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IDENTIFICATION OF GENETIC COMPONENTS OF β-RYR ASSOCIATED WITH PSE CHARACTERISTICS IN TURKEYS

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Introduction

The modern turkey industry has been successful in producing fast-growing birds with large breast muscle due to intensive genetic selection. However, these improvements in growth rate and size of birds have led to an increased incidence of pale, soft and exudative (PSE) meat (Toelle et al., 1991; Pietrzak et al., 1997).

Since the term PSE was first coined to describe a meat quality problem in pork (Wismer-Pederson, 1959), there have been extensive studies on the mechanism of PSE meat development. The development of PSE pork, in part, results from an inherited skeletal muscle disorder in swine known as porcine stress syndrome (PSS). A single point mutation, Arg615Cys, has been identified in the skeletal muscle calcium release channel (ryanodine receptor 1, RyR1) which is responsible for PSS (Fujii et al., 1991) and, in turn, is associated with the development of PSE meat. As there are many striking similarities in the development of PSE meat between turkey and pork (Sosnicki et al. (1996) and Pietrzak et al. (1997)), it has been suggested that there is a genetic defect in turkey skeletal muscle RyR which alters the channel activity and causes the development of PSE turkey (Owens et al., 2000).

The ryanodine binding assay has been utilized as a method to measure the channel activity of pig RyR (Mickelson et al., 1986, 1988). Our laboratory has optimized the ryanodine binding assay to assess RyR channel activity in turkey skeletal muscle (Zhang, 2000). Sarcoplasmic reticulum (SR) vesicles from genetically improved turkeys showed a two-fold greater ryanodine affinity compared to the unimproved turkeys (K_d = 8.4 vs. 16.0 nM, respectively). However, another line of genetically improved turkeys showed similar calcium channel properties to those of random-bred turkeys (K_d = 19.1 vs. 16.0 nM, respectively; Zhang, 2000). This suggests that there is a diversity of the calcium channel activity among different turkey populations.

Despite similarities in development of PSE pork and turkey, we cannot assume that the mutation associated with development of PSE pork would also be present in turkey. More than 20 mutations in RyR1 have been associated with malignant hyperthermia, the human analog of PSS (Jurkat-Rott et al, 2000). Moreover, a key difference between turkey and pig skeletal muscle is that there are two ryanodine receptor isoforms (α and β) expressed in avian skeletal muscle, while there is only one major isoform (RyR1) in

mammalian skeletal muscle (Airey et al., 1993). Therefore, there could be alteration(s) in either or both isoforms which could contribute to the PSE turkey meat problem.

Although both RyR isoforms are expressed in equal abundance in turkey skeletal muscle, the observed ryanodine binding is solely the result of β-RyR activity, since α–RyR activity is almost completely suppressed under the assay conditions (Murayama and Ogawa, 2001). Therefore, we hypothesize that the ryanodine binding assay can be used as a phenotype reflecting the β-RyR genotype.

Objectives

The overall goal of this project is to test the hypothesis that mutations in β-RyR of turkey skeletal muscle are associated with the development of PSE turkey meat.

Methodology

Turkey breast muscle sampling and meat quality evaluation

Genetically unimproved random-bred control (RBC1) turkeys (n=40) were obtained from Dr. K. Nestor (Ohio Agricultural Research and Development Center, Wooster OH). Growth selected turkeys (n=40) were a gift of Hybrid Turkeys, Inc (Kitchener, ON). Turkeys were slaughtered according to standard industry practices. The pectoralis major muscle from one side was collected within 5 min of death, and was immediately sectioned, snap frozen in liquid nitrogen and stored at –80 ºC for total RNA, genomic DNA extraction and SR vesicle preparation. The remaining pectoralis muscle was used to evaluate meat quality indicators, including pH_{15min} and color (L*) at 24-h post mortem.

Isolation of SR vesicles and [^{3}H]ryanodine binding

Crude sarcoplasmic reticulum vesicles were purified as previously described (Zhang, 2000). Briefly, fifty μg of CSR protein was incubated in buffer containing 0.1 M NaCl, 0.5 M sucrose, 10 mM MOPS (pH 7.2) and a $CaCl_2$-EGTA-nitrilotriacetic acid buffer to give a $[Ca^{2+}]_{free}$ of 10 μM. [^{3}H] ryanodine (2-70 nM as final concentration) was added to each tube. Triplicate samples of each [^{3}H] ryanodine concentration were incubated for 6 h at 37 ^{o}C, and filtered through Whatman GF/B filters. Filters were washed three times with 5 mL of ice-cold wash buffer (10 mM MOPS, 0.1 M NaCl, 50 μM $CaCl_2$, pH 7.0), and the amount of [^{3}H] ryanodine retained on the filter was determined by scintillation counting. Non-specific binding of ryanodine was determined by adding unlabeled ryanodine at 1000 times the [^{3}H] ryanodine in the incubation buffer. Specific ryanodine binding was determined by subtracting the non-specific binding from the total binding. The K_d (ryanodine binding affinity) and B_{max} (maximum binding capacity) values for ryanodine binding were analyzed by Scatchard analysis.

Screening for polymorphism(s) in turkey β–RyR

Ryanodine binding and meat quality data from 77 turkeys were segregated based on their K_d values and meat quality traits as illustrated in Table 1. On this basis, sixteen muscle samples were selected for β–RyR polymorphism screening.

Total RNA from turkey skeletal muscle was extracted using *Trizol* reagent (Invitrogen; Carlsbad, CA). The target cDNA sequence was amplified through RT-PCR using Access RT-PCR system (Promega; Madison, WI) and the PCR products were sequenced directly. The sequencing was done in both directions using the MSU-DOE DNA sequencing facility. The entire coding region of β-RyR was screened.

Statistical Analysis

The GLM procedure of SAS (SAS Inst. Inc., Cary, NC, 2000) was used to analyze data.

Results & Discussion

Measurement of calcium channel activity

β-RyR channel activity was measured by ryanodine binding assay. Dissociation constants (K_d) for ryanodine binding for 77 birds were distributed over a broad range (20~74 nM) and did not show any significant correlation with other meat quality traits. Genetically improved turkeys and random-bred turkeys did not show significant difference in K_d from each other ($p>0.05$). Because there was no significant correlation between K_d and meat quality indices, the data from the 77 turkeys were combined for further analyses. Muscles from ten birds were selected based on the functional properties as typical PSE (+) meat and PSE (-) meat: pH < 5.8 and L* > 52.0 and pH > 5.8 and L* < 50.0, respectively (Table 2). Channel activity of PSE (+) turkeys was significantly higher than that of PSE (-) turkeys (Table 3).

Screening of the coding region of β-RyR gene

As a result of polymorphism screening of cDNA of β-RyR, 10 single nucleotide polymorphisms (SNPs) were identified (Table 4). All of them were synonymous and therefore the primary structure of the protein was not changed. None of these SNPs or haplotypes was correlated with meat quality indices or K_d.

The cDNA screening revealed that the primary structure of β-RyR protein was conserved for the 16 birds, but their channel activity data were distributed over a broad range. It is possible that post-translational modification of the protein played a role in the large variation in channel activity among turkeys with the same amino acid sequence.

Conclusions

Based on channel activity differences in turkey muscle β-RyR, 16 birds were selected for screening for polymorphisms in the β-RyR cDNA. Ten SNPs were identified but none of them was correlated with meat quality traits or channel activity. Although β-RyR is very well-conserved, the channel activity of turkeys was distributed over a broad range; the variation may result from the post-translational modification. It has been reported that there are two different genomic alleles for α-RyR and several transcript variants (Chiang et al., 2004). Therefore we suggest that α-RyR channel activity is highly adaptable to environmental stress while the coding region of β-RyR is highly conserved.

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Tables and Figures

	High K_d	**Low K_d**
Better meat quality (high pH, low L*)	**N=4 (K_d 57.60 nM) (pH 6.21, L* 51.23)**	**N=5 (K_d 29.53 nM) (pH 6.24, L* 51.50)**
Poorer meat quality (low pH, high L*)	**N=3 (K_d 57.24 nM) (pH 5.79, L*53.12)**	**N=4 (K_d 26.65 nM) (pH 5.77, L*55.43)**

Table 1. Sixteen turkeys selected for screening of β-RyR cDNA.

PSE (-)	pH	L*	K_d
29-11	6.63	47.42	38.64
31-13	6.42	48.53	30.03
29-15	6.31	47.45	44.05
29-21	6.28	46.68	37.04
29-37	6.01	49.72	51.28
29-03	5.96	47.18	41.15
31-26	5.9	49.56	26.46
31-03	5.9	45.01	39.22
29-40	5.86	49.72	59.52
29-34	5.86	49.08	45.05

PSE (+)	pH	L*	K_d
31-33	5.8	55.13	28.65
31-28	5.79	56.58	27.1
31-25	5.76	55.07	21.01
31-36	5.75	52.03	24.57
31-19	5.74	52.24	30.86
31-32	5.73	54.95	29.85
31-22	5.73	53.69	41.67
31-20	5.72	53.45	30.67
29-08	5.71	56.25	39.22
31-12	5.64	54.31	44.25

Table 2. Selected turkey samples for channel activity comparison

Group	LSMean of K_d (nM)
PSE (-) (N=10)	31.785
PSE (+) (N=10)	41.244

$P<0.05$

Table 3. Mean K_d of turkeys in PSE and non-PSE groups

SNP ID	Base	Amino acid
2352	A/G	Val
9111	G/A	Ser
9681	A/G	Lys
9762	C/T	Leu
10434	C/T	His
11094	C/T	Asn
11097	G/A	Ala
12159	C/T	Ser
12675	T/C	Asn
14076	A/G	Lys

Table 4. Ten SNPs identified in the entire coding region of β-RyR.