Published April 25, 2019

2018 Reciprocal Meat Conference – Muscle and Lipid Biology and Biochemistry

Meat and Muscle Biology™

Impact of PRRS Challenge on Calpain and Calpastatin Activity in Skeletal Muscle of Young Pigs

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Keywords: calpain, calpastatin, proteolysis, PRRS
Meat and Muscle Biology 2(2):172 **doi:10.221751/rmc2018.150**

Objectives

Porcine Reproductive and Respiratory Syndrome (PRRS) virus challenges in growing pigs are known to negatively affect growth performance. One hypothesis to explain this observation is that an increase in calpain system initiated protein degradation in muscle contributes to the reduction of muscle protein accretion. Calpain-1 and –2 are calcium dependent cysteine proteinases that are regulated by an endogenous inhibitor, calpastatin. The objective of this experiment was to define the extent to which PRRS challenge influences skeletal muscle calpain and calpastatin activity at 2 stages of infection (viremia and seroconversion).

Materials and Methods

Thirty-three pigs (11.3 ± 1.54 kg body weight (BW), approximately 6 wk of age) were assigned to a contemporary group (3 pigs per group) based on age and weight. Pigs were housed in individual pens in separate rooms depending on treatment. One pig per group was assigned to 1 of 3 treatments, 1)PRRS [PRRS challenged with ORF5 RFLP1–3-4 isolate (n = 11)], 2)Pair-fed [Non-challenged, daily feed intake matched to challenged pigs (n = 11)], and 3)Ad-lib [Non-challenged, Ad libitum fed (n = 11)]. Weekly BW, feed disappearance, and feed efficiency were assessed over each period. At days post inoculation (dpi) 9–10 (7 groups; viremia period) and 16 to 17 (4 groups; seroconversion period), pigs were euthanized and a 50-g sample of longissimus muscle was collected and homogenized in prerigor extraction buffer (100 mM Tris-HCl pH 8.3, 10 mM EDTA, 100 mg/L trypsin inhibitor, 2 µM E-64, and 0.1% 2-mercaptoethanol). Calpains and calpastatins were separated using anion exchange chromatography and quantified using casein as a substrate. Data from each time period were analyzed as a separate experiment. Data were analyzed using the mixed procedure in JMP Pro version 13.1 with fixed effects of treatment and a random effect of contemporary group. Means separations were conducted via Student t test with $P < 0.05$ considered significantly different.

Results

At both time points, PRRS pigs had reduced ADG and G:F compared with Ad-lib and Pair-fed pigs ($P < 0.01$). Additionally, ADFI was reduced in PRRS and Pair-fed pigs compared with Ad-lib pigs ($P < 0.001$). At dpi 9–10, PRRS challenge resulted in greater calpastatin-2 (1.04) and total calpastatin (1.70) activity compared with the Pair-fed pigs (0.58, 1.06 respectively; $P < 0.05$). By contrast, at dpi 16 to 17, PRRS challenge resulted in greater calpastatin-1 (1.08) and total calpastatin activity (1.98) compared with Pair-fed controls (0.54, 0.89, respectively; $P < 0.05$). Calpain-1 and –2 were not significantly altered by treatment at either time point. The ratio of calpain-2 to total calpastatin activity was significantly ($P < 0.05$) less in muscle from PRRS challenged pigs (1.16) compared with muscle from the Pair-fed (2.47), but not the Ad-lib pigs (1.58).

Conclusion

The results demonstrate that a decline in voluntary feed intake was not singularly responsible for the observed response to PRRS challenge. These results are counter to the hypothesis that PRRS challenge would decrease calpastatin activity. Moreover, the results of this study indicate a difference in calpastatin response to PRRS challenge, notably that PRRS challenge increased calpastatin-2 activity at dpi 9 to 10 and calpastatin-1 activity at dpi 16 to 17. The switch indicates a temporal response in skeletal muscle proteolysis due to PRRS virus challenge.

