

# Ileal alkaline phosphatase is upregulated in *Salmonella*-challenged pigs fed functional AA

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## SUMMARY

Functional amino acid (FAA) supplementation improves growth performance and immune status of *Salmonella* Typhimurium (ST)-challenged pigs, which was further improved by a longer adaptation period. It is expected that the effects are associated with increased activity of intestinal alkaline phosphatase (IAP). The objective of this study was to evaluate the effects of FAA supplementation and adaptation period on the ileal, cecal, and colonic activity of IAP in weaned pigs challenged with ST. In experiment 1, a total of 32 mixed sex weanling pigs were randomly assigned to dietary treatments in a 2 × 2 factorial arrangement with low (LP) or high protein (HP) content and basal (FAA-) or FAA profile (FAA+; Thr, Met, and Trp at 120% of requirements) as factors. In experiment 2, a total of 32 mixed-sex weanling pigs were randomly assigned to one of four dietary treatments, being FAA- fed throughout the experimental period (FAA-) or an FAA profile fed only in the post-inoculation (FAA+0), for 1 wk pre- and post-inoculation (FAA+1), or throughout the experimental period (FAA+2). In experiments 1 and 2, after a 7- and 14-d adaptation period, respectively, pigs were inoculated with saline solution containing ST (3.3 and 2.2 × 10<sup>9</sup> CFU/mL, respectively). Plasma alkaline phosphatase was measured on days 0 and 7 post-inoculation in experiment 1, and IAP (ileum, cecum, and colon) was measured in experiments 1 and 2.

In experiment 1, plasma alkaline phosphatase was decreased with ST inoculation and the overall content was increased in LP-FAA+ compared with LP-FAA- ( $P < 0.05$ ). Moreover, ileal IAP was increased in FAA+ compared with FAA- pigs in both studies ( $P < 0.05$ ) regardless of adaptation time ( $P > 0.05$ ). Ileal IAP activity correlated with systemic markers of antioxidant defense, which highlights the enzyme's role in attenuating systemic infection. These results demonstrate a positive effect of FAA supplementation, but not adaptation period, on ileal alkaline phosphatase activity in *Salmonella*-challenged pigs, which may be associated with improvements in antioxidant balance.

## INTRODUCTION

Intestinal alkaline phosphatase (IAP) is a brush border enzyme associated with positive effects on gastrointestinal health. Increased IAP activity may limit the translocation of pathogenic gut bacteria to surrounding lymphoid tissues. Amino acids have been shown to perform several functional roles, particularly in supporting gut health and development, which may be associated with improved IAP activity. We recently reported that pigs fed functional amino acids (FAAs; Thr, Met, and Trp at 120% of requirements) were better equipped to cope with a *Salmonella* challenge and the benefits were greater when pigs were fed FAA for a longer period pre-challenge. As the positive effects were associated with improved gut health and reduced pathogen colonization, we hypothesized that they may be mediated by or associated with increased IAP activity. The objective of this study was to determine the effects of FAA supplementation and adaptation period on IAP activity in *Salmonella*-challenged pigs.

## EXPERIMENTAL PROCEDURES

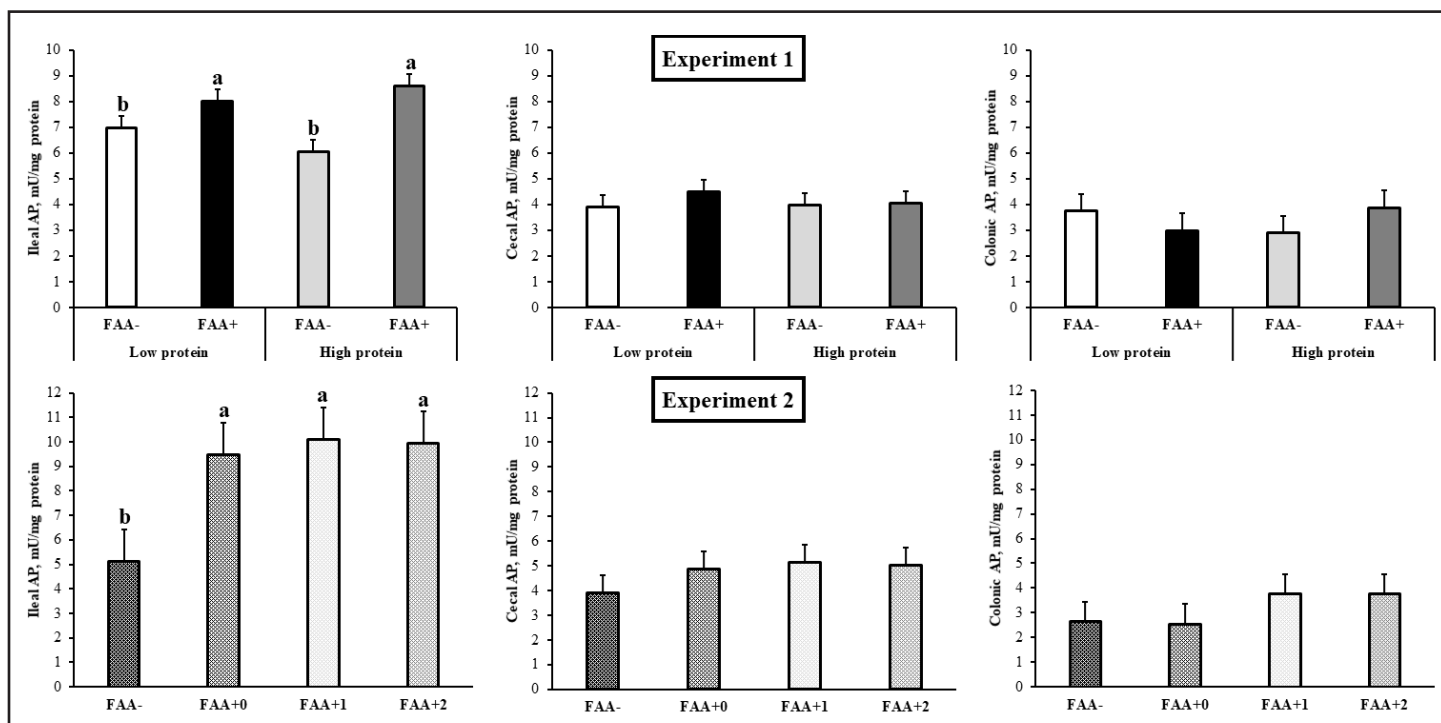
**Experiment 1:** After weaning, 32 mixed-sex piglets (13.9 ± 0.8 kg initial body weight [BW]) were individually housed and randomly assigned to 1 of 4 treatments in a 2 × 2 factorial arrangement in a randomized complete block design (RCBD). Dietary treatments consisted of a low (LP) or high protein (HP) content and basal (FAA-) or functional AA profile (FAA+; Thr, Met, and Trp at 120% of requirements) as factors. After a 7-d adaptation period pigs were inoculated with solution containing *Salmonella* Typhimurium (ST; 3.3 × 10<sup>9</sup> CFU/mL).

**Experiment 2:** A total of 32 mixed-sex weanling (weaning age: 25 d) pigs (11.6 ± 0.30 kg) were housed individually and randomly assigned to 1 of 4 treatments in an RCBD for 21 d. Treatments consisted of FAA- fed throughout the experimental period (FAA-), FAA+ fed only in the postinoculation (FAA+0), for 1 wk pre- and post-inoculation (FAA+1), or throughout the experimental period (FAA+2). After a 14-d adaptation period pigs were inoculated with ST (2.2 × 10<sup>9</sup> CFU/mL).

Plasma alkaline phosphatase was measured on days 0 and 7 post-inoculation in experiment 1, and IAP (ileum, cecum, and colon) was measured on d 7 post-inoculation in experiments 1 and 2. Correlations among ileal IAP and serum albumin and haptoglobin, plasma superoxide dismutase (SOD), malondialdehyde (MDA), and reduced:oxidized glutathione, ileal myeloperoxidase, ST shedding and ileal colonization, and post-inoculation average daily gain, feed intake (ADFI), and gain:feed were also analyzed.

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**Figure 1.** Intestinal alkaline phosphatase (IAP) activity in ileum, cecum, and colon of *Salmonella*-challenged pigs in Expt. 1 and 2. Within intestinal section, bars with different letters differ significantly ( $P < 0.05$ ). Values are least squares means;  $n=8$  pigs/treatment.

## RESULTS AND DISCUSSION

Plasma alkaline phosphatase was decreased (day 0 vs. 7;  $152.0 \pm 9.4$  vs.  $67.6 \pm 10.0$  IU/L) post-inoculation with ST ( $P < 0.05$ ). Overall, pigs fed LP-FAA+ had increased plasma alkaline phosphatase activity compared with pigs fed LP-FAA- ( $151.8 \pm 14.4$  vs.  $82.2 \pm 13.8$  IU/L) with HP-FAA- ( $100.8 \pm 13.4$  IU/L) and HP-FAA+ ( $104.2 \pm 13.8$  IU/L) pigs being intermediate ( $P < 0.05$ ).

IAP results for experiments 1 and 2 are shown in Figure 1. In experiment 1, pigs fed FAA+ had greater IAP activity in ileum tissue compared with pigs fed FAA- regardless of dietary CP content ( $P < 0.05$ ). There was no effect of FAA supplementation or dietary CP content on IAP activity in cecum and colon tissues ( $P > 0.10$ ). In experiment 2, FAA- pigs had lower IAP activity in ileum tissue compared with FAA+0, FAA+1, and FAA+2 pigs ( $P < 0.05$ ). Likewise, there was no effect of treatments on IAP activity in cecum and colon tissues ( $P > 0.10$ ).

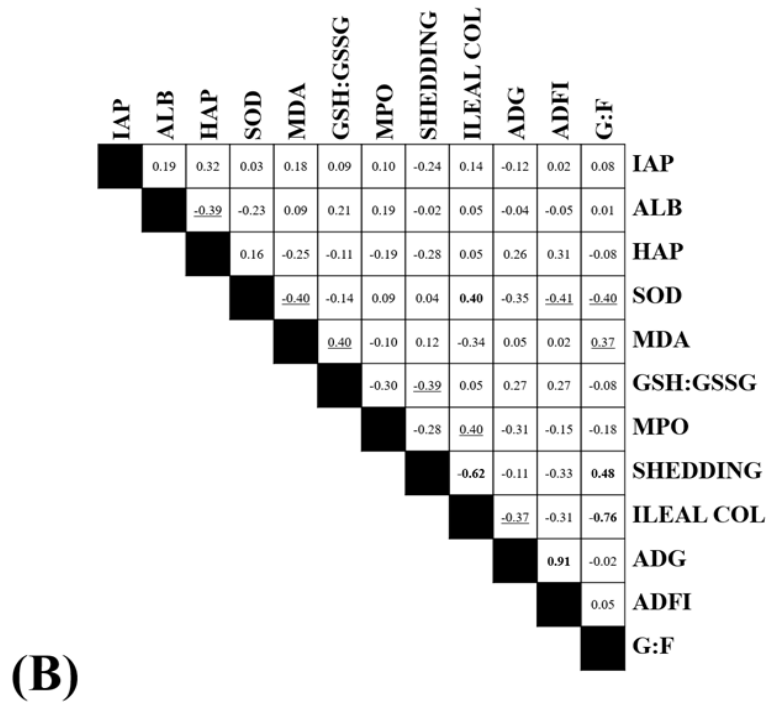
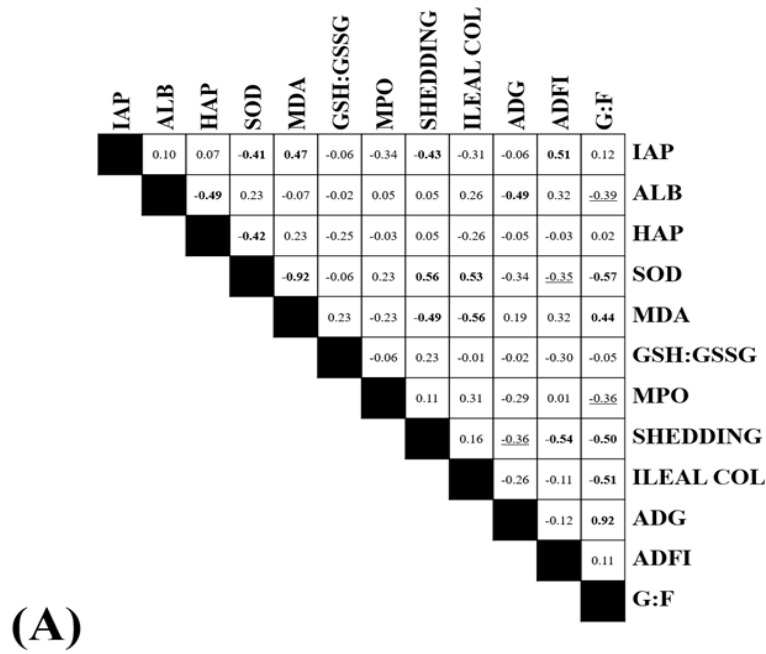
In experiment 1, IAP was positively correlated with MDA and ADFI and negatively correlated with SOD and ST shedding (Figure 2A). Other correlations among the measured parameters in both experiments can be found in Figure 2.

## IMPLICATIONS

Collectively, our findings shed light on IAP modulation by diet and its relationship with systemic homeostasis. This study is the first to identify that ileal IAP activity is increased following FAA supplementation in ST-challenged pigs, regardless of adaptation period. The lack of effect of FAA adaptation period on IAP activity may be explained by antioxidant balance being more associated with AA intake than length of feeding. This study also showed that ileal IAP activity correlated with systemic markers of antioxidant defense, which highlights the enzyme's role in attenuating systemic infection. Overall, the development of feeding strategies with positive effects on IAP activity is of interest, due to the enzyme's central role on the gut and whole-body homeostasis and health.

## ACKNOWLEDGEMENTS

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**Figure 2.** Correlation matrix among intestinal alkaline phosphatase (IAP), serum albumin (ALB) and haptoglobin (HAP), plasma superoxide dismutase (SOD), malondialdehyde (MDA), and reduced:oxidized glutathione (GSH:GSSG), ileal myeloperoxidase (MPO), Salmonella shedding (SHEDDING) and ileal colonization (ILEAL COL), average daily gain (ADG), average daily feed intake (ADFI), and gain:feed (G:F) in Salmonella-challenged pigs in Expt. 1 (A) and 2 (B). Values are the Pearson correlation coefficient (r value); n=32 pigs. Bold values are statistically significant (P < 0.05) and underlined values tend to be statistically significant (P < 0.10).