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Effects of the Chicken Sex-linked Dwarf Gene on Growth and Muscle Development

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ABSTRACT : The aim of this study was to analyze the effects on growth and muscle development during the growing period of the sex-linked dwarf gene in the background of a Taiwan Country chicken strain, L2, selected for egg production. Eight crossbred males, heterozygous for the *DW*DW* mutation, were each backcrossed to six females of the L2 strain to produce two genotypes of BC females, either normal (*DW*N*$^{+}$/-) or dwarf (*DW*DW*/-). The experiment included 251 normal and 207 dwarf pullets. The effect of the dwarf gene on body weight and shank length was highly significant from 2 weeks of age. The reduction of body weight by the dwarf gene reached 34.8% and 37.4% as compared to normal sibs at 16 and 20 weeks of age, respectively. Parameters of the growth curve were estimated: the age at inflection (TI) was higher in normal pullets (66.9 days) than in dwarf pullets (61.2 days). A significant effect of the dwarf gene on single muscle fiber cross-section area was found from 12 weeks of age onwards, whereas the dwarf gene had no effect on the total number of muscle fibers. Comparing the effect of the dwarf gene on shank length at different ages revealed an earlier effect on skeleton growth, observed from 2 weeks of age, than on muscle development, which was affected from 8 to 12 weeks of age. (**Key Words** : Growth Curve, Muscle Development, Sex-linked Dwarf Gene, Chicken)

INTRODUCTION

It is known that some genes could reduce the chicken's body size. The thyroid dwarfism (td) gene was a hypothyroidism (Landauer, 1929). The creeper (Cp) gene was a form of chondrodystrophy caused by an abnormal cartilage growth (Landauer and Dunn, 1930). Two sex-linked genes are known to cause dwarfism. A recessive gene for smaller body size in Bantam breeds (dw^{B}) was found by Godfrey (1953). The other gene, described by Hutt (1959), and known as the dwarfism (dw) gene resulted in a reduction of body weight, representing about 30-40% of the normal female weight, depending on age and genetic background, as reviewed by Mérat, 1984. During postnatal growth, the increase in skeletal muscle mass is mainly due to muscle fiber hypertrophy. This process is accompanied by the proliferating activity of satellite cells (Rehfeldt et al., 2000; Nierobisz and Mozdziak, 2008). Furthermore, as muscle tissue constitutes over 40% of lean body weight, growth and development of skeletal muscle tissue are a major component of body growth. The sex-linked dwarf gene has been widely used by broiler breeders to reduce the body size of dam lines in order to improve their egg production and feed efficiency (Mérat, 1984; Islam, 2005), and it has been also used for the dam line of the 'Label rouge' slow-growing chickens in France. Besides an interest in the gene from an economical point of view, there was also a great deal of interest for the understanding of muscle development, considering this dwarf gene as a good model to use in the study of the growth curve and tissue development. The aim of this study was to compare the muscle growth of normal and dwarf pullets during the growing period, in the background of Taiwan Country Chicken, which is a slow-growing breed, with high quality meat.

MATERIALS AND METHODS

Bird genetic background and husbandry

Animal care and experimental protocol were reviewed and approved by the Ethics Committee of Laboratory Animals at the National Chung-Hsing University, Taiwan. The parental stock used sex-linked dwarf male chickens from a strain developed at the French National Institute for Agricultural Research (INRA) and selected for a high clutch

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Table 1. Number of chickens according to slaughter age, genotype and hatch

Hatch	1	2	3	4	5	6	Total
Slaughter age (wk)	20	16	12	8	4	0	
Number of pullets							
Normal pullets	52	36	44	36	38	45	251
Dwarf pullets	31	31	29	29	55	32	207

length. These males were mated with normal-size female chickens from the National Chung-Hsing University L2 strain. This mating produced an F1 generation consisting in dwarf females and heterozygote normal males. Eight F1 males, heterozygotes for the *DW*DW* mutation, were backcrossed each to six females of strain L2 to produce two genotypes of females (Backcrossed pullets, BC), either normal (*DW*N*$^{+}$/-) or dwarf (*DW*DW*/-). The BC birds were hatched in six batches. Table 1 shows the number of experimental pullets according to slaughter age and batch. In total, this experiment used BC full-sisters, 251 normal and 207 dwarf pullets. At hatch, the female chicks were reared in floor pens until slaughter. Water and food were supplied *ad libitum*. A starter diet containing 19.1% crude protein (CP) and 2,830 Kcal/kg metabolizable energy (ME) was given between 0 to 8 weeks of age, followed by a finisher diet containing 16.1% CP and 2800 Kcal/kg ME from 9 to 20 weeks of age.

Genotyping

In order to be sure of the dwarf genotype of young chicks before 8 weeks of age, a molecular diagnostic test was performed to identify the genotype of the birds. The mutation of the INRA line was found to be identical to the deletion identified by Agarwal et al. (1994). Two pairs of primers were produced based on the chicken growth hormone receptor gene. Blood was collected from BC pullets and genomic DNA was extracted and prepared for a polymerase chain reaction (PCR) test.Primer set GHR-B: 5'-TTG TTC ACT CTC CAC AAG GC-3' and GHR-C: 5'-GAT TCT CCT GGC AGA ATC TC-3' were designed to amplify a 253-bp PCR product specific of the dwarf allele, in the absence of the deletion, the expected fragment is too long to be amplified in standard PCR conditions. Primer set GHR-F2: 5'-CTT TGT GAC CAA GGG GAT CAG G-3' and GHR-E: 5'-GAA TGC TCG ATT CTC CTG GCA G-3' were designed to amplify a 463-bp PCR specific of the normal allele (Figure 1).

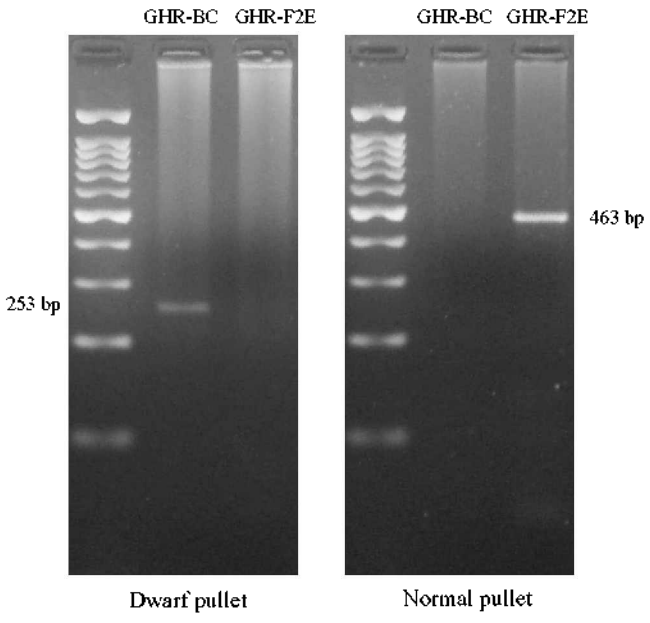

Figure 1. Illustration of electrophoresis agarose gel of dwarf and normal pullets.

Growth development

The birds were weighed and shank length was measured every two weeks from birth to slaughter age to determine the growth rate.

Parameters of the growth curve were estimated with the Gompertz equation (Laird et al., 1965) as already applied to slow growing chickens by N'dri et al. (2006).

$$BWt = BW_0 \times \exp\left(\frac{L}{K}(1-\exp(-Kt))\right)$$

where BWt is the recorded body weight at age t, BW_0 the estimated weight at hatching, L the initial specific growth rate and K the maturation rate. Parameters A, the asymptotic body weight at infinite age, and TI, the age at inflexion at which the growth rate is maximum, was calculated as follows (Mignon-Grasteau et al., 1999):

$$A = BW_0 \times \exp^{\frac{L}{K}}; \quad TI = \left(\frac{1}{K}\right)\ln\left|\frac{L}{K}\right|$$

These parameters were estimated by non-linear regression with NLIN procedure of SAS (SAS Institute, 2002).

Single muscle fiber cross-section area and number of muscle fibers

Muscle fiber number and cross-sectional areas characteristics of *M. gastrocnemius* were measured for dwarf and normal pullets during growth period every four weeks from hatching to 20 weeks of age. The whole muscle connected to the tibial bone was fixed in Formalin for 48 h.

Table 2. Least-squares means and standard error of body weight and growth curve parameter

Trait[1]	N	Genotype		Reduction[2] (%)	Significance[3]
		Normal	Dwarf		
BW0 (g)	458	31.4±0.1	31.5±0.2	-0.3	NS
BW2 (g)	381	119±2	103±2	13.4	***
BW4 (g)	381	254±3	208±4	18.1	***
BW6 (g)	288	485±6	377±8	22.3	***
BW8 (g)	288	742±10	554±11	25.3	***
BW10 (g)	223	1,029±9	714±12	30.6	***
BW12 (g)	223	1,222±10	838±12	31.4	***
BW14 (g)	150	1,446±16	983±19	32.0	***
BW16 (g)	150	1,693±18	1,103±22	34.8	***
BW18 (g)	83	1,944±31	1,231±43	36.7	***
BW20 (g)	83	2,107±30	1,319±42	37.4	***
A (g)	83	2,529±38	1,547±54	38.8	***
L (/d)	83	0.087±0.001	0.082±0.002	-	*
K (/d)	83	0.0212±0.0003	0.0219±0.0004	-	NS
TI (/d)	83	66.9±1.2	61.2±1.6	-	**

[1] BW0, BW2, BW4, BW6, BW8, BW10, BW12, BW14, BW16, BW18, and BW20 = body weight at hatching, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 weeks of age, respectively; A = asymptotic body weight at an infinite age; L = Initial specific growth rate; K = Maturation rate; TI = Age at inflection.

[2] (Normal-dwarf)/normal×100%.

[3] Significant difference between normal and dwarf means: NS = Not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

The sampling muscle was cut at the 1/3 of its length towards the joint with Femur, and then the sample was paraffin-embedded and sectioned. The measurement of muscle fiber cross-section area, and the calculation of the number of muscle fibers, were done following Rémignon et al. (1995) with a digital camera (Nikon coolpix 5000, Japan) connected with microscope to take photomicrographs of *M. gastrocnemius*. The average cross-section area was calculated from 500-800 fibers for each sample (pullet). Afterwards, using Photoshop 6.0 software, these pictures were combined with photomicrograph of hemocytometer in the same magnification, in order to standardize these pictures, to measure muscle fiber cross-section area, and to calculate number of muscle fibers.

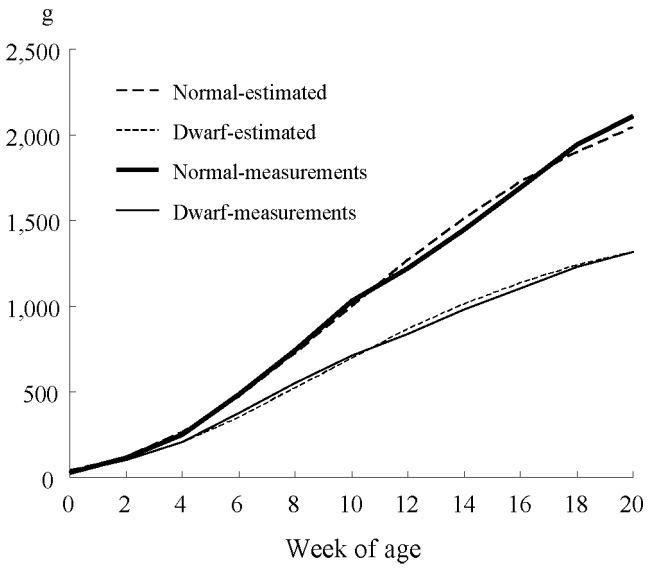

Figure 2. Observed and estimated growth curves for pullets of each genotype.

Statistical analysis

The model used for analysis of effects of dwarf genotype, dam, sire on muscle develop was as follows:

$$X_{ijklm} = \mu+S_i+D_{ij}+B_k+G_l+\varepsilon_{ijklm}$$

where X_{ijkl} is the performance of the m^{th} animal; S_i is the fixed effect of i^{th} sire; D_{ij} is the fixed effect of i^{th} sire mating to j^{th} dam; B_k is the fixed effect of k^{th} batch; G_l is the fixed effect to l^{th} genotype; ε_{ijklm} is random error, expected to have an average of zero. All statistical analyses were conducted by using SAS software (SAS Institute, 2002).

RESULTS

Growth curve and shank length

The body weights at two weeks interval are given in Table 2. The dwarf genotype significantly affected the body weight from 2 weeks of age. The reduction of body weight by the dwarf gene reached 34.8 and 37.4 of normal pullets body weight at 16 and 20 weeks of age, respectively. The observed growth curves and the Gompertz curves fitted for each genotype are shown in Figure 2. Both curves were very similar until 20 weeks of age within genotype. The estimated parameters of growth curve are given in Table 2. The sexual maturity for asymptotic body weights (A) at infinite age were 2,529 g and 1,547 g for normal and dwarf

Table 3. Least-squares means and standard error of shank length according to genotype

Trait[1]	N	Genotype		Reduction[2] (%)	Significance[3]
		Normal	Dwarf		
SL0 (mm)	458	26.3±0.1	26.1±0.1	0.8	*
SL2 (mm)	381	39.5±0.2	37.7±0.2	4.6	***
SL4 (mm)	381	53.3±0.4	48.5±0.4	9.0	***
SL6 (mm)	288	69.3±0.4	60.9±0.5	12.1	***
SL8 (mm)	288	81.9±0.5	70.3±0.6	14.2	***
SL10 (mm)	223	93.6±0.4	75.4±0.5	19.4	***
SL12 (mm)	223	99.3±0.4	77.8±0.5	21.7	***
SL14 (mm)	150	102.6±0.4	79.2±0.5	22.8	***
SL16 (mm)	150	103.4±0.4	80.0±0.5	22.6	***
SL18 (mm)	83	104.5±0.6	81.2±0.8	22.3	***
SL20 (mm)	83	104.9±0.6	82.1±0.8	21.7	***

[1] SL0, SL2, SL4, SL6, SL8, SL10, SL12, SL14, SL16, SL18, and SL20 = Shank length at hatching, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 weeks of age, respectively.

[2] (Normal-dwarf)/normal×100%.

[3] Significant difference between normal and dwarf means: * $p<0.1$; *** $p<0.001$.

pullets, respectively. The initial specific growth rate (L) was significantly higher in normal pullets than in dwarf pullets. Genotype did not significantly affect the maturation rate (K). Age at inflection (TI) was higher in normal pullets than in dwarf pullets, 66.9 and 61.2 days for normal and dwarf pullets, respectively.

The effect of dwarf gene on shank length was also significant from 2 weeks of age (Table 3). Although its influence was not as great as on the body weight, the reduction of shank length reached 19.4 and 21.7% of shank length of the normal chicken at 10 and 20 weeks of age respectively.

Single muscle fiber cross-section area and number of muscle fibers

Figure 3 shows the single muscle fiber cross-section area and muscle fiber numbers in the *M. gastrocnemius* for the two genotypes. Muscle fibers grow in size toward a plateau, whereas fiber number remains almost constant. The effect of dwarf gene on single muscle fiber cross-section area of chickens started to appear at 8 weeks of age ($p<0.1$). After 12 weeks, the muscle fiber cross-section area in dwarf chickens was significantly smaller than in normal chickens ($p<0.01$).

DISCUSSION

The objective of the present study was to compare the muscle growth of normal and dwarf pullets during the growing period, in the background of Taiwan Country Chicken, which is a slow-growing breed, with high quality meat. In this study, the reduction of body weight by the dwarf gene reached 34.8 and 37.4 of normal pullets body weight at 16 and 20 weeks of age, respectively. This was similar to the findings of Mérat (1990), which has summarized from many literatures that the reduction in weight of dwarf chickens compared with normal was about 31-39%. The rate of reduction may vary according to the genetic background and age. Reddy and Siegel (1977) reported the body weight was less depressed by dwarfing gene in a line selected for high body weight than in a line selected in opposite direction.

The evaluation of growth and development has been based on body weight at different ages. Otherwise, the growth curve was also available to result from differences in precocity between two genotypes, that is, differences in physiological ages between normal and dwarf pullets at the same chronological age. The precocity of dwarf genotype was similarly altered, as two weeks delayed in age at first

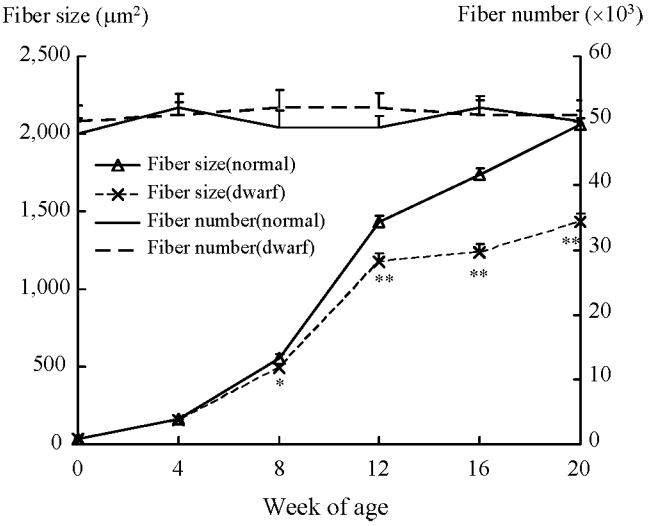

Figure 3. Postnatal development of the mean cross-section area of single fiber and total number of *M. gastrocnemius* for pullets of each genotype (significant difference between normal and dwarf means: * $p<0.1$; ** $p<0.01$).

egg for dwarf layer relative to normal layer (Mérat, 1984; Decuypere et al., 1991). TI is defined the age at inflexion at which the growth rate is maximum. At the point of inflexion, normal and dwarf pullets were the same physiological ages. In present result, dwarf pullets exhibited an earlier physiological age than normal pullets (61.2 d vs. 66.9 d). Mignon-Grasteau et al. (1999) reported that lines selected for high adult body weight at 36 weeks had higher ages at inflection (TI) whereas lines selected for juvenile body weight had larger initial specific growth rates (L) and lower estimates of TI. In current study, the dwarf pullets exhibited a lower initial specific growth rates (L) as compared to normal pullets.

The measurement of shank length was used to evaluate the skeletal development for the chicken. The effect of dwarf gene on shank length was also significant from 2 weeks of age. In fact, the use of the molecular diagnostic test made it possible to classify birds at a very young age and revealed that shank length presented a significant difference between the two genotypes already at two weeks of age. The influence of dwarf gene was not as great as on the body weight, the reduction of shank length reached 21.7% of shank length of the normal chicken at 20 weeks of age, and the change of reduction was small after 10 weeks of age (19.4% to 21.7%). Usually, the dwarf phenotype may be distinguished visually from the normal only from 8 to 10 weeks of age.

The dwarf gene effect on muscle fibers development seems to begin in the interval between 8 and 12 weeks of age. However, the effect of dwarf gene on the muscle development and shank length was at the different age. The body weights differed significantly, from the age of 2 weeks on, between normal and dwarf pullets, although there was no significant difference in the growth of muscle before the age of 8 weeks. It has been reported that the development of skeleton is earlier than those of muscle and feathers in chick after hatching (Rémignon et al., 1995). Therefore, the differences in body weight could result from different growth rates of skeleton according to the the genotype. In addition, in this study, the dwarf gene had no effect on the total number of muscle fibers (Figure 3). This result was consistent with the theory that muscle growth is mostly due to the enlargement of muscle fibers for a constant number of fibers. After birth, total muscle fiber number has been reported to remain unchanged in mammals and birds by most authors (reviewed by Rehfeldt et al., 2004).

In the current study, the reductions of single muscle fiber cross-section area by the dwarf gene were 10.9% and 30.1% at 8 and 20 weeks of age, respectively. It was close to the 12% reduction found for normal and dwarf White Plymouth Rock females at 9 weeks of age, as reported by Knizetova (1993). During postnatal growth, the increase in skeletal muscle is normally due to an increase in muscle hypertrophy. Growth hormone (GH) and insulin-like growth factor-I (IGF-I) regulate both satellite cell proliferation and protein synthesis in muscle. As reviewed by Decuypere et al. (1991), the sex-linked dwarf mutation is characterized by high circulating GH levels, low serum levels of IGF-I and a low GH-binding activity (Decuypere et al., 1991). These effects are a consequence of the deletion in the GH receptor gene which may be seen as equivalent to a knock-out mutation of the GH receptor. The lower proliferating activity of satellite cells may be considered as another consequence of this impaired activity of GH receptor in dwarf chickens.

In conclusion, the effect of dwarf gene on skeleton started at 2 weeks of age, quite earlier than that on the muscle. The reduction of muscle growth was caused by a lower muscle fiber size, without any effect on the fiber number.

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