VIRAL CHALLENGES AUGMENT SKELETAL MUSCLE PROTEOLYSIS IN GROWING PIGS

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Abstract – The objective of the experiment was to determine the impact of viral challenge on the calpain system components in skeletal muscle. Gilts (20-40 kg; n=6 per treatment) were assigned to the following treatments: 1) control (no infection), 2) challenged with porcine reproductive and respiratory syndrome virus (PRRSV), 3) were infected with porcine endemic diarrhea virus (PEDV) 15 days post infection with PRRSV (PRRSV+PEDV). Calpain and calpstatin activity were measured in longissimus muscle samples collected at euthanasia. Moisture, lipid, and protein content were determined in longissimus muscle. Infection significantly reduced longissimus lipid content. In addition both challenge treatments resulted in an increase in μ-calpain activity and tended to increase m-calpain activity. Co-infection (PRRSV+PEDV) caused a marked decrease in calpastatin activity in calpastatin peak 2). A challenge specific increase in μ-calpain activity, combined with a decrease in calpastatin activity demonstrates a metabolic adjustment to mobilize more protein by calpain dependent degradation of muscle proteins.

Key Words – Calpain system, Skeletal muscle, Viral challenge

I. INTRODUCTION

Just as improvement of swine muscle growth efficiency is a benefit to producers and consumers, factors that impede conversion of feedstuffs to pork are a threat to productivity, profitability, and consumer prices. Swine health is a known impediment to growth efficiency. The impacts of Porcine Endemic Diarrhea (PED) and Porcine Reproductive and Respiratory Syndrome (PRRS) viruses on the swine industry are well documented. To the U.S. pork industry, PRRSV and PEDV are two significant threats to efficient and sustainable pork production. Alone, PRRSV is estimated to cost the U.S. swine industry more than $664 million annually (Holtkamp et al., 2013). In addition, PEDV has been estimated to have cost the U.S. pork industry approximately $1 billion since it was first observed in the U.S. in 2013 (Paarlberg, 2014). Although PRRSV targets to the respiratory system in growing pigs and PEDV impacts the gastrointestinal tract, what remains undefined is how these viruses may interact to impact overall metabolism and growth efficiency. A key component that has not been examined is how these two virus infections impact the maintenance of muscle mass and/or the mobilization of protein from skeletal muscle.

The skeletal muscle calpain system is linked to different models of improved growth efficiency achieved by genetic selection (Cruzen et al. 2013). The calpain system has been proposed to work in concert with the proteasome system to achieve protein turnover (Goll et al., 2008). The protease μ-calpain is hypothesized to initiate the turnover of myofibrillar proteins by making precise cleavages in proteins around the myofibril (for example, desmin) and within the myofibril (titin, nebulin). In some cases, calpain activation has been observed in response to need to mobilize amino acids and energy from skeletal muscle. What is not defined is the involvement of the calpain system in skeletal muscle protein turnover in the event of a viral health challenges. Moreover, little is known about the interaction of PRRSV and PEDV on skeletal muscle metabolism and composition. The objective of this experiment was to define the response of the skeletal muscle calpain system to a PRRSV infection and a combined infection of PRRSV and PEDV.

II. MATERIALS AND METHODS

All animal work was approved by the Iowa State University Institutional Animal Care and Use

Committee (IACUC# 1-14-7710-S). Three treatments were assigned to gilts (16 ± 0.98 kg body weight). Treatments included 1) Control PRRSV and PEDV naïve (n=6); 2) PRRS inoculated for 21 days (n=6); and 3) gilts (n=6) co-infected with PRRSV (day 0) and PEDV (day 15). Pigs in treatment 2 and 3 were inoculated with a live field strain of PRRSV (ORF5 1-18-4 Wild type), in which 500 genomic units was inoculated intramuscularly and 500 genomic units nasally. Treatments 1 received a saline solution sham. PEDV was inoculated intra-gastrically inoculated with 10^3 plaque forming units of an isolate plaque-cloned PEDV P6 (USA/Iowa/18984/2013), while treatments 1 and 2 received a saline sham. At the end of the 21 day test period, all gilts were euthanized by barbiturate overdose and exsanguination, and longisimus dorsi samples were immediately taken and prepared for calpastatin and calpain quantification as described by Cruzen et al. (2013). Muscle samples in buffer were homogenized using a Polytron PT 3100 (Lucerne, Switzerland), in three 30 s bursts. The homogenate was centrifuged at 25,000 x g for 20 min, and the supernatant dialyzed against 40 vol of 40 mM Tris-HCl pH 7.4, 1 mM EDTA, and 0.1% 2-mercaptoethanol (TEM). The dialysate was loaded onto a 20 ml Q-Sepharose Fast Flow anion exchange column equilibrated with TEM (40 mM Tris, 1 mM EDTA, 0.1% 2-mercaptoethanol). After washing, calpastatin, µ-calpain, and m-calpain were eluted using a linear gradient of 0 to 400 mM KCl in TEM. Calpastatin can be eluted in two separate, distinct peaks (calpastatin I and II, at 50 to 90 mM KCl and 120 to 190 mM KCl, respectively), followed by µ-calpain (180 to 240 mM KCl) and m-calpain (300 to 400 mM KCl). The activities of µ- or m- calpain or calpastatin-containing fractions were determined using casein as a substrate. The calpain and calpastatin activity assays were conducted on fresh (never frozen) tissue. Longissimus dorsi from each carcass snap frozen until preparation for proximate analysis as described by Arkfeld et al. (2013). Data were analyzed with a treatment as the sole fixed effect. When appropriate, means were separated with a Student's t test.

III. RESULTS AND DISCUSSION

Compared to the control gilts, both virus challenges severely altered pig performance (data not shown). Initial examination of proteolysis changes in response to the health challenge in the skeletal muscle showed µ-calpain activity increased with infection. Further, a trend toward an increase in m-calpain activity with infection is noted (Table 1). Co-infection resulted in an attenuated calpastatin activity (Table 1). These data indicate that increased protein turnover could potentially be caused by PRRSV and PEDV infection and be related to reduced feed efficiency. Although protein and moisture content of the longissimus dorsi was not altered due to either PRRSV or PRRSV challenges, these health challenges clearly reduced intramuscular neutral lipid (Table 2). This can be interpreted as an attempt to mobilize energy stores, or perhaps simply a response to decreased feed intake that was observed over the 21 day challenge period (data not shown).

Table 1. Effect of viral infection on calpain and calpastatin activities in longissimus muscle[1]

	Control	PRRSV	PRRSV PEDV	SEM	P Value
Calpastatin 1	0.60	0.87	1.07	0.20	0.29
Calpastatin 2	0.81[a]	0.91[a]	0.57[b]	0.08	0.04
Total calpastatin	1.41	1.77	1.64	0.21	0.22
µ-Calpain	0.64[b]	0.87[a]	0.86[a]	0.06	0.03
m-Calpain	1.62	1.79	1.91	0.10	0.18

[1]n=6 pigs per treatment. Units per gram of tissue. One unit of calpain is equivalent to the amount necessary to increase absorbance (at 278 nm) of TCA soluble casein peptides by 1.0 unit. One unit of calpastatin activity is the amount necessary to inhibit one unit of m-calpain activity (Cruzen et al. 2013).

Table 2. Viral infection on proximate composition of longissimus muscle (n=6 per treatment).

	Control	PRRSV	PRRSV+PEDV	SEM	P Value
Moisture %	77.91	77.88	77.59	0.21	0.52
Protein %	21.37	21.43	22.05	0.31	0.27
Lipid %	0.72[a]	0.36[b]	0.20[b]	0.07	<0.01

Moisture, protein and lipid determined as described by Arkfeld et al. (2015). Lipid content represents a % of total neutral lipid.

IV. CONCLUSION

In the Midwest, it is inevitable that a pig will be immunologically challenged at some point in their

life, whether it is from vaccination or natural infection. Gilts exposed to PRRSV or co-infection with PRRSV and PEDV had in a significant reduction in lipid content in longisimus dorsi muscle. A challenge specific increase in μ-calpain activity, combined with a decrease in calpastatin activity, demonstrates a metabolic adjustment to mobilize more protein by calpain dependent degradation of muscle proteins. This is assumed to support the amino acid and energy needs of the immune system. These data have implications for muscle and meat quality.

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