113 Sequence analysis, synthesis, and antibacterial activity of a proline-arginine-rich peptide from porcine neutrophils. J. Shi, C. R. Ross, M. M. Chengappa, and F. Blecha. Kansas State University, Manhattan.

Antibacterial proteins are rudimentary elements of host defense that are found on mucosal surfaces, in body fluids and in phagocytic leukocytes. The objective of this study was to characterize low molecular weight antimicrobial peptides from porcine leukocytes. Neutrophils were isolated from 6- to 9-month-old crossbred gilts and protein was extracted by sonication and treatment with 20% acetic acid in the presence of protease inhibitors. Crude supernatants were subjected to gel filtration on a Bio-gel P10 column and fractions were evaluated for bactericidal activity with a bacterial-lawn spotting assay using Streptococcus suis, Staphylococcus aureus and Escherichia coli. Because we were interested in isolating low molecular weight, cationic antimicrobial peptides, a modified gel-overlay assay then was used on the gel-filtration fractions that showed antibacterial activity. Fractions that contained cationic, antibacterial peptides were determined from acid-urea polyacrylamide gel electrophoresis gels that were overlaid with E. coli. Positive fractions were subjected to reversed-phase high-performance liquid chromatography and peaks with bactericidal activity were sequenced using automatic Edman degradation. The protein that eluted at 22 to 24 min indicated a proline-arginine (PR)-rich peptide. The first 34 residues of the NH₂-terminus of this peptide was 97% identical to the first 34 residues of PR-39, an antibacterial peptide that has been previously isolated from the porcine small intestine. Mass spectrometry indicated a molecular mass of 17,127 Da; very similar to PR-39 (4720 Da). Synthesis of PR-39 and a truncated peptide, PR-26, was performed and both synthetic peptides displayed antibacterial activity against E. coli, including serotype 0157:H7. These data suggest that porcine neutrophils contain a homolog to PR-39 which may contribute significantly to systemic as well as intestinal host defense.

Key Words: porcine, leukocyte, antimicrobial

NONRUMINANT NUTRITION


A total of 350 crossbred (Newsham) pigs (9 to 2.5 d old and 3.8 ± 0.9 kg BW) was used to determine the appropriate methionine/cystine ratio in diets for the segregated early-weaned pig. Two lysine levels (1.4 and 1.5%) and five methionine levels within each lysine level were used in a 2 X 5 factorial arrangement. Dietary methionine levels in relation to lysine ranged from 0.5 to 0.75%. Blocks were based on initial weight, with pigs from the same blocks designated for the same treatment. Pigs were housed in an environmentally-regulated off-farm nursery. From d 0 to 17 postweaning, all diets contained 25% fried wheat, 12% lactose, 7.5% spray-dried porcine plasma, 6.0% select midden fish meal, and 1.75% spray-dried blood meal. The basal diets containing 1.4 and 1.5% lysine were formulated to contain 30% and 18% dietary methionine, respectively. Corn starch was replaced by Almit (55% aqueous solution of DL-7-hydroxy-4-methylthio- butanone) to provide the four additional experimental methionine concentrations for each lysine level. Cystine content of all diets within each lysine level were identical at 52 or 53% of the 1.4 and 1.5% lysine diets, respectively. All other amino acids were formulated on a digestible basis to ensure methionine was first limiting. There were no methionine + cystine interactions noted in this experiment.

Key Words: methionine, cystine, early-weaned pig.