# Developing an indigestible protein index to investigate the effects of dietary protein in pigs

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# **APPLICATION FOR PRODUCERS**

Ask your nutritionist to look at indigestible protein rather than total protein content of your nursery diets to reduce potential negative effects of high protein content, especially when feeding plant-based diets.

## **SUMMARY**

It is unclear what causes the inconsistent negative effects of high protein diets in nursery pigs, but it could be due to protein source or indigestible protein (IDP) content. A literature search and meta-analysis was done that included ninety-four studies reporting growth performance [average daily gain (ADG), average daily feed intake (ADFI), gain:feed (GF), initial and final body weight (BW)] and duodenal histomorphometrics [villus height (VH), crypt depth(CD), and villus height:crypt depth ratio (VCR)] variables of pigs fed different protein content (CP) and sources in the diet. Diets were recalculated to generate the IDP content from each study. Studies were grouped by similarity in performance in two clusters [C1= mid-late nursery phase (9-18 kg) and C2= immediate post-weaning phase (6-9 kg)]. Quadratic response plateau (QRP) models were fitted to assess the interrelationships between ADG, ADFI, GF, VH, CD, and VCR with the dietary CP or calculated IDP content within cluster. The results suggest that dietary IDP may be a better indicator of potential negative dietary effects than total protein, particularly in the immediate post-weaning phase. Dietary IDP is affected more by the inclusion of plant-based ingredients than animalbased ingredients, most likely as a result of lower digestibility of protein in plant-based ingredients.

# **INTRODUCTION**

Enteric pathogens, such as E. coli and Salmonella, are a major cause of post-weaning diarrhea. High protein diets likely increase susceptibility to enteric pathogens and are a predisposing factor in the development of post-weaning diarrhea. While much work has been done on the concept of dietary protein content and performance and health of nursery pigs, it is still not clear what factors are primarily responsible for the negative response to high protein diets. While there is a general trend for reduced incidence of diarrhea with decreasing dietary protein content, this is not consistent across studies when examining the same protein content, suggesting a factor other than simply total dietary protein content is involved, such as protein type or indigestible protein (IDP) content. Dietary indigestible protein content (i.e., protein not absorbed in the small intestine) is available for microbial fermentation in the hindgut and may have negative effects on gut health. Having said that, while the production/presence of fermentation metabolites have been suggested as a potential mechanism for the negative effects of protein, their actual contribution to intestinal health remains unclear in addition to a lack of consensus on how to evaluate intestinal health in general.

The concept of indigestible protein content is relatively new and, therefore, has not been specifically examined in past studies. Currently, diet formulations are based on meeting nutrient requirements and, in the case of nursery pig diets, to limit crude protein content. The objective of this study was to develop and validate, by investigating performance and gut structure outcomes, an index of indigestible protein content (IDP) in weaned pigs. This study was the first to characterize and validate the effect of indigestible protein on measures of animal health and performance. We hypothesized that the dietary IDP would be more predictive of performance and gut health outcomes than dietary CP content, as the IDP portion of dietary protein intake is available for fermentation and production of harmful metabolites.

The overall goal of this and upcoming studies is to provide mitigation strategies to mitigate negative effects of indigestible protein and enhance the ability to utilize feedstuffs common in Saskatchewan while improving sustainability of pork production.

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#### **EXPERIMENTAL PROCEDURES**

A thorough literature search provided ninety-four studies of pigs fed different protein content (CP) and sources in the diet that reported growth performance [average daily gain (ADG), average daily feed intake (ADFI), gain:feed (GF), initial body weight (BW) and final BW] and duodenal histomorphometrics [villus height (VH), crypt depth (CD), and villus height:crypt depth ratio (VCR)]. Ingredient composition of each diet within each study was incorporated into a common dataset of ingredient nutrient composition, with values obtained from NRC (2012) and Evonik AminoDat. Nutrients reported by studies included dry matter (DM), CP, fiber (CF), standard ileal digestible Lys (SID Lys), and net (NE) energy. The same nutrients were recalculated (DM-c, CP-c, CF-c, SID Lys-c, and NE-c). An index of IDP was generated by subtracting the standard ileal digestible protein (SID CP-c) from CP-c. The inclusion of plant and animal protein sources as well as synthetic amino acids were recorded.

Studies were grouped by similarity in performance in two clusters (C1 and C2) by hierarchical clustering on principal components (HCPC). The effects of cluster were investigated. Quadratic response plateau (QRP) models were fitted to assess the interrelationships between ADG, ADFI, GF, VH, CD, and VCR with the dietary CP or calculated IDP content within cluster.

#### RESULTS AND DISCUSSION

Pigs in cluster 1 and 2 had an average initial BW of 9.78 and 6.54 kg, respectively, corresponding to piglets in the mid-late nursery phase (9-18 kg) and the immediate post-weaning phase (6-9 kg), respectively. Growth performance parameters such as ADG, ADFI, and GF of pigs in C2 were lower than pigs in C1, and duodenal VH and CD were higher in C2 pigs than in C1 pigs because pigs in C2 were younger and the intestinal morphology changes as pigs grow older.

Figure 1 shows the expected linear relationship between the reported dietary CP content in each study and the CP-c and SID CP-c. A linear and quadratic relationship was observed between CP content in each study and calculated IDP content in diets, indicating that IDP increased with greater dietary CP content, regardless of the source of protein. Dietary CP content above 23 % resulted in a marked increase in IDP content of diets. However, it is uncommon for commercial swine diets to have CP inclusion levels above 22 % due to the high cost of protein-rich ingredients. When sources of IDP in diets were investigated, plant-based protein sources (e.g., soybean meal, wheat, barley) contributed the highest proportion of IDP in diets vs. animal-based protein sources and synthetic amino acids.

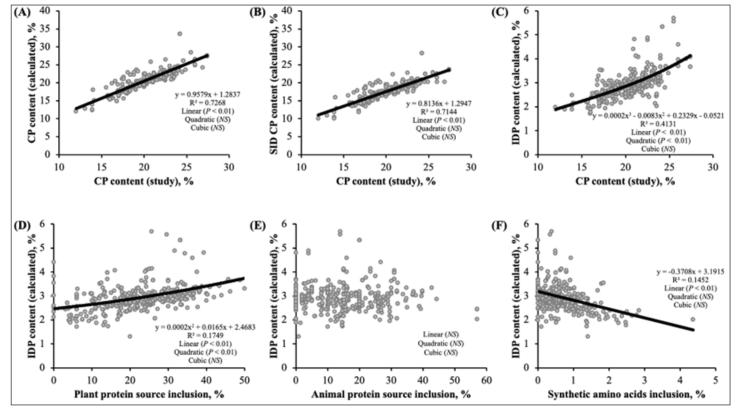


Figure 1. Calculated dietary protein (CP; A), standard ileal digestible CP (SID CP; B) and indigestible protein (IDP; C) contents according to CP content reported by individual studies. Calculated IDP content according to the inclusion of plant-based protein sources (D), animal-based protein sources (E), and synthetic amino acids (F).

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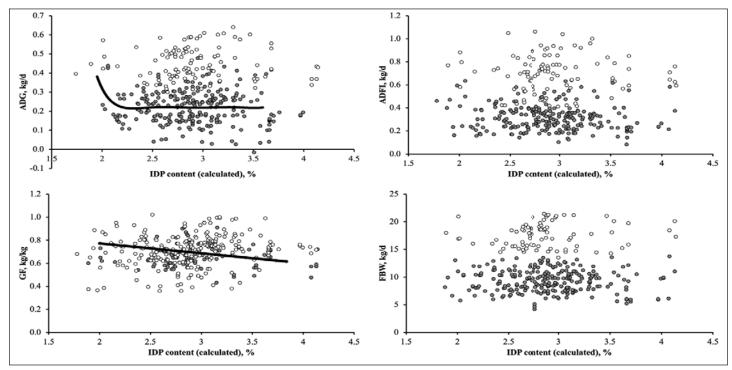


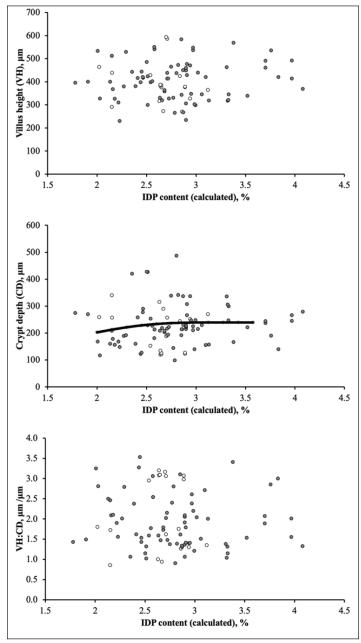
Figure 2. The quadratic break-point model analysis estimates for performance variables as a function of calculated indigestible protein content (IDP) in Cluster 1 (white circles) and 2 (dark grey circles) pigs. The quadratic break-point model was significant (y = 2.68x2 - 11.75x + 13.11) for average daily gain (ADG; solid black line; P < 0.05) in Cluster 2 pigs only, with breakpoint of 2.20 % IDP content for minimum ADG at 0.22 kg/d (P < 0.01). There was no breakpoint or plateau achieved (P > 0.05) for gain:feed ratio (GF), which was significant for linear term in Cluster 2 pigs only (P < 0.09x2 + 0.09x2 +

There was a tendency for CP content to affect ADG and CD in pigs. An increase in CP gradually reduced ADG until a plateau was reached at 23.8% CP and 0.27 kg/d ADG. Our linear mixed model and QRP did not reveal any effects of CP content on other performance parameters. There was no relationship between dietary CP and duodenal morphology except for CD. An increase in CP resulted in a gradual reduction in CD until a plateau was reached at 17.4% CP and 223.8 µm crypt depth.

Based on IDP content, there was an interaction between IDP and cluster for ADG and GF, where an increase in the IDP content resulted in a sharp decline in ADG of pigs in C2 with a breakpoint at 2.2 % and a linear decrease in GF with no plateau (Figure 2). This suggests that the IDP index is more accurate than CP content in detecting relationships between negative effects of protein and performance parameters, particularly in piglets in the immediate post-weaning phase. An interaction was observed between IDP content and cluster for CD, where increasing IDP resulted in an increase in the duodenal CD in C2 pigs (Figure 3). No effects were observed on VH and VCR. An increase in CD is indicative of stress in the gut environment. The inclusion of high IDP diets during the immediate post-weaning phase could lead to the microbial breakdown of proteins in the hindgut resulting in the production of harmful metabolites such as branched-chain fatty acids, ammonia, biogenic amines, phenols, and hydrogen sulphide.

"An increase in dietary indigestible protein suggests a greater amount of protein is available as substrate for microbial fermentation in the hindgut."





**Figure 3.** The quadratic break-point model analysis estimates for duodenal histomorphometric variables as a function of calculated indigestible protein content (IDP) in Cluster 1 (white circles) and 2 (dark grey circles) pigs. The quadratic break-point model was significant (y = -38.45x2 + 228.90x – 101.60) for crypt depth (CD; solid black line; P < 0.05) in Cluster 2 pigs only, with breakpoint of 2.97 % IDP content for maximum CD at 239.10  $\mu$ m (P < 0.01). There was no breakpoint or plateau achieved (P > 0.05) for villus height (VH) or VCR in Cluster 1 or 2 pigs which were not significant for linear, quadratic, or cubic term (P > 0.05).

#### **IMPLICATIONS**

- Dietary IDP may be a better indicator of potential negative dietary effects than total protein, however, data on protein effects in the hindgut are limited. Considering IDP is a measure of the amount of protein that is not digested prior to the terminal ileum, an increase in dietary IDP suggests a greater amount of protein that is available as substrate for microbial fermentation in the hindgut.
- Dietary IDP is affected more by the inclusion of plant-based ingredients than animal-based ingredients, most likely as a result of lower digestibility of protein in plant-based ingredients.
- It appears that younger animals (i.e., immediately postweaning) are more likely to experience negative effects of IDP.

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