DIFFERENCE IN SKELETAL MUSCLE PEROXIREDOXIN-2 IN PIGS DIVERGENTLY SELECTED FOR RESIDUAL FEED INTAKE IN RESPONSE TO DISEASE CHALLENGE

Brian M. Patterson[1 *], Amanda C. Outhouse[1], Emma T. Helm[1], Jack C. M. Dekkers[1], Kent J. Schwartz[2], Nicholas K. Gabler[1] and Elisabeth Huff-Lonergan[1], and Steven M. Lonergan[1]

[1]Department of Animal Science, Iowa State University, Ames, Iowa;

[2]School of Veterinary Medicine, Iowa State University, Ames, Iowa;

*Corresponding author email: bmp@iastate.edu

Abstract – Barrows divergently selected for high and low residual feed intake (eleventh generation of selection) were subjected to a respiratory and enteric infection with *Mycoplasma hyopneumoniae* and *Lawsonia intracellularis*. Close to peak infection, 21 days post inoculation, littermate pairs of challenged and non-challenged pigs were euthanized and necropsied. Longissimus muscle samples were collected for reducing and nonreducing sarcoplasmic protein analysis. Peroxiredoxin-2, an antioxidant protein in muscle, was analyzed using western blotting. The fastest migrating immunoreactive band was found to compose a greater percentage of the total immunoreactive protein per lane in the infected high residual feed intake (less efficient) pigs compared to other treatment combinations. This difference may indicate that less efficient lines of pigs respond differently to oxidative stress.

Key Words – feed efficiency, oxidative stress, peroxiredoxin

I. INTRODUCTION

Skeletal muscle accretion can be antagonized by health challenges and oxidative stress. Oxidative stress and the antioxidant proteins livestock species use to combat it play a role in both livestock performance and meat quality. Peroxiredoxin-2 (Prdx 2) is an ubiquitously expressed antioxidant protein which aides in an animal's response to oxidative stress by converting peroxides into water using cysteine residues [1]. Some forms of Prdx 2 have been seen to be increased in the blood serum [2], and decreased in semitendinosus [3] and liver [4] of pigs exposed to different forms of oxidative stress. Prdx 2 has also been shown to be differentially abundant in meat differing in in quality [5]. Residual feed intake (RFI) is a measure of feed efficiency representing the difference between actual feed consumption and expected feed consumption for observed weight gain with low RFI (LRFI) pigs being more efficient than high RFI (HRFI) pigs. The objective of this study was to determine the impact of dual respiratory and enteric bacterial infection on the Prdx 2 profile in the skeletal muscle of LRFI and high HRFI pigs. It was hypothesized that the effects of RFI line and infection applied as a stressor would affect Prdx 2 profile. By better understanding the mechanisms of how animals respond to health challenges and oxidative stress, progress may be made towards reducing its impact on livestock performance and potentially negative effects on meat quality may be avoided.

II. MATERIALS AND METHODS

A 2x2 factorial design utilizing one hundred barrows from the eleventh generation of the Iowa State University RFI Project was used in this study. At approximately 50 kg, pigs were assigned to treatment groups and placed in individual pens in adjacent rooms. After a three week acclimation period, one room of pigs was inoculated with *Mycoplasma hyopneumoniae* and *Lawsonia intracellularis*. Twelve pigs with the poorest growth rates were selected from the infected room along with twelve of their littermate pigs from the control room. Selected pigs were euthanized and necropsied at the expected peak of infection (21 days post inoculation) and longissimus samples were immediately excised, frozen in liquid nitrogen, and stored at -80°C. Nonreducing and reducing sarcoplasmic protein extracts were prepared, diluted to 4mg/ml, and run on 15% (reducing) and 12% (nonreducing) SDS-PAGE gels. Western blot analysis was performed with anti- Prdx 2 antibody (Abcam#109367). Samples were run in duplicate. Nonreducing gel results were determined by calculating the percentage of the fastest migrating Prdx 2 band relative to the total anti-Prdx 2 immunoreactive bands in the lane. Reducing gel results were determined by comparing sample Prdx 2 content to a reference sample from outside the sample set. Data were analyzed using the mixed procedure of SAS version 9.4 with gel repetition included as a random effect and line and infection status as fixed effects. Figure 1 provides a representative nonreducing western blot.

III. RESULTS AND DISCUSSION

No significant differences in total Prdx 2 content were seen for reducing gels. For nonreducing gels, no significant difference was seen in the percentage of the faster migrating Prdx 2 band for the main effect of RFI line (main effect *P*-value=0.807). The main effects of infection status (main effect *P*-value=0.014) and RFI line*infection status interaction (*P*-value=0.012) were significant for the percentage of Prdx 2 in the faster migrating band in the nonreducing gels. Table 1 shows the LS Means values for percentage of Prdx 2 in the faster migrating band relative to total immunoreactive bands in the lane for each RFI line and infection status combination.

Table 1. Least Squares Means of the Percentage Prdx 2 in the Faster Migrating Band

RFI Line	Infection Status	Least Squares Mean	Standard Error
LRFI	Control	50.73a	1.428
LRFI	Infected	50.69a	1.508
HRFI	Control	48.35a	1.467
HRFI	Infected	53.57b	1.439

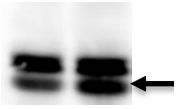

Figure 1.
Nonreducing Prdx 2 Western Blot

The difference in the migration patterns of nonreducing Prdx 2 among the treatment groups is likely due to a posttranslational modification event. One possibility is glutathionylation. The tripeptide glutathione plays a role in the Prdx 2 immune response by forming mixed disulfides with cysteine groups through a process known as glutathionylation. Immune challenge in other systems can result in a similar migration pattern that has been shown to be the result of glutathionylation [6]. The faster migrating band of Prdx 2 found in this study may be a glutathionylated Prdx 2. The significant RFI line*infection status documents that infection did not influence Prdx modification in the LRFI pigs but infection did result in an increase in the modified Prdx 2 in the HRFI pigs.

IV. CONCLUSION

Differences in Prdx 2 profile in response to disease challenge were seen between divergently selected LRFI and HRFI pigs. This could be attributed to differences in biochemical response to oxidative stress between pigs divergently selected for feed efficiency. Differences may exist in oxidative stress response, Prdx 2 profile, and Prdx 2 posttranslational modification between high and low feed efficiency pigs.

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