ONTARIO PORK RESEARCH PROPOSAL FINAL REPORT

1. **Project Leader:** C. de Lange
2. **Project Title:** Impact of immune system stimulation on nutrient utilization and response to dietary methionine plus cysteine intake in growing pigs

**Objectives of the Research Proposal:**
To determine the impact of immune system stimulation (ISS; disease) in growing pigs on:
1. fecal digestibility of energy yielding nutrients and ileal digestibility of amino acids,
2. utilization of methionine plus cysteine intake for whole body protein deposition,
3. the efficiency of trans-sulfuration of methionine to cysteine, and
4. the contribution of glutathione synthesis to irreversible cysteine loss (to be conducted with matching funds that are to be obtained from the NSERC CRD program).

**Benefit of Research to the Ontario Pork Industry:** Sub-clinical disease in growing-finishing pigs can reduce feed efficiency, and thus production costs, by as much as 30%. By minimizing the negative impact of immune system stimulation on nutrient utilization and animal productivity, and by more closely meeting the pigs’ nutrient requirements, the economic losses due to immune system activation can be reduced. A mere 1% improvement in feed utilization efficiency represents a savings of more than $0.50 per pig or more than $3,000,000 for the Ontario Swine industry. In addition, a reduction in immune system stimulation will benefit well-being of pigs and reduce nutrient losses into the environment.
Impact of immune system stimulation on nutrient utilization and response to dietary methionine plus cysteine intake in growing pigs

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Executive summary

In commercial production systems pigs frequently experience sub-clinical levels of disease which leads to stimulation of the immune system. Exposure to sub-clinical level of disease has a substantial negative impact on productivity of growing pigs, and thus on nutrient utilization and profitability in commercial pork production. The overall aim of this research program was to explore nutritional means to reduce the negative impact of immune system stimulation (ISS; disease) on growth performance of pigs. The emphasis was on dietary amino acids that are known to play a role in the immune system: methionine, cysteine and tryptophan. Aspects of nutrient utilization that were considered were nutrient digestibility, reductions in the animals growth performance potential (expressed in units of whole body protein gain; closely associated with muscle tissue gain), and key aspects of post-absorptive metabolism of these amino acids. In these studies a controlled level of ISS was achieved based on a repeated injection of increasing doses of inactivated microbial wall material (lipo-polysaccharides from coliform bacteria). Various indicators of ISS, including body temperature, were monitored to confirm effectiveness of ISS. The research showed that ISS does not influence nutrient digestibility, but reduces the pig’s growth performance potential. It was also demonstrated that the dietary requirements for methionine plus cysteine and for tryptophan for supporting body maintenance functions were increased during ISS. In metabolism and gene expression studies it was shown that ISS up-regulates metabolic pathways that utilize methionine and cysteine, reducing the availability of these amino acids for supporting accretion of body protein (e.g. muscle mass). Based on this research practical recommendations can be made to adjust intake of these three key amino acids to optimize the pig’s response to sub-clinical levels of disease.

Background & Introduction

Exposure to sub-clinical level of disease has a substantial negative impact on productivity of growing pigs, and thus on nutrient utilization and profitability in commercial pork production (e.g. Williams et al., 1997; Dionissopoulos et al., 2006). According to Williams et al. (1997), exposure to sub-clinical levels of disease can reduce lean tissue growth and feed efficiency by more than 25%. Clearly, it is critical to minimize exposure of pigs to disease causing organisms in commercial pork production. However, in spite of extensive bio-security measures in the Ontario pork industry, we are still faced with disease challenges caused by pathogens such as PRRS, swine influenza and circo-virus. When managing pigs exposed to sub-clinical levels of disease, two key questions are (1)
whether we can manipulate the pigs’ immune response and (2) to what extent disease alters the pigs’ dietary nutrient requirements.

A series on nitrogen (N) balance and nutrient metabolism studies were conducted to determine the impact of immune system stimulation in growing pigs on:

1. fecal digestibility of energy yielding nutrients and ileal digestibility of amino acids,
2. utilization of methionine plus cysteine intake for whole body protein deposition,
3. the efficiency of trans-sulfuration of methionine to cysteine, and
4. the contribution of glutathione (GSH) synthesis to irreversible cysteine loss (to be conducted with matching funds that are to be obtained from the NSERC CRD program).


Research activities and key findings

Objectives 1 & 2
In two nutrient balance studies it was shown that a repeated injection with increasing amounts of LPS represents an effective means of inducing chronic ISS in growing pigs, as indicated by increased liver weight (P <0.01), body temperature (40.2 versus 39.8 oC; SE 0.07; P<0.05) and blood plasma levels of acute phase proteins (P <0.01).

In the two nutrient balance experiments (initial BW 21.5 and 23.0 kg), apparent ileal and fecal digestibility of crude protein and amino acids were not affected by ISS (P >0.10) in pigs fed varying levels of methionine plus cysteine (SAA) intake.

In the first nutrient balance experiment, pre-challenge body protein deposition (PD)(P <0.01), but not PD during ISS (P >0.10), was increased with increasing intake of methionine plus cysteine (SAA; 3.6 vs 4.7 g/d). Administration of LPS reduced PD at both SAA intake levels (P <0.01). No interactive effects between ISS and SAA intake on PD were found (P >0.10). However, the reduction in PD due to ISS was smaller at the high SAA intake level than at the low SAA intake level (16.4 ± 1.9 versus 10.1 ± 1.6 g/d; P <0.03), indicating that additional SAA intake reduces the negative effects of ISS on PD. In the second nutrient balance experiment, pigs (initial BW 21.5 + 3.5 kg) were fed at 3 levels of SAA intake (L1 vs L2 vs L3; 0.95 vs 2.1 vs 3.1 g/d). Both urinary N and S excretion increased with SAA intake level (L1 vs L3: 74.4 ± 15.9 vs. 203.6 ± 15.9 and 19.4 ± 0.4 vs. 21.0 ± 0.4 g/kg BW/d for N and S, respectively, P <0.01). Only urinary N was elevated by ISS (110.1 ± 16.4 vs. 167.9 ± 15.6 g/kg BW/d; P <0.01). ISS and SAA reduced the whole-body N/S-balance (14.8 ± 0.8 vs. 10.7 ± 0.7 in ISS; 17.0 ± 0.7 at L2 vs. 7.8 ± 0.7 at L1; P <0.002). Based on regression analyses, the marginal efficiency of utilizing SAA for PD was not influenced by ISS (P >0.10). However, estimated maintenance SAA requirements (intercept in regression analyses) were increased by ISS.
(P <0.05). At low levels of methionine plus cysteine intake and during immune system stimulation of growing pigs, methionine plus cysteine is preferentially preserved or repartitioned in favor of non-protein body stores. Additional dietary intake of methionine plus cysteine can reduce the adverse effects of immune system stimulation on whole body protein deposition in growing pigs, and probably accelerates the recovery.

With additional support from NSERC a study was conducted to explore utilization of tryptophan during ISS. This N balance study was conducted using 36 growing pigs (20 ± 1.2 kg initial BW) fed 1 of 4 diets with decreasing dietary tryptophan (Trp) content (1.3, 1.1, 0.8, and 0.6 g/kg). The balance among essential AA was held constant across treatments and Trp was formulated to be first limiting in all diets. Feed was provided at 85 g/kg BW0.75. Pigs were subjected injections of lipopolysaccharide (LPS, 0.02 ml/kg, increasing 15% each injection) at 48 h intervals to induce a chronic immune response. Whole body N retention was measured before and during administration of LPS (pre-challenge and challenge period, respectively). An inflammatory response was induced as evidenced by a significant increase in plasma levels of acute phase proteins. There were no interactive effects of dietary Trp level and immune challenge. Nitrogen retention decreased linearly (p < 0.0001; 12.3, 9.2, 5.2, and 3.9 g/d) with decreasing Trp intake, due primarily to an increase in urinary N excretion (p < 0.05). Immune challenge increased (p < 0.05) urinary N excretion (3.2 g/d), compared to the pre-challenge period (2.6 g/d), resulting in a decrease in N retention (p < 0.001; 6.4 vs 7.2 g/d). Fecal N excretion was not affected by immune challenge but was decreased (p < 0.05) as Trp intake decreased. The repeated LPS-injection protocol induced an inflammatory response. The increased urinary N during an immune challenge suggests increased Trp catabolism, reducing the AA pool for protein deposition. In pigs fed diets limiting in Trp N-balance was reduced during an immune challenge, indicating increased Trp requirements for mounting an immune response during clinical or sub-clinical disease.

Objectives 3
An N balance study was conducted to assess the efficiency of trans-sulfuration of methionine to cysteine, and thus the optimal methionine to methionine plus cysteine ratio (M:M+C) during ISS in 20 kg pigs. Thirty-six pigs were fed 800 g/d of one of five SAA-limiting diets, containing graded levels of M:M+C (42, 47, 52, 57 and 62%) and supplying 2.5 g/d of SAA. After adaptation, N balances were determined sequentially during a 5-d pre-ISS period and two ISS periods of 3 and 4 d, respectively. To induce ISS, pigs were injected intramuscularly with repeated and increasing doses of E. coli lipopolysaccharide. Eye temperature and blood parameters confirmed effective ISS. In the first ISS period, ISS reduced the mean N-balance more severely than in the second ISS period (8.6±0.2 vs. 9.6±0.3 g/d) and was lower than the pre-ISS period (10.0±0.2 g/d). An interactive effect of ISS and diet on N balance was observed (P<0.001). Quadratic-plateau regression analysis determined the optimal dietary M:M+C to be 56.6±2.6% and 58.8±2.4% for pre-ISS, and ISS period 2, respectively. The optimal dietary M:M+C for ISS period 1 was found to be greater than the highest level in the present experiment (62%), suggesting that the optimal M:M+C is greater during
initial ISS. It is suggested that this may be a result of toxic properties of cysteine, and preferential use of methionine during ISS. This is supported by the elevated transsulfuration rate previously noted during ISS mainly due to the enhanced cystathionine β-synthase and cystathionase enzyme activity. In this study ISS resulted in an increase in the optimal dietary M:M+C in growing pigs.

Objectives 4.
The multiple tracer approach to simultaneously measure to conversion of methionine to cysteine (using a 14C serine tracer; measuring 14C tracer appearance in cysteine), the use of cysteine for production of GSH and other immune compounds, and total irreversible cysteine losses (using 35S cysteine; measuring appearance of 35S tracer in glutathionine and urinary S) within pigs was not successful. Using HPLC we separated serine, cysteine and GSH from various tissues and have recovered measurable radioactivity in these isolated compounds. However, in plasma cysteine counts of radioactivity from 14C could not be separated reliably from 35S. The protocol for infusing isotope tracers was changed.

In a subsequent and modified isotope tracer studies, we achieved steady state conditions within the first h of the five h infusion period in terms of 35S specific radio-activity (SRA) in plasma cysteine. Therefore, this approach allowed measurement of cysteine flux and of the fractional synthesis rate of GSH and other cysteine containing compounds. ISS increased the plasma cysteine flux and reduced the plasma cysteine and total SAA concentration indicating increase in utilization of cysteine during ISS. However, absolute rate of appearance of end products of cysteine catabolism (taurine and SO4) remained unaffected by ISS indicating increased cysteine utilization during ISS was not due to cysteine catabolism. In a subsequent isotope tracer study ISS increased the rate of synthesis of GSH in liver, the gastrointestinal tract, lung, muscle and heart, implying that increased cysteine utilization during ISS, and consequently increased maintenance SAA requirements, can mainly be attributed mainly to increased GSH synthesis.

Scientific significance of the results

In these studies a controlled level of immune system stimulation (ISS; disease) was achieved based on a repeated injection of increasing doses of inactivated microbial wall material (lipo-polysaccharides from coliform bacteria). Various indicators of ISS, including body temperature, were monitored to confirm effectiveness of ISS. The research showed that ISS does not influence nutrient digestibility, but reduces the pig’s growth performance potential. It was also demonstrated that the dietary requirements for methionine plus cysteine and for tryptophan for supporting body maintenance functions were increased during ISS. In metabolism and gene expression studies it was shown that ISS up-regulates metabolic pathways that utilize methionine and cysteine, reducing the availability of these amino acids for supporting accretion of body protein (e.g. muscle growth). Based on this research practical recommendations can be made to adjust intake of these three key amino acids to optimize the pig’s response to sub-clinical levels of disease.

Brief discussion of the potential benefits to Canada.
Sub-clinical disease in growing-finishing pigs can reduce feed efficiency, and thus production costs, by as much as 30%. By minimizing the disease induced negative impact of immune system stimulation on nutrient utilization and animal productivity, and by more closely meeting the pigs’ nutrient requirements, the economic losses due to immune system activation can be reduced. In this research it was demonstrated that the dietary requirements for methionine plus cysteine and for tryptophan for supporting body maintenance functions are increased during disease and that the impact of disease on pig productivity can be reduced by increasing the dietary supply of these key amino acids. A mere 1% improvement in feed utilization efficiency represents a savings of more than $0.50 per pig or more than $3,000,000 for the Ontario Swine industry. In addition, a reduction in immune system stimulation benefits well-being of pigs and reduce nutrient losses into the environment.

Publications

1. Peer reviewed manuscript

2. Submitted manuscripts

3. Abstracts presented at scientific meetings


4. Technical reports and extension activities


