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## Assessing the effect of dietary inulin supplementation on gastrointestinal fermentation, digestibility and growth in pigs: A meta-analysis

B.U. Metzler-Zebeli<sup>a,\*</sup>, P. Trevisi<sup>b</sup>, J.A.M. Prates<sup>c</sup>, S. Tanghe<sup>d</sup>, P. Bosi<sup>b</sup>, N. Canibe<sup>e</sup>, L. Montagne<sup>f</sup>, J. Freire<sup>g</sup>, Q. Zebeli<sup>h</sup>

<sup>a</sup> University Clinic for Swine, University of Veterinary Medicine Vienna, 1210 Vienna, Austria

<sup>b</sup> Department of Agricultural and Food Science (DISTAL) - University of Bologna, 40127 Bologna, Italy

<sup>c</sup> CIISA, Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, Alto da Ajuda, 1300-477 Lisbon, Portugal

<sup>d</sup> Institute of Animal Nutrition and Functional Plant Compounds, University of Veterinary Medicine Vienna, 1210 Vienna, Austria

<sup>e</sup> Department of Animal Science, Aarhus University, 8830 Tjele, Denmark

<sup>f</sup> PEGASE, Agrocampus Ouest, INRA, 35590, Saint-Gilles, France

<sup>g</sup> LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

h Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, Vetmeduni Vienna,

Veterinaerplatz 1, 1210 Vienna, Austria

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#### ABSTRACT

Inulin has been reported to improve the homeostasis in the gastrointestinal tract (GIT) of pigs by modulating the intestinal microbiota and fermentation. The present study aimed to quantify the relationship between dietary inulin and microbial response variables in digesta from the GIT and feces of weaned, growing and finishing pigs using a meta-analytical approach. We further examined the effect of dietary inulin on the coefficients of ileal (CIAD) and total tract apparent digestibility (CTTAD) of nutrients and ADG. Pig's starting body weight was considered the main inclusion criterion. Missing information about explanatory variables and few values available for response variables reduced the number of studies included. From the 33 included articles published between 2000 and 2016, individual sub-datasets for fermentation metabolites, bacterial abundances, CIAD, CTTAD and performance were built. Prediction models on the effect on inulin were computed accounting for inter- and intra-study variability. Dietary inulin levels ranged from 0.1 to 25.8%, whereby the median and mean inulin levels were 0.1-2% and 3-4%, respectively. Few of the investigated fermentation response variables were influenced by dietary inulin. Strong negative relationships were found between dietary inulin and gastric pH in weaned pigs ( $R^2 = 0.81$ ; P < 0.001; n = 12), colonic enterobacteria ( $R^2 = 0.50$ ; P < 0.001; n = 19) and fecal lactobacilli ( $R^2 = 0.41$ ; P < 0.001; n = 26) throughout all production phases, whereas observed negative relationships between inulin and colonic bifidobacteria and fecal enterobacteria and *Escherichia coli* were of minor physiological relevance (P < 0.05). Moreover, increasing inulin levels negatively correlated with the CTTAD of crude protein ( $R^2 = 0.83$ ; P < 0.001; n = 15), but they did not influence average daily gain of pigs. Best-fit models indicated that dietary crude protein amplified the effect of inulin on CTTAD of crude protein and gastric pH, but counteracted the inulin effect on fecal E. coli (P < 0.05). Accordingly, both pig's

\* Corresponding author.

E-mail address: barbara.metzler@vetmeduni.ac.at (B.U. Metzler-Zebeli).

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Abbreviations: ADG, average daily gain; BW, body weight; CFU, colony forming units; CIAD, coefficient of ileal apparent digestibility; CP, crude protein; CTTAD, coefficient of total tract apparent digestibility; DM, dry matter; FISH, fluorescence-in-situ-hybridization; GIT, gastrointestinal tract; NDF, neutral detergent fiber; RMSE, root mean square error; SAS, statistical analysis system; SE, standard error; VIF, variance inflation factor; VFA, volatile fatty acids

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body weight and inulin decreased gastric pH and fecal lactobacilli but counteracted the inulin effect on colonic bifidobacteria and fecal *E. coli* (P < 0.05). In conclusion, this study supported a stimulatory effect of dietary inulin on gastric acid secretion, which may be favorable GIT health in weaned pigs. However, due to limiting information provided in the original studies, like dietary fructans or fibers, low numbers of observation and low inulin levels, relationships should be regarded as trends.

#### 1. Introduction

The ban of antimicrobial growth promoters in the EU has caused an overall high interest in alternative feeding concepts and products to enhance disease resistance and support growth performance in pig production (Metzler et al., 2005; Gallois et al., 2009). Especially, dietary inclusion of functional ingredients and supplements, such as prebiotics, are of persistent interest to maintain production efficiency in pigs (de Lange et al., 2010; Pluske, 2013). Among others, considerable attention has been paid to the nondigestible oligosaccharide inulin for which health benefits around weaning have been reported (Modesto et al., 2009; Jensen et al., 2011). Inulin encompasses all  $\beta$ -(2,1)-linear fructans of varying chain lengths (Roberfroid, 2007) and can be found in several fruits and vegetables, like asparagus, leek, onions, banana, wheat and garlic, and in higher concentrations in chicory (Compositae family) and Jerusalem artichoke (Helianthus tuberosus). Industrially, inulin is predominantly extracted from chicory (Roberfroid, 2005; Kleessen et al., 2007; Ramnani et al., 2010). Inulin-type fructans are resistant to hydrolysis by enzymes in the small intestine, but are rapidly fermented by saccharolytic bacteria including bifidobacteria and lactobacilli (Konstantinov et al., 2004; Kleessen et al., 2007; Kolida and Gibson, 2007; Liu et al., 2016). Promotion of these bacterial genera by dietary inulin may suppress the growth of enterotoxigenic Escherichia coli, thereby lowering the risk for post-weaning diarrhoea in piglets (Halas et al., 2009). Although inulin has been consistently shown to exert prebiotic functions in the human hindgut from infants to the elderly (Kelly, 2008; Stiverson et al., 2014; Liu et al., 2016), the reported effects in pigs were more contradictory (e.g., Verdonk et al., 2005; Loh et al., 2006). Analysis of digesta from various segments of the small and large intestines revealed measureable inulin concentrations in the jejunum and ileum, but not in the cecum and colon of pigs (Branner et al., 2004; Böhmer et al., 2005), which may indicate a reduced capacity of inulin to modify porcine hindgut fermentation. Yet, beneficial effects on the microbial composition in the colon or feces were found (e.g., Janczyk et al., 2010; Gao et al., 2015). Likewise, modulation of the gastrointestinal tract (GIT) microbiota by dietary inulin has been assumed to be most effective in newly weaned pigs (Konstantinov et al., 2004; Janczyk et al., 2010); however, enhanced hindgut fermentation was lately reported for finishing pigs receiving a diet with 5% inulin (Gao et al., 2015).

In general, qualitative reviews on alternative feed additives have repeatedly addressed the effect of dietary supplementation of inulin on GIT health in weaned and growing pigs (e.g., Verdonk et al., 2005; de Lange et al., 2010). Changes in direct (type and dose) and indirect factors (e.g., age of the animal) can cause varying results across research studies which cannot be considered in qualitative reviews (Sales, 2014). Also, it is difficult to examine all potential influencing factors in one single experiment. To address this complexity, a meta-analysis of published studies is an efficient way to evaluate different factors by generalizing the overall treatment effect (Charbonneau et al., 2006). So far, results for inulin research in pigs were not investigated using a meta-analytical approach to summarize results across individual experiments and therefore across a wide range of experimental conditions. With the inconsistency obtained in empirical studies on the effects of inulin on GIT fermentation, the current meta-analysis was designed to quantify the effect of dietary inulin supplementation on fermentation metabolites and bacterial abundances in the GIT of weaned, growing and finishing pigs. Additionally, effects of inulin on growth performance and coefficients of ileal (CIAD) and total tract apparent digestibility (CTTAD) of nutrients and dry matter (DM) were assessed using data from the studies included in the datasets for microbial fermentation and abundances.

#### 2. Materials and methods

#### 2.1. Literature search

A literature search was conducted using the public search generators Pubmed, Google Scholar, Web of Science, and Scopus. The main aim of the present study was the impact of dietary inulin supplementation on microbial abundances and fermentation metabolites in the GIT of pigs. For that reason, research articles in scientific journals on controlled experiments investigating the effect of inulin supplementation from purified or natural sources on intestinal fermentation and bacterial abundance that appeared between the years 2000 and January 2016 were primarily considered for data extraction. The following search terms in different combinations were applied to identify adequate articles: inulin, chicory, chicory root, Jerusalem artichokes, pig, piglet, swine, gut, large intestine or individual segments, stomach, fermentation, microbial metabolites, volatile fatty acids (VFA) and short-chain fatty acids, lactate, bacteria, microbiota, microflora, and microbiome.

#### 2.2. Selection of studies

Stringent criteria were in place whether published experiments were included or excluded in this study. Quality assessment criteria included information about dietary composition, inulin level and source (purified concentrate or natural source), type of pigs,

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