Manuscript Details

Manuscript number	ANIFEE_2016_564
Title	Assessing the effect of dietary inulin supplementation on gastrointestinal fermentation, digestibility and growth in pigs: a meta-analysis
Article type	Research Paper

Abstract

Inulin has been reported to improve the homeostasis in the gastrointestinal tract (GIT) of pigs by stimulating modulating the intestinal microbiota and fermentation. The aim of this study was 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 to 2% and 3 to 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 ($R2 = 0.81$; $P < 0.001$; $n = 12$), colonic enterobacteria ($R2 = 0.50$; $P < 0.001$; $n = 19$) and fecal lactobacilli ($R2 = 0.41$; $P < 0.001$; $n = 26$) 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 ($R2 = 0.83$; $P < 0.001$; $n = 15$), 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 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 that dietary inulin can stimulate gastric acid secretion which may be favorable GIT health in weaned pigs. However, meta-regressions did not support that inulin promotes the bacterial groups previously associated with porcine GIT health, such as lactobacilli and bifidobacteria.

Keywords	Inulin; gastro-intestinal tract; Fermentation; microbiota; meta-Analysis; pig
Taxonomy	Domestic Animals, Non-Ruminant Nutrition, Animal Dietary Supplement
Manuscript category	Non-ruminant

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Suggested reviewers	Jos Houdijk

Submission Files Included in this PDF

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Cover letter_ANIFEE_2016_564_R1.docx [Cover Letter]

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ANIFEE_2016_564_Author reply.docx [Response to Reviewers]

ANIFEE_2016_564_revision_1.pdf.docx [Manuscript File]

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Vienna, 23rd of March, 2017

Re: Submission of revised manuscript ANIFEE_2016_564

Dear Dr. de Blas,

We are grateful for the opportunity to revise our manuscript ANIFEE_2016_564 entitled "Assessing the effect of dietary inulin supplementation on gastrointestinal fermentation, digestibility and growth in pigs: a meta-analysis".

All reviewer comments were addressed point-by-point in the author reply and line numbers in the revised manuscript were provided where changes can be found. Changes made in the manuscript text are highlighted in yellow. We hope that we could improve our manuscript to meet the high standards of Animal Feed Science and Technology.

Sincerely yours,

Barbara Metzler-Zebeli

Declaration of interests

The authors declare no conflict of interest. Moreover, all authors listed have contributed to the work, all authors have read and approved the final manuscript submitted to Animal Feed Science and Technology. No part of the work has been published before and is not under consideration for publication elsewhere.

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ANIFEE_2016_564

AUTHORS: Dear Dr. de Blas,

We are grateful for the opportunity to revise our manuscript. All reviewer comments were addressed point-by-point in the author reply and line numbers in the revised manuscript were provided where changes can be found. Changes made in the manuscript text are highlighted in yellow. We hope that we could improve our manuscript to meet the high standards of Animal Feed Science and Technology.

Sincerely yours,
Barbara Metzler-Zebeli

Reviewer 1:
General comments:
This is an interesting paper, comprehensively statistically analysed, examining the trans-study effects of added inulin in diets for pigs on a number of different variables.

AUTHORS: We would like to thank the reviewer for the helpful comments.

Major comments are as follows:
The main weakness of the paper is the lack of statistical accountability reflecting endogenous fructans levels in the diets used. Whilst the authors' duly acknowledge this (L353-355), strong doubt remains concerning the veracity of the data derived without taking endogenous fructans levels into account. The authors have made other assumptions (L181-183) in the paper; hence, the authors should calculate (based on 'best-bet' book or feed matrix values) endogenous fructans levels in the diets used in the studies analysed, and redo the analyses.

AUTHORS: Thank you for this comment. The strength of a meta-analysis is that it takes into account effects within treatments of a study and between studies. Each study had a 'control treatment' and the 'supplemented inulin treatment'. Our meta-analytical approach weighted the treatment to the corresponding control within the individual studies. So, the fructan content of the basal diet did not matter as each treatment effect was weighted to the respective control. In this way, the 'native' fructan content of the diet was balanced as it is clear that the native fructan content of the basal diet in the different studies varied. We modified the sentence in the Discussion to clarify that we could not distinguish the effect of the supplemented inulin from that of the naturally occurring fructan levels in the basal diet due to the fact that insufficient information was provided in the original studies (New Line 350-354)

As alluded to below (L119), the authors only searched for 'microbiota' whereas the terms flora and microbiome are often used interchangeably with microbiota. The authors need to ensure that no publications were omitted because of this.

AUTHORS: The literature was searched using other terms for "microbiota" such as microflora, and microbiome. In addition, literature was searched again using only the

search terms inulin and pig. According to this search, our original datasets comprised all relevant papers. This additional search was amended in the Materials and Methods section 2.1 Literature search (New Line 120).

As alluded to below (L151), the authors don't provide sound justification for using dietary crude protein content as a major criterion.

AUTHORS: A justification for using dietary crude protein content as a major criterion was provided (New Line 151-153). The main reason was that studies used different protein levels despite similar production stages and starting BW. Since the dietary protein content can influence the intestinal fermentation, digestibility and growth performance, it was one of the few prediction variables that were available for the studies included.

Specific comments:

1. L32-L33: '..appears to improve..', and then '…inconsistent results..'. These two parts of the sentence are in congruence, i.e., one cannot say that inulin improves but then that improvement is inconsistent. This needs rewriting.

AUTHORS: Thank you for pointing this out. The sentence was modified (New Line 32-33).

2. L32, and throughout paper: the authors need to use a consistent terminology for gastrointestinal tract/gut/intestinal etc. There are numerous 'versions' used in the paper. Suggest use gastrointestinal tract (GIT), abbreviating to GIT thereafter, unless there's more specific detail provided.

AUTHORS: Gastrointestinal tract (GIT) was used as consistent term throughout the revised manuscript.

3. L67, L86 etc.: what is meant by 'beneficial'? This is a term used loosely in the scientific literature, and is generally unhelpful and sometimes meaningless. The authors are strongly encouraged to define this more precisely, or use different language altogether.

AUTHORS: The term 'beneficial bacteria' has been removed.

4. L84: isn't it the fructans that are measured, rather than inulin?

AUTHORS: Indeed fructans are measured. However, in the studies used in this meta-analysis, the inulin level was reported, but in most cases not the fructan content or chain length of the inulin used. This is why we used 'inulin level' and not 'fructan level'.

5. L119: did the authors also use the terms flora and microbiome? These terms are often used interchangeably with microbiota. I think this is a key issue, and one that may require the authors to research the literature.

AUTHORS: Please see our comment above. We double-checked the literature with other search terms for microbiota and bacteria to ensure that we did not miss an original article.

6. L146, L159 etc., and throughout: the authors (in L131) define CIAD and CTTAD, but then use nutrient digestibility. Moreover, DM is not a nutrient. The authors should use consistent terminology throughput the paper.

AUTHORS: Consistent terminology using CIAD and CTTAD was used throughout the manuscript. CIAD and CTTAD of DM was mentioned in addition to those of nutrients.

7. L151: why was dietary crude protein content chosen? This seemingly is a major determinant of the outcomes and conclusions (e.g., L459) yet the reader is provided with little reasoning for its inclusion.

AUTHORS: Please see our reply under "major comments".

8. L176: what serotype(s) of *E. coli*? Please define.

AUTHORS: In most studies used in this meat-analysis, culturing was done using selective media for the enumeration of *E. coli* but no further culturing on strain/serotype-selective media was performed.

9. L181-183: evidence must be provided by the authors' to substantiate the assumption that CFUs and gene copy numbers 'approximately correspond to each other'.

AUTHORS: Evidence was provided (New Line 182-184).

10. L204: write as, ' MEANS procedure of..'

AUTHORS: Done as suggested (New Line 204-205).

11. L238-239: further to previous comments; I am unconvinced that just because dietary crude protein content was available for all response variables, then it should be used. Where is the hypothesis justifying its inclusion? A physiological basis for its inclusion needs to be provided.

AUTHORS: Please see our reply under "major comments". The dietary CP level varied within production phases despite similar starting BW. As the CP level can influence all of the dependent variables investigated in the present meta-analysis, the dietary CP level was considered.

12. L353-355: see General comments.

13. L429: pathogenic serotypes?

AUTHORS: Please see our comment above. *Escherichia coli* was enumerated on selective medium but no further differentiation using selective media for the various

serotypes was done in the studies used in this meta-analysis. Also, only 'total *E. coli*' counts were used in this meta-analysis if specific serotypes were determined.

14. Table 1: in line with previous comments, the (base) diet formulation(s) may have a profound impact on the variables' responses. Perhaps total fructans levels should be used rather than added fructans levels?

AUTHORS: It would have been great if the fructan contents were reported in the studies used in this meta-analysis. As this was not the case for many studies, we focused on the supplemented inulin level. Please see also our reply under the "major comments" as the meta-analysis accounted for variation in the basal fructan content of the diets by taking into account the control treatment and the supplemented inulin content when estimating the relationship between a dependent variable and the inulin level.

Reviewer 2

The authors report a meta-analysis designed to establish the benefits of inulin supplementation for pigs on a number of performance and fermentation characteristics. The study is well executed, including the critical approach of paper quality to be included, and the resulting overall insight into inulin benefits is relevant for publication. However, a number of queries have arisen during my review, which I list below for the authors to consider.

AUTHORS: Thank you for the helpful comments to improve the quality of our manuscript.

General

The text contains a number of grammar inconsistencies arising from translation challenges. I consider it not to be the task of a reviewer to list these but encourage the authors to have the manuscript read by native English speakers, with experience in publication in this area of research.

AUTHORS: Thank you for pointing out grammar inconsistencies. The manuscript was checked by a native American English speaker and we hope that this person did not oversee typos and grammar issues.

Major comments

2. L218-242. Two approaches were used to assess impact of start BW, diet CP and inulin level, i.e. by inclusion as random effects and through backward elimination. It is not clear what the benefit is of doing both, especially because outcomes seem to vary. Would the conclusions be any less if only one of them is chosen and presented?

AUTHORS: The rationale behind assessing the impact of the start BW was to consider maturational changes from weaned to finisher pigs. Because studies used different crude protein (CP) levels within the same production stage and for similar starting BW, the dietary CP content was included as another predictor variable as the dietary CP content can influence the intestinal fermentation, digestibility and growth performance. Taking both effects into account in the modeling leads to a more powerful estimation of the response variables. We used the VIF in order to avoid potential multi-collinarity among predictor variables, start BW, dietary CP, dietary inulin level and quadratic inulin level.

Also, the backward elimination analysis demonstrated quadratic relationships between inulin level and a number of read outs. What is the biological meaning of this quadratic relation ((L312, 318)?

AUTHORS: The biological meaning of a quadratic relation is that there is a maximum supplementation level above which no further increase or decrease in the response variable can be expected (asymptotic approximation).

4. Where significant relations were found between level of inulin and read outs, the prediction of the read out at 3% inulin was presented. What was the rational of choosing 3%, given that the vast majority of studies as you indicated had levels of inulin between 0.1 and 2%?

AUTHORS: It is true that median levels of inulin ranged between 0.1 and 2 %. The mean (which was about 3 to 4 % inulin) rather than median supplementation level was used for the prediction as very low levels of inulin may be insufficient to produce an effect.

6. Related to the above, where a significant slope was observed, it would be useful to speculate whether the magnitude of effect is also biologically relevant. For example, would a reduction of 0.19 log units in faecal E. coli be of relevance?

AUTHORS: It was indicated in the Discussion if the magnitude was of significance relevance (New Line 380 and 429-433).

8. You state that "an insufficient level of inulin supplementation to modulate gastrointestinal fermentation and bacterial abundances was probably a crucial factor determining the results obtained". Whilst this is not disputed, could your data indicate what level would be sufficient to observe responses?

AUTHORS: A sentence was added to discuss at which inulin level responses could be expected (New Line 346-350).

10. Inulin consists of variable numbers of fructose units, as clearly indicated. This would also include the shorter chain fructo-oligosaccharides. Some of the outcomes of the effects of inulin reported and discussed here accord

with effects of fructo-oligosaccharides, including effects on gastric pH and ileal lactic acid. It would be advisable to bring this up during the discussion.

AUTHORS: Thank you for this comment. Indeed, there are some consistencies when comparing the present relationships for inulin and reports for short-chain fructo-oligosaccharides. However, as the main focus of the present meta-analysis is the long-chain fructan inulin, we prefer mainly comparing the present results with those previously reported for inulin in individual studies.

12. The conclusion that inulin is more effective in younger pigs compared to older ones (L365) is acceptable but it would be appropriate for the authors to speculate why this would be.

AUTHORS: These sentences were modified and some reasoning was provided why younger pigs may benefit more from inulin feeding than older pigs (New Line 355-359).

14. L440. The authors state here that no relation between dietary inulin and ADG could be established. Is it not better to state that the data support the view that "ADG is not sensitive to dietary inulin levels".

AUTHORS: Modified as suggested (New Line 437-438).

Minor comments

Title

L1. The order in which the results are reported are not in line with the order of the parameters in the title. As a consequence, based on the title one would expect greater emphasis on performance, then digestibility and lastly fermentation characteristics. I would suggest to amend the title to reflect this, moreover because the outcome is that inulin did not really affect growth performance in the first place.

AUTHORS: The title was modified accordingly.

Abstract

L32. Please amend abstract as per suggestions from main text where needed.

AUTHORS: The abstract was amended after revising the main text.

Introduction

L62. It is of interest to note that the ban on AGP in Europe is supported by references from Canada and Western Australia. Can a similar reference supporting this position be added coming from an EU-led review?

AUTHORS: References were replaced by references from European authors (New Line 65).

L89. Please include relevant references after "weaned pigs"; the way how this sentence is constructed requests it to be referenced.

AUTHORS: Relevant references were added (New Line 88-89).

Materials and methods

L133. It might be useful to consider how the inulin was included in the test diets, i.e. whether it was exchanged against some ingredients, or diluted a basal diet, or otherwise. This may add some insight into its variation in response.

AUTHORS: The dietary inulin most often replaced one of the main energy feedstuffs, but did not dilute the diets.

L156. A comment or observation rather than anything else but I was rather surprised to learn that 25% of articles had to be excluded as they would not report initial body weight. Could you have considered using these studies for the analyses that did not rely on BW?

AUTHORS: All dependent variables investigated in the present meta-analysis depended either on the age or the BW of the pigs. Therefore, it was obligatory that this information was provided.

L182. What do you mean with "approximately correspond"?

AUTHORS: This sentence was modified for clarification (New Line 182-184)

L198. Is it not better to say that Breed and Sex were not included in the model due to inconsistency of reporting, rather than assuming responses were not affected by Breed and Sex?

AUTHORS: This was modified accordingly (New Line 200-201).

L218. Predictor variables tested were study ? Something missing here.

AUTHORS: This was corrected and the other predictor variables tested were added (New Line 220).

Discussion

L336. Please split the list of authors into those relevant for the small intestine statement and those relevant for the large intestine statement.

AUTHORS: As suggested, the list of authors was split into those relevant for the small intestine and those relevant for the large intestine (New Line 333-335).

L356. I think the first line is not needed, as it destracts from what you want to say; start with "It was".

AUTHORS: The beginning of this sentence has been modified (New Line 360).

L388. This line is not clear. What does "effects of BW and diet CP in conjunction with inulin" mean?

AUTHORS: This sentence has been modified for clarification (New Line 383)

L396. Here you seem to underpin the outcome of the meta-analyses with that of individual studies, which likely were part of the meta-analysis. Is that acceptable? Or were there other reasons to refer to these specific studies?

AUTHORS: It is acceptable to compare the outcome of the meta-analysis with the individual studies included.

L424. In Table 8 and L319, the relationship between faecal lactobacilli is negative. Please check for consistency.

AUTHORS: Thank you. The sentence was corrected (New Line 417).

L421. To what extent it the effect of "maturation" confounded by the effect of increased feed intake?

AUTHORS: The increased feed intake but also the changing dietary composition will have contributed to the maturation of the microbiota. Because the feed intake level was not provided in many studies, we prefer not to speculate about the impact of the feed intake.

L432. Replace "If" with "Whether".

AUTHORS: Changed as suggested (New Line 425).

L435. This is consistent with a large body of evidence from across the world that CP levels can modify faecal coli counts. Perhaps worth highlighting.

AUTHORS: Due the rather low biological relevance of the changes observed, this finding should not be overemphasized.

L441. Something is missing here ". ….. between increasing dietary CIAD of DM……"; something seems missing after "increasing" and before "dietary", as dietary CIAD does not make sense?

AUTHORS: Thank you. “dietary” was deleted to correct the sentence (New Line 439-440).

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

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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,

24 statistical analysis system; SE, standard error; VIF, variance inflation factor; VFA, volatile fatty
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30

ABSTRACT (400 words)

Inulin has been reported to improve the homeostasis in the gastrointestinal tract (GIT) of pigs by stimulating modulating the intestinal microbiota and fermentation. The aim of this study was 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 to 2% and 3 to 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 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 that dietary inulin can stimulate gastric acid secretion which may be favorable GIT health in weaned pigs. However, meta-regressions did not support that inulin promotes the bacterial groups previously associated with porcine GIT health, such as lactobacilli and bifidobacteria.

Keywords: inulin, gastro-intestinal tract, fermentation, microbiota, meta-analysis, pig

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 non-digestible oligosaccharide inulin for which health benefits around weaning have been reported (Modesto et al., 2009; Jensen et al., 2011). Inulin encompasses all β-(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

86 microbial composition in the colon or feces were found (e.g., Janczyk et al., 2010; Gao et al.,
87 2015). Likewise, modulation of the gastrointestinal tract (GIT) microbiota by dietary inulin has
88 been assumed to be most effective in newly weaned pigs (Konstantinov et al., 2004; Janczyk et
89 al., 2010); however, enhanced hindgut fermentation was lately reported for finishing pigs
90 receiving a diet with 5% inulin (Gao et al., 2015).
91 In general, qualitative reviews on alternative feed additives have repeatedly addressed the
92 effect of dietary supplementation of inulin on GIT health in weaned and growing pigs (e.g.,
93 Verdonk et al., 2005; de Lange et al., 2010). Changes in direct (type and dose) and indirect
94 factors (e.g., age of the animal) can cause varying results across research studies which cannot
95 be considered in qualitative reviews (Sales, 2014). Also, it is difficult to examine all potential
96 influencing factors in one single experiment. To address this complexity, a meta-analysis of
97 published studies is an efficient way to evaluate different factors by generalizing the overall
98 treatment effect (Charbonneau et al., 2006). So far, results for inulin research in pigs were not
99 investigated using a meta-analytical approach to summarize results across individual
100 experiments and therefore across a wide range of experimental conditions. With the
101 inconsistency obtained in empirical studies on the effects of inulin on GIT fermentation, the
102 current meta-analysis was designed to quantify the effect of dietary inulin supplementation on
103 fermentation metabolites and bacterial abundances in the GIT of weaned, growing and finishing
104 pigs. Additionally, effects of inulin on growth performance and coefficients of ileal (CIAD) and
105 total tract apparent digestibility (CTTAD) of nutrients and dry matter (DM) were assessed using
106 data from the studies included in the datasets for microbial fermentation and abundances.
107
108 **2. Materials and methods**
109 *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, small 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, body weight (BW) and age of the pigs, number of pigs within treatment groups, duration of the experimental period, experimental design including randomization of treatment groups, description of statistical analysis, and intra-study error (if standard deviation was provided, it was converted into standard error), as well as fermentation metabolites (i.e., volatile fatty acids (VFA) and lactate), pH, and bacterial abundances in digesta of stomach, ileum, cecum, proximal, mid and distal colon and rectum or feces. Studies were also included that investigated the combined effects of inulin with another treatments on the search parameters. From those studies, data for the control without any treatment and the sole inulin treatment were considered, or, if the basal (control) diet already

contained the other alternative feed additive, data for this basal diet without inulin and with inulin were included. Published research studies on in-vitro experiments were excluded.

2.3. Construction of Database

Our search found 45 articles that were eligible for the present meta-analysis by meeting a sufficient number of above mentioned eligibility criteria. Beside the dietary inulin level as main prediction variable and dependent variables microbial abundance, pH and fermentation metabolites in the various GIT segments, given details on pig (breed, age, BW, gender, production stage), experimental design, housing condition, dietary ingredients and chemical composition of diets were extracted from the 45 articles to be considered as probable additional prediction variables in the regression analysis. If provided, average daily feed intake, average daily weight gain (ADG) and CIAD and CTTAD of DM and nutrients were extracted as well.

Careful examination and quality assessment of the dataset, however, showed that predictor variables and dependent variables of interest were not always available across all studies or ill-defined, leading to a large number of missing data. The main criterion to be considered in this meta-analysis was "age" and "start BW" at the beginning of the experiment in order to use these variables as additional predictor variable to consider maturational changes from weaned to finisher pigs. Studies used different crude protein (CP) levels within the same production stage and for similar starting BW. Since the dietary CP content can influence the intestinal fermentation, digestibility and growth performance, this variable was included as further predictor variable. Because "age" and "start BW" of pigs were not provided in all studies but more often "start BW" than "age" was given, we decided to set "start BW" as the required information needed to be provided in the study to remain in the dataset for analysis. Studies that did not provide "start BW" at the beginning of the experimental period were removed from the

158 dataset. Due to this, twelve articles had to be excluded and a total of 33 studies formed the
159 "filtered" dataset which was used to compile the sub-datasets for GIT fermentation metabolites
160 and digesta characteristics (pH and DM), absolute bacterial abundances, growth performance,
161 CIAD and CTTAD of DM and nutrients with data from weaned, growing and finishing pigs. The
162 list of publications from which sub-dataset were built is provided in Table 1. The sub-datasets for
163 GIT fermentation metabolites, pH and absolute bacterial abundances were divided further; one
164 sub-dataset was created for each GIT site.
165 As minimum, three studies were set as requirement to quantify a combined effect size
166 (Lipsey and Wilson, 2001). In addition, a minimum of single observations (treatment means) of
167 10 per dependent variable as well as the respective standard error (SE) of each variable were set
168 as further requirement to measure the combined effect size. According to this requirement,
169 sufficient numbers of studies and observations were available to evaluate ADG, and CIAD of
170 DM and CP, and CTTAD of DM, CP, ash and neutral detergent fiber (NDF) as dependent
171 variables for performance. Luminal pH, total and individual VFA (i.e., acetate, propionate,
172 butyrate, isobutyrate, valerate and isovalerate) and lactate in gastric, ileal, cecal and colonic
173 digesta and feces were response variables related to microbial action. Data of fermentation
174 metabolites in digesta from proximal and distal colon were also extracted. However, they did not
175 fulfill the minimum requirement of 10 single observations (treatment means). As dependent
176 variables for absolute bacterial abundances, sufficient numbers of observations were only
177 available for lactobacilli, bifidobacteria, enterobacteria and *E. coli*; however, not for all GIT
178 segments. Although studies using quantitative PCR and fluorescence-in-situ-hybridization (FISH)
179 quantified the abundances of other bacterial groups which are difficult to culture, the number of
180 observations was often too small and primer and probe sets not equivalent, thereby hampering the
181 comparison of data. Results of bacterial abundances originated from both culturing and

quantitative molecular approaches. Although these are different methods of quantification, it was assumed that colony forming units (CFU) correlate to gene copy numbers per gram of sample (Hein et al., 2001). Bacterial data were expressed as CFU/g digesta or fecal sample. If provided on DM basis, fermentation metabolites and bacterial abundances were converted to fresh matter basis. Data reported for the rectum were included in the "feces dataset" for fermentation metabolites, pH, digesta DM and absolute bacterial abundances. Fermentation and bacterial data reported to be collected from the colon were allocated to the "mid-colon dataset" for fermentation, pH and digesta.

Taken together, the recorded information from the research articles that matched the inclusion criteria included authors, year of publication, dietary inulin level and source (i.e., purified or natural source), experimental design, sex, type and start BW of pig, breed, housing (individual or pen), number of pigs per treatment, duration of feeding period, number of experimental periods, and dietary main cereals and protein feedstuffs, and dietary CP level as well as the dependent variables. The chain length of inulin was not provided in most research articles and could therefore not be considered. Other dietary fibrous components might interact in the inulin effects on fermentation and bacterial variables and should be considered. However, the dietary level of fibrous components and the fructan content of the basal diet were not provided in all studies or different fiber analytical methodologies were applied (e.g., crude fiber, total dietary fiber, neutral-detergent fiber), thereby hindering comparisons among studies. Moreover, due to the inconsistencies in reporting, breed and sex were also not included in the analysis.

2.4. Data Analysis

Descriptive statistics on predictor and dependent variables was performed using the MEANS procedure of SAS (SAS Inst. Inc., version 9.4). Microbial, CIAD, CTTAD and performance data

were subjected to mixed modeling analysis using the MIXED procedure according to the following algorithm (St-Pierre, 2001):

$Y_{ij} = \alpha_0 + \beta_1 X_{ij} + s_i + b_i X_{ij} + e_{ij}$

where Y_{ij} = expected outcome for the dependent variable *Y* observed at level *j* (j = 2,…, n) of the predictor variable *X* in the study *i*, whereas *n* is the number of treatment means in study *i*, α_0 = overall intercept across all studies (fixed effect), β_1 = overall regression coefficient of Y on X across all studies (fixed effect), X_{ij} = the value *j* of continuous variable X in study *i*, s_i = random effect of the study *i* (*i* = 1,…), b_i = the random effect of study *i* on the regression coefficient of *Y* on *X* in study *i*, and e_{ij} = the unexplained error. Thus, the random effect components of the model include $s_i + b_i X_{ij} + e_{ij}$, and the distributions are shown below:

$e_{ij} \sim iid\ N(0, \sigma_e^2)$ and $\begin{bmatrix} s_i \\ b_i \end{bmatrix} \sim iid\ N\left[\left(\frac{0}{0}\right), \sum\right]$, which assumes that e_{ij} is normally distributed with a mean of 0 and constant variance, and that s_i and b_i are normally distributed, have means of 0 and $\sum$ is their variance-covariance matrix: $\sum = \begin{bmatrix} \sigma_s^2 & \sigma_{sb} \\ \sigma_{sb} & \sigma_b^2 \end{bmatrix}$.

Predictor variables tested were study, start BW, the dietary CP and inulin level. The slope and intercept by study, start BW, the dietary CP and inulin level were initially included as random effects and an unstructured variance-covariance matrix (type = UN) was used to avoid a positive correlation between intercepts and slopes (St-Pierre, 2001). To take the unequal variance among studies into account, the dependent variable was weighted by the inverse of its squared SE (SE of treatment means were taken directly from studies). When a predictor variable was significant ($P < 0.05$), its squared term was included in the model to test any quadratic relationship. In this case, the variance-covariance matrix was modeled as variance components (TYPE = VC). Significant

quadratic relationships did not exist, only linear relationships between predictor and response variables for the present datasets. Trends were discussed at $0.05 < P < 0.10$. Data were visualized using the GPLOT procedure. Estimates, root mean square error (RMSE) and R^2 were computed and used to evaluate the goodness of fit. For established relationships, changes in the quantity of dependent variables caused by dietary inulin supplementation were illustrated for an assumed dietary inulin level of 3%.

To obtain a more precise prediction of influencing factors on dependent variables that were influenced by the dietary inulin level, we used backward elimination analysis (Zebeli et al., 2008). In doing so, we concurrently evaluated the effects of the predictor variables dietary inulin level, squared dietary inulin level, start BW as well as the dietary CP level on the response variables. Start BW was used as indicative for maturational changes from weaner to finishing period. Changes in the dietary composition, such as the dietary CP content, affect gastrointestinal microbial action. The dietary CP content varied among studies within one production phase and for similar starting BW. Therefore, the effect of dietary CP was taken into consideration. Model overparameterization was limited by considering a variance inflation factor less than 10 (which assumes no significant multicollinearity among predictor variables tested) for every continuous independent variable tested (Neter et al., 1996).

3. Results

3.1. Database description

Table 1 presents the main characteristics of the 33 studies between the years 2000 and January 2016 included in this meta-analysis. In eight studies the inulin supplement originated from chicory root (extract, powder or fiber) or Jerusalem artichokes, and in 25 studies from commercially available purified inulin concentrates (Table 1). The experimental diets were

mainly composed of wheat, barley, and corn, with soybean meal, fish meal, skimmed milk powder, whey protein and soy protein concentrate as protein feedstuffs (Table 1).

Results of the descriptive statistics for the response variables of fermentation metabolites, pH and digesta DM, bacterial abundances as well as ADG, CIAD and CTTAD of DM and nutrients are presented in Table 2, 3 and 4. Inulin supplementation levels ranged from 0.1 to 25.8% with means for the various categories of response variables averaging around 3 to 4% inulin (as-fed; Tables 2, 3 and 4). Only in one study the effect of a very high dietary supplementation level of 25.8% inulin was investigated; this study was included in the performance, CIAD and CTTAD sub-dataset. In the other sub-datasets, maximum inulin levels were 15 to 20% (Tables 3 and 4). Median values, however, showed that most data were available for low dietary inulin levels of 0.1 to 2% (Tables 2, 3 and 4). Body weight of pigs ranged from 5.9 to 112 kg (Tables 2, 3 and 4). With regards to the response variables for ADG, CIAD and CTTAD, cecal and mid-colonic fermentation metabolites as well as for colonic and fecal bacterial abundances, minimum and maximum BW values indicated that all production phases were covered in this study (Tables 2, 3 and 4), whereas the influence of inulin on gastric pH only included data from weaned pigs (Table 3). Ileal and fecal fermentation metabolites and ileal bacterial abundances encompassed data from weaned and growing pigs (Table 2 and 3). According to the means and median values throughout all categories of response variables, the data originated mostly from weaned and growing pigs. The dietary CP levels ranged from 13.7 to 24.5% DM and had mean and median values of about 20% DM (Tables 2, 3 and 4).

3.2. Inulin Effects on Digesta pH and Fermentation Metabolites along the GIT

In weaned pigs, gastric pH showed a negative linear relationship with increasing dietary inulin levels ($R^2 = 0.81$; $P < 0.001$; Table 5). Accordingly, a dietary inclusion level of 3% would

decrease gastric pH by 0.12 units, whereas the digesta pH in ileum, cecum, colon and feces was not affected by the dietary inulin level. The ileal lactate concentration tended to increase with more inulin in the diet (R^2 = 0.28; P = 0.062), which would amount to 4.5 mmol/kg with 3% inulin. In contrast, there was a small negative relationship between the cecal concentration of acetate and increasing dietary inulin levels (R^2 = 0.13; P = 0.080). Fermentation metabolites in colonic digesta and feces, in turn, were not influenced by the dietary inulin level.

3.3. Inulin Effects on Ileal, Colonic and Fecal Bacterial Abundances

Ileal abundances of lactobacilli and bifidobacteria were independent of the dietary inulin level (Table 6). Likewise, an increasing dietary inulin level from 0 to 20% did not modify the absolute abundance of lactobacilli in colonic digesta. By contrast, higher dietary inulin levels lowered the colonic abundance of bifidobacteria and enterobacteria, whereby the inhibiting effect of inulin was twice as strong for enterobacteria (-0.55 log units with a dietary inulin level of 3%; R^2 = 0.50; P < 0.001) as for bifidobacteria (-0.29 log units with a dietary inulin level of 3%; R^2 = 0.37; P = 0.022). In feces, increasing inulin levels reduced the abundance of lactobacilli (R^2 = 0.61; P < 0.001) which amounted to a reduction in lactobacilli numbers of 1.69 log units with a dietary inulin level of 3%, whereas bifidobacteria tended to be slightly enhanced by dietary inulin (R^2 = 0.29; P = 0.086). Increasing dietary inulin levels reduced the absolute enterobacteria abundance (R^2 = 0.23; P = 0.006) and the abundance of *E. coli* in feces (R^2 = 0.55; P < 0.001). Accordingly, a dietary inulin supplementation of 3% reduced the enterobacteria and *E. coli* numbers by 0.32 and 0.19 log units, respectively.

3.4. Inulin Effects on ADG, CIAD and CTTAD

298 Average daily weight gain was not affected by dietary inulin when inulin was supplemented
299 in the range from 0 to 25.8% (R^2 = 0.05; P = 0.311; Table 7). There was a tendency for a linear
300 effect that increasing dietary inulin levels decreased CIAD of DM (R^2 = 0.24; P = 0.091) which
301 corresponded to a decrease in CIAD of 7.4% with a dietary inulin level of 3%. The CTTAD of
302 CP also linearly decreased with increasing inulin levels (R^2 = 0.83; P < 0.001), amounting to a
303 1.3%-decrease with a dietary supplementation level of inulin of 3%.
304
305 *3.5. Effects of pig's BW, dietary CP and inulin level*
306 Including the dietary CP and starting BW of pigs in the same model as the dietary inulin
307 level enhanced the prediction accuracy for several variables when compared to the analysis of
308 the dietary inulin level alone (Tables 8). As such, gastric pH linearly decreased with increasing
309 BW and dietary inulin level, but it was also negatively correlated with increasing dietary CP
310 levels (R^2 = 0.98; $P \leq 0.003$). According to the equation derived from these associations, the
311 effect of BW was the strongest on gastric pH. Ileal lactate concentration showed a square effect
312 for dietary inulin level, thereby indicating that the positive relationship was asymptotic. As a
313 tendency, backward elimination showed that cecal acetate was mainly negatively associated with
314 dietary inulin level (R^2 = 0.35; P = 0.071) but not influenced by other predictor variables tested.
315 Backward elimination further showed that increasing BW positively affected the colonic
316 abundance of bifidobacteria which was stronger and opposite to the dietary inulin effect (R^2 =
317 0.74; P < 0.01). Colonic enterobacteria were only affected by inulin (R^2 = 0.57; P < 0.001);
318 however, the square effect of inulin indicated an asymptotic approximation. Fecal abundance of
319 lactobacilli was negatively associated with both increasing BW and increasing dietary inulin
320 level (R^2 = 0.77; P < 0.01). Moreover, backward elimination analysis indicated that increasing
321 BW and dietary inulin were the main factors influencing bifidobacteria abundance in feces (R^2 =

322 0.54; $P < 0.05$). Likewise, *E. coli* abundance in feces was not only negatively affected by
323 increasing dietary inulin levels, but it was positively correlated to the dietary protein level and
324 pig's BW ($R^2 = 0.80$; $P < 0.05$). Furthermore, backward elimination showed a strong positive
325 relation between the CIAD of DM and increasing dietary CP levels as well ($R^2 = 0.89$; $P <$
326 0.001), whereas the CTTAD of CP was not only negatively related with the dietary inulin level
327 ($P < 0.001$) but also with the dietary CP level ($P = 0.029$).
328
329 **4. Discussion**
330 Published research showed inconsistent results for the microbiota-modulating abilities of
331 inulin in the GIT of pigs (e.g., Böhmer et al., 2005; Gao et al., 2015). Similarly, the GIT region
332 where dietary inulin would be most effective was not clear since some authors reported
333 alterations in the small intestine (ileum; Böhmer et al., 2005; Loh et al., 2006), whereas others
334 observed inulin-related changes in the large intestine (Loh et al., 2006; Janczyk et al., 2010; Gao
335 et al., 2015). Therefore, the present meta-analysis aimed at investigating and quantifying the
336 effects of dietary inulin supplementation on fermentation metabolites and absolute bacterial
337 abundances along the GIT and feces, together with effects on performance and CIAD and
338 CTTAD of DM and nutrients. Data of the included studies covered a wide range of experimental
339 conditions; therefore, models derived from these data may yield relevant predictions to assist in
340 the conclusion of effects of the target factors (Sauvant et al., 2008). Overall, the current results
341 provided insights into the discussion of the usefulness of inulin supplementation in pig diets and
342 confirm that inulin can be effective along the GIT.
343 The level of inclusion is one of the critical factors for measurable inulin effects in the GIT of
344 pigs. The medians of the dietary inulin levels of 0.1 to 2% inulin showed that the dietary
345 supplementation level may explain the small effect of inulin on fermentation metabolites

observed in the individual studies and in the present meta-analysis. According to the established relationships between the dietary inulin supplementation and dependent variables (e.g., gastric pH, ileal lactate, and colonic and fecal enterobacteria) a minimum supplementation level of inulin of 3 to 5% may be advisable to modulate physiological and microbial parameters in the GIT of pigs. Most studies used wheat and barley as main cereals in the diet. These cereals naturally contain fructans in a range of 0.2 to 4% in wheat and 0.5 to 1% in barley (Moshfegh et al., 1999). As the endogenous fructan levels of the basal diets were not provided in most studies, the effect of them on the observed effects of the supplemented inulin could not be distinguished in the present meta-analysis. Moreover, median values for the BW at the start of the experiment indicate that most data originated from studies in weaned and growing pigs. This is consistent with the general assumption that alternative feed additives, such as prebiotics, are more effective in young pigs due to their immature GIT functions and microbial community (e.g., de Lange et al., 2010). Relationships established in the present meta-analysis may be therefore more applicable to young pigs.

Increasing dietary inulin levels negatively affected CTTAD of CP, gastric pH, bifidobacteria and enterobacteria in colonic digesta, and lactobacilli and *E. coli* in feces, whereas VFA concentrations along the GIT and feces appeared to be mostly unaffected by inulin. A specific stimulation of lactic acid producing bacteria in the small intestine, such as lactobacilli and bifidobacteria (Van Loo, 2004) may have been indicated by the positive relationship between dietary inulin level and ileal lactate. In humans, a general positive relationship between the daily consumed amount of inulin and the abundance of bifidobacteria in stool exists (Van Loo, 2004). Since pigs have a higher microbial activity in the small intestine (Jensen and Jørgensen, 1994) and a lower abundance of bifidobacteria in the GIT than humans (Loh et al., 2006), the proposed prebiotic effect of inulin in humans cannot be extrapolated to pigs. Unfortunately, CIAD and

370 CTTAD of inulin were not adequately provided in the research papers to link inulin availability
371 in digesta and microbial numbers along the GIT. It is generally estimated that about 50% of
372 inulin are prececally fermented in pigs (Graham and Åman, 1986; Böhmer et al., 2005), whereby
373 the reported range of prececal CIAD of inulin ranges from 50 to 98% in the literature (e.g.,
374 Branner et al., 2004; Böhmer et al., 2005). Differences in the degree of fermentation may be
375 associated with the source of inulin (natural versus purified), degree of polymerization and the
376 maturation of the porcine GIT microbiota. Theoretically, inulin should lead to a decrease in the
377 pH along the GIT due to stimulation of fermentation and hence VFA and lactate production
378 (e.g., Böhmer et al., 2005). This assumption may be supported by the present negative and
379 positive relationships between dietary inulin level and gastric pH and, as trend, ileal lactate,
380 respectively, whereby at least 3% inulin should be supplemented to achieve physiological
381 changes. Aside from fermentation, higher water-holding properties of non-absorbable sugars
382 such as inulin may have reduced the gastric passage, thereby enhancing the acidification of the
383 gastric content (Wiggins, 1984). However, it needs to be considered that the data for gastric pH
384 originated only from three studies in weaned pigs.
385 Lactic acid producing *Bifidobacterium* and *Lactobacillus* strains encode β-
386 fructofuranosidases with different activities towards short- and long-chain fructans (e.g., Janer et
387 al., 2004; Ryan et al., 2005; Saulnier et al., 2007). Present regression models, however, did not
388 show an enhancing effect on absolute abundances of bifidobacteria and lactobacilli in ileal
389 digesta of weaned and early growing pigs, thereby confirming findings from individual research
390 studies (e.g., Böhmer et al., 2005; Janczyk et al., 2010). This was contrary to our findings for
391 ileal lactate and may indicate that changes were more at metabolic level. In addition, a certain
392 bias from the combination of the different methodologies used for bacterial quantification (i.e.,
393 culturing versus PCR-based approaches) cannot be excluded. Moreover, multiple regression

394 models demonstrated that dietary inulin can modulate the abundance of lactobacilli and
395 bifidobacteria in the large intestine; thereby supporting the observations of many individual
396 studies (e.g., Loh et al., 2006; Halas et al., 2009; Janczyk et al., 2010; Yan et al., 2013). In
397 contrast to the general assumption but in conformity with some literature findings (e.g., Vhile et
398 al., 2012), colonic and fecal lactobacilli and bifidobacteria abundances were not always
399 positively correlated with the dietary inulin level. In fact, increasing inulin levels decreased
400 colonic bifidobacteria and fecal lactobacilli numbers. This raises the question if the present
401 observations were direct effects of inulin or related to changes in substrate availability in digesta
402 of the large intestine, microbial cross-feeding and other microbe-to-microbe interactions (Flint et
403 al., 2012). Accordingly, cross-feeding of lactate produced by bacterial inulin fructan
404 fermentation has been reported to increase *Megasphaera elsdenii* in the colon of growing pigs
405 (Mølback et al., 2007). Aside from lactobacilli and bifidobacteria strains, inulin-degraders are
406 widespread among other bacterial genera, such as *Roseburia* and *Blautia* within *Clostridium*
407 cluster XIVa (Eckburg et al., 2005; Manderson et al., 2005). Also, *Catenibacterium* and
408 *Bacteroides* appear to have growth advantages in cecal digesta of pigs fed inulin supplemented
409 diets (Yan et al., 2013). Since most inulin entering the large intestine would be available to the
410 cecal bacterial community, the cecal bacterial responses to inulin might provide the link between
411 small and large intestines. In spite of the fact that about <10 to 50% of the ingested inulin
412 reaches the cecum, we could only establish a small trend for a negative relationship between
413 dietary inulin and acetate concentration in cecal digesta, indicating alterations in the substrate
414 available for microbial fermentation. Unfortunately, regression models for bacterial abundances
415 in cecal digesta could not be developed due to ill-definition of variables in the respective
416 literature. Maturational changes in the abundances of bifidobacteria in colonic digesta, and
417 lactobacilli and *E. coli* in feces were indicated by their relationships with increasing BW and

418 thus the age of the pigs which either counteracted or strengthened the observed inulin effect.
419 Although the family *Enterobacteriaceae* belongs to the commensal microbiota in the porcine
420 GIT (Mach et al., 2015; Metzler-Zebeli et al., 2015; Zhao et al., 2015), it contains some common
421 etiological agents of diarrhea including enterotoxigenic *E. coli* (Fairbrother et al., 2005).
422 Nutritional attempts to control the intestinal numbers of *E. coli*, especially in the early
423 postweaning period, have therefore received considerable attention (de Lange et al., 2010;
424 Pluske, 2013). The current regression models indicated that dietary inulin might have the ability
425 to control colonic and fecal numbers of enterobacteria and fecal *E. coli* numbers. Whether this
426 reduction can be linked to the increased abundance of bifidobacteria as often presumed remains
427 open due to the complexity of the fecal bacterial community (e.g., Mach et al., 2015). However,
428 meta-regression results also showed that the dietary CP level should be concurrently controlled
429 in order not to counteract a potential inhibiting effect of inulin on *E. coli* in feces. Finally, it
430 should be considered that only changes in bacterial abundances of more than 0.5 log units may
431 be of physiological relevance. Therefore, higher dietary supplementation levels of more than 6 to
432 8% are necessary to impair colonic bifidobacteria and to be effective against *E. coli* in feces
433 according to the present meta-regressions.
434 One selection criterion for alternative feed additives is their effect on growth performance
435 and feed efficiency. Although non-digestible carbohydrates may have a negative impact on pig's
436 performance (Grieshop et al., 2001), this may not be applicable for most of the studies included
437 in this meta-analysis due to the low dietary inulin level. Accordingly, ADG appeared not be
438 sensitive to dietary inulin levels as no relation between dietary inulin and ADG could be
439 established. This was despite the fact that negative relationships between increasing CIAD of
440 DM (as trend) and CTTAD of CP were found. Reduced CIAD of DM, but not of CP, with
441 increasing dietary inulin levels may indicate inulin residuals in ileal digesta. According to the

best-fit models, higher dietary CP levels could counteract the inulin effect on CIAD of DM. This may be related to the fact that dietary CP can elongate the retention time of the feed in the stomach, thereby allowing luminal bacteria more time to ferment dietary components (Wiggins, 1984). The CTTAD of CP was reduced with more inulin in the diet and, in contrast to the CIAD of DM, this effect was greater with increasing dietary CP levels and can likely be associated with enhanced microbial protein synthesis in the large intestine due to greater substrate availability.

5. Conclusion

This meta-analysis showed that dietary inulin supplementation may have the ability to lower gastric pH in weaned pigs. Together with the trend for higher ileal lactate with increasing dietary inulin levels, this may support an increased microbial activity in the upper GIT. Despite the negative relation between dietary inulin and bifidobacteria in the colon and lactobacilli in feces, the observed inhibition of enterobacteria numbers in feces with higher dietary inulin levels may be favorable for porcine GIT health postweaning. However, pig's BW and the dietary CP level were other sources of variation which may act synergistically and counteract the inulin effect. Finally, since some results were based on low numbers of observations and often low dietary inulin levels were tested, established relationships should be regarded as universal trends and may be more applicable for weaned and early growing pigs.

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683

684 **Table 1**

685 List of references and the respective experimental variables included in the meta-analysis.

Reference	Inulin[a]	Pig	Feeding level[b]	Basal diet[c]	Gastro-intestinal sites[d]					Variables[e]		
					Sto	Ile	Cec	Col	Fec	Ferm-metab	Bact-abund	Perf./ Dig.
Branner et al., 2004	p	grower	restr	corn, wheat, barley, soybean meal		x			x	x	x	x
Rideout et al., 2004	p	grower	s-ad lib	corn, corn starch, soybean meal					x	x		x
Böhmer et al., 2005	p	grower	restr	corn, wheat, barley, soybean meal		x			x	x	x	x
Pierce et al., 2005	p	weaner	ad lib	wheat, soybean meal	x		x	x	x	x	x	x
Yasuda et al., 2006	p	weaner	ad lib	corn, soybean meal					x	x		x
Lynch et al., 2007	p	finisher	restr	wheat, soybean meal			x	x		x		x
Mølback et al., 2007	na	grower	restr	triticale, barley, potato protein								
Tako et al., 2008	p	weaner	ad lib	corn, soybean meal								
Wellock et al., 2008	p	weaner	ad lib	oat, wheat, fish meal	x	x	x	x		x	x	x
Lynch et al., 2009	p	weaner	ad lib	wheat, soybean meal					x	x	x	x
Metzler-Zebeli et al., 2009	p	weaner	restr	barley, wheat, soybean meal		x			x		x	
Ratriyanto et al., 2009	p	weaner	restr	barley, wheat, soybean meal		x			x	x		x
Yasuda et al., 2009	p	grower	ad lib	corn, soybean meal								x
Patterson et al., 2010	p	weaner	ad lib	corn, soybean meal								
Halas et al., 2010	p	weaner	ad lib	wheat, soybean meal			x	x		x		
Hedemann and Bach Knudsen, 2010	na	grower	restr	wheat, soybean meal								x
Kjøs et al., 2010	na	finisher	restr	barley, wheat, soybean meal					x		x	
Mair et al., 2010	p	weaner	ad lib	barley, corn, soy concentrate	x	x		x		x	x	
Varley et al., 2010	p	finisher	restr	wheat, soybean meal		x	x	x		x	x	x
Aufreiter et al., 2011	p	weaner	restr	skim milk powder, whey powder, soy oil				x			x	
Øverland et al., 2011	na	grower	restr	barley, wheat, soybean meal			x	x	x	x	x	x
Boudry et al., 2012	na	grower	restr	wheat, soybean meal								
Ivarsson et al., 2012	na	grower	ad lib	wheat, barley, potato					x		x	x
Jolliff and Mahan, 2012	p	weaner	ad lib	corn, soybean meal								x
Liu et al., 2012	na	grower	ad lib	wheat, barley, potato		x		x	x	x		x

O'Shea et al., 2012	p	grower	ad lib	wheat, soybean meal				x		x	x
Vhile et al., 2012	na	finisher	restr	wheat, soybean meal			x	x	x	x	
Rodrigues et al., 2013	p	weaner	restr	corn, soybean meal							x
Yan et al., 2013	p	grower	-	corn, soybean meal		x			x		x
Grela et al., 2014	p	finisher	ad lib	wheat, barley, corn soybean meal							
Brambillasca et al., 2015	p	weaner	ad lib	corn, soybean meal	x		x	x	x		x
Gao et al., 2015	p	grower	ad lib	corn, soybean meal		x		x	x		x
Sobolewska and Grela, 2015	p	grower	ad lib	wheat, barley soybean meal		x	x		x		

[a] Inulin source: p, purified; na, natural.

[b] Feeding level: restr, restrictive feeding; ad lib, ad libitum feeding; s-ad lib, semi-ad libitum feeding.

[c] Main energy and protein feedstuffs of basal diet.

[d] Intestinal sites: Sto, stomach; Ile, ileum; Cec, cecum; Col, colon; Fec, feces.

[e] Response variables: Ferm met, fermentation metabolites; Bact-abund, absolute bacterial abundances; Perf./Dig., performance and digestibility variables.

Table 2

Descriptive statistics for dietary inulin and crude protein, start body weight, pH and fermentation metabolites in gastric, ileal, cecal and colonic digesta and feces of pigs in the respective datasets.

Item[a]	n_{Treat}[b,c]	Mean	SE[d]	Min.	Max.	Median
Gastric digesta						
Start BW (kg)	12	9.1	0.13	8.1	9.3	9.3
Dietary inulin (%)	12	3.5	1.64	0.0	15.0	0.2
Dietary CP (%)	12	21.9	0.72	17.8	24.0	23.2
pH	12	3.4	0.10	2.8	3.9	3.4
Ileal digesta						
Start BW (kg)	27	17.3	2.63	6.7	51.0	9.3
Dietary inulin (%)	27	4.3	1.22	0.0	20.0	0.4
Dietary CP (%)	27	20.8	0.55	16.6	24.0	22.2
pH	25	6.7	0.07	6.1	7.4	6.6
Total VFA (mmol/kg)	17	24.0	4.48	6.3	78.8	16.7
Acetate (mmol/kg)	15	15.5	2.15	5.0	39.4	14.2
Butyrate (mmol/kg)	13	2.1	0.69	0.0	8.2	1.1
Lactate (mmol/kg)	13	35.3	8.87	0.3	96.2	25.1
Cecal digesta						
Start BW (kg)	35	22.9	3.73	5.9	74.5	10.2
Dietary inulin (%)	35	3.6	0.82	0.0	20.0	2.0
Dietary CP (%)	20	21.2	0.55	16.6	24.0	21.8
pH	16	5.8	0.08	5.1	6.2	5.8
Total VFA (mmol/kg)	27	110.4	12.63	19.0	229.0	109.5
Acetate (mmol/kg)	25	51.0	7.80	10.7	138.8	48.1
Propionate (mmol/kg)	25	23.1	2.74	5.2	50.4	24.5
Butyrate (mmol/kg)	25	11.3	1.87	0.5	31.4	10.8
Iso-butyrate (mmol/kg)	17	1.0	0.16	0.2	2.3	0.8
Valerate (mmol/kg)	21	2.4	0.38	0.5	5.9	2.2
Iso-valerate (mmol/kg)	12	2.0	0.33	0.5	4.0	1.8
Lactate (mmol/kg)	10	6.2	1.87	0.0	15.1	6.7
Colonic digesta						
Start BW (kg)	26	43.2	8.02	5.9	112.0	30.0
Dietary inulin (%)	26	2.9	0.73	0.0	16.0	1.8
Dietary CP (%)	18	20.3	0.54	16.6	23.6	19.7
pH	18	5.8	0.08	5.2	6.3	5.8
Total VFA (mmol/kg)	19	133.9	15.28	45.1	243.6	146.6
Acetate (mmol/kg)	19	68.7	9.56	17.1	140.5	64.6

Propionate (mmol/kg)	19	31.8	3.37	11.3	52.6	26.5
Butyrate (mmol/kg)	19	14.1	2.62	1.1	38.2	11.5
Iso-butyrate (mmol/kg)	17	1.7	0.23	0.6	3.2	1.4
Valerate (mmol/kg)	19	3.2	0.33	1.6	5.9	2.7
Iso-valerate (mmol/kg)	14	3.2	0.59	0.4	6.1	3.5
Feces						
Start BW (kg)	26	17.0	2.16	6.0	36.5	12.7
Dietary inulin (%)	26	2.5	0.74	0.0	16.0	1.5
Dietary CP (%)	26	19.9	0.50	16.6	24.5	19.3
pH	20	6.8	0.13	6.0	8.1	6.6
Dry matter content (%)	16	24.2	0.97	17.1	33.0	23.8
Total VFA (mmol/kg)	16	88.4	15.98	7.3	181.7	92.1
Acetate (mmol/kg)	16	52.6	9.53	1.9	108.8	54.6
Propionate (mmol/kg)	16	19.1	3.12	3.5	35.7	19.9
Butyrate (mmol/kg)	16	10.1	1.94	0.8	19.6	10.1
Valerate (mmol/kg)	12	4.5	0.65	0.7	6.9	5.4

694 [a] BW, body weight; CP; crude protein; n_{Treat} = number of treatment means included; SE = standard error; VFA,

695 volatile fatty acids.

696 [b] Separate datasets for response variables in gastric, ileal, cecal and colonic digesta and feces were built.

697

Table 3

Descriptive statistics for dietary inulin and crude protein, start body weight, and absolute abundances of bacterial groups in ileal and colonic digesta and feces of pigs in the respective datasets.

Item[a]	n_{Treat}[b]	Mean	SE	Min.	Max.	Median
Ileal digesta						
Start BW (kg)	14	22.6	4.65	6.7	51.0	9.3
Dietary inulin (%)	14	3.2	1.68	0.0	20.0	0.1
Dietary CP (%)	14	19.4	0.78	16.6	24.0	18.0
Lactobacilli (CFU/g)	12	8.5	0.14	7.7	9.3	8.5
Bifidobacteria (CFU/g)	10	7.3	0.70	3.3	9.3	8.2
Colonic digesta						
Start BW (kg)	39	27.9	4.14	8.1	74.5	9.3
Dietary inulin (%)	39	3.7	0.86	0	20	1.25
Dietary CP (%)	39	21.0	0.44	16.6	24	22.8
Lactobacilli (CFU/g)	30	8.6	0.13	6.7	9.5	8.9
Bifidobacteria (CFU/g)	14	8.1	0.23	6.3	8.8	8.5
Enterobacteria (CFU/g)	19	6.5	0.30	2.7	8.0	6.7
Feces						
Start BW (kg)	39	25.2	3.04	2.4	67.0	21.9
Dietary inulin (%)	39	3.1	0.68	0.0	16.0	1.5
Dietary CP (%)	39	19.6	0.45	16.5	24.5	18.7
Lactobacilli (CFU/g)	26	8.7	0.31	4.2	10.7	9.2
Bifidobacteria (CFU/g)	13	8.2	0.42	5.0	9.6	8.6
Enterobacteria (CFU/g)	20	7.4	0.31	5.2	9.8	7.0
Escherichia coli (CFU/g)	19	6.6	0.12	5.8	8.0	6.6

[a] BW, body weight; CFU, colony forming units; CP, crude protein; n_{Treat} = number of treatment means included; SE = standard error.

[b] Separate datasets for response variables in ileal, and colonic digesta and feces were built.

Table 4

Descriptive statistics for dietary inulin and crude protein, start body weight, average daily gain, and coefficients of apparent ileal and total tract digestibility of pigs in the respective dataset.

Item [a]	n_{Treat}	Mean	SE	Min.	Max.	Median
Start BW (kg)	61	21.8	2.28	6.0	74.5	15.5
Dietary inulin (%)	61	4.2	0.80	0	25.8	1.5
Dietary CP (%)	61	20.4	0.34	13.7	24.5	19.9
ADG (g)	25	611	63.3	75.0	981.5	623
CIAD of DM (%)	13	79.2	2.05	67.9	87.8	76.0
CIAD of CP (%)	11	73.8	0.58	71.0	78.5	73.5
CTTAD of DM (%)	27	87.5	0.41	83.6	91.0	87.0
CTTAD of CP (%)	15	77.4	5.15	74.3	86.0	83.5
CTTAD of ash (%)	15	59.8	1.94	46.1	68.7	62.9
CTTAD of NDF (%)	19	59.2	1.71	39.1	69.8	61.2

[a] ADG, average daily gain; BW, body weight; CIAD, coefficient of ileal apparent digestibility; CP, crude protein; CTTAD, coefficient of total tract apparent digestibility; DM, dry matter; NDF, neutral detergent fiber; n_{Treat} = number of treatment means included; SE = standard error.

Table 5

Prediction of pH and fermentation metabolites in gastric, ileal, cecal and colonic digesta and feces as affected by supplementation dose of inulin (%) in pig diets for all production phases.

		Parameter estimates				Model statistics		
Response variable (Y)[a]	n_{Treat}	Intercept	$SE_{Intercept}$	Slope	SE_{Slope}	RMSE	R^2	P-value
Gastric digesta								
pH	12	3.51	0.039	-0.040	0.006	0.115	0.81	<0.001
Ileal digesta								
pH	25	6.62	0.073	0.015	0.009	0.293	0.01	0.116
Total VFA (mmol/kg)	17	22.94	4.086	-0.130	0.520	14.018	0.00	0.806
Acetate (mmol/kg)	15	16.27	2.093	-0.343	0.318	6.783	0.08	0.300
Butyrate (mmol/kg)	13	1.87	0.561	-0.061	0.079	1.641	0.05	0.456
Lactate (mmol/kg)	13	21.09	5.051	1.486	0.715	14.926	0.28	0.062
Cecal digesta								
pH	16	5.82	0.077	-0.017	0.010	0.262	0.16	0.130
Total VFA (mmol/kg)	27	109.46	15.887	0.111	2.988	66.103	0.00	0.971
Acetate (mmol/kg)	25	63.80	10.227	-4.920	2.683	36.892	0.13	0.080
Propionate (mmol/kg)	25	26.40	3.688	-1.296	0.967	13.302	0.07	0.193
Butyrate (mmol/kg)	25	13.76	2.432	-1.005	0.638	8.772	0.10	0.129
Valerate (mmol/kg)	21	2.12	0.516	0.096	0.130	1.699	0.03	0.470
Mid colonic digesta								
pH	18	5.83	0.092	-0.023	0.019	0.321	0.09	0.234
Total VFA (mmol/kg)	19	135.20	19.083	-0.542	3.941	68.374	0.00	0.892
Acetate (mmol/kg)	19	71.25	11.885	-0.974	2.454	42.592	0.01	0.696
Propionate (mmol/kg)	19	31.60	4.186	0.018	0.864	0.983	0.00	0.983
Butyrate (mmol/kg)	19	15.06	3.249	-0.338	0.671	11.642	0.02	0.621
Iso-butyrate (mmol/kg)	17	1.84	0.269	-0.059	0.052	0.888	0.08	0.276
Valerate (mmol/kg)	19	3.10	0.403	0.029	0.083	1.445	0.01	0.736
Iso-valerate (mmol/kg)	14	2.83	0.707	0.121	0.131	2.234	0.07	0.375
Feces								
pH	20	6.82	0.151	-0.024	0.034	0.574	0.03	0.487
Dry matter content (%)	16	23.74	1.251	0.217	0.389	3.959	0.02	0.586
Total VFA (mmol/kg)	16	88.18	20.433	0.131	6.391	65.969	0.00	0.984
Acetate (mmol/kg)	16	52.82	12.179	-0.209	3.809	39.320	0.00	0.957
Propionate (mmol/kg)	16	18.36	3.978	0.406	1.244	12.844	0.01	0.749
Butyrate (mmol/kg)	16	9.47	2.474	0.327	0.774	7.989	0.01	0.679
Valerate (mmol/kg)	12	4.30	0.834	0.087	0.232	2.296	0.01	0.716

714 [a] VFA, volatile fatty acids; n_{Treat} = number of treatment means included; RMSE = root mean square error.

715

716 **Table 6**

717 Prediction of absolute abundances of bacterial groups in ileal and colonic digesta and feces as

718 affected by supplementation dose of inulin (%) in pig diets for all production classes.

		Parameter estimates				Model statistics		
Response variable (Y)[a]	n_{Treat}	Intercept	$SE_{Intercept}$	Slope	SE_{Slope}	RMSE	R^2	P-value
Ileal digesta								
Lactobacilli (CFU/g)	12	8.53	0.155	-0.010	0.033	0.483	0.01	0.771
Bifidobacteria (CFU/g)	10	7.38	0.801	-0.017	0.125	2.339	0.00	0.893
Colonic digesta								
Lactobacilli (CFU/g)	30	8.62	0.140	0.005	0.020	0.652	0.00	0.821
Bifidobacteria (CFU/g)	14	8.28	0.195	-0.095	0.036	0.679	0.37	0.022
Enterobacteria (CFU/g)	19	7.07	0.256	-0.184	0.044	0.923	0.50	<0.001
Feces								
Lactobacilli (CFU/g)	26	9.33	0.262	-0.562	0.102	1.0317	0.61	<0.001
Bifidobacteria (CFU/g)	13	7.47	0.558	0.140	0.073	1.455	0.29	0.086
Enterobacteria (CFU/g)	20	7.18	0.193	-0.108	0.037	0.889	0.23	0.006
Escherichia coli (CFU/g)	19	6.86	0.096	-0.063	0.014	0.303	0.55	<0.001

719 [a] CFU, colony forming units; n_{Treat} = number of treatment means included; RMSE = root mean square error.

720

Table 7

Prediction of growth performance and coefficients of apparent ileal and total tract digestibility as affected by supplementation dose of inulin (%) in pig diets for all production classes.

		Parameter estimates				Model statistics		
Response variable (Y)[a]	n_{Treat}	Intercept	$SE_{Intercept}$	Slope	SE_{Slope}	RMSE	R^2	P-value
ADG (g)	25	606.99	76.404	-9.017	8.695	256.122	0.05	0.311
CIAD of DM (%)	13	81.18	2.146	-2.473	1.334	6.691	0.24	0.091
CIAD of CP (%)	11	73.68	0.658	0.024	0.067	1.808	0.01	0.727
CTTAD of DM (%)	27	87.29	0.421	0.145	0.102	2.017	0.08	0.168
CTTAD of CP (%)	15	84.51	0.467	-0.443	0.055	1.555	0.83	<0.001
CTTAD of ash (%)	15	58.52	1.332	0.320	0.247	4.732	0.11	0.218
CTTAD of NDF (%)	19	58.73	1.850	0.234	0.382	7.396	0.02	0.548

[a] ADG, average daily gain; CIAD, coefficient of ileal apparent digestibility; CP, crude protein; CTTAD, coefficient of total tract apparent digestibility; DM, dry matter; NDF, neutral detergent fiber; n_{Treat} = number of treatment means included; RMSE = root mean square error.

728 **Table 8**

729 Best-fit equations showing the coefficients of apparent ileal and total tract digestibility and microbial response variables in relation to

730 increasing dietary inulin and crude protein level, and pig's start body weight using backward elimination technique.

			Parameter estimates				Model statistics			
Response variable (*Y*)[a]	Predictor (*X*)	n_{Treat}	Intercept	$SE_{Intercept}$	Slope	SE_{Slope}	RMSE	R^2	VIF	*P*-value
Gastric pH		15	5.28	0.228			0.037	0.98		
	BW (kg)				-0.140	0.030			1.52	0.002
	dietary CP (%)				-0.024	0.006			1.52	0.003
	Inulin (%)				-0.034	0.002			1.09	<0.001
Ileal lactate (mmol/kg)		13	20.24	4.201			13.274	0.61		
	Inulin-square (%)				0.1674	0.041			1.00	0.002
Cecal acetate (mmol/kg)		25	92.64	18.476			46.434	0.35		
	Inulin (%)				-10.620	5.100			1.00	0.071
Colonic bifidobacteria (CFU/g)		14	7.39	0.253			0.455	0.74		
	BW (kg)				0.0163	0.004			1.00	0.003
	Inulin (%)				-0.005	0.001			1.00	0.002
Colonic enterobacteria (CFU/g)		19	6.82	0.210			0.857	0.57		
	Inulin-square (%)				-0.011	0.002			1.00	<0.001
Fecal Lactobacilli (CFU/g)		26	10.30	0.247			0.764	0.79		
	BW (kg)				-0.045	0.008			1.22	<0.001
	Inulin (%)				-0.255	0.078			1.22	0.004
Fecal bifidobacteria (CFU/g)		13	5.81	0.837			1.113	0.54		
	BW (kg)				0.068	0.0278			1.150	0.034
	Inulin (%)				0.181	0.0578			1.150	0.011
Fecal *Escherichia coli* (CFU/g)		19	2.59	1.249			0.216	0.80		
	BW (kg)				0.063	0.0149			6.10	<0.001
	dietary CP (%)				0.134	0.0472			6.31	0.012
	Inulin (%)				-0.044	0.013			1.67	0.004

AID of DM (%)		13	24.43	5.918			2.587	0.89		
	dietary CP (%)				3.098	0.333			1.00	<0.001
ATTD of CP (%)		15	89.59	2.149			1.238	0.89		
	dietary CP (%)				-0.284	0.115			1.02	0.029
	Inulin (%)				-0.420	0.046			1.02	<.0001

[a] BW, body weight; CIAD, coefficient of ileal apparent digestibility; CFU, colony forming units; CP, crude protein; CTTAD, coefficient of total tract apparent digestibility; DM, dry matter; n_{Treat} = number of treatment means included; RMSE, root mean square error; SE, standard error; VIF, variance inflation factor.

Highlights

Meta-regressions showed potential of dietary inulin to lower gastric pH in weaned pigs.

Meta-regressions indicated an inhibitory effect of dietary inulin on *Escherichia coli* in feces.

Meta-regressions did not confirm a stimulatory effect of dietary inulin on intestinal lactobacilli and bifidobacteria throughout the intestinal tract.

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