO vs. 46.2% ± 1.5 for the WT, $P < 0.01$], Figure 1B). Whereas, the numbers of heavier eggs (over 11 g) were significantly greater in the HO compared to the WT hens ([in the ranges of 11 to 12 g, 19.6% ± 3.1 for the HO vs. 3.6% ± 0.8 for the WT, $P < 0.001$, or in the ranges of over 12 g, 2.0% ± 0.6 for the HO vs. 0% for the WT, $P < 0.01$], Figure 1B). Although it is generally known that heavier chicken breeds lay heavier eggs (Bish et al., 1985), it is the first time to show that heavier body weight resulted from Mstn mutation in quail led to producing greater egg weight compared to the eggs from the WT.

Similar to the data of egg weight in Figure 1A, the weight of fertilized eggs was significantly heavier in the HO than the WT (10.18 g ± 0.07 vs. 9.69 g ± 0.05, $P < 0.001$, Figure 1C). To compare the effect of EW to BW at the day of hatch (W0), BW was measured at W0. BW was heavier in the HO hatchlings than the WT (7.28 g ± 0.06 vs. 6.72 g ± 0.05, $P < 0.001$, Figure 1D). These data suggest that Mstn mutation resulted in increased BW of hatchlings due to a major effect of laying heavier eggs. These findings could be partially supported by a previous study which reported chicks having greater body weight can be hatched out from the heavier eggs (Ulmer-Franco et al., 2010). In addition, due to more nutrition contents in eggs from the HO (Lee et al., 2021), developing embryos in HO eggs might use more nutrition for their growth requirements compared to embryos in WT eggs.

The BW of hatchlings relative to EW was also higher ($P < 0.001$, Figure 1E) in the HO (71.44% ± 0.26) compared to WT hatchlings (70.22% ± 0.25). These data

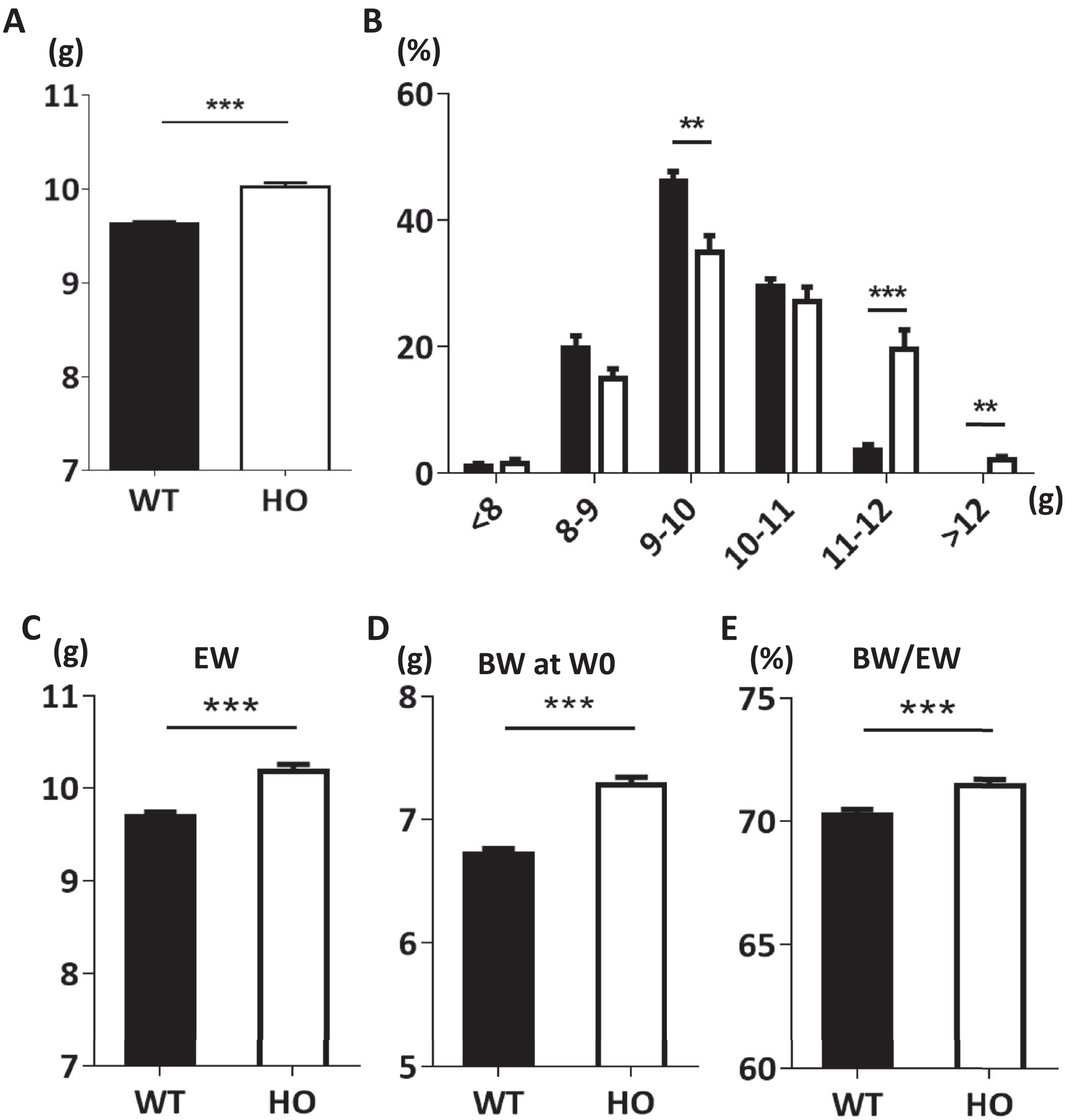

Figure 1. Comparisons of egg weights and body weights at hatch. Comparisons of weights of total collected eggs (A) and distributions of egg weights (B). Total numbers of eggs were 609 for WT, and 586 for HO. Comparisons of fertilized egg weight (EW), body weight (BW) at hatch, and percentages of BW compared to EW between WT and HO (C, D, and E, respectively). The numbers of fertilized eggs were 239 for WT, and 136 for HO. Statistical analyses were performed using the SAS 9.4 software package. **: $P < 0.01$ and ***: $P < 0.001$. Abbreviations: HO, homozygote mutant quail; WT, wild-type quail.

suggest that Mstn mutation resulted in increased chick: egg weight ratio, being interpreted as a positive effect of Mstn mutation on hatchling weight from the same size of eggs. However, this percentage increase (1.22%) seems to be significant but comparatively smaller than those in cattle (33%), pigs (15%), and sheep (23%) (Casas et al., 1999; Wang et al., 2015,2016). In mammals, the phenotypic differences between WT and Mstn knockout (**KO**) are evident at birth perhaps due to the continuous maternal supply of nutrients and energy during fetal development in mammals. However, because avian embryogenesis occurs (independent of the hen) in an egg consisting of a fixed size and containing a limited supply of nutrients, the BW of hatchlings might be influenced by genetic factors. Therefore, the genetic potential for increasing BW by MSTN mutation might result in only a 1.2% increase in BW under the confined environments with limited nutrients in avian species.

To compare differences of BW between the WT and HO in the male or female during growth, BW was measured every 2-wk after hatch (Figure 2A). BW of the HO group was significantly higher in both males and females ($P < 0.001$) compared to the WT at W0, W4, and W6, however, there was no significant difference at W2 (Figure 2A). Our previous study proved that folds of weight gain of *pectoralis major* muscle (**PM**) were

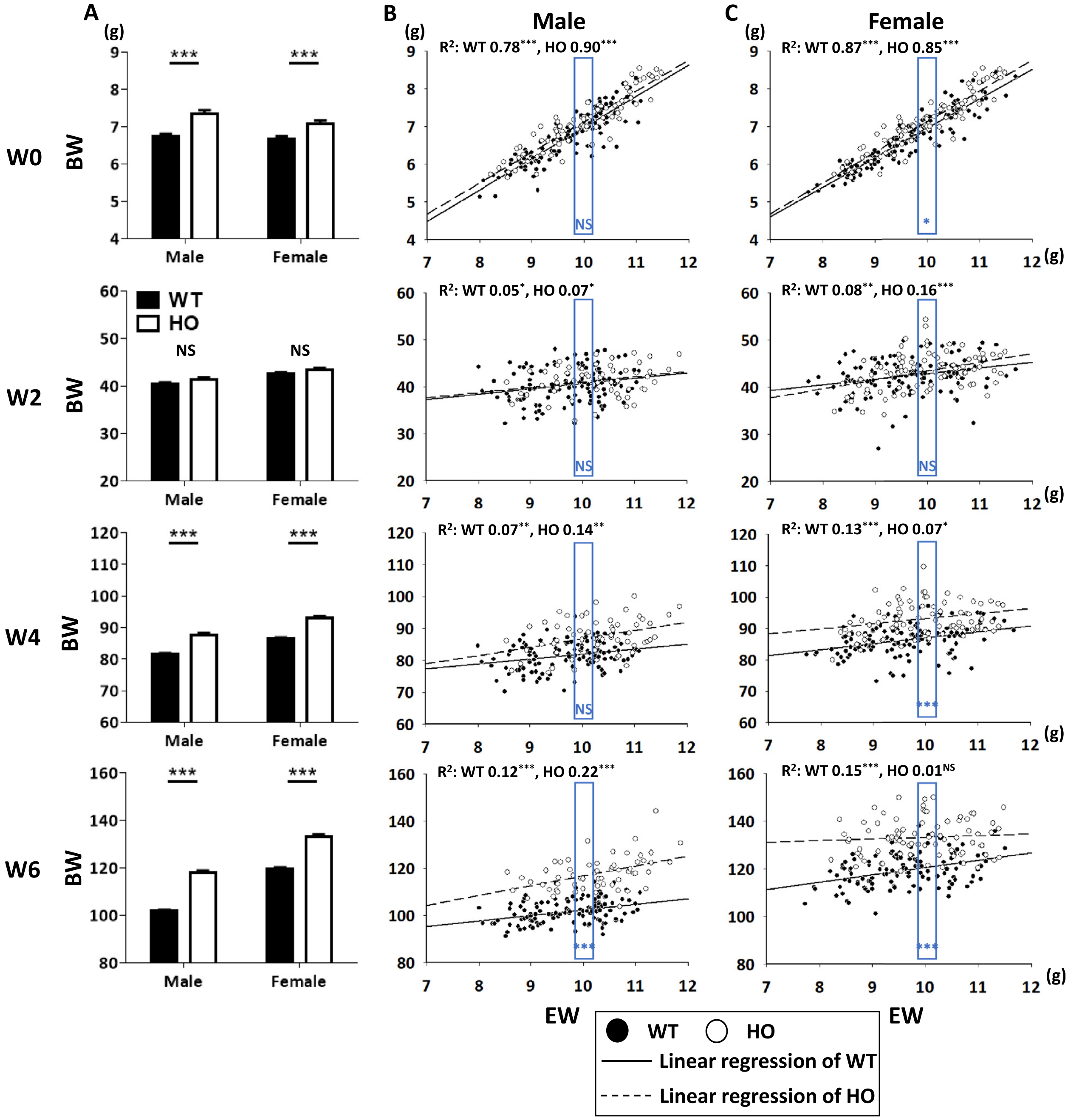

Figure 2. Relationship between egg weight and body weight during growth. Effect of Mstn mutation on average body weights (A). Average BWs were measured every 2 wk and compared between WT and HO in male and female quail. Comparisons of linear regression analysis between BW and EW for the WT and HO in male (B) and female (C) quail. Blue box indicates a range of egg weights (10.1 g ± 0.2). For this study, total numbers of quail were 118 for WT male, 57 for HO male, 120 for WT female, and 80 for HO female. Statistical analyses were performed using the SAS 9.4 software package. *: $P < 0.05$, **: $P < 0.01$ and ***: $P < 0.001$. NS means no significant difference. Abbreviations: HO, homozygote mutant quail; WT, wild-type quail.

the most highly increased during the first week after hatch in quail (Kim et al., 2020a). Also, BW and PM weight gains were gradually increased until W2, but decreased after W2 (Kim et al., 2021). The current study also shows that folds of BW gain are the highest during W0 to W2 in both males and females (6.0-folds for WT and 6.4-folds for HO in male, 5.6-folds for WT and 6.1-folds for HO in female); however, under 2-folds were exhibited in other growth ranges, W2 to W4 and W4 to W6, in all groups (Figure 2A). Based on the previous and current study, there was no significant difference in BW at W2 between the WT and HO and might be partially explained by physiological factors and environments governing rapid growth during the first 2 wk after hatch and potentially overriding effects of Mstn mutation. Additionally, relatively very low expression levels of Mstn at early post-hatch in quail (Kim et al., 2020a,2021) might result in ignorable effects of Mstn knock-out on growth of young quail.

Using multiple linear regression models, further investigation was performed to analyze whether body growth is correlated with EW in WT and Mstn mutant quail.

The present study showed a very strong correlation between EW and BW in WT and HO males at W0 ([R^2: 0.78 and 0.9, respectively, $P < 0.001$], Figure 2B). The positive correlation was also shown at W2, W4, and W6 in males ([R^2: 0.05, $P < 0.05$ for WT and 0.07, $P < 0.05$ for HO at W2; R^2: 0.07, $P < 0.01$ for WT and 0.14, $P < 0.01$ for HO at W4; R^2: 0.12, $P < 0.001$ for WT and 0.22, $P < 0.001$ for HO at W6], Figure 2B). In Figure 2C, similar with the male, females at W0 showed the highest positive correlation between EW and BW (R^2: 0.87 for WT and 0.85 for HO, $P < 0.001$ on both), and positive correlations were maintained at W2 and W4 (R^2: 0.08, $P < 0.001$ for WT and 0.16, $P < 0.001$ for HO at W2; R^2: 0.13, $P < 0.001$ for WT and 0.07, $P < 0.05$ for HO at W4). Interestingly, the correlation between EW and BW was not shown in HO female quail at W6 (R^2: 0.01, $P > 0.05$), although positive correlation was maintained in WT female quail at the same age (R^2: 0.15, $P < 0.001$; Figure 2C). To the best of our knowledge, it is the first report clearly showing a positive relationship between EW and BW in WT and Mstn mutant quail. Overall, chicks hatched from large egg sizes could have heavier body weights during growth compared to those from small sizes.

Average sizes of eggs from Mstn mutant hens are bigger, thus leading to greater body weights during the post-hatch ages (Figures 1C and 2A). However, it is interesting to investigate whether Mstn mutation can cause greater body growth compared to the WT quail originated from the same size of eggs. Therefore, eggs within the tight range (10.1 g ± 0.2) near average weights were selected to have similar weights of pre-incubated eggs between the WT and HO in both males and females. At hatching of those eggs, BW in the female was significantly greater in HO compared to those in WT ($P < 0.05$, Figure 2C). At W4, although there was no significant difference in male quail between WT and HO quail (Figure 2B), BW of female quail were significantly heavier in the HO group compared to those of the WT ($P < 0.001$, Figure 2C). In addition, Mstn mutation significantly increased BW compared to the WT male and female quail at W6 originated from eggs with similar weights ($P < 0.001$, Figures 2B and 2C, respectively). Our previous studies reported that gains of BW are increased the most during the first 2-wk after hatch, W0 to W2 (6-folds), compared to other growth periods, W2 to W4 (2-folds) and W4 to W6 (1.2-folds), in quail (Kim et al., 2020a, 2021). At W2, the similar BW between WT and HO quail at W2 originated from similar egg weights might be due to the physiological conditions associated with rapid growth from W0 to W2 (Figures 2B and 2C). Overall, these data could indicate that Mstn mutation could increase BW of mature quail originated from the same size of eggs.

Taken together, the positive role of Mstn knockout on growth in the avian model was studied through the association between egg weight, and chick weight and growth. This study proved that increased EW by Mstn mutation led to significantly increased BW of chicks and exhibited a positive correlation with body growth. In addition, increased BW in Mstn mutant quail originated from the similar size of eggs further indicates growth promoting activity of Mstn mutation independent of increasing egg sizes. These findings encourage identifying Mstn as a desirable genetic factor for traditional selection or a candidate for gene editing to improve poultry production.

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DISCLOSURES

The authors declare that they have no competing interests.

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