Changes in the Protein Profile of Porcine Liver in Response to Immune System Stimulation

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Summary and Implications

Immune system function has a direct influence on swine growth. Using lipopolysaccharide (LPS) to stimulate the immune system of pigs provides insight on how they handle immunological stress. Several proteins were shown to be part of the liver's response to LPS. These proteins included heat shock protein (HSP) 60, HSP70, and peroxiredoxin-2. Changes in the abundance of these proteins indicate the extent to which an animal can respond to this immune system stimulation (ISS). Proteins responsible for cellular rescue were found to be increased in abundance in pigs with stimulated immune systems.

Introduction

Understanding how immune system simulation impacts the biology of an animal will contribute to a better understanding of how to manage animals during an immune challenge. This in turn will allow increases in efficiency of production.

Lipopolysaccharide, a component of bacteria, can be used to stimulate an animal's immune system. How an individual animal responds to immune system stimulation may also be linked to feed efficiency. Residual feed intake (RFI) is a measure of feed efficiency. Pigs with a low RFI consume less feed and are more efficient than pigs with a high RFI, while keeping average daily gain and back fat as constant as possible. Using pigs genetically selected for divergent levels of RFI and stimulating their immune systems results in a better understanding of how an immune challenge changes the biology of a pig.

Table 1: Proteins identified that were changed in response to LPS treatment.

Protein	Change (%)	P-Value
	*with LPS	
	stimulation	
Heat Shock Protein 60	27	0.019
Heat Shock Protein 70	20	0.0026
Peroxiredoxin-2	-42	0.00021

The response to immune system stimulation involves many biological pathways, including those related to cellular rescue and antioxidant pathways. The liver has a high degree of metabolic activity and plays a key role in the innate immune system through the acute phase response. Determining differences in the protein profile of the liver may provide an indication of the overall biology of an immune system stimulation.

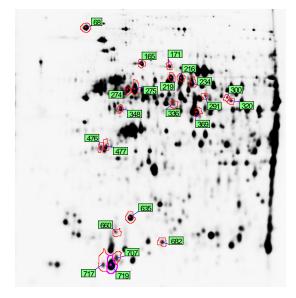
Materials and Methods

Pigs divergently selected for low RFI (8 generations) were more efficient than their high RFI contemporaries (3 generations). Six (3/RFI line) of 12 gilts (BW 63 \pm 4 kg) were injected intramuscularly every 48 h for 7 d, with increasing amounts of *E. coli* lipopolysaccharide. The remaining six pigs were injected with saline (3/RFI line). Pigs were then euthanized and liver tissue collected. The cytoplasmic (soluble) protein fraction of the liver was used to determine the protein profile differences between the ISS pigs and the RFI lines. Two-dimensional difference in-gel electrophoresis, a technique that allows the comparison of two individual samples in a single two dimensional SDS-PAGE gel, was used to determine the difference in protein profile.

Results and Discussion

A total of 563 protein spots were resolved on the twodimensional difference in-gel electrophoresis gel. Of these

Figure 1: Proteins spots that were changed in response to LPS treatment.



proteins, the abundance of 78 were different in response to ISS and 36 were different between RFI lines. Twenty-two of these spots were selected for mass spectrometry analysis to determine the identity of the protein.

Three of the proteins identified were heat shock protein (HSP) 60, HSP70, and peroxiredoxin-2 (Table 1). These identified proteins were not influenced by RFI line. Protein identities were confirmed through two-dimensional western blotting using antibodies specific for the proteins identified. Both HSP60 and HSP70 were increased in abundance during the ISS. Both proteins are known for their ability to protect cells during times of physiological stress (such as heat stress or an immune challenge). Peroxiredoxin-2 was

decreased in abundance in the ISS pigs. Peroxiredoxin-2 is an antioxidant enzyme that is responsible for the reduction of harmful compounds such as hydrogen peroxide in the cells. The reduction of peroxiredoxin-2 abundance in the liver could be an indication of movement of the protein from the liver in response to the increase in physiological stress. These data show that ISS alters the protein profile of the liver specifically proteins responsible for cellular rescue.

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