

# Functional amino acids in plant-based nursery diets in pigs subsequently challenged with Salmonella

L.A. Rodrigues<sup>1,2</sup>, J.C. Panisson<sup>1,2</sup>, A.G. Van Kessel<sup>2</sup>, and D.A. Columbus<sup>1,2</sup>



Lucas Rodrigues



Dan Columbus

## SUMMARY

Functional amino acids (FAA) attenuate the effects of Salmonella challenge in pigs. However, this may be affected by protein source (PS). The objective of the present study was to determine the effects of nursery dietary PS and FAA supplementation on growth performance and immune status of pigs subsequently challenged with Salmonella Typhimurium (ST). Thirty-two weanling pigs ( $8.7 \pm 0.23$  kg) were assigned to a feeding program for 31 d in a  $2 \times 2$  factorial arrangement. Factors were dietary PS (plant-based [PB] vs. animal-based [AB]) and FAA profile (basal [FAA-] or supplemented [FAA+; Thr, Met, and Trp at 120% of requirements]). Pigs were subsequently placed on common grower diet and, after a 7-d adaptation, were inoculated with ST and monitored for 7 d post-inoculation. Growth performance, rectal temperature, fecal score, gut health, ST shedding score, intestinal colonization and translocation, and blood parameters of acute-phase response and antioxidant balance were measured pre- and post-inoculation.

Post-inoculation fecal score was worse, ST shedding, cecal myeloperoxidase, and cecal and colonic ST colonization were greater in PB compared to AB pigs ( $P < 0.05$ ). Translocation of ST to spleen was decreased by FAA+ ( $P < 0.05$ ), regardless of dietary protein source. Post-inoculation, AB pigs had greater average daily gain compared to PB-FAA- ( $P < 0.05$ ). Pigs fed AB-FAA- showed increased average daily feed intake compared to PB-FAA- pigs ( $P < 0.05$ ) and feed efficiency was increased in AB-FAA+ compared to PB-FAA- pigs ( $P < 0.05$ ). Feeding PB ingredients in nursery diets seems to increase susceptibility of pigs to Salmonella. Moreover, FAA supplementation partially attenuated the negative effects of PB diets on the response of pigs to ST challenge.

## INTRODUCTION

There is reduced societal acceptance of feeding livestock diets containing animal-based (AB) ingredients. Feeding plant-based (PB) protein sources (PS) during the post-weaning phase may not affect overall growth performance to market weight. However, a previous study showed reduced growth performance and increased mortality in pigs fed PB diets when an unexpected disease challenge occurred. This indicates that pigs fed a PB vs. AB diet in the nursery may be more susceptible to subsequent disease challenges.

The positive effects of dietary amino acids (AA) on overall health, recently regarded as 'functional' roles, are mainly associated with improvements in intestinal mucosal barrier, antioxidant defense, and immune molecule synthesis. Previous work has shown that dietary supplementation with key functional amino acids (FAA) improves growth performance and immune status of disease-challenged pigs. It is not known if supplementation with FAA may mitigate the negative effects of plant-based nursery diets.

The objective of the present study was to determine the effects of provision of PB or AB diets supplemented or not with FAA in the nursery period on growth performance and immune status of weaned pigs subsequently challenged with Salmonella. We hypothesized that removal of AB ingredients in nursery diets would impair gut development and decrease pig robustness, increasing susceptibility to Salmonella challenge. We further hypothesized that FAA supplementation would improve animal response to Salmonella challenge, regardless of dietary proteins source.

## EXPERIMENTAL PROCEDURES

After weaning, 32 mixed-sex piglets ( $8.7 \pm 0.2$  kg initial body weight [BW]) were individually housed for 45 days, including a 38-day adaptation period (no inoculation) and a 7-day post-inoculation period. Pigs were randomly assigned to 1 of 4 treatments in a  $2 \times 2$  factorial arrangement. Factors were dietary PS (plant-based [PB] vs. animal-based [AB]) and FAA profile (basal [FAA-] or supplemented [FAA+]). The FAA- profile met the standardized ileal digestible (SID) AA requirements according to NRC (2012) and the FAA+ profile contained Thr, Met, and Trp at 120% of requirements for growth. Pigs were fed the experimental dietary treatments from d 0 to 31 post-weaning. Experimental diets were wheat-barley based with either canola meal (PB) or meat meal-fish meal-blood meal (AB) as protein source. At d 31, pigs were placed onto a common grower diet and, after a 7-d adaptation period, were inoculated with Salmonella Typhimurium (ST;  $3.05 \times 10^9$  colony-forming units/mL) and monitored for 7-d post inoculation.

Growth performance, rectal temperature, fecal score, indicators of gut health, ST shedding score in feces, intestinal ST colonization and translocation, and blood parameters of acute-phase response and antioxidant balance were measured pre- and post inoculation.

1 Prairie Swine Centre Inc, PO Box 21057, 2105 – 8th Street East, Saskatoon, SK S7H 5N9

2 Department of Animal and Poultry Science, University of Saskatchewan, 51 Campus Dr, Saskatoon, SK S7N 5A8

RESULTS AND DISCUSSION

Overall rectal temperature tended to be reduced in FAA+ compared to FAA- pigs, regardless of protein source ( $P < 0.10$ ; Figure 1A). Overall fecal score was negatively impacted in PB- compared to AB-fed pigs ( $P < 0.05$ ; Figure 1B). Overall fecal ST shedding was reduced in AB pigs compared to PB pigs, regardless of FAA supplementation ( $P < 0.05$ ). Pigs fed FAA+ tended to have reduced ST colonization in ileum ( $P < 0.10$ ), regardless of protein source, and pigs fed AB had decreased ST colonization in cecum and colon ( $P < 0.05$ ), regardless of FAA supplementation (Table 1). Translocation of ST to spleen was reduced by FAA+, regardless of protein source ( $P < 0.05$ ).

Post-inoculation, AB pigs had greater average daily gain compared to PB-FAA- with PB-FAA+ being intermediate ( $P < 0.05$ ; Table 2), which indicates a clear attenuation of the negative effects of PB by FAA supplementation during a subsequent enteric infection. Pigs fed AB-FAA- showed increased average daily feed intake compared to PB-FAA- pigs ( $P < 0.05$ ) and feed efficiency was increased in AB-FAA+ compared to PB-FAA- pigs ( $P < 0.05$ ; Table 2).

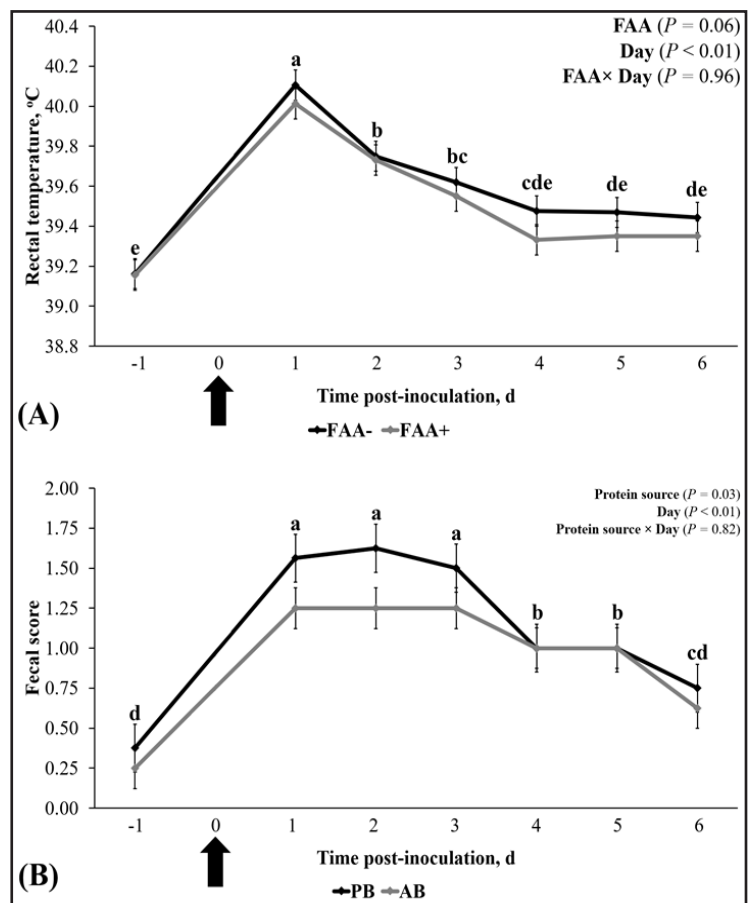
Serum albumin tended to increase in FAA+ compared to FAA- pigs, regardless of protein source ( $P < 0.10$ ). There was no effect of protein source or FAA supplementation on other blood parameters (haptoglobin, SAC, SOD, GSH, GSSG or GSH:GSSG;  $P > 0.10$ ). Pigs fed FAA+ diets tended to have reduced ileal myeloperoxidase (MPO) compared to pigs fed FAA- regardless of protein source ( $P < 0.05$ ). Cecal MPO was reduced in AB pigs compared to PB pigs regardless of FAA supplementation ( $P < 0.05$ ; Table 3).

IMPLICATIONS

Taken together, our findings show that a plant-based feeding program in the nursery period predisposed pigs to a subsequent enteric challenge, mainly through increased Salmonella intestinal colonization and fecal shedding, and deteriorated fecal score. Further, when plant-based diets were supplemented with FAA, specifically with Thr, Met, and Trp, above estimated requirements for growth, the negative effects of Salmonella on growth performance were attenuated, despite no effect on systemic markers of acute-phase response and antioxidant balance. This, combined with a lack of effect of FAA supplementation in animal-based diets during a subsequent Salmonella challenge, indicates that FAA may be a valuable strategy to mitigate the enteric disturbances caused by plant-based nursery diets.

ACKNOWLEDGEMENTS

Funding for this project has been provided by Swine Innovation Porc, Evonik Nutrition & Care GmbH, and Mitacs. The authors would also like to acknowledge the strategic program funding provided by Sask Pork, Alberta Pork, Ontario Pork, the Manitoba Pork Council, and the Saskatchewan Agriculture Development Fund. In addition, we wish to acknowledge the support of the production and research technicians at Prairie Swine Centre and the Animal Care Unit at the Western College of Veterinary Medicine that make it possible to conduct this research.



**Figure 1.** Rectal temperature (A) and fecal score (B) of Salmonella-inoculated pigs (indicated by arrow). Normal consistency feces were given a score of 0, semisolid feces without blood were given a score of 1, watery feces without blood were given a score of 2 and blood-tinged feces were given a score of 3. Within days, points with different superscripts differ ( $P < 0.05$ ). Values are least squares means;  $n=16$  pigs/treatment.

**Table 1.** Salmonella Typhimurium var. Copenhagen quantification in intestinal contents (Log<sub>10</sub> CFU/g; d 7 post-inoculation) of Salmonella-inoculated pigs fed plant or animal-based nursery diets with or without functional amino acids supplementation<sup>1</sup>

Item	Plant based		Animal based		SEM	P-value		
	FAA-	FAA+	FAA-	FAA+		PS	FAA	PS×FAA
Ileum	3.49	2.87	3.36	2.13	0.625	0.41	0.09	0.56
Cecum	2.73	2.27	1.89	1.49	0.389	0.03	0.24	0.93
Colon	2.67	2.99	2.17	1.93	0.345	0.04	0.90	0.45

FAA-, Basal amino acid profile; FAA+, Functional amino acid profile (Thr, Met, and Trp at 120% of requirements for growth). SEM, standard error of the mean. PS, protein source.

<sup>1</sup> Values are least squares means;  $n=8$  pigs/treatment.

**Table 2.** Post-inoculation (day 38-45) growth performance of pigs fed plant or animal-based nursery diets with or without functional amino acids supplementation<sup>1</sup>

Item (kg)	Plant based		Animal based		SEM	P-value		
	FAA-	FAA+	FAA-	FAA+		PS	FAA	PS×FAA
Initial body weight	26.63	26.74	26.17	25.49	2.342	0.63	0.77	0.86
Average daily gain	0.516b	0.605ab	0.726a	0.716a	0.065	0.60	0.40	0.04
Average daily feed intake	1.173b	1.315ab	1.452a	1.325ab	0.084	0.35	0.74	0.04
Gain:Feed	0.44b	0.46ab	0.50ab	0.54a	0.047	0.49	0.59	0.05
Final body weight	30.34	30.98	31.25	30.50	1.371	0.74	0.98	0.44

FAA-, Basal amino acid profile; FAA+, Functional amino acid profile (Thr, Met, and Trp at 120% of requirements for growth). SEM, standard error of the mean. PS, protein source.

<sup>1</sup> Values are least squares means; n=8 pigs/treatment.

a–b Means within a row with different letters differ ( $P \leq 0.05$ ).

**Table 3.** Myeloperoxidase in intestinal contents ( $\mu\text{U}/\text{mL}$ ; d 7 post-inoculation) of Salmonella-inoculated pigs fed plant or animal-based nursery diets with or without functional amino acids supplementation<sup>1</sup>

Item	Plant based		Animal based		SEM	P-value		
	FAA-	FAA+	FAA-	FAA+		PS	FAA	PS×FAA
Ileum	4.56	2.77	4.38	2.95	0.964	0.99	0.09	0.85
Cecum	2.64	2.65	2.00	1.83	0.372	0.05	0.82	0.80
Colon	2.24	2.41	2.51	2.63	0.225	0.27	0.51	0.90

FAA-, Basal amino acid profile; FAA+, Functional amino acid profile (Thr, Met, and Trp at 120% of requirements for growth). SEM, standard error of the mean. PS, protein source.

<sup>1</sup> Values are least squares means; n=8 pigs/treatment.

a–b Means within a row with different letters differ ( $P \leq 0.05$ ).