Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

# Genetic Polymorphisms of *Mc4R* and *IGF2* Gene Association with Feed Conversion Efficiency Traits in Beef Cattle

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ARTICLE HISTORY

Received: November 10, 2012
Revised: January 09, 2013
Accepted: March 25, 2013

**Key words:**
Beef cattle
Feed conversion efficiency
*IGF2*
MALDI-TOF-MS
*MC4R*

**ABSTRACT**

Melanocortin-4 receptor (*MC4R*) gene is part of the central melanocortin pathway located in the hypothalamus, an area of the brain in which appetite is regulated. Insulin-like growth factor 2 (*IGF2*) gene plays a role in muscle growth, myoblast proliferation and differentiation. Thus, they are candidate genes for feed conversion efficiency (FCE). The study was to investigate the effects of variants in cattle *MC4R* and *IGF2* gene on FCE traits including residual feed intake (RFI), feed conversion ratio (FCR) and average daily gain (ADG). We screened single nucleotide polymorphisms (SNPs) of the two genes in 118 Simmental bulls by DNA-pool sequencing and genotyped by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis. *C1069G* locus of *MC4R* and four SNPs (*C2209T*, *G18587C*, *A22950T* and *G26920T*) of *IGF2* were identified in the population. The $\chi 2$ test showed that only *MC4R-C1069G, IGF2-C2209T* and *IGF2-G18587C* loci fitted with Hardy-Weinberg equilibrium ($P>0.05$). General linear model (GLM) was used to analyze differences between genotypes. The results showed that only *IGF2-G18587C* locus has a significant effect on ADG ($P<0.05$), but has no significant effect on RFI or FCR ($P>0.05$). CC and GG genotypes were the dominant genotypes; individual with CC or GG genotype had a larger ADG than GC ($P<0.05$).



**To Cite This Article:** Du XH, C Chen, ZR Yuan, LM Zhang, XJ Chen, YH Wang, X Gao, LP Zhang, HJ Gao, JY Li and SZ Xu, 2013. Genetic polymorphisms of *Mc4R* and *IGF2* gene association with feed conversion efficiency traits in beef cattle. Pak Vet J, 33(4): 418-422.

## INTRODUCTION

Feed conversion efficiency (FCE) has been an important indicator as a measure of economic efficiency in livestock. Traditionally, it is measured as feed conversion ratio (FCR), which is the ratio of feed to gain. However, the FCR value of livestock individual with the same feed intake (FI) but different genetic background is not always the same. Residual feed intake (RFI) was proposed and introduced to livestock breeding and defined as the difference value of the actual FI and expected FI (Kennedy *et al.,* 1993). Compared with FCR, RFI is independent of weight and based on energy intake and balance. Therefore, it could be a more accurate method to evaluate FCE.

Melanocortin-4 receptor (*MC4R*) is a G-protein coupled receptor that is highly expressed in the hypothalamus, a region of the brain intimately involved in appetite regulation (Yeo *et al.,* 1998). *MC4R* plays a role in energy homeostasis and body-weight regulation (Benoit *et al.,* 2000). Huszar *et al.* (1997) showed *MC4R* knockout mouse has symptoms of polyphagia, obesity and more insulin secretion. Bovine *MC4R* gene is an intron-less gene with a transcript of 1,808bp and located on chromosome 24 (Haegeman *et al.,* 2001). *MC4R* has been shown to be associated with ultrasonic backfat depth and FCE in pigs (Houston *et al.,* 2004). In cattle, some SNPs of *MC4R* have been discovered: 5 SNPs were detected at position 19(C/A), 20(A/T), 83(T/C), 128(G/A) and 1069(G/C) in eight cattle breeds, only the 1069(G/C) locus was significantly associated with backfat thickness (BF) (Huang *et al.,* 2010). From the previous studies, we got that *MC4R-C1069G* was an important SNP and significantly associated with BF, fat class, live weight and carcass weight in different cattle breeds (Zhang *et al.,* 2009; Liu *et al.,* 2010; Huang *et al.,* 2010; Gill *et al.,* 2010; Seong *et al.,*

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2012). Yet, few researches have reported on the relationship between the locus and FCE traits.

Insulin-like growth factor 2 (*IGF2*) gene is a member of insulin-like growth factor family and encodes a fetal mitogenic protein structurally related to insulin, which plays a role in muscle growth, myoblast proliferation, and differentiation (Dafgard *et al.,* 1985) and has a positive correlation between gene content and growth rate of young boars (Nezer *et al.,* 2003). Bovine *IGF2* gene is an expression of imprinted genes and located on chromosome 29 (Schmutz *et al.,* 1996). In addition, gene expression level between normal and abnormally large bovine fetuses is different (Listrat *et al.,* 1999). In previous studies, some SNPs of *IGF2* gene were found in different cattle breeds, for example, C→T transition in exon 2, A→G mutation in exon 2 and G→T transversion in exon 10 (Goodall and Schmutz, 2003; Han *et al.,* 2008; Bagnicka *et al.,* 2010). Moreover, the association analysis of the *IGF2* gene was mostly correlated to the meat quality and growth traits. However, studies concerning the relationship between polymorphism and FCE traits were little.

Hence, we chose *MC4R* and *IGF2* as FCE candidate genes. The objectives of this study were to investigate SNPs in the two genes and to evaluate whether these polymorphisms affected FCE traits in Simmental bulls.

## MATERIALS AND METHODS

**Animals and data collection:** 118 animals (414±27 kg, 14±1 months) were randomly selected from Simmental bull population, which were bolted and fed in single-slot with the same feeds in Beijing Jinwei Animal Husbandry Co., Ltd. According to the National Research Council (2000) animal nutrition standards, the feeds were composed of corn silage, bread crumbs, soybean meal, brewer's grains and feedlot supplement, which was 27.2% dry matter and supplied 2.87 mj/kg of net energy, 8.96% crude protein, 0.06% calcium and 0.25% phosphorus. The bulls were fed twice a day at 5:30 and 16:30 and drunk freely. All experimental procedures were performed according to authorization granted by the Chinese Ministry of Agriculture.

The formal feeding trail lasted for three months after one week pre-experiment. The supply and surplus of the feeds were measured three times a week, and the differential of the two values was FI. The empty body weight was measured in the morning on the 1$^{st}$, 45$^{th}$, 90$^{th}$ days of the formal experiment, which were recorded as W1, W2 and W3, respectively. Dry matter intake (DMI) means the intake of dry matter of each individual on an average day, which was the sum total FI of individual test record divided by the record times.

Average daily gain (ADG) was calculated by the following formula: ADG=((W2-W1)/45 + (W3-W2)/45) /2. FCR was calculated as the ratio of DMI to ADG, which was derived using linear regression of weight measurements taken throughout the test period (Sherman *et al.,* 2008a). RFI was the difference value between the actual value and expected value. The expected value was calculated by the following formula (Nkrumah *et al.,* 2007): RFI (expected)=$\beta_0+\beta_1X_1+\beta_2X_2$, where $\beta_0$: equation intercept, β1 and β2: equation coefficients, X1: metabolic weight ($BW^{0.75}$), X2: ADG.

**SNPs detection and genotyping:** Genomic DNA was extracted from 118 blood samples according to Mullenbach *et al.* (1989). Based on the DNA sequence of bovine *MC4R* gene (GenBank: AF265221.1) and *IGF2* gene (GenBank: EU518675.1), primers (Table 1) were designed by Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA). PCR amplifications were performed in 20μL solution containing 50ng DNA template, 1×buffer (Tris-HCl 100mmol/L, pH 8.3; KCl 500mmol/L), 0.25μmol/L primers, 2.0mmol/L MgCl2, 0.25mmol/L dNTPs, and 0.5U Taq DNA polymerase (TaKaRa, Dalian, P. R.China). The PCR protocol was 94°C for 5 min, followed by 35 cycles of 94°C for 30s, annealing for 30s and 72°C for 30s, and a final extension at 72°C for 10min. The products were purified using Wizard Prep PCR purification kit (Shanghai Bioasia Biotechnology Co., Ltd. P. R. China).

Three mixing samples, which containing five individuals selected randomly from the population, were used to detect SNPs by DNA sequencing (Applied Biosystems 377 DNA sequencer, Foster city, CA, USA). We validated the SNPs of each individual by the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Then, SNP genotyping was performed on the Sequenom MassArray platform (MassArray Compact System, Sequenom, San Diego, CA) using the iPlex Reagent Kit according to the manufacturer's instructions.

**Statistical analyses:** Differences in genotypic and allelic frequencies of *MC4R* and *IGF2* gene in the population were analyzed by Popgene3 (shareware for population genetic analysis, University of Alberta, 1997). Hardy-Weinberg equilibrium of the SNPs was determined by $\chi 2$ test. The haplotype probabilities of *IGF2* gene was constructed by Phase V2.0 (http://www.stat.washington.edu/ stephens/software.html) (Stephens *et al.,* 2001). The linkage disequilibrium (LD) was analyzed by Haploview 4.2 (Barrett *et al.,* 2005). Analysis of associations between the genotypes of SNPs loci and FCE traits was carried out with the GLM procedure, using SAS software (Statistical Analysis System 9.1, SAS Institute Inc) by the following statistical linear model: $Y_{ijkl}=\mu+M_i+G_j+W_k+e_{ijk.}$ Where $Y_{ijk.}$ phenotypic values, μ: global mean, $M_i$: month effect, $G_j$: genotype effect, $W_k$: covariate of birth weight, $e_{ijk}$: random error.

## RESULTS

**Identification and genotyping of SNPs:** By DNA mixed-pool sequencing analysis, *MC4R-C1069G* locus was identified, which was located in exon 1, causing leucine into valine; and four SNPs of *IGF2* were identified including *C2209T*, *G18587C*, *A22950T* and *G26920T*, which were located in 5' regulatory region, intron 3, intron 7, exon 10, respectively. The SNPs were determined by MALDI-TOF-MS (Fig. 1 and Fig. 2) and the genotypic and allelic frequencies were presented in Table 2. For *IGF2-C2209T*, *IGF2-A22950T* and *IGF2-G26920T* loci, A allele was preponderant; and AA was the preponderant genotype. For *MC4R-C1069G* locus and *IGF2-A22950T* locus, A and B allelic frequency was both 0.5252, 0.4746, respectively.

**Table 1:** Primer sequence, PCR size and location of candidate genes

| Genes | Primer Name | Primer sequence ( 5'→3' ) | $T_m$(℃) | Region | Size(bp) |
|---|---|---|---|---|---|
| *MC4R* | *MC4R* | F:TGGAGCGCATAGAAGATAGTG<br>R:AAGTGAGAACAAAAGAGCAAGC | 59 | exon 1 | 731 |
| *IGF2* | *IGF2 -1* | FAGACCCCTTTCTGTTCTCACTGCGT<br>R:GAGTCTGTTGGCACCTGAGGGG | 66 | 5'regulatory region | 787 |
| | *IGF2-2* | F:TTAGACCCTTTGCCCTTTGC<br>R:AGAGAACGAAGGTCCTGAAGTGG | 48 | intron 3 | 302 |
| | *IGF2-3* | F:TGGGCTTGCAGAAAGATGGA<br>R:TAAATCGTGTAGGAAATCAGAGGAC | 53 | intron 7 | 239 |
| | *IGF2-4* | F:ACGTTGGATGTCCCCACGTCAGGCGAAT<br>R:ACGTTGGATGGAGATGTTGTTCTGATCCC | 54 | exon 10 | 85 |

**Table 2:** Allele and genotype frequencies of the polymorphisms in *MC4R* and *IGF2* gene

| Gene | Locus | Genotype | | | Frequency of genotype | | | Frequency of alleles | |
|---|---|---|---|---|---|---|---|---|---|
| | | AA | AB | BB | AA | AB | BB | A | B |
| *MC4R* | *MC4R-C1069G* | 36 | 52 | 30 | 0.3051 | 0.4407 | 0.2542 | 0.5254 | 0.4746 |
| *IGF2* | *IGF2- C2209T* | 80 | 30 | 8 | 0.678 | 0.2542 | 0.0678 | 0.8051 | 0.1949 |
| | *IGF2-G18587C* | 39 | 46 | 33 | 0.3305 | 0.3898 | 0.2797 | 0.5254 | 0.4746 |
| | *IGF2-A22950T* | 98 | 16 | 4 | 0.8305 | 0.1365 | 0.0339 | 0.8983 | 0.1017 |
| | *IGF2-G26920T* | 106 | 9 | 3 | 0.8983 | 0.0763 | 0.0254 | 0.9364 | 0.0636 |

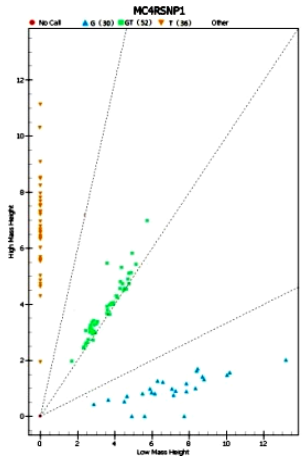


**Fig. 1:** SNP genotype of *MC4R* gene by MALDI-TOF-MS

**Genetic polymorphisms of *MC4R* and *IGF2* and χ2 test:** The value of the five SNPs were determined for gene homozygosity (Ho), heterozygosity (He), effective number of alleles (Ne), polymorphism information content (PIC) and χ2 value (Table 3). For *MC4R-C1069G* locus, PIC value was 0.37, indicating moderate polymorphic in the population (0.25<PIC<0.5). For *IGF2* gene, *C2209T* and *G18587C* loci were also moderate polymorphic (0.25<PIC<0.5); and the other two SNPs were low polymorphic (PIC<0.25).

The χ2 values of *MC4R-C1069G*, *IGF2-C2209T* and *IGF2-G18587C* loci were less than 5.991, which indicated that these loci fitted with Hardy-Weinberg equilibrium in the population (P>0.05). But the χ2 value of *IGF2-A22950T* was 7.8461 (>5.991), which indicated the locus did not fit with Hardy-Weinberg equilibrium (P<0.05); the χ2 value of *IGF2-G26920T* was 15.2314 (>9.21), which also indicated the locus did not fit with Hardy-Weinberg equilibrium (P<0.01).

**Association of the SNPs with FCE traits:** The effects of the five SNPs on cattle FCE traits were evaluated (Table 4). For *MC4R-C1069G* locus, no significant association was detected between the genotypes and the traits (P>0.05). For *IGF2* gene, only *IGF2-G18587C* locus had a significant effect on ADG (P<0.05), but no significant effect on RFI or FCR. CC and GG were the dominant genotypes; and individual with CC or GG genotype had a larger ADG than GC (P<0.05), but no difference existed between them. For the other SNPs, no statistically significant differences were detected between the genotypes and FCE traits.

The haplotype blocks and LD analysis showed that no haplotype combinations was existed among the four SNPs of *IGF2* gene (Fig. 3) and the linkage degree of the four SNPs was low ($r^2$=0.013).

## DISCUSSION

**Correlation between *MC4R* polymorphism and FCE traits:** The *MC4R* signaling is important for mediating the effect of leptin on FI and energy homeostasis (Seeley *et al.*, 1997). In humans, the SNPs loci of *MC4R* gene were associated with obesity, energy homeostasis and control of feeding behavior (Rosmond *et al.*, 2001; MacKenzie, 2006). Furthermore, the association of *MC4R* variants has been extensively studied in livestock: reports presented significant associations between Asp298Asn locus and growth rate and FI traits in pig (Kim *et al.*, 2000); 4 SNPs in 3'-UTR had significant associations with birth weight and 45d-weaning weight in sheep (Song *et al.*, 2012). In our study, we deteched the *MC4R-C1069G* SNP, which has been detected in different cattle breeds. For example, Liu *et al.* (2010) showed the locus had significant association with BF, live weight and carcass weight in Qinchuan cattle; Huang *et al.* (2010) suggested the locus was significantly associated with BF in eight commercial breeds; and Seong *et al.* (2012) got the similar result in Korean cattle. Nevertheless, our study was different from most of the previous researches. We analyzed the locus with FCE traits. However, the correlation analysis showed that no significant differences were existed between the SNP and RFI, FCR and ADG (Table 4). It was similar to the report of Zhang *et al.* (2009) that they found no significant association with ADG in Nanyang cattle aged 24 month, nevertheless, it had significant association with ADG in cattle aged 6 month. It was also different from the study of Fan *et al.* (2009) that they found Arg236His and Asp298Asn loci had significant associations with ADG. It may be because the different mechanism on different

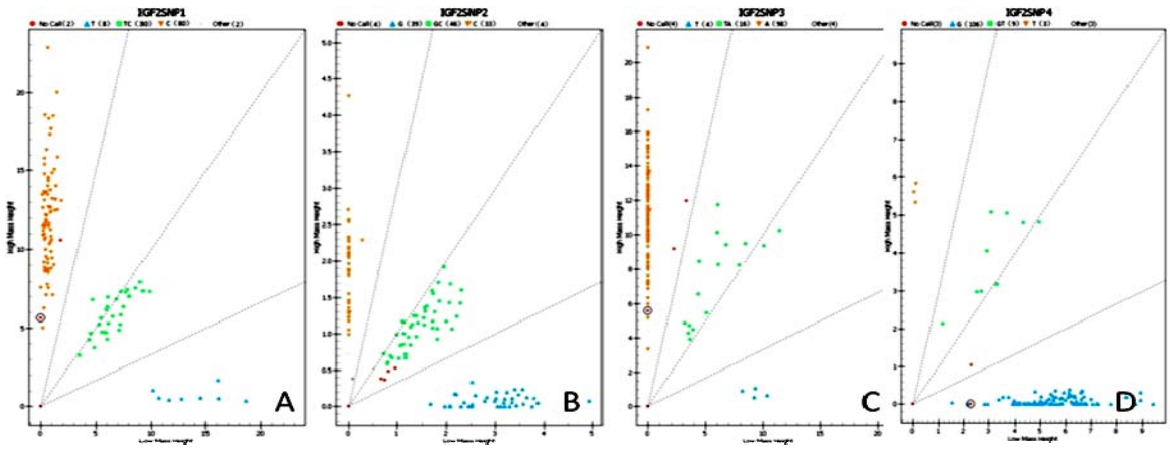


**Fig. 2:** SNP genotype of *IGF2* gene by MALDI-TOF-MS; A: *IGF2-C2209T* locus; B: *IGF2-G18587C* locus; C: *IGF2-A22950T* locus; D: *IGF2-G26920T* locus

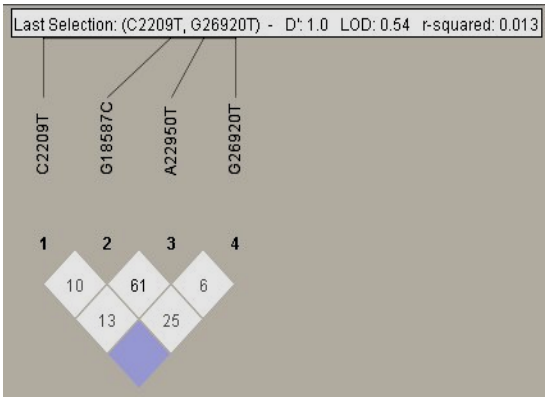


**Fig. 3:** Haplotype and LD analysis for *IGF2* gene SNPs

**Table 3:** Data of Genetic diversity of the polymorphisms of *MC4R* and *IGF2* gene

| Locus | Homozygosity | Heterozygosity | Ne | PIC | $\chi^2$ Test |
|---|---|---|---|---|---|
| *MC4R-C1069G* | 0.5013 | 0.4987 | 1.9948 | 0.37 | 1.5977 |
| *IGF2-C2209T* | 0.6862 | 0.3138 | 1.4574 | 0.26 | 4.2567 |
| *IGF2-G18587C* | 0.5013 | 0.4987 | 1.9948 | 0.37 | 5.6242 |
| *IGF2-A22950T* | 0.8173 | 0.1827 | 1.2236 | 0.17 | 7.8461* |
| *IGF2-G26920T* | 0.8810 | 0.1190 | 1.1351 | 0.11 | 15.2314** |

*(P<0.05), $\chi_{0.05}^2$=5.991; **(P<0.01), $\chi_{0.01}^2$=9.21.

**Table 4:** Associations of *MC4R* and *IGF2* polymorphisms with feed conversion traits

| Gene | Locus | Genotype | Traits | | |
|---|---|---|---|---|---|
| | | | RFI (Kg/d) | FCR (%) | ADG (Kg/d) |
| | | CC | -0.04±0.07 | 0.0752±0.36 | 1.17±0.03 |
| *MC4R* | *MC4R-C1069G* | CG | -0.03±0.18 | 0.0809±0.66 | 1.13±0.05 |
| | | GG | 0.02±0.16 | 0.0830±1.60 | 1.07±0.05 |
| | | CC | 0.16±0.04 | 0.7498±0.33 | 1.15±0.03 |
| | *IGF2-C2209T* | TC | 0.14±0.12 | 0.8193±1.32 | 1.07±0.04 |
| | | TT | -0.04±0.13 | 0.8092±0.40 | 0.84±0.15 |
| | | CC | -0.02±0.06 | 0.0774±0.42 | 1.32±0.03[a] |
| | *IGF2-G18587C* | GC | -0.18±0.10 | 0.0855±1.60 | 0.99±0.06[b] |
| | | GG | 0.05±0.18 | 0.0752±0.45 | 1.19±0.06[a] |
| *IGF2* | | AA | 0.03±0.11 | 0.0842±0.13 | 1.20±0.02 |
| | *IGF2-A22950T* | AT | 0.19±0.30 | 0.0739±0.65 | 1.17±0.06 |
| | | TT | -0.17±0.21 | 0.0794±0.71 | 0.97±0.11 |
| | | GG | 0.13±0.04 | 0.0831±0.55 | 1.11±0.03 |
| | *IGF2-G26920T* | GT | 0.09±0.02 | 0.7999±0.48 | 1.15±0.07 |
| | | TT | -0.07±0.04 | 0.7947±0.17 | 1.00±0.07 |

[ab]stands for means with different superscripts were significantly different (P<0.05)

varieties or the limited number of the test population. Thus, study of *MC4R* gene in cattle FCE needs to explore further.

**Correlation between *IGF2* polymorphisms and FCE traits:** *IGF2* gene has been the most extensively studied imprinted mammalian gene owing to its pivotal role in the regulation of embryonic development (Berkowicz *et al.*, 2011). So far, polymorphisms in the *IGF2* gene have been associated with many production traits, including growth rate, FCE, muscle mass and fat deposition traits in different livestock species, including pig (Van Laere *et al.*, 2003; Fontanesi *et al.*, 2010) and cattle (Sherman *et al.*, 2008b). For cattle, the SNPs of *IGF2* gene have been reported in different breeds and focused on the corelation with the growth and meat quality traits. Goodall and Schmutz (2007) identified *C292T* SNP in non-translated region, which was associated with loin-eye muscle area (LMA) and percent fat in beef cattle. Han *et al.* (2008) found C→T mutation and A→G mutation in exon 2, which was associated with slaughter weight, carcass weight, carcass length, carcass chest depth and LMA in Qinchuan cattle (P<0.05). Nevertheless, the studies about FCE were few. In our study, four SNPs of *IGF2* gene were analyzed for effect on FCE in Simmental bulls, including *C2209T* locus, *G18587C* locus, *A22950T* locus and *G26920T*. *G18587C* locus was a novel polymorphic locus discovered in intron 3 and significant correlated to ADG. CC and GG were dominant genotypes and individuals with CC and GG genotypes had a larger ADG than GC (P<0.05). The results were consistent with the function of the gene. In addition, it was similar to the result of Sherman *et al.* (2008b) that they showed a C→T mutation in exon 2, which most strongly affected ADG and FCR (P<0.05). For other three loci, no differences existed between the loci and the FCE traits. It was similar to the study of Magee *et al.* (2010) that they re-sequenced the *IGF2* gene and found 15 SNPs, which was not significantly associated with any of the performance traits evaluated except SNP-1 and SNP-15; SNP-1 was associated with ADG (P<0.05) and SNP-15 was associated with FCR (P<0.05).

In conclusion, the research on polymorphism of *IGF2* gene revealed a new SNP that has potential effect on FCE traits. However, further studies will be needed before the SNP used for marker-assisted selection, including increasing the number of the population or the breeds of cattle or expanding the time of feeding trail to get enough data etc.

**Acknowledgement:** This research was financially supported by the New Variety of Transgenic Organism Great Breeding Program (No. 2011ZX08007-002-009), the Twelfth "Five-Year" National Science and Technology Support Project (No. 2011BAD28B04), the Agriculture Ministry (Project No. CARS-38), National Natural Science Foundation of China (No. 31201768), Beijing Natural Science Foundation (No. 6133033) and China Postdoctoral Science Foundation funded project (No. 2012M510011).

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