INFLUENCE OF HORMONES ON mRNA ABUNDANCE OF FATTY ACID SYNTHETASE IN LONISSINUS DORSI MUSCLE OF PIG IN VITRO

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Introduction

Flavour and tenderness are key components contributing to a good eating experience. Intramuscular fat extracted from the *Longissimus* muscle was related to flavour and juiciness, with marbling related to percent intramuscular fat. Sutdies showed that one of Chinese local species, Rongchang, had higher intramuscular fat content in muscle(Pang et al.,2006).

Muscle tissues and cells undergo a regulated growth and differentiation processes, as well as substrate utilization and energy partitioning, were affected by a range of factors.Recently, the (signaling) interaction between myogenic cells and adipocytes had been found which was considered as a significant role in regulation of the rate and extent of adipogenesis,myogenesis,and lipogenesis/lipolysis (Boone et al., 2000). Studies showed that insulin (INS), somatotropin (ST),epinephrine (Epi) and glucagon (GCG) were hormones participating in the processes of lipogenesis/ lipolysis in animals.

Materials and Methods

Longissimus dorsi muscle isolation and Incubations. Three Rongchang barrows,which body weight was 100.5±5.3 kg,were exsanguinated and slaughtered by a sterile scalpel and samples of *Longissimus dorsi* muscle were immediately collected from the 10^{th} rib on the right side and rapidly placed into an incubator vessel containing sterile Hank's buffer.*Longissimus dorsi* muscle explants were maintained in incubator at 37℃ in a humidified, 5% CO2 atmosphere with culture of DMEM/F12 containing 20% fetal bovine serum (FBS).When explants surrounded by cells, cultures were changed to DMEM/F12 containing 10% FBS. Until 80% cells confluency,culture then was shifted to serum–free media for 6 h, supplemented with different hormone including insulin (INS),somatotropin (ST),epinephrine (Epi) and glucagon (GCG) according to a orthogonal design with a $L_9(3^4)$ (Table 1).

Table 1. The orthogonal experiment design of INS,ST,Epi and GCG mol/L

No. of treatments	Factors			
	INS	ST	Epi	GCG
Ⅰ	10^{-7}	5×10^{-11}	10^{-8}	10^{-8}
Ⅱ	10^{-7}	5×10^{-10}	10^{-7}	10^{-7}
Ⅲ	10^{-7}	5×10^{-9}	10^{-6}	10^{-6}
Ⅳ	10^{-6}	5×10^{-11}	10^{-7}	10^{-6}
Ⅴ	10^{-6}	5×10^{-10}	10^{-6}	10^{-8}
Ⅵ	10^{-6}	5×10^{-9}	10^{-8}	10^{-7}
Ⅶ	10^{-5}	5×10^{-11}	10^{-6}	10^{-7}
Ⅷ	10^{-5}	5×10^{-10}	10^{-8}	10^{-6}
Ⅸ	10^{-5}	5×10^{-9}	10^{-7}	10^{-8}

*Quantitative real–time–PCR.*RNA was isolated extracted and cDNA was synthesized following the protocol recommended by the manufacturer.

The primer and TaqMan detection probe was:beta actin(accession No. U07786): forward primer, 5′-CTCT TCCAGCCCTCCTTCCT-3′,reverse primer,5′-GATGTCCACGTCGCACTTCA-3′, probe, 5′- (FAM) TCCTG CGGCATCCACGAGACCACC (Eclipse)-3′, which product of amplification was 87 bp; fatty acid synthetase (FAS,accession No.AY952929):forward primer,5′-CTACCTTGTGGATCACTGCATAGA-3′,reverse primer, 5′ -GGCGTCTCCTCCAAGTTCTG-3′,probe,5′-(FAM)CGTGCCAGCGTCTTCCAGGTCAGC(Eclipse)-3′, which product of amplification was 114 bp.

Real–time quantitative–PCR was used to measure the quantity of FAS mRNA relative to the quantity of beta actin in total RNA isolated from *Longissimus* muscle tissue of pigs.The thermal cycling parameters was as following:10 s at 95 ºC, followed by 45 cycles of 5 s at 95ºC,15 s at 57.5ºC,6 s at 72ºCand 1 s at 80ºC.Relative expression of FAS was normalized with the beta actin endogenous control and expressed in arbitrary units.

Data were analyzed by ANOVA with the General Linear Model (GLM) procedure of SAS software (SAS Inst., Inc., Cary, NC).

Results and Discussion

FAS, the enzyme complex involved in the *de novo* synthesis was of long chain fatty acids,which was a key intermediate at the branch point between the glycolytic pathway and triacylglycerol synthesis. The effects of insulin,somatotropin,epinephrine and glucagon on mRNA level of FAS were shown in Table2 and Table 3.The degree of effect on FAS mRNA was ranged with ST>GCG>INS>Epi. Somatotropin(P<0.01) significantly affected FAS mRNA level.The optimal combination of was INS:1×10^{-7}M,ST:5×10^{-9} M, Epi:1×10^{-6} M,GCG:1×10^{-6}M.

Fatty acid synthase (FAS) was a key enzyme in the lipogenic pathway that catalyzes all the reactions in the conversion of acetyl-CoA and malonyl-CoA to palmitic acid. Fatty acid synthase activity, mRNA levels, and gene transcription are exquisitely sensitive to nutritional and hormonal manipulations (Hillgartner et al., 1995). For example, our previous studies indicate that somatotropin (ST) dramatically attenuates the stimulatory effect of insulin on FAS mRNA abundance and gene transcription in rat liver and in 3T3-F442A adipocytes (Yin et al., 1998). An impressive body of evidence indicates that ST dramatically decreases lipogenesis in adipose tissue of growing pigs.This decrease occurs as the result of a ST-dependent reduction in insulin sensitivity and responsiveness in adipocytes (Etherton and Bauman, 1998).

Table 2. Analysis of variances of the orthogonal experimental results of FAS mRNA $\times10^{-3}$

Source	Sum of Squares	df	Mean Square	F	*P–Value*
Corrected model	296.029	8	37.004	3.185	0.020
Intercept	571.044	1	571.044	49.148	0.000
INS	37.449	2	18.724	1.612	0.227
ST	175.227	2	87.613	7.541	0.004
Epi	24.327	2	12.163	1.047	0.371
GCG	59.026	2	29.513	2.540	0.107
Error	209.141	18	11.619		
Total	1076.214	27			
Corrected total	505.170	26			

Table 3. Single factor investigation of the orthogonal experimental results of FAS mRNA $\times10^{-3}$

Factor	hormonal concentrations(M)	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
INS	1×10^{-7}	5.823	1.136	3.436	8.210
	1×10^{-6}	4.964	1.136	2.577	7.352
	1×10^{-5}	3.009	1.136	0.622	5.396
ST	5×10^{-11}	2.704	1.136	0.317	5.092
	5×10^{-10}	2.892	1.136	0.505	5.279
	5×10^{-9}	8.200	1.136	5.813	10.587
Epi	1×10^{-8}	3.790	1.136	1.403	6.177
	1×10^{-7}	4.076	1.136	1.688	6.463
	1×10^{-6}	5.931	1.136	3.544	8.318
GCG	1×10^{-8}	5.486	1.136	3.098	7.873
	1×10^{-7}	2.516	1.136	.128	4.903
	1×10^{-6}	5.796	1.136	3.408	8.183

Conclusions

In conclusion, our results suggested that the combination of hormones (INS: 1×10^{-7}M, ST: 5×10^{-9} M, Epi: 1×10^{-6} M, GCG: 1×10^{-6} M) could increase IMF content by increasing FAS mRNA expression.

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