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Myostatin mRNA expression and its association with body weight and carcass traits in Yunnan Wuding chicken

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ABSTRACT. Myostatin (*MSTN*) is expressed in the myotome and developing skeletal muscles, and acts to regulate the number of muscle fibers. Wuding chicken large body, developed muscle, high disease resistance, and tender, delicious meat, and are not selected for fast growth. Broiler chickens (Avian broiler) are selected for fast growth and have a large body size and high muscle mass. Here, 240 one-day-old chickens (120 Wuding chickens and 120 broilers) were examined. Twenty chickens from each breed were sacrificed at days 1, 30, 60, 90,

120, and 150. Breast and leg muscle samples were collected within 20 min of sacrifice to investigate the effects of *MSTN* gene expression on growth performance and carcass traits. Body weight, carcass traits, and skeletal muscle mass in Wuding chickens were significantly ($P < 0.05$) lower than those in broiler chickens at all time points. Breast muscle *MSTN* mRNA was lower in Wuding chickens than in broilers before day 30 ($P < 0.05$). After day 30, breast muscle *MSTN* expression was higher in Wuding chicken than in broilers ($P < 0.05$). Leg muscle *MSTN* mRNA expression was higher in Wuding chicken than in broilers at all ages except for day 60 ($P < 0.05$). Correlation analysis revealed that breast muscle MSTN expression has a greater effect in slow growing Wuding chickens than in the fast growing broilers. In contract, leg muscle *MSTN* mRNA level has a greater effect in broilers than in Wuding chickens. *MSTN* regulates growth performance and carcass traits in chickens.

Key words: Wuding Chicken; Myostatin gene; Body weight; Carcass traits

INTRODUCTION

Muscle growth is regulated through the proliferation and differentiation of myoblasts that express myogenic transcription factors. Myostatin (*MSTN*), also known as growth differentiation factor 8 (GDF-8), is a member of the transforming growth factor-β (TGF-β) superfamily, which plays an important role in the regulation of skeletal muscle growth. During embryogenesis, *MSTN* is expressed in the myotome and developing skeletal muscles (McPherron et al., 1997; Langley et al., 2002), and acts to regulate the final number of muscle fibers. Many studies in mice have shown that *MSTN* negatively regulates the growth of muscle cells by inhibiting the transcriptional activity of myogenic differentiation antigen (MyoD) (Langley et al., 2002) family members and C2C12 cells (Taylor et al., 2001). Mutations in the *MSTN* gene in mice prevent its expression resulting in the loss of function on muscle growth inhibition, which leads to excessive muscle development (Lee and McPherron, 1999). Mutations in the *MSTN* gene are associated with muscle hypertrophy (McPherron and Lee, 1997; Grobet et al., 1998), an increase in abdominal fat, abdominal fat percentage, birth weight, and breast muscle percentage in bovine (Karim et al., 2000), with muscle mass, carcass fat percentage, weight gain, and lamb carcass classification in sheep (Boman and Våge, 2009; Boman et al., 2010), and with *MSTN* expression, growth, muscle mass, and carcass composition traits in pigs (Stinckens et al., 2008). Moreover, naturally occurring mutations in the *MSTN* gene cause a similar double-muscled phenotype in cattle breeds, which exhibit a 20% increase in muscle mass (Grobet et al., 1998; Kambadur et al., 1997; McPherron et al., 1997).

In chicken, previous studies have shown that *MSTN* gene expression is increased in skeletal muscles during the second half of embryonic development and decline on hatching day (Kocamis and Killefer, 2002). The low levels of MSTN measured during the first weeks of life may contribute to the onset of skeletal muscle development in newly hatched chicks, which is dependent of the rapid initiation of neonatal metabolism (Mott and Ivarie, 2002). Nutrient supply regulates *MSTN* mRNA levels in chicken skeletal muscle (Guernec et al., 2004).

However, how *MSTN* mRNA expression regulates growth, carcass traits, and development of skeletal muscle in poultry has not been elucidated. The expression of *MSTN* mRNA and its association with carcass composition trait and body weight have not been reported in chickens.

Wuding chicken is a local broiler-type breed in Yunnan, famous for its large body, attractive appearance, developed muscle, high disease resistance, and tender, delicious meat. Broiler chickens (Avian broiler) are selected for their fast growth rates, and they have a large body size and high muscle mass. Therefore, the objective of the present study was to investigate *MSTN* mRNA expression and to determine its association with body weight and carcass traits, using Wuding chicken and Avian broiler chicken as a model system.

MATERIAL AND METHODS

All procedures involving chickens received prior approval by the Animal Care and Use Committee of the Yunnan Province of China. The study was conducted in Yunnan Agricultural University of China.

Ethics statement

All experiments complied with the requirements of the Directory Proposals on the Ethical Treatment of Experimental Animals in China.

Animals

One-day-old Wuding chickens (local native breed of Yunnan Province of China) were purchased from the Chicken Farm of Yunnan Agricultural University. One-day-old broiler chicks (Avian) were purchased from the Chicken Farm of Kunming Zhengda Group. The chickens were sacrificed, the samples were collected, and the data were analyzed in the Key Laboratory of Animal Nutrition and Feed in Yunnan Agricultural University.

Experimental design and dietary nutrient level

The diet content was consistent with the formulation recommended by the National Research Council (1994) and Chinese Chicken Feeding Standard (2004) recommendations. A total of 240 one-day-old chicks, including 120 Wuding chicks and 120 broiler chicks (Avian) as a control were used in this study. Twenty chickens from each breed were sacrificed at day 0 and the remaining 100 chicks of each breed were reared under standard conditions on starter diets to day 30, and then on adult chicken diets to day 150. The composition of diets is provided in Table 1.

Feeding and management

The chickens had free access to feed and water during the entire rearing period. The chicks were reared in an environmentally controlled room. The brooding temperature was maintained at 35°C for the first 2 days, and then decreased gradually to 22°C until 30 days and thereafter maintained until the end of the experiment (day 60). At 30 days old, the chickens were randomly allocated to individual metabolism cages in an enclosed room, with ambient temperatures varying from 21°-24°C, under artificial fluorescent light on a light:dark cycle of 12:12 h.

Table 1. Compositions and nutrient levels in the diets.

Diet composition (%)	Chick diet	Adult chicken diet
Corn	64.70	67.90
Soy protein	30.2	18.1
Wheat bran	0.00	10.00
Soya oil	1.10	0.00
Calcium hydrogen phosphate	1.50	1.50
Stone meal	0.70	0.60
Midding flour	0.41	0.46
Met	0.08	0.07
Salt	0.35	0.35
Compound premix[1]	1.00	1.00
Total	100	100
Nutrient levels		
Metabolism energy (kcal/kg)	2900	2780
Crude protein (%)	19.30	15.50
Calcium (%)	0.85	0.80
Total phosphorus (%)	0.61	0.64
Available phosphorus (%)	0.37	0.37
Salt (%)	0.37	0.37
Lys (%)	0.98	0.75
Met (%)	0.39	0.32
Methionine + Cystinol (%)	0.73	0.60

[1]Supplied per kilogram of diet: vitamin A, 15,000 IU; vitamin D3, 3300 IU; vitamin E, 62.5 mg; vitamin K, 3.6 mg; vitamin B1, 3 mg; vitamin B2, 9 mg; vitamin B6, 6 mg; vitamin B12, 0.03 mg; niacin, 60 mg; calcium pantothenate, 18 mg; folic acid, 1.5 mg; biotin, 0.36 mg; choline chloride, 600 mg; Fe, 80 mg; Cu, 12 mg; Zn, 75 mg; Mn, 60 mg; I, 0.35 mg; Se, 0.15 mg; growth promoting agent, 30 mg; and antioxidant, 100 mg.

Measurement of carcass traits

Body weight (BW) was determined in the morning following a 16 h-fast on days 0, 30, 60, 90,120, and 150. The chickens were weighed by transfer to a transport box, which was placed on a tared digital scale (Shanghai Yizhan Weighing Apparatus Ltd, YZ 0.01 g to 10 kg, China) to determine the body weight of conscious animals.

Carcass traits were measured on days 90, 120, and 150. The estimated values of several important economic traits were determined, including carcass weight (CW), eviscerated weight (EW), semi-eviscerated weight (SEW), breast muscle weight (BMW), leg muscle weight (LMW), and abdominal fat weight (AFW). CW was measured using the live body with the blood and feathers removed. SEW was measured using the carcass with the esophagus, trachea, gastrointestinal tract, pancreas, spleen, and gonad removed. EW was measured using the SEW following the removal of the head, heart, claws, liver, glandular stomach, gizzard, and abdominal fat. The proportions of each of the above traits were calculated as dressing percentage (DP), eviscerated percentage (EP), semi-eviscerated percentage, breast muscle percentage, leg muscle percentage, and abdominal fat percentage, respectively (Zhang et al., 2009; Zhou et al., 2010). All the experiments were complied with the requirements of the Directory Proposals on the Ethical Treatment of Experimental Animals of China and animal care guidelines.

Expression of *MSTN* mRNA

Real-time PCR was performed to determine the expression of *MSTN* mRNA in muscle as described previously (Li et al., 2013). Breast and leg muscle samples were collected within

20 min of the experimental chickens being sacrificed on days 0, 30, 60, 90, 120, and 150. Small samples of breast muscle and leg muscle were placed in sterile tubes (RNase-free) and immediately immersed in liquid nitrogen prior to storage at -80°C pending subsequent analyses. Tissues were homogenized in Trizol-Reagent (Invitrogen Corporation, Carlsbad, CA, USA) and total RNA was isolated according to the manufacturer protocol. Kits provided by Trans Gen Biotech, Beijing, were used to reverse-transcribe mRNA into cDNA; a 10-μL reaction system was used, and the manufacturer instructions were followed. Real-time RT-PCR analysis was performed to determine the expression of *MSTN* mRNA and 18S using the iCycler Real Time Detection System (Bio-Rad Laboratories Inc., USA) and SYBR Green master mix [iQTM SYBR-Green® Supermix, TaKaRa Biotechnology (Dalian) Co. Ltd. Add]. Quantitative PCR was performed in a volume of 25 μL, following the manufacturer instructions. The relevant gene sequences of *Gallus gallus* deposited in GenBank (ID: 373964) were used to design *MSTN* primers by primer premier 5.0. Primers specific for the *MSTN* gene were 5'-GCTTTTGATGAGACTGGACGAG-3' and 5'-AGCGGGTAGCGACAACATC-3' and the annealing temperature was 60°C. Primers specific for chicken 18S were 5'-CGCGTGCATTTATCAGACCA-3' and 5'-ACCCGTGGTCACCATGGTA-3', used as a reference, with an annealing temperature of 58°C. The primers were synthesized by Shanghai Biological Engineering Co. Ltd., China. The specificity of the amplified product was verified by electrophoresis on 0.8% agarose gel and by DNA sequencing. Expression of all test genes was determined relative to that of 18S, which was used as a control gene. Gene expression data for muscle were obtained from 20 chickens of each breed and each age, and all assays were performed in triplicate. To compare the effects of different treatments on tissues, we performed *t*-tests on cycle threshold (Ct) values of target *MSTN* gene expression normalized to 18S threshold values.

Statistical analysis

All data were analyzed using Microsoft Excel (Office 2013) and the statistical package SPSS 21.0. For the analysis of carcass traits, data are reported as means ± standard error from two breeds. For analysis of gene expression data, differences in the Ct values of 18S and MSTN was calculated using a two-sample *t*-test. Delta Ct values for each treatment group were calculated as ΔCT = CT (MSTN) - CT (18S). The fold change was calculated as 2 to the power -ΔCt. Alpha = 0.05 was used to determine statistical significance. To determine the significance of the correlation data, a single factor test was used.

RESULTS

Body weight

Data for BW gain over days 0 to 150 are summarized in Table 2.

Carcass traits

Data for carcass traits at 90, 120, 150 days are summarized in Tables 3, 4, and 5, respectively.

Table 2. Mean body weight of different chickens from 0 to 150 days of age.

Day	Wuding chickens BW (g)	Broilers BW (g)
0	$31.63 \pm 2.64^{a*}$	48.19 ± 4.02^{a}
30	$228.65 \pm 19.05^{b*}$	733.55 ± 61.13^{b}
60	$662.11 \pm 110.35^{c*}$	2377.56 ± 96.26^{c}
90	$1264.71 \pm 35.84^{d*}$	3244.42 ± 82.01^{d}
120	$1832.16 \pm 124.36^{e*}$	3960.50 ± 72.46^{e}
150	$2157.55 \pm 85.59^{e*}$	4577.50 ± 98.68^{e}

BW, body weight. Means with different superscript letters differ between ages at $P < 0.05$. *Means with different superscript letters differ between breeds at $P < 0.05$. Throughout the whole growth period, the body weights of Wuding chickens were significantly ($P < 0.05$) lower than those Broilers ($P < 0.05$). The body weight of the two breeds increased significantly with age ($P < 0.05$).

Table 3. Carcass traits of different chicken breeds from 90 days of age.

Trait	Wuding chickens	Broilers
CW (g)	1090.83 ± 30.73^{a}	2935.87 ± 89.88^{b}
SEW (g)	931.55 ± 26.64^{a}	2653.71 ± 86.06^{b}
EW (g)	788.92 ± 26.16^{a}	2365.80 ± 68.74^{b}
BMW (g)	144.25 ± 6.87^{a}	726.66 ± 30.37^{b}
LMW(g)	198.96 ± 8.88^{a}	615.26 ± 31.49^{b}
DP	86.28 ± 0.69	90.25 ± 0.67
SEP	73.70 ± 0.82^{a}	81.52 ± 0.88^{b}
EP	62.46 ± 1.23^{a}	72.79 ± 0.40^{b}
BMP	18.25 ± 0.55^{a}	30.51 ± 0.46^{b}
LMP	25.17 ± 0.58	25.70 ± 0.64

CW, carcass weight; SEW, semi-eviscerated weight; EW, eviscerated weight; BMW, breast muscle weight; LMW, leg muscle weight; DP, dressing percentage; SEP, semi-eviscerated percentage; EP, eviscerated percentage; BMP, breast muscle percentage; LMP, leg muscle percentage. Means with different superscripts differ between breeds at $P < 0.05$.

Table 4. Carcass traits of different chicken breeds from 120 days of age.

	Mini chickens	Broilers
CW (g)	1586.64 ± 109.79^{a}	3576.19 ± 71.30^{b}
SEW (g)	1355.15 ± 95.87^{a}	3240.44 ± 62.54^{b}
EW (g)	1140.41 ± 84.13^{a}	2789.80 ± 57.48^{b}
BMW (g)	221.78 ± 19.14^{a}	914.25 ± 26.82^{b}
LMW(g)	308.20 ± 29.97^{a}	782.69 ± 26.66^{b}
DP	86.51 ± 0.64^{a}	90.27 ± 0.55^{b}
SEP	73.77 ± 0.67^{a}	81.85 ± 0.45^{b}
EP	61.87 ± 0.76^{a}	70.41 ± 0.50^{b}
BMP	19.37 ± 0.59^{a}	32.72 ± 0.60^{b}
LMP	26.38 ± 0.81	28.06 ± 0.75

CW, carcass weight; SEW, semi-eviscerated weight; EW, eviscerated weight; BMW, breast muscle weight; LMW, leg muscle weight; DP, dressing percentage; SEP, semi-eviscerated percentage; EP, eviscerated percentage; BMP, breast muscle percentage; LMP, leg muscle percentage. Means with different superscripts differ between breeds at $P < 0.05$.

The CW, SEW, EW, BMW, LMW, and AFW, and the ratios of each of the above traits in Wuding chicken were significantly ($P < 0.05$) lower than those in Broilers at 90, 120, and 150 days.

MSTN expression levels

The expression of *MSTN* mRNA is summarized in Figure 1. *MSTN* mRNA expression levels were lower in breast muscle from Wuding chicken than in that from broilers before

day 30 ($P < 0.05$). After day 30, the expression levels in the breast muscle were higher in Wuding chicken than in broilers ($P < 0.05$). Moreover, *MSTN* mRNA expression levels in the leg muscle were greater in Wuding chicken than in broilers at all ages, with the exception of day 60 ($P < 0.05$).

Table 5. Carcass traits in different chicken breeds from 150 days of age.

	Mini chickens	Broilers
CW (g)	1880.48 ± 76.79[a]	4160.29 ± 96.05[b]
SEW (g)	1620.33 ± 81.00[a]	3829.43 ± 89.54[b]
EW (g)	1337.24 ± 69.50[a]	3356.18 ± 84.15[b]
BMW (g)	288.49 ± 13.44[a]	1109.55 ± 33.51[b]
LMW(g)	367.97 ± 25.40[a]	993.47 ± 42.99[b]
DP	87.14 ± 0.54[a]	91.15 ± 0.31[b]
SEP	74.84 ± 1.67[a]	83.89 ± 0.31[b]
EP	61.67 ± 1.41[a]	73.48 ± 0.45[b]
BMP	21.76 ± 0.35[a]	33.03 ± 0.48[b]
LMP	27.16± 0.61	29.38 ± 0.62

CW, carcass weight; SEW, semi-eviscerated weight; EW, eviscerated weight; BMW, breast muscle weight; LMW, leg muscle weight; DP, dressing percentage; SEP, semi-eviscerated percentage; EP, eviscerated percentage; BMP, breast muscle percentage; LMP, leg muscle percentage. Means with different superscripts differ between breeds at $P < 0.05$.

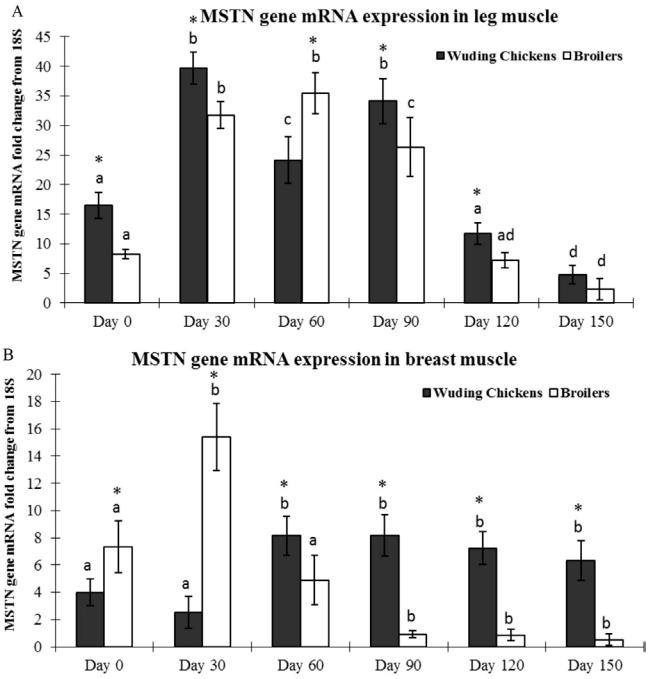

Figure 1. mRNA abundance based on the extraction of total RNA and subsequent real-time PCR analysis for MSTN in breast muscle (**A**) and leg muscle (**B**) from two chicken breeds at 0, 30, 60, 90, and 120 days. Data are reported as mean ratio ± standard error of *MSTN* mRNA: 18S rRNA for chickens from each breed in each age or each diet group from two breeds. Lowercase letters: means with different superscripts differ between age at $P < 0.05$; *means with different superscripts differ between breeds at $P < 0.05$.

Correlation analysis

Correlation analyses of BW, carcass traits, and *MSTN* mRNA expression in Wuding chicken and broilers are summarized in Tables 6 and 7. There was a positive correlation between BW and carcass traits in the two breeds. Discrepancies in the association between tissue-specific *MSTN* mRNA expression and body carcass traits were observed in the two breeds. Thus, the expression of breast muscle *MSTN* mRNA in Wuding chicken was negatively correlated ($P < 0.05$) with CW, SEW, EW, BMW, and LMW. Leg muscle *MSTN* mRNA expression in Wuding chicken was negatively correlated with carcass traits, but no differences were observed ($P < 0.05$). *MSTN* mRNA expression in breast muscle of broilers was negatively correlated with BW ($P < 0.05$). Leg muscle *MSTN* mRNA expression in broilers was negatively correlated with CW, SEW, EW, BMW, LMW, and AFW ($P < 0.05$).

Table 6. Correlation analysis between LW, carcass traits, and *MSTN* mRNA expression in Wuding chickens.

	LW	CW	SEW	EW	BMW	LMW	MSTN-BM	MSTN-LM
LW	1							
CW	0.994**	1						
SEW	0.969**	0.982**	1					
EW	0.961**	0.976**	0.992**	1				
BMW	0.890**	0.924**	0.941**	0.945**	1			
LMW	0.880**	0.913**	0.952**	0.966**	0.966**	1		
MSTN-BM[a]	-0.413**	-0.394**	-0.372**	-0.369**	-0.393**	-0.377**	1	
MSTN-LM[b]	-0.724**	-0.712**	-0.682**	-0.655**	-0.663**	-0.577**	0.512**	1

LW, mean live weight; CW, carcass weight; SEW, semi-eviscerated weight; EW, eviscerated weight; BMW, breast muscle weight; LMW, leg muscle weight. [a]Mean MSTN expression in breast muscle. [b]Mean MSTN expression in leg muscle. **Means with extremely significant correlation ($P < 0.01$).

Table 7. Correlation analysis between mean live weight (LW), carcass traits, and *MSTN* mRNA expression in Broilers.

	LW	CW	SEW	EW	BMW	LMW	MSTN-BM	MSTN-LM
LW	1							
CW	0.992**	1						
SEW	0.990**	0.996**	1					
EW	0.984**	0.985**	0.990**	1				
BMW	0.925**	0.938**	0.946**	0.953**	1			
LMW	0.929**	0.943**	0.940**	0.940**	0.893**	1		
MSTN-BM[a]	-0.295*	-0.310*	-0.317*	-0.327*	-0.338**	-0.306*	1	
MSTN-LM[b]	-0.666**	-0.644**	-0.643**	-0.613**	-0.613**	-0.562**	0.199	1

Carcass traits: CW, carcass weight; SEW, semi-eviscerated weight; EW eviscerated weight; BMW, breast muscle weight; LMW, leg muscle weight. [a]Mean MSTN expression in breast muscle. [b]Mean MSTN expression in leg muscle. *Means differ at $P < 0.05$. **Means differ at $P < 0.01$.

DISCUSSION

Several factors affect production and carcass performance in chicken, including breed or strain, sex, nutrition, housing, and stocking rate. However, breed plays an important role in determining carcass characteristics and muscle mass. Breed significantly affects BW (Shahin and Elazeem, 2005; Jaturasitha et al., 2008; Bhattacharya et al., 2015), carcass weight (Ojedapo et al., 2008; Olawumi and Fagbuaro, 2011), breast and leg muscle weight fat and edible giblet weight (Ojedapo et al., 2008), and back and drumstick weights (Ojedapo et al., 2008) in

chickens. Consistent with those reports, CW, BMW, LMW, AFW, DP, and EP were significantly lower in Wuding chickens than in broilers. Compared to Jingle fowl and Wuding chickens, broiler chickens have been selected for their fast growth rate, which has significantly increased the productive and carcass performance and has been associated with changes in morphology and physiology (Rong et al., 2011). Domestic animals have evolved genetic adaptations to the farm environment, and have been subjected to strong human-driven selection resulting to marked phenotypic changes in their morphology, physiology, and behavior (Andersson, 2001). Identifying the genetic changes underlying these developments provides new insight into the mechanisms by which genetic variation shapes phenotypic diversity (Rubin et al., 2010). Indeed, the genetic diversity present in modern commercial pure lines has been estimated at just 50% of that present in ancestral breeds in jungle fowl (Muir et al., 2008).

In general, DP and EP are considered the main indicators of meat yield in chicken (Lehmann, 2006). DP exceeding 80% and EP up to 60% are considered good signs of meat performance (Zhang, 2004). In the present study, broiler chickens had high muscle mass and excellent carcass characteristics than Wuding chickens. DP and EP have been shown to be 86.28 and 62.46% for Wuding chickens, 90.25 and 72.79% for broilers, respectively. Wuding chickens might carry potential excellent genetic genes for breast meat yield due to their high percentage of muscle mass (Rong et al., 2011). The correlation analysis in the present study showed that BW was positively associated with CW, SEW, and EW in both breeds. In agreement with other reports, there was high correlation between BW and carcass traits in the present study (Chen et al., 2014).

Myostatin is a highly conserved, potent regulator of growth and differentiation of skeletal muscle in many species, from teleost fishes to humans, although its mechanisms of action are incompletely understood (Morissette et al., 2006). *MSTN* mRNA expression was detected in a wide range of tissues in chicken, and its tissue-specific expression was similar to that reported in fishes, but different to that reported in mammals. During the embryonic stage in chicken, MSTN was shown to be expressed even before the establishment of the myogenic lineage (Kocamis and Killefer, 2002). It was suggested that chicken MSTN might play a major role in the morphogenesis of chicken liver, heart, brain, and intestine (Sundaresan et al., 2008). In the present study, expression of *MSTN* mRNA in breast muscle was greater in broiler chickens than in Wuding chickens on day 30. Wuding chicken is reared solely as a local breed and has not been selected for fast growth (Rong et al., 2011). Lower *MSTN* mRNA in muscle of 30-day-old Wuding chickens suggested that the chicken *MSTN* gene might play an important role in the regulation of muscle fiber development.

Myostatin is a potent growth and differentiation factor involved in skeletal muscle formation in vertebrates (Castelhano-Barbosa et al., 2005). During the early embryonic stages, myostatin is restricted to the myotome compartment of the developing somites, and myostatin has been proposed to play an essential role in skeletal muscle growth and development (McPherron et al., 1997). Myostatin inhibits myoblast proliferation (Bass et al., 1999) by preventing their progression from the G1 to the S phase of the cell cycle (Thomas et al., 2000). In chicken, the developmental pattern of myostatin mRNA expression coincides roughly with the progression of muscle fiber formation (Kocamis and Killefer, 2002). In the present study, *MSTN* mRNA in breast muscle was expressed at lower levels in Wuding chicken than in broilers before day 30. After day 30, *MSTN* mRNA in breast muscle was higher in Wuding chicken than in broilers. Expression of *MSTN* mRNA in leg muscle was higher in Wuding chicken than in broilers at all ages, with the exception of day 60. Broilers selected for growth

performance and muscle yield might have led to changes in the *MSTN* gene sequence and regulation. Consistent with previous reports (Zhang et al., 2012), a negative effect of the chicken *MSTN* gene on the regulation of growth performance and carcass traits was observed in the present study.

The *MSTN* gene has been closely linked with the growth and development of animals, and its expression is negatively correlated with muscle weight in channel catfish (Weber et al., 2005). Variation in the *MSTN* gene is associated with meat production in New Zealand Romney sheep, skeletal muscle growth, DP, leg muscle rate, and other carcass traits in sheep (Boman and Våge, 2009; Wiener et al., 2009; Hickford et al., 2010). Mutations in the *MSTN* gene significantly affected BW in Bian chicken, and the correlation between growth rate and mortality rate in broiler chickens (Zhang et al., 2012). *MSTN* mRNA expression in muscle was negatively associated with BW and carcass traits in both breeds in present study. Consistent with a previous report (Guimaraes et al., 2007) that *MSTN* mRNA expression was negatively correlated with growth rate and meat quality traits in pig. *MSTN* mRNA expression in pig has a greater effect in fast-growing animals than in slow-growing animals. However, there were differences noted in the correlation of tissue-specific *MSTN* mRNA expression with body carcass traits in the two breeds. *MSTN* mRNA expression in breast muscle was negatively correlated with CW, SEW, EW, BMW, and LMW in Wuding chickens and with BW in broilers. *MSTN* mRNA expression in leg muscle in broiler chicken was negatively correlated with CW, SEW, EW, BMW, and LMW. The present data suggest that expression of *MSTN* mRNA in breast and leg muscle has more of an effect in slow growing Wuding chickens than in fast growing broilers.

It is clear that the *MSTN* gene has an important role in the regulation of growth performance and skeletal muscle mass in chickens. Broiler chickens selected for fast growth exhibit decreased *MSTN* mRNA expression in skeletal muscle, which is associated with an increase in muscle mass. The results of the present study further strengthen the notion that the *MSTN* gene may be useful in molecular breeding in chicken owing to its negative regulatory effect on muscle mass.

Conflicts of interest

The authors declare no conflict of interest.

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