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Original Article

Polymorphisms of candidate genes associated with feed efficiency and growth traits in commercial crossbred pigs

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Abstract

Feed efficiency is an important trait in the swine industry, but it is difficult to measure individually for genetic improvement. A total of 184 commercial pigs (50% Duroc, 25% Large White, and 25% Landrace) were collected for blood and genotyped. The average daily gain, daily feed intake (DFI), feed conversion ratio (FCR), gain per feed (G/F) and residual feed intake (RFI) were used in the analysis. The PCR-RFLP was used for the genotyping of candidate gene markers. The associations of gene polymorphism (cholecystokinin type A receptor (*CCKAR*), melanocortin 4 receptor (*MC4R*), leptin gene (*LEP*), porcine cathepsin with feed efficiency and growth traits in commercial pigs were investigated. The AA genotype of *MC4R* was significantly associated with the DFI at P=0.01. The polymorphism in porcine cathepsin D (*CTSD*) and cathepsin Z (*CTSZ*) were significantly associated with FCR, G/F, and RFI at P<0.05. The low FCR was associated with GG genotype in *CTSZ* and *CTSD*.

Keywords: growth, feed efficiency, markers associated, pigs

1. Introduction

Over the past few years, the cost of feed has increased considerably, and genetic improvement of feed efficiency has become more important for the swine industry. On the other hand, it is difficult to measure feed efficiency (feed consumption) of an individual pig because 20 or more pigs are housed together and they fed from a common pen. A direct method called Feed Intake Recording Equipment (FIRE) system is used by some seedstock suppliers to measure feed intake by an individual pig housed in a common rearing pen (Sadler, Johnson, Newton, Stalder, & Dekkers, 2009). The FIRE method allows the direct measurement of feed efficiency in an animal, which can increase the rate of genetic improvement. Feed efficiency is estimated from the feed conversion ratio (FCR) and residual feed intake (RFI), i.e. the difference between the actual feed intake and expected feed intake based on growth and maintenance. FCR is the traditional ratio trait, which is often set to terminal line index with backfat thickness (Kuhlers *et al.*, 2003). On the other hand, residual feed intake is a linear index which is an

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alternative measurement of feed efficiency (Hoque *et al.*, (2007).

The genetic markers approach has become a popular method for those traits where it is difficult to measure the phenotype (Onteru *et al.*, 2013). Based on a genome-wide association study, numerous gene markers are involved with significant variation in the feed efficiency and growth traits of pigs. In purebred Yorkshires, the genes involved in insulin release were significantly associated with average daily feed intake (DFI) and RFI (Onteru *et al.*, 2013). Moreover, RFI was revealed to be associated with 12-15 loci in Duroc pigs as reported by Do *et al.* (2014). The average daily gain (ADG) in purebred Yorkshires was reported to be associated with genes involved in muscle growth and energy homeostasis (Onteru *et al.*, 2013). Generally, the candidate gene approach may be used to screen animals for pre-selection before any direct measurement of difficult traits such as feed efficiency and carcass traits. However, these traits are controlled by several genes and those genes are known by their biological functions.

The association of genes with feed efficiency has been investigated in previous reports. Cholecystokinin type A receptor (*CCKAR*) is associated with appetite and satiety and have influence over feed intake and ADG in pigs (Moran, 2000; Houston *et al.*, 2006). The melanocortin 4 receptor (*MC4R*) gene codes for G-protein-coupled receptor which is involved with leptin signaling (Wikberg *et al.*, 2000). The leptin gene controls energy homeostasis, thus it affects feed intake, body weight gain, fattening and carcass composition in pigs (Houston, Cameron, & Rance, 2004; Óvilo, Fernández, Rodríguez, Nieto, & Silio, 2006). Moreover, the leptin gene is used as a candidate gene for feed intake in pigs (Barb, Hausman, & Houseknecht, 2001).

The cathepsin gene synthesizes lysosomal proteinase and this enzyme is involved with proteolytic activity or protein degradation (Russo *et al.*, 2008). Several members of the cathepsin genes are closely located to the QTL region of growth, carcass, and feed efficiency in pigs (Houston, Haley, Archibald, & Rance, 2005; Thomsen & Kejariwal, 2004; Ramos, Helm, Sherwood, Rocha, & Rothschild, 2006). However, porcine cathepsin D (*CTSD*), cathepsin H (*CTSH*), and cathepsin Z (*CTSZ*) are members of the cathepsin gene that were reported to be associated with feed efficiency trait (Russo *et al.*, 2008). Therefore, this study was conducted to investigate the association of gene polymorphisms (*MC4R*, *CCKAR*, leptin gene, and porcine cathepsin genes) with feed efficiency and growth traits in commercial pigs.

2. Materials and Methods

2.1 Animals

A total of 184 fattening commercial pigs (60–90 days old) of the same genetic line (50% Duroc, 25% Large White, and 25% Landrace) were used. They were reared under the same management conditions, evaporative cooling housing with automatic water/feeding/weighing system and were randomly sampled for genomic DNA extraction and genotyping. The initial live body weights of the sampled pigs ranged from 30 to 60 kg and they were reared in common pens to observe the growth performance. The pigs were fed *ad libitum* under the same rations as the test station. The individual feed intake and body weight were recorded from the FIRE system. The performances of the pigs were recorded until the slaughter weight was 92-157 kg.

The RFI was estimated as the difference between observed DFI and predicted DFI (pDFI). The pDFI was estimated using a regression model.

$$DFI_{ijk} = \mu + S_i + B_j + S * B_{(ij)} + \varepsilon_{ijk} \quad (1)$$

$$pDFI_{ij} = \mu + S_i + B_j + S * B_{(ij)} + b_1(ADG) + b_2(AWM) \quad (2)$$

$$RFI = DFI - pDFI \quad (3)$$

where *pDFI* = predicted feed intake, S_i = the effect of sex , B_j = the effect of station, *S*B* = interaction between sex and station, *ADG* = average daily gain (g/d), *AMW* = average metabolic body weight (Noblet, Karege, Dubois, & van Milgen, 1999), b_1 and b_2 = partial regression coefficients and ε_{ijk} = the residual error.

2.2 PCR-RFLP genotyping

The primer characteristics of each candidate gene are shown in Table 1. The PCR amplification of each candidate gene was amplified in a total reaction volume of 10 µL containing 1 µL 50 ng of genomic DNA, 1.5 µL 10X buffer, 0.8 µL 25 mM $MgCl_2$, 1 µL 1 mM of each dNTP (Promega, USA), 1 µL 3 µM of each primer, and 0.1 µL 0.25 U *Taq* Polymerase (RBC®, Taiwan). The volume was adjusted to 10 µL with water. Amplification conditions were as follows: 95 °C for 5 min followed by 35 cycles at 95 °C for 45 sec, melting temperature (Table 1) for 30-45 sec, 72 °C for 60 sec, and a final extension of 72 °C for 1.30 min. PCR fragments were separated by 2% agarose gel. Thereafter, the PCR products were digested with the appropriate restriction enzyme, incubation temperature, and time (Table 1). The digested products were separated by electrophoresis through a 2% agarose gel.

2.3 Statistical analysis

Genotypic frequencies were estimated according to the Falconer and Mackay (1996) formula. The linear mixed model was used to analyze the association between each gene and trait, i.e. ADG, DFI, FCR, gain per feed (G/F), and RFI. Sex, genotype and covariate of initial weight were considered as fixed effects while sire was the random effect in the model.

$$y_{ijk} = \mu + S_i + G_j + B_0(WT0) + Sire_k + \varepsilon_{ijk} \quad (4)$$

where y_{ijk} = the phenotypic of traits, μ = the overall mean, S_i = the effect of sex, G_j = the effect of genotype of gene, $B_0(WT_0)$ = the regression of the initial weight, $Sire_k$ = the random effect of sire, and ε_{ijk} = the residual error.

3. Results and Discussion

About 196 kg of feed was consumed by each pig to gain body weight from the initial weight (54 kg) to the slaughter weight (127 kg). The weekly body weight gain was directly proportional to (Table 2). The poor FCR with age may

Table 1. Details of gene primers of candidate gene markers for feed efficiencies and growth trait.

Genes	Regions	Primers[2]	Annealing/PCR Product sizes	Restricted enzymes
MC4R	exon	F: ACAGTTAAGCGGGTTGGAAT R: CAGGGGATAGCAACAGATGA	56 °C/ 483 bp	*Taq* I /65 °C, 6 h
CTSD	EST	F: GCTGTGCACCCTAGGAACC R: TCGTCAGGTCCAGGACAAAC	56 °C/184 bp	*Msc*I /37 °C, overnight
CTSH	3'UTR	F: AATCTTGCCCTGGAGGAAGT R: GGTTAAAAATCACGCCCAAG	56 °C /177bp	*Bst*UI /60 °C, 6 hrs
CTSZ	EST	F: GGCCTCATGAGTACCTGTCC R: ATGTGCTGGTTCCTGGTGAC	56 °C/100bp	*Scr*FI /37 °C, overnight
CCKAR	5'UTR	F: CTTGGGAGACTCTGCAGTCC R:GGGCTGATCCAAACAGAAAA	56 °C/200bp	H*py*8I /37 °C, overnight
LEP	Exon 3	F:CTGTCTCCTCCAAACAGAGGGTCA R:CAGCAGCCAGGGCTGAGGTCCA	56 °C/355bp	H*inf*I /37 °C, overnight

Abbreviations: *CTSD*, cathepsin D; *CTSH*, cathepsin H; *CTSZ* cathepsin Z (Russo *et al.*, 2008); *CCKAR*, cholecystokinin type A receptor (Houston *et al.*, 2006); *MC4R* melanocortin-4 receptor (Jokubka, Maak, Kerziene, and Swalve, 2005); *LEP*, leptin gene (Villalba *et al.*, 2009).

Table 2. Means and standard deviations for growth traits and feed efficiency on a weekly basis.

Weeks	Traits					
	BW (kg)	DFI (kg)	ADG (kg/d)	FCR (kg/kg/d)	G/F (kg/kg/d)	RFI (kg/d)
1	54.58±5.79	14.19±2.19	1.54±0.24	1.32±0.23	0.67±0.13	-0.49±0.23
2	62.69±6.02	30.01±2.13	1.35±0.17	1.59±0.22	0.59±0.09	0.89±0.21
3	69.40±6.43	45.78±2.32	1.22±0.15	1.79±0.23	0.53±0.07	-0.59±0.18
4	75.96±7.09	61.78±2.97	1.15±0.14	1.92±0.23	0.50±0.06	0.35±0.18
5	82.26±7.82	78.08±3.52	1.10±0.14	2.03±0.23	0.48±0.06	-0.02±0.18
6	88.96±8.37	95.46±2.90	1.08±0.14	2.11±0.23	0.46±0.05	0.59±0.18
7	95.27±8.24	113.37±3.03	1.05±0.12	2.20±0.20	0.50±0.05	1.33±0.17
8	101.76±8.57	132.51±2.85	1.04±0.11	2.29±0.23	0.43±0.04	-0.62±0.17
9	109.27±9.46	152.63±3.24	1.04±0.11	2.33±0.24	0.42±0.04	0.13±0.18
10	115.07±9.90	171.84±4.01	1.02±0.11	2.41±0.23	0.41±0.04	0.80±0.18
11	121.66±11.00	192.35±3.75	1.01±0.12	2.47±0.47	0.40±0.05	-0.17±0.21
12	127.15±9.77	210.28±4.51	1.00±0.10	2.52±0.28	0.38±0.09	0.13±0.20

Abbreviations: BW, body weight; DFI, daily feed intake; ADG, average daily gain; FCR, feed conversion ratio; G/F, gain per feed; RFI, residual feed intake.

be explained by the extra feed required for maintenance as the pigs matured. Therefore, the heavy pigs should be removed from the pens to improve FCR and improve the growth rates of the other pigs (Decker *et al.*, 2005). Boars were significantly better than the gilts in terms of FCR and ADG at $P<0.05$ (Table 3). On the other hand, the gilts had higher G/F than the boars ($P<0.05$). In addition, the effect of sex was not significant on DFI and RFI ($P>0.05$).

3.1 Genotype frequencies

Genotype frequencies are shown in Table 4. All of the genes studied showed variations. However, the GG (*g.122G/G*) genotype of *CTSH* gene was not detected in our study. A common allele in the pigs was the most frequent genotype of *CTSH* gene (AA), which was consistent with Russo *et al.* (2008) who studied the Italian Large White population at the same position (g.122A>G). Therefore, we suggest that the *CTSH* gene in this position should not be used for an associated analysis in pigs.

3.2 Association analysis of candidate genes on growth and feed efficiency

3.2.1 Association with ADG

All six candidate genes had no significant effect on ADG (Table 4). Nevertheless, *MC4R* and *CTSD* may be tentatively considered as significantly associated with ADG ($P\leq0.15$). Our report supports the findings of Russo *et al.* (2008) who reported that the *CTSD* was associated with the estimated breeding value of ADG in the Italian Large white breed.

In the *CCKAR* gene, it seems that the allele g.179A may be negatively associated with growth, and this allele may be lost while selecting for growth (Houston *et al.*, 2006). We found that *CCKAR* was not significantly associated with ADG, which disagreed with the report by Houston *et al.* (2006). They found a significant association with ADG and feed intake in Meishan x Large White. Generally, in seed stock production, the selection often focuses on purebred,

Table 3. Gender effects on feed efficiencies and growth traits.

Traits	Genders		SEM	P-value
	Male	Female		
ADG (kg/d)	1.01[a]	0.97[b]	0.01	0.01
DFI (kg)	2.48	2.51	0.04	0.62
FCR (kg/kg/d)	2.46[a]	2.58[b]	0.04	0.01
G/F (kg/kg/d)	0.41[b]	0.39[a]	0.005	0.03
RFI (kg/d)	0.011	0.006	0.032	0.86

Abbreviations: ADG, average daily gain; DFI, daily feed intake; FCR, feed conversion ratio; G/F, gain per feed; RFI, residual feed intake. [a,b] Means within a row of each trait without common letters are significant difference (P<0.05).

Table 4. Gene frequencies and associations between gene markers and feed efficiency and growth traits.

Gene	Genotype	No#	Fre.	ADG		DFI		FCR		G/F		RFI	
				lsmeans	SE	lsmeans	SE	lsmeans	SE	lsmeans	SE	lsmeans	SE
CTSD	AA	127	0.69	0.96	0.04	2.58[a]	0.09	2.70[a]	0.08	0.37[a]	0.01	0.14[a]	0.07
	AG	48	0.26	0.93	0.04	2.47[b]	0.10	2.70[a]	0.09	0.37[a]	0.01	0.11[a]	0.07
	GG	9	0.05	0.99	0.06	2.36[b]	0.14	2.44[b]	0.12	0.42[b]	0.02	-0.11[b]	0.10
	P-value				0.15		0.02		0.02		0.005		0.01
CTSH	AA	175	0.98	0.98	0.03	2.45	0.07	2.53	0.06	0.40	0.01	-0.01	0.06
	AG	3	0.02	0.93	0.06	2.49	0.15	2.69	0.13	0.38	0.02	0.10	0.12
	P-value				0.44		0.80		0.19		0.23		0.28
CTSZ	AA	26	0.14	0.93	0.04	2.48[ab]	0.11	2.68[a]	0.09	0.38[a]	0.01	0.08[a]	0.08
	AG	64	0.35	0.97	0.04	2.52[a]	0.11	2.65[a]	0.09	0.38[a]	0.01	0.09[a]	0.08
	GG	94	0.51	0.97	0.04	2.40[b]	0.10	2.51[b]	0.08	0.41[b]	0.01	-0.05[b]	0.07
	P-value				0.29		0.05		0.003		0.001		0.001
CCKAR	AA	5	0.03	0.91	0.06	2.37	0.14	2.63	0.12	0.38	0.02	0.02	0.10
	AB	41	0.22	0.97	0.04	2.51	0.10	2.63	0.09	0.39	0.01	0.07	0.08
	BB	137	0.75	0.99	0.04	2.52	0.10	2.57	0.08	0.40	0.01	0.04	0.07
	P-value				0.21		0.45		0.36		0.19		0.58
MC4R	AA	64	0.35	0.98	0.04	2.56[a]	0.10	2.65	0.09	0.39	0.01	0.10[a]	0.08
	AG	90	0.49	0.95	0.04	2.43[b]	0.10	2.60	0.09	0.39	0.01	0.02[b]	0.07
	GG	28	0.15	0.94	0.04	2.42[b]	0.11	2.59	0.09	0.39	0.01	0.01[b]	0.08
	P-value				0.11		0.01		0.39		0.52		0.055
LEP	GG	5	0.03	0.97	0.07	2.56	0.17	2.67	0.15	0.38	0.02	0.11	0.13
	TG	64	0.35	0.95	0.04	2.45	0.09	2.60	0.08	0.39	0.01	0.03	0.07
	TT	113	0.62	0.95	0.03	2.40	0.08	2.57	0.07	0.40	0.01	-0.01	0.06
	P-value				0.94		0.28		0.52		0.44		0.30

Abbreviations: ADG, average daily gain; DFI, daily feed intake; FCR, feed conversion ratio; G/F, gain per feed; RFI, residual feed intake; *CTSD*, cathepsin D; *CTSH*, cathepsin H; *CTSZ*, cathepsin Z; *CCKAR*, choleccystokinin type A receptor; *MC4R*, melanocortin-4 receptor; *LEP*, leptin gene. [a,b] within a column in each candidate genes are significant difference (P<0.05).

while the majority of pork production is crossbred pigs which reduces production costs (Godinho *et al.*, 2018). In addition, the study of gene markers and genotype in the offspring can be referred to the parents for selection of boars or sows or both.

3.2.2 Association with feed intake and feed efficiency

In the association analysis, a homozygous mutant (*Asn298Asn* or AA) genotype of melanocortin 4 receptor (*MC4R*) was significantly associated with higher DFI (Table 4). The result was inconsistent with several research studies (Houston, Cameron, & Rance, 2004; Davoli *et al.*, 2012; Onteru *et al.*, 2013; Reyer *et al.*, 2017). Several research reports described the *MC4R* gene codes for a G-protein-coupled receptor which plays a key role in leptin signaling which affects feed intake, fattening, carcass composition, body weight, and composition in pigs (Davoli *et al.*, 2012; Meidtner *et al.*, 2006; Óvilo, Fernández, Rodríguez, Nieto, & Silio, 2005; Reyer *et al.*, 2017; Wikberg *et al.*, 2000). In addition, the SNP mutation caused a change in amino acids from aspartic acid to asparagine acid (*Asp298Asn*), and consequently changes the feed intake (Kim, Larsen, & Rothschild, 2000). Furthermore, the highest feed intake of homozygous mutant (AA) of *MC4R* gene was not significantly associated with FCR which was consistent with Hoston, Cameron, and Rance (2004) and Jokubka, Maak, Kerziene, and Swalve (2005). Consequently, the AA genotype may be undesirable in swine production.

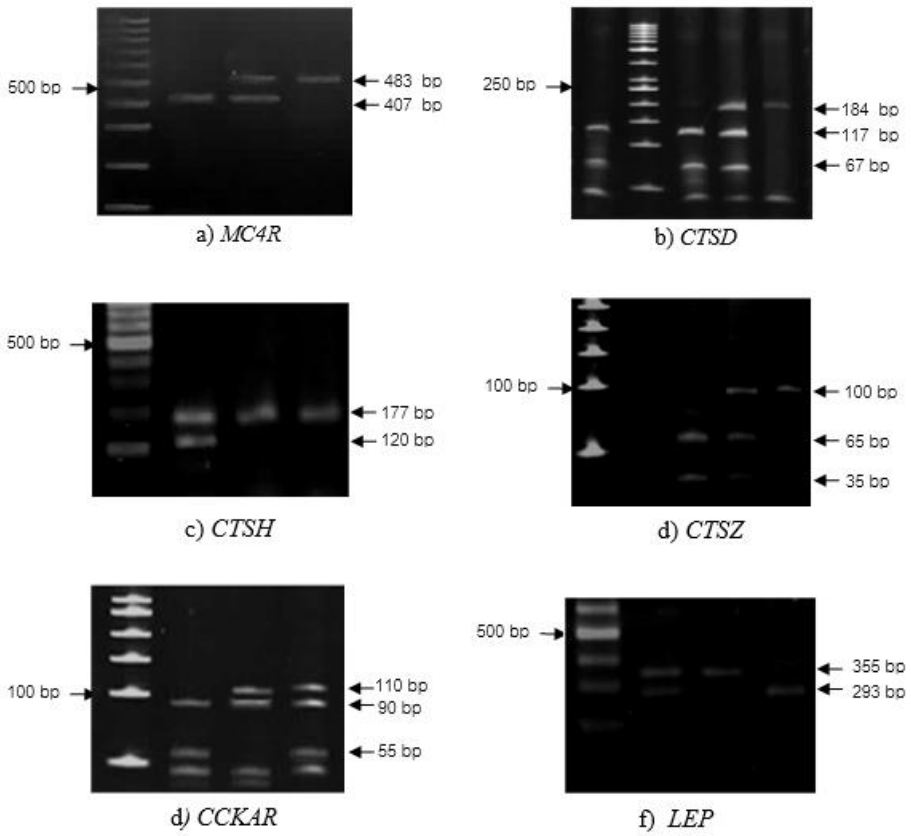

Figure 1. Genetic polymorphisms of candidate genes after amplification with allele specific primers and cutting with restriction enzyme: a) Melanocortin 4 receptor (*MC4R*)/*Taq*I, allele A (483 bp) and allele G (407, 76 bp); b) Cathepsin D/ *Msc*I; allele G (184 bp) and allele A (117, 67 bp); c) Cathepsin H (*CTSH*)/*Bst*UI; allele A (177 bp) and allele G (120, 57 bp); d) Cathepsin Z (*CTSZ*)/*Scr*FI, allele A (100 bp) and allele G (65, 35 bp); e) Porcine choleccystokinin type A receptor (*CCKAR*)/*Hpy*8I, allele A (110, 90 bp) and allele G (90, 55, 55 bp); f) Leptin gene (*LEP*)/*Hinf* I, allele T (355 bp) and C (293, 62 bp).

The leptin gene (*LEP*) had a direct effect on feed intake but there was no significant association with DFI in our study. Moreover, the C allele frequency was smaller than T, which supports the earlier report in Duroc, Landrace, and Yorkshire (Kennes, Murphy, Pothier, & Palin, 2001; Villalba *et al.*, 2009). The mutant allele (allele C) seems to be associated with decreased growth rate and increased feed intake (Kennes, Murphy, Pothier, & Palin, 2001), and commercial pigs have been selected for higher feed efficiency while culling unfavorable alleles.

In addition, for feed efficiency traits, the porcine *CTSD* and *CTSZ* were associated with FCR and RFI, except *MC4R* gene for only RFI (Table 4). The good FCR and RFI would be related with the same genotypes. Perhaps it is because of a highly positive genetic correlation between FCR and RFI (0.84 to 0.86) which was reported by Hoque, Suzuki, Kadowaki, Shibata, and Oikawa (2007). The low FCR was related with GG genotype of *CTSZ*, which was inconsistent with Russo *et al.* (2008) who studied the same SNP position (g.37A>G) in Italian Large white. It can be implied that the allele G has an additive genetic effect on FCR, while the high FCR is affected by dominance deviation of allele A which is similar to a report by Russo *et al.* (2008). The mechanism of the *CTSZ* effect on feed efficiency is unclear, but the *CTSZ* gene was located approximately at 80-110 cM and linkage-mapped with QTL for FCR (Ramos, Helm, Sherwood, Rocha, & Rothschild, 2006; Thomsen & Kejariwal, 2004).

The *CTSD* affected the FCR and DFI (Table 4). The GG genotype (at g.70G>A) was significantly associated with low FCR which was in agreement with previous reports in Duroc and Italian Large white breed (Fontanesi *et al.*, 2010; Russo *et al.*, 2008). This result confirmed that *CTSD* was linked mapped with QTL region (SSC2) for feed efficiency as reported by Houston, Haley, Archibald, and Rance (2005) and Russo *et al.* (2008). However, GG genotype consisted of only 5% in this population which confirmed a previous report in Italian Large white breed (Russo *et al.*, 2008). Therefore, GG genotype must be selected by mating between heterozygous genotype. The application of *CTSZ* seems straightforward to implement in a breeding program compared to *CTSD* due to the unbalance of genotype distribution. In this case, an association study of *CTSD* by mating among heterozygous genotype (AG) to get more GG genotype needs to be confirmed for a particular population. Moreover, this study confirmed that the *CCKAR* gene was not significantly associated with FCR and RFI, which was in agreement with the report by Houston *et al.* (2006) for crossbred pigs.

4. Conclusions

Homozygous mutant (AA) genotype of *MC4R* was significantly associated with higher DFI and RFI. The *CTSZ* and *CTSD* were significantly associated with FCR, RFI, and G/F and the feed efficiency was related with the GG genotype in both genes. Therefore, AA genotype of *MC4R* can be used for marker assisted selection to improve the feed intake of a genetic line. Also, the GG genotype of *CTSZ* is the most appropriate as pre-selection for high feed efficiency in replacement seed stock.

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