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Effects of different dietary intake on mRNA levels of *MSTN*, *IGF-I*, and *IGF-II* in the keletal muscle of Dorper and Hu sheep hybrid F_1 rams

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ABSTRACT. *MSTN*, *IGF-I* (insulin-like growth factor-I) and *IGF-II* (insulin-like growth factor-II) regulate skeletal muscle growth. This study investigated the effects of different dietary intake levels on skeletal muscles. Sheep was randomly assigned to 3 feeding groups: 1) the maintenance diet (M), 2) 1.4 x the maintenance diet (1.4M), and 3) 2.15 x the maintenance diet (2.15M). Before slaughtering the animals, blood samples were collected to measure plasma urea, growth hormone, and insulin concentrations. After slaughtering, the longissimus dorsi, semitendinosus, semimembranosus, gastrocnemius, soleus, and chest muscle were removed to record various parameters, including the mRNA expression levels of *MSTN* and *IGFs*, in addition to skeletal muscle fiber diameter and cross-sectional area. The result showed that as dietary intake improved, the mRNA expression levels

of *MSTN* and *IGF-II* decreased, whereas *IGF-I* expression increased. The mRNA expression levels of *MSTN* and *IGFs* were significantly different in the same skeletal muscle under different dietary intake. The skeletal muscle fiber diameter and cross-sectional area increased with greater dietary intake, as observed for the mRNA expression of *IGF-I*; however, it contrasted to that observed for the mRNA expression of *MSTN* and *IGF-II*. In conclusion, dietary intake levels have a certain influence on *MSTN* and *IGFs* mRNA expression levels, in addition to skeletal muscle fiber diameter and cross-sectional area. This study contributes valuable information for enhancing the molecular-based breeding of sheep.

Key words: *MSTN*; *IGF-I*; *IGF-II*; Dietary intake; Skeletal muscle; Sheep

INTRODUCTION

MSTN has been identified as a member of the transforming growth factor-β superfamily, which is predominantly expressed in muscle tissue, and acts as a negative regulator of muscle development (McPherron et al., 1997; Lee and McPherron, 1999; Kocamis and Killefer, 2002). *MSTN* is expressed in the early development of skeletal muscle, and continues to be expressed in adult skeletal muscle (McPherron et al., 1997; McCroskery et al., 2003). In general, fetal or postnatal inactivation of *MSTN* does not affect the number of fibers, but results in muscle fiber hypertrophy (Bogdanovich et al., 2002; Whittemore et al., 2003). Mice carrying a targeted disruption of the *MSTN* gene exhibit a significant increase in muscle mass, which is the result of a combination of muscle fiber hyperplasia and hypertrophy (Szabó et al., 1998).

IGFs (insulin-like growth factors), including *IGF-I* and *IGF-II*, are structurally similar polypeptides that are important in regulating the proliferation and differentiation of a variety of somatic cell types (Froesch et al., 1985). *IGFs* are essential for the growth and development of all vertebrates (Wood et al., 2005). According to published reports, *IGF-II* plays a significant role in embryonic development, while *IGF-I* plays a fundamental role in postnatal development (Gerrard et al., 1998; Vary, 2006). However, *IGF-II* has also been reported to be an important regulator of muscle mass in rats after birth (Lalani et al., 2000). *IGF-I* and *IGF-II* knockout mice have about 60% lower birth weight compared with their wild-type littermates (Baker et al., 1993; Liu et al., 1993). The body weight of both *IGF-I* and *IGF-II* null mutation mice was 30% lower compared with their wild-type littermates at birth; however, they invariably died shortly after birth (Baker et al., 1993; Liu et al., 1993).

Molecular biology provides a powerful tool to modify livestock growth rates, body composition, and meat quality. To date, this tool has been mainly used to meet the market requirements of livestock production (Guernec et al., 2003). The development of skeletal muscle has been an important subject of scientific research for many years in a number of animal species (Rehfeldt et al., 2011). The expressions of insulin-like growth factor system genes and *MSTN* are closely related to the development of skeletal muscle tissue, which is a significant contributor to postnatal growth rates, body composition, and meat quality (Peng et al., 1996;

McPherron et al., 1997; Tilley et al., 2007). However, there has been controversy over the effect of nutrition level on the expression of *MSTN*, *IGF-I*, and *IGF- II* in skeletal muscle.

According to Hirai et al. (2004), protein deficiency does not affect *MSTN* mRNA abundance in the muscle. In contrast, it also has been reported that *IGF-I* and *MSTN* mRNA are reduced when animals are underfed (Jeanplong et al., 2003). Previous experiments have shown that circulating levels of *IGFs* in plasma are regulated by nutrition, with severe protein deficiency causing a decline in *IGF-I* concentrations. However, other studies indicate that the expression of *IGF-I* in skeletal muscle is relatively insensitive to short-term fasting (Oldham et al., 1996, 1999; Jeanplong et al., 2003).

During the late fattening period, animal feeding management aims to enhance body composition and meat quality. The present study aimed to determine whether dietary intake nutrition levels influence the contribution of *MSTN*, *IGF-I*, and *IGF-II* mRNAs to muscle deposition and quality during the late fattening period.

MATERIAL AND METHODS

Animals, feeding, and tissue collection

The experiment was conducted at the Research Centre of Haimen Goat of Nanjing Agriculture University (Haimen, Jiangsu, China) (longitude 121.28°N, latitude 31.89°E) from May to July 2011. The experiment used 18 Dorper sheep and Hu sheep hybrid F_1 rams of similar age, body weight (35.83 ± 0.87 kg), and body composition. During the experiment, the animals were given free access to water, maintained under a natural photoperiod (about 7.6 h per day), and received 2 equal allotments of feed at 8:00 am and 16:00 pm. From day 1 to day 25 of the experiment, all animals were pre-fed a 2.15 x maintenance diet until the weight reached about 42 ± 0.96 kg. Then, the animals were randomly assigned to 3 groups: 1) the maintenance diet group (M), 2) the 1.4 x maintenance diet group (1.4M), and 3) the 2.15 x maintenance diet group (2.15M). According to a previous report (Nie et al., 2012), all sheep received a total mixed ration diet (shown in Table 1), whereby the M group received a diet of 1000 g/day per sheep, the 1.4M group received a diet of 1400 g/day per sheep, and the 2.15M group received a diet of 2150 g/day per sheep, to achieve daily weight gain of 0 g, 150 g, and 350 g, respectively. When the weight of the sheep in the 2.15M diet group reached about 50 kg (50.01 ± 1.11 kg), the animals were slaughtered by jugular vein bloodletting. All experimental procedures were performed according to the guide for animal care and use of laboratory animals of the Institutional Animal Care and use Committee of Nanjing Agricultural University (Ying et al., 2011).

At the end of the experiment, before the animals were slaughtered, blood (5 mL) was sampled from the jugular vein by venipuncture, and immediately placed in heparinized tubes, maintained on ice, separated by centrifugation at 4°C, and stored at -20°C for the subsequent measurement of plasma urea, growth hormone (GH), and insulin concentrations. Within 5 min of death, 6 pieces of skeletal muscle from various parts of the body (including longissimus dorsi, semitendinosus, semimembranosus, gastrocnemius, soleus, and chest muscle) were quick frozen and stored in liquid nitrogen, until RNA extraction. Small blocks of tissue (2 x 1 x 1 cm^3 in size) were cut off, placed in physiological saline, and stored in 4% paraformaldehyde, until subsequent analyses.

Table 1. Composition and nutrient contents of basal diets.

Ingredients	Percentage of dry matter
Corn (%)	42.83
Soybean meal (%)	16.04
Soy straw (%)	40.02
Limestone (%)	0.2
$CaHPO_4$ (%)	0.4
Salt (%)	0.4
Trace mineral and vitamin Premix (%)[a]	0.11
Chemical composition	
DE (MJ/day)	11.7
ME (MJ/day)	9.7
CP (g/kg)	152.8
CF (g/kg)	26.6
Neutral detergent fiber (g/kg)	491.7
Acid detergent fiber (g/kg)	208.9
Ca (%)	7.8
P (%)	3.9

[a]Composition/kg of trace mineral and vitamin Premix: 56 mg Fe, 15 mg Cu, 30 mg Mn, 40 mg Zn, 1.5 mg I, 0.2 mg Se, 0.25 mg Co, 3.2 g S, 2150 IU vitamin A, 170 IU vitamin D, and 131 IU vitamin E, 2.7 g super-concentrated Yuan Kangbao, 1.6 g monensin (2%), 10.1 g sodium sulfare.

RNA isolation and cDNA synthesis

Total RNA was extracted from the skeletal muscle tissues by using the RNAprep pure tissue kit (Tiangen, Beijing, China), following manufacturer protocols. The extracted RNA was resuspended in diethyl pyrocarbonate (DEPC)-treated water, and the final concentration was assessed spectrophotometrically by a NanoDrop 1000 Spectrophotometer (Thermo, Shanghai, China). A quantity of 8 µL total RNA from each sample was separated on 1.2% agarose gel, with electrophoresis showing intact 28 and 18s RNA.

First-strand cDNA was synthesized in a total volume containing 2.5 µg total RNA from each sample, 10 µL 5X PrimerScript Buffer (for real-time analysis) (Takara, Dalian, China), and then was made up to a volume of 50 µL with DEPC-treated water. The mixture was heated to 37°C for 15 min and then 85°C for 5 s, and stored at -20°C until use.

Expression analysis of *MSTN* and *IGFs* by real-time polymerase chain reaction (PCR)

Real-time PCR assessment was performed with 1 µL cDNA, 0.6 µL (10 mM) of each primer, and 10 µL FastStart Universal SYBR Green Master (ROX; Roche, Shanghai, China) in a total reaction volume of 20 µL. The ABI 7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) was used. The specific primers of *MSTN* (GenBank accession No. AF019622), *IGF-II* (GenBank accession No. AH005355S7), and *β-actin* (GenBank accession No. OAU39357) transcripts have been reported previously (Hirai et al., 2004; Ying et al., 2011). The specific primers used for the detection of *IGF-I* (GenBank accession No. M30653) were designed using the Primer Premier Software (version 5.0; Premier Biosoft International, Palo Alto, CA, USA). Real-time PCR assessment for *MSTN*, *IGF-I*, *IGF-II*, and *β-actin* was programmed to conduct 40 cycles (a single cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s). Each sample was added to a melt curve at the end of the cycles to test the specificity of the primers. All samples were assayed in triplicate to improve the accuracy of the results. The primers

designed for quantifying the mRNA expression of *MSTN*, *IGF-I*, *IGF-II*, and *β-actin* genes are listed in Table 2.

Table 2. Primers used for quantifying mRNA expression of *MSTN*, *IGF-I*, *IGF-II*, and *β-actin* genes in sheep.

Gene name	Oligonucleotides (5'→3')	Product size (bp)[a]
MSTN	GAACCAGGAGAAGAAGGACT GCACAAGATGGGTATGAGG	256
β-actin	GCAGTTGTGGATCAGCAAGC GGGCAATCTCATCTCGTTTTC	144
IGF-I	TATTTCAACAAGCCCACG ACATCTCCAGCCTCCTCA	109
IGF-II	TTCTTCCAATCTGACACCTG AGGCAGGGCGATCAGCGGACGGTGA	150

All primers were synthesized by Invitrogen (Shanghai, China). [a]The product length is the length of cDNA sequence without introns.

Blood sample determination

The concentration of plasma urea was determined using a commercial kit (Diagnostic kit; Shanghai Kehua Bio-Engineering Co., Ltd., Shanghai, China). Plasma GH and plasma insulin concentrations were determined by a commercial kit (Diagnostic Product, Beijing North Institute of Biological Technology). All experimental techniques followed manufacturer protocols.

Measurement of muscle fiber diameter and cross-sectional area

Pieces of skeletal muscle tissue (2 x 1 x 1 cm^3 in size) collected after the animals were slaughtered were fixed with 4% paraformaldehyde, dehydrated, embedded, and stained to measure the skeletal muscle fiber diameter and cross-sectional area with a digital microscope (Version 80I; Nikon, Tokyo, Japan) at 400X magnification routinely. The tissue slices were resected for hematoxylin/eosin staining by a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), following the manufacturer protocol.

The measurement of the muscle fiber diameter and cross-sectional area was conducted by the Image-Pro Plus software program (version 6.0; Media Cybernetics, San Francisco, CA, USA).

Statistical analysis

The relative quantification of real-time data of cDNA allowed the Ct values of each gene to be determined. These values were measured to create standard curves as a basis for calculating whether the amplicons have the same PCR efficiency, by observing the ΔCt variation with dilution. To use the comparative Ct method, the absolute slope of the target and house-keeping gene should be close to zero, so that the assumption of equal PCR efficiencies is met (Livak and Schmittgen, 2001). The expression of each gene was analyzed using the $2^{-\Delta\Delta Ct}$ method, as reported previously (Wang et al., 2012).

Statistical analyses were performed by the SPSS statistical software program (version 13.0; SPSS, Chicago, IL, USA). All data are reported as means ± SD. Outcomes were con-

sidered to be statistically significant when $P < 0.05$, and extremely significant when $P < 0.01$. The data distribution was first confirmed by the Kolmogorov-Smirnov goodness-of-fit test for data with a normal distribution. Non-normally distributed data were tested by the equivalent nonparametric test. Data were analyzed for treatment effects by the independent samples *t*-test for each tissue (Wang et al., 2013).

RESULTS

Daily intake, daily weight gain, and muscle weight

Figure 1 and Table 3 show the daily intake (g), daily weight gain (g), and muscle weight (kg) throughout the experiment. There were highly significant differences ($P < 0.01$) in daily intake (g), daily weight gain (g), and muscle weight (kg) among the 3 different intake treatments. Increased daily intake was found to improve daily weight gain (g) under the present experiment design.

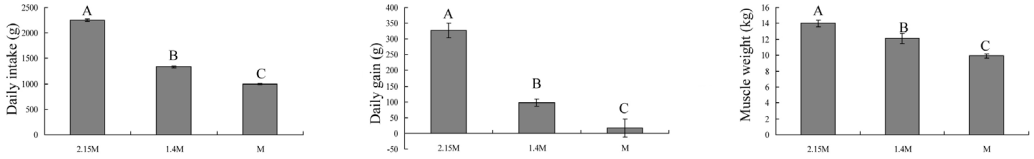

Figure 1. Daily intake, daily gain and muscle weight. Different capital letters mean significant differences at $P < 0.01$.

Table 3. Daily intake (g), daily gain (g) and muscle weight (kg) throughout the experiment.

	2.15M	1.4M	M
Daily intake (g)	2248.2 ± 22.1^{A}	1333.7 ± 15.33^{B}	994.7 ± 16.5^{C}
Daily gain (g)	327.2 ± 22.7^{A}	97.0 ± 11.1^{B}	17.5 ± 29.05^{C}
Muscle weight (kg)	14.0 ± 0.42^{A}	12.1 ± 0.62^{B}	9.9 ± 0.26^{C}

Different capital letters in the same row mean significant differences at $P < 0.01$.

Expressions of *MSTN* and *IGFs* in skeletal muscle under different dietary intake levels

Expression analysis with real-time fluorescence quantitative PCR showed that the expressions of *MSTN* and *IGFs* in skeletal muscle varied under the 3 different intake levels. In general, the expression of *MSTN* decreased with increasing intake level. The expression of *MSTN* in the soleus of the M group was significantly higher compared with the 2.15M and the 1.4M diet groups (Table 4 and Figure 2; $P < 0.05$ and $P < 0.01$, respectively). The *MSTN* mRNA expression of the chest muscle was higher in the 1.4M group ($P < 0.05$) compared with

the 2.15M group (Table 4 and Figure 2). As intake levels improved, *MSTN* and *IGF-II* mRNA expression decreased, while *IGF-I* mRNA expression increased. *IGF-I* mRNA expression was highest in the 2.15M group, followed by the M and 1.4M groups, as shown in Table 4 and Figure 2. The mRNA of *MSTN* and *IGF-II* was negatively correlated with *IGF-I*, whereas the mRNA of *MSTN* was positively correlated with *IGF-II*. The *IGF-I* and *IGF-II* mRNAs had different significant effects on skeletal muscle, with different intake levels affecting the abundance of the *IGF-I* and *IGF-II* mRNAs (Table 4 and Figure 2).

Table 4. Effect of different intake levels on skeletal tissue differential expression.

Gene name	Skeletal tissue	2.15M	1.4M	M
MSTN	Longissimus dorsi	0.87 ± 0.34	0.84 ± 0.13	1.20 ± 0.21
	Semitendinosus	0.61 ± 0.19	0.82 ± 0.17	0.79 ± 0.19
	Semimembranosus	1.53 ± 0.08	1.17 ± 0.40	1.34 ± 0.32
	Gastrocnemius	0.29 ± 0.11	0.43 ± 0.13	0.37 ± 0.09
	Soleus	0.44 ± 0.09^{A}	0.64 ± 0.17^{a}	1.00 ± 0.13^{Bb}
	Chest muscle	0.53 ± 0.07^{a}	1.14 ± 0.16^{b}	0.96 ± 0.26
IGF-I	Longissimus dorsi	0.99 ± 0.14^{a}	0.89 ± 0.06^{A}	0.94 ± 0.15^{Bb}
	Semitendinosus	1.50 ± 0.30^{Aa}	0.81 ± 0.09^{Ba}	1.15 ± 0.07^{b}
	Semimembranosus	1.07 ± 0.28^{a}	0.65 ± 0.22^{b}	0.86 ± 0.16^{a}
	Gastrocnemius	1.04 ± 0.10	0.79 ± 0.17	0.92 ± 0.09
	Soleus	1.27 ± 0.14^{a}	0.75 ± 0.21^{b}	1.01 ± 0.21^{a}
	Chest muscle	1.47 ± 0.41	1.07 ± 0.16	1.27 ± 0.22
IGF-II	Longissimus dorsi	1.90 ± 0.52^{a}	0.75 ± 0.04^{Ab}	2.72 ± 0.11^{B}
	Semitendinosus	1.58 ± 0.28^{a}	0.84 ± 0.11^{B}	2.31 ± 0.08^{Bb}
	Semimembranosus	2.84 ± 0.23^{A}	0.77 ± 0.01^{A}	2.99 ± 0.18^{A}
	Gastrocnemius	1.93 ± 0.35^{a}	1.25 ± 0.22^{Ab}	2.17 ± 0.18^{B}
	Soleus	1.43 ± 0.43^{Aa}	0.74 ± 0.02^{Ba}	2.55 ± 0.36^{b}
	Chest muscle	2.83 ± 0.58^{a}	1.81 ± 0.32	1.52 ± 0.12^{b}

Different capital letters in the same row mean significant differences at $P < 0.01$. Different lowercase letters in the same row mean significant differences at $P < 0.05$.

Plasma urea, GH, and insulin concentrations

The concentration of plasma urea, GH, and insulin differed in relation to different intake levels. Plasma urea and insulin concentrations increased with increasing intake levels, whereas plasma GH concentrations decreased. Significantly higher plasma urea levels were obtained for the 2.15M group compared with the 1.4M ($P < 0.01$) and M ($P < 0.01$) groups. Significantly higher plasma GH was obtained for the M group compared with the 2.15M group ($P < 0.01$). Significantly lower plasma insulin was obtained in the M group compared with the 2.15M ($P < 0.01$) and 1.4M ($P < 0.05$) groups (Table 5 and Figure 3).

Effects of dietary intake level on muscle fiber diameter and cross-sectional area

The muscle fiber diameter and cross-sectional area of the skeletal muscle increased significantly with higher dietary intake levels, as shown in Table 6 and Figure 4. This trend was similar to that observed for the expression of *IGF-I*, but was the opposite to that observed for the expression of *MSTN* and *IGF- II*.

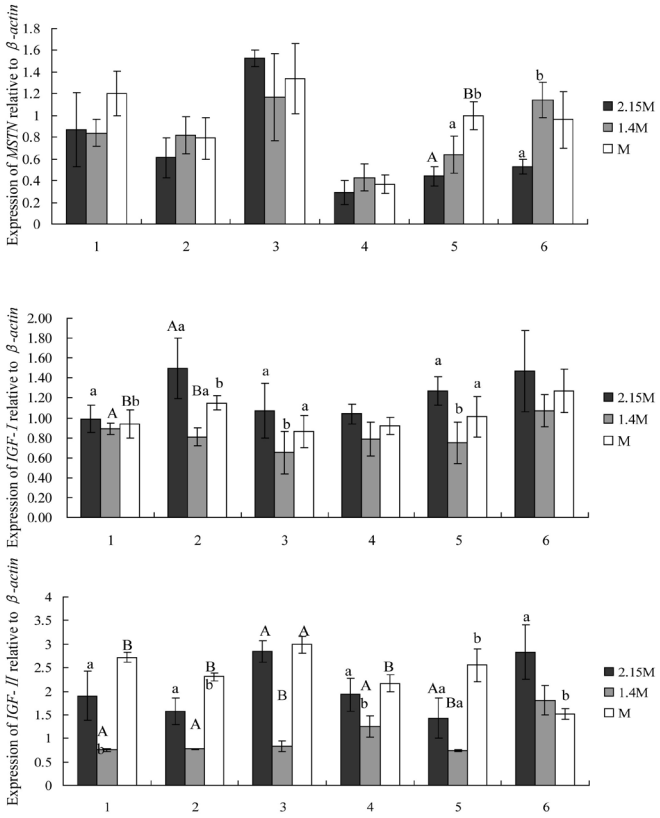

Figure 2. Gene expression of *MSTN*, *IGF-I*, and *IGF-II* mRNA in skeletal tissue. Different capital letters mean significant differences at $P < 0.01$. Different lowercase letters mean significant differences at $P < 0.05$. The numbers in the x-axis 1-6 represent longissimus dorsi, semitendinosus, semimembranosus, gastrocnemius, soleus, and chest muscle, respectively.

Table 5. Concentration of serum urea, GH and insulin.

	2.15M	1.4M	M
Urea (mM)	13.00 ± 0.68^{A}	9.28 ± 0.68^{B}	8.87 ± 0.62^{B}
GH (ng/mL)	0.63 ± 0.05^{a}	0.71 ± 0.05	0.73 ± 0.05^{b}
Insulin (μU/mL)	14.44 ± 1.04^{A}	13.88 ± 1.96^{a}	6.87 ± 0.67^{Bb}

Different capital letters in the same row mean significant differences at $P < 0.01$. Different lowercase letters in the same row mean significant differences at $P < 0.05$.

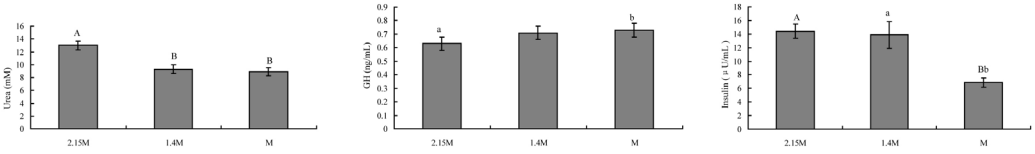

Figure 3. Concentration of serum urea, GH and insulin. Different capital letters mean significant differences at $P < 0.01$. Different lowercase letters mean significant differences at $P < 0.05$.

Table 6. Measurement of muscle fiber diameter and cross-sectional area under different dietary intake.

Measurement	Skeletal tissue	2.15M	1.4M	M
Fiber diameter (μm)	Longissimus dorsi	32.88 ± 1.60^{a}	28.30 ± 1.97	25.39 ± 0.11^{b}
	Semitendinosus	35.32 ± 0.89^{a}	30.16 ± 1.91^{a}	28.06 ± 0.55^{b}
	Semimembranosus	34.26 ± 0.90^{Aa}	29.46 ± 1.12^{b}	26.23 ± 0.12^{Ba}
	Gastrocnemius	33.49 ± 1.52^{a}	30.52 ± 2.98	26.96 ± 0.69^{b}
	Soleus	34.99 ± 1.10^{a}	31.63 ± 2.54	27.16 ± 0.93^{b}
	Chest muscle	36.20 ± 2.46^{Aa}	32.74 ± 2.20^{b}	26.79 ± 0.59^{B}
Cross-sectional area (μm^2)	Longissimus dorsi	826.07 ± 44.30^{A}	780.14 ± 16.63^{B}	567.06 ± 9.42^{B}
	Semitendinosus	902.35 ± 27.81^{Aa}	858.77 ± 25.80^{b}	637.77 ± 16.14^{B}
	Semimembranosus	836.08 ± 22.52^{a}	805.90 ± 23.20^{b}	602.79 ± 15.50^{b}
	Gastrocnemius	830.35 ± 18.37^{A}	850.71 ± 47.43^{b}	633.18 ± 14.34^{B}
	Soleus	880.13 ± 18.38^{Aa}	862.41 ± 24.69^{b}	667.61 ± 7.48^{B}
	Chest muscle	891.42 ± 19.36^{Aa}	861.71 ± 30.03^{b}	666.91 ± 10.26^{B}

Different capital letters in the same row mean significant differences at $P < 0.01$. Different lowercase letters in the same row mean significant differences at $P < 0.05$.

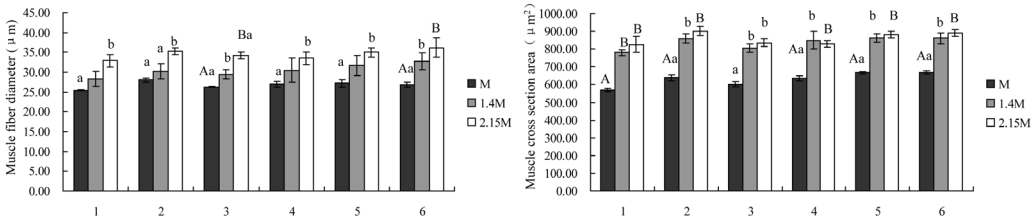

Figure 4. Measurement of muscle fiber diameter and cross-sectional area. Different capital letters mean significant differences at $P < 0.01$. Different lowercase letters mean significant differences at $P < 0.05$.

DISCUSSION

Previous research on double-muscled, *MSTN* knockout mice demonstrated that a decrease in *MSTN* mRNA resulted in an increase in the expression of *IGF-II*, with the mice exhibiting improved insulin sensitivity when fed both standard chow and a high-fat diet (Kocamis et al., 2002; Guo et al., 2009). As a result, it was hypothesized that the ratio of mRNA concentrations between *MSTN* and *IGFs* might serve as indicators of muscle mass (Stinckens et al., 2009). In this study, we determined the expression patterns of *MSTN* and *IGF* genes in the skeletal muscle of sheep. As mouse *MSTN* gene caused a significant increase in muscle mass, because of a combination of muscle fiber hyperplasia and hypertrophy (Szabó et al., 1998), we also measured the muscle fiber diameter and cross-sectional area of sheep. Plasma urea was measured to confirm the presence of any deficiency in dietary intake levels, while plasma GH and insulin concentrations are associated with livestock growth and development factors. The concentration of plasma urea increased with increasing intake levels. This result showed that sheep with high dietary intake had high plasma urea concentrations.

During the late fattening period, feeding management programs focus on enhancing body composition and meat quality, rather than daily weight gain. The expressions of *MSTN* and *IGFs* are associated with muscle weight, muscle fiber diameter and cross-sectional area. These findings provide the basis on which to develop further research about the meat qual-

ity by controlling the expression of *MSTN* and *IGFs*. Skeletal muscle sections were taken to measure the mRNA expression of *MSTN* and *IGFs*, in addition to muscle fiber diameter and cross-sectional area. Muscles from different parts of the carcass have different function, and hence meat quality. Plasma insulin and GH concentrations were also determined. The same trend was observed for these hormones as for daily weight gain and muscle weight gain.

β-actin was selected as the internal standard for the expression analysis with real-time fluorescence quantitative PCR. In brief, dietary intake levels affected the expression of *MSTN* and *IGFs*. Previous research suggested that *MSTN* is a negative modulator for muscle mass, whereas *IGF-I* and *IGF-II* represent known positive regulators of muscle growth. In the current study, with the improvement of the dietary intake, the expression of *MSTN* and *IGF-II* mRNAs decreased, whereas that of *IGF-I* increased. The mRNAs of *MSTN* and *IGF-II* were negatively correlated with *IGF-I*, whereas the mRNA of *MSTN* was positively correlated with *IGF-II.* The lowest *IGF-I* and *IGF-II* levels were obtained in the 1.4M group compared with the other 2 groups. This finding differed to previous reports stating that protein deficiency does not affect *IGF-I* mRNA expression in the muscle. Instead, the reduction of *IGF-I* mRNA protein consistently led to a decline in the quantity of skeletal muscle of lambs (Grant et al., 1991; Guo et al., 2009).

The present study showed that increased dietary intake promoted an increase in both muscle fiber diameter and cross-sectional area. The mRNA of *MSTN* was negatively correlated with muscle fiber diameter and cross-sectional area. The results also showed that a decline in *MSTN* mRNA causes muscle fiber hyperplasia and hypertrophy. The reason for *IGF-I* and *IGF-II* mRNA causing an increase in muscle fiber was not determined.

Overall, the 2.15M group had the highest *IGF-I* mRNA expression, lowest *IGF-II* mRNA expression, and largest muscle fiber diameter and cross-sectional area compared with the other 2 groups. In comparison, the 1.4M group had the lowest *IGF-I* expression, while the M group had the smallest muscle fiber diameter and cross-sectional area. Further research required to determine the relationship between the expression of the 2 types of *IGFs* with muscle fiber diameter and cross-sectional area.

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