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 m n indicated a proline-arginine (PR)-rich peptide. The first 34 residues of the NH_2 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 4717 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 defenses in the pig. The implications of this research are twofold: synthetic analogs of PR-39 may provide new agents to prevent and/or treat porcine diseases, and upregulation of this natural antibiotic may reduce the reliance on feed-additive antimicrobials. Supported in part by USDA NRI Competitive Grant 93-37206-9351.

Key Words: porcine, leukocyte, antimicrobial

NONRUMINANT NUTRITION

114 **Appropriate methionine:lysine ratio in diets for the segregated early-weaned pig** K.Q. Owen*, R.D. Goodband, J.L. Nelssen, M.D. Tokach, S.S. Dritz, B.T. Richert, and K.G. Friesen, Kansas State University, Manhattan.

A total of 350 crossbred (Newsham) pigs (9.0 ± 2 d old and 3.8 ± .9 kg BW) was used to determine the appropriate methionine:lysine ratio in diets for the segregated early-weaned pig. Two lysine levels (1.8 and 1.4%) 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 21.5 to 33.5%. Blocks were based on initial weight, with 5 pigs/pen and 7 pens/treatment. Pigs were housed in an environmentally-regulated off-site nursery. From d 0 to 21 postweaning, all diets contained 25% dried whey, 12% lactose, 7.5% spray dried porcine plasma, 6.0% select menhaden fish meal, and 1.75% spray-dried blood meal. The basal diets containing 1.4 and 1.8% lysine were formulated to contain .301 and .387% dietary methionine, respectively. Corn starch was replaced by Alimet (an 88% aqueous solution of DL-2-hydroxy-4-(methylthio) butanoic acid) 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 .66% for the 1.4 and 1.8% lysine diets, respectively. All other amino acids were formulated on a digestible basis to ensure methionine was first limiting. There were no methionine × lysine interactions ($P > .10$) observed throughout the 21 day experiment. From d 0 to 7 postweaning, increasing dietary methionine improved (quadratic, $P < .01$) ADG and ADFI regardless of dietary lysine. Inflection point analysis projected 95% of maximum ADG at a methionine:lysine ratio of 27%. Increasing dietary lysine improved ($P < .01$) ADG and gain/feed (G/F) from d 0 to 7 postweaning with pigs fed 1.8% lysine having 10 and 7% greater ADG and G/F than those fed 1.4% lysine, respectively. From d 0 to 14 postweaning, increasing dietary methionine improved ADG (quadratic, $P < .01$), ADFI (quadratic, $P = .02$) and G/F (quadratic, $P = .10$). Inflection point analysis projected 95% of maximum ADG at a methionine:lysine ratio of 27 and 27.5% for pigs fed the 1.4 and 1.8% lysine, respectively. Cumulative (d 0 to 21 postweaning) ADG, ADFI, and G/F were improved (quadratic, $P < .05$) by increasing dietary methionine. Inflection point analysis projected 95% of maximal ADG at a methionine:lysine ratio of 28% for pigs fed either 1.4 or 1.8% lysine and maximum G/F at a ratio of 27.2 and 28.2% for pigs on the 1.4 and 1.8% lysine treatment, respectively. Increasing dietary lysine improved ($P < .01$) ADG and G/F from d 0 to 14 and for the overall experiment. In conclusion, regardless of dietary lysine, maintaining methionine at 27.5% of lysine was required to maximize growth from d 0 to 21 postweaning.

	1.8% Lysine					1.4% Lysine					
Met, %	.387	.441	.495	.549	.603	.301	.343	.385	.427	.469	
Met:Lys,%	21.5	24.5	27.5	30.5	33.5	21.5	24.5	27.5	30.5	33.5	CV
D 0 to 7											
ADG,g[ac]	205	225	244	210	205	191	198	203	212	180	17.2
G/F[c]	1.08	1.09	1.13	1.08	1.07	1.03	.97	1.03	1.02	1.01	9.1
D 0 to 14											
ADG,g[ac]	284	309	329	307	298	252	274	291	294	272	10.4
G/F[bc]	.94	.99	1.01	1.01	.97	.85	.86	.90	.89	.89	5.2

[ab]Quadratic effect of dietary methionine ($P < .01$ and $P < .10$, respectively)
[c]Lysine effect ($P < .01$)

Key Words: Methionine, Lysine, Pigs, Growth performance.

115 **Dietary lysine requirements of segregated early-weaned pigs** K.Q. Owen*, J.L. Nelssen, R.D. Goodband, M.D. Tokach, B.T. Richert, K.G. Friesen, J.W. Smith, J.R. Bergstrom, and S.S. Dritz, Kansas State University, Manhattan.

A total of 320 (160 barrows and 160 gilts) 14- to 18-d old pigs (PIC, C15 X 326, initially 4.6 ± .8 kg) was used to determine the optimal level of dietary lysine needed for the segregated early weaned pig. Pigs were housed in an environmentally-regulated off-site nursery with 4 pigs per pen and 6 pens per treatment. Two diet formulation methods were used with 6 dietary lysine levels within each formulation method resulting in a 2 × 6 factorial arrangement of treatments. The first formulation method (FM1) consisted of a basal diet that contained 1.95% lysine. Increasing levels of corn starch replaced L-lysine HCl to achieve the other 5 dietary treatments (1.2, 1.35, 1.50, 1.65, and 1.80% dietary lysine). All other amino acids in each diet were maintained at the same level as in the 1.95% lysine treatment. The second formulation method (FM2) consisted of a basal diet (1.20% lysine) with the five additional treatments achieved by adding synthetic lysine and other essential amino acids maintaining an ideal amino acid ratio relative to lysine. The first formulation was used to determine if the ratio of other amino acids relative to lysine is important for maximizing growth performance because the diets containing the higher level of lysine will be approaching the appropriate amino acid ratio. The second formulation was used to minimize excess amino acids. All diets contained 20% dried whey, 10% lactose, 7.5% spray-dried porcine plasma, 5.0% spray-dried wheat gluten, 5.0% select menhaden fish meal, 5.0% soybean oil, and 1.75% spray-dried blood meal. There were no lysine × formulation method interactions for ADG or ADFI throughout the 28 d period ($P > .10$). However, a lysine × formulation method interaction was observed for G/F from d 0 to 7, d 0 to 14, and d 0 to 28. From d 0 to 7 postweaning, ADG was improved (quadratic, $P < .01$) as dietary lysine increased, with ADG appearing to be maximized between 1.65 and 1.80% dietary lysine. Feed efficiency, from d 0 to 7 postweaning, was improved (quadratic $P < .01$, linear, $P < .01$) for FM1 and FM2, respectively, with G/F highest for pigs fed 1.80% lysine for FM1 and 1.95% lysine for pigs fed the FM2. From d 0 to 14 postweaning, ADG was improved (quadratic, $P < .01$) by increasing dietary lysine with ADG being maximized at approximately 1.65% dietary lysine. A lysine × formulation method interaction was observed for G/F from d 0 to 14 with G/F improved (quadratic, $P < .01$; quadratic, $P < .05$) for FM1 and FM2, respectively. For the overall experiment (d 0 to 28 postweaning), ADG and G/F were improved (quadratic, $P < .01$) with increasing dietary lysine. However, ADFI was not effected during the 28 d experiment. These data suggest that segregated early weaned pigs require approximately 5.2 to 6.2 g/d of lysine from d 0 to 7 and d 0 to 14 postweaning, respectively, to optimize growth performance.

	FM1, Lysine, %						FM2, Lysine, %						
Item	1.20	1.35	1.50	1.65	1.80	1.95	1.20	1.35	1.50	1.65	1.80	1.95	CV
D 0 to 7													
ADG,g[a]	254	302	330	338	350	321	277	316	335	351	346	374	12.2
G/F[bc]	.89	1.04	1.15	1.20	1.23	1.13	1.00	1.01	1.06	1.12	1.19	1.21	8.1
D 0 to 14													
ADG,g[a]	287	347	373	388	388	393	331	362	382	408	394	402	9.1
G/F[bd]	.77	.91	1.01	1.06	1.05	1.04	.87	.90	.93	1.03	1.03	1.03	5.9

[a]Quadratic effect of lysine ($P < .01$) [b]Diet X formulation method interaction ($P < .01$)
[c]Quadratic and linear effect of lysine within FM1 and FM2 ($P < .01$), respectivley.
[d]Quadratic effect of lysine with FM1 and FM2 ($P < .01$ and $P < .05$, respectively).

Key Words: Lysine, Pigs, Growth performance.