AARHUS
UNIVERSITET
DCA - NATIONALT CENTER FOR FØDEVARER OG JORDBRUG

Til Fødevarestyrelsen

Genfremsendelse af bestillingen: "Vidensyntese om muligheder for forebyggelse af fravænningsdiarré hos grise".

DCA - Nationalt Center for Fødevarer og Jordbrug

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Specialkonsulent

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Fødevarestyrelsen har i en bestilling fremsendt d. 4. maj 2016 via Miljø- og Fødevareministeriets forskningsbank bedt DCA – Nationalt Center for Fødevarer og Jordbrug – om at udarbejde en vidensyntese om muligheder for forebyggelse af fravænningsdiarré hos grise i såvel økologisk og konventionel produktion.

Bestillingen blev leveret den 24. april 2017, og Fødevarestyrelsen har efterfølgende bedt om at få lavet ændringer til bestillingen. I denne version er der derfor rettet en fejl i referencerne, og udtrykket cost-benefit er erstattet med cost-effectiveness, som bruges af andre danske forskere.

Den reviderede vidensyntese, der følger nedenfor, er jf projektbeskrivelsen udarbejdet på engelsk med et dansk resumé. Den er udarbejdet af Sektionsleder Charlotte Lauridsen, Seniorforsker Ole Højbjerg, Videnskabelig assistent Hanne Kongsted og Seniorforsker Nuria Canibe alle fra Institut for Husdyrvidenskab, Aarhus Universitet.

Besvarelsen er udarbejdet som led i "Aftale mellem Aarhus Universitet og Fødevareministeriet om udførelse af forskningsbaseret myndighedsbetjening m.v. ved Aarhus Universitet, DCA – Nationalt Center for Fødevarer og Jordbrug, 2016-2019 (punkt BH203 i Aftalens Bilag 2 samt B14 i Arbejdsprogram 2017 for Ydelsesaftale for Husdyrproduktion, 2017-2020)".

Venlig hilsen

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A critical review on alternatives to antibiotics and pharmacological zinc for prevention of diarrhoea in pigs post-weaning

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Dansk resumé

Diarre associeret med *Escherichia coli* (*E. coli*) forekommer de første 7-10 dage efter fravænning og kan behandles med antibiotika, men problematikken omkring udvikling af resistens betyder, at forbruget af antibiotika skal reduceres. Som alternativ anvendes medicinsk zink (**Zn**) i form af zinkoxid , som ordineres med 2500 ppm Zn i 14 dage efter fravænning hos både konventionelle og økologiske besætninger. Anvendelsen af zinkoxid bevirker en øget mængde zink i svinegyllen, og i forbindelse med MRSA risikovurderingen er det blevet anbefalet, at zink bør anvendes mere restriktivt. Der er derfor behov for at finde alternativer til både antibiotika og medicinsk zink til forebyggelse af fravænningsdiarre hos grise.

På denne baggrund har Fødevarestyrelsen anmodet DCA – Nationalt Center for Fødevarer og Jordbrug om at foranledige udarbejdelse af en vidensyntese ved Aarhus Universitet. Rapporten er led i 'Aftale om forskningsbaseret myndighedsbetjening mellem AU og Fødevareministeriet'. Formålet var at undersøge muligheder for forebyggelse af fravænningsdiarre hos grise i såvel økologisk som konventionel produktion baseret på litteratur fra både ind- og udland. Et andet formål var at fremlægge rammer og oversigt af muligheder for forebyggelse af fravænningsdiarre, der kunne danne udgangspunkt for en cost-effectiveness vurdering, som skulle gennemføres sideløbende ved IFRO, Københavns universitet.

Vores litteraturgennemgang tager udgangspunkt i de ændringer, der forekommer i tarmkanalen omkring fravænning, efterfulgt af en beskrivelse af patogenese og diagnosticering af fravænningsdiarre, samt af de faktorer, der kan fremme/hæmme fravænningsdiarre forårsaget af *E. coli*, såsom genetik, management og miljø samt ernæring og fodring. Blandt disse faktorer er der kun et begrænset antal undersøgelser, der har angivet effektiviteten af en given intervention eller dennes potentielle effektivitet i relation til forebyggelse af fravænningsdiarre, og undersøgelserne er fortrinsvist gennemført med henblik på at finde alternativer til antibiotika. I litteraturen er medicinsk zink hidtil betragtet som et effektivt alternativ til antibiotika. På baggrund af vores litteraturundersøgelse konkluderede vi, at 1) reduktion af foderets proteinindhold; 2) øgning i

fravænningsalder, og 3) optimering af miljøets hygiejnestatus for de fravænnede grise er strategier, som kunne være relevante at underkaste en cost-benefit analyse. Strategierne er af interesse for både konventionel og økologisk griseproduktion, men der er nogle særlige udfordringer, der gør, at alle tre strategier måske ikke er lige relevante i de to produktionsformer: En begrænsning af foderets proteinindhold medfører overvejelse af foderets aminosyresammensætning i forhold til dyrets vækst. Det er ikke tilladt i økologisk svineproduktion at tilsætte syntetiske aminosyrer. I økologisk svineproduktion anvendes i forvejen en højere fravænningsalder (7 uger), hvorfor 5 i stedet for 4 ugers fravænningsalder ikke er relevant. Det skal her nævnes, at pludselig fravænning muligvis er det mest udfordrende for grisen, uanset om alderen er 4 eller 7 uger. Gradvis fravænning (forlænget dieperiode) er derfor relevant at undersøge nærmere med henblik på reduktion af fravænningsdiarre i relation til økologisk svineproduktion. Vi har beskrevet effekten af organiske syrer og fermenteret vådfoder på forekomsten af fravænningsdiarre, samt muligheden for anvendelse af antistoffer oprenset fra blodplasma eller æggepulver, men det er vores vurdering, at der skal tilvejebringes yderligere forskning for at udvikle produkterne og vurdere deres effekt, førend de underkastes en cost-effectiveness analyse.

1. Summary

Post-weaning diarrhoea (**PWD**) is a significant enteric disease causing considerable economic losses for the pig industry. Among several etiological risk factors, enterotoxigenic *Escherichia coli* (**ETEC**) is considered to be a major cause, i.e. colibacillosis. After being routinely used for several decades as growth promoters and to control bacterial disease outbreaks in piglets, the use of antibiotics at sub-therapeutic concentrations has been banned in the European Union since 1 January 2006 due to the increasing prevalence of resistance to antibiotics in pigs. The removal of in-feed antibiotics from piglet diets has negative economic consequences as it dramatically increases the rate of morbidity and mortality due to ETEC as well as the use of antibiotics for therapeutic purposes. One of the used substitutes for in-feed antibiotics in some European countries, including Denmark, in which this practice is allowed, is pharmacological levels of dietary zinc (2500 ppm during the first two weeks post-weaning following veterinary prescription), but its high excretion has a negative impact on the environment. Thus, alternatives to antibiotics and to high levels of heavy metals that can control ETEC infections in piglets post-weaning will be of great advantage, also because of the risk of cross resistance between antibiotics and heavy metals. A number of nutritional, genetic and management strategies have been reported in the literature as alternatives to in-feed antibiotics to prevent PWD,

but, in most studies, the reduction of PWD has not been addressed as a primary response parameter. The present review addresses the possible mode of action by which nutrition and feed additives, breeding and rearing/management may potentially prevent and/or reduce ETEC diarrhoea in pigs (in conventional and organic production systems) during the post-weaning period, and as such, could be considered as alternative strategies to in-feed antibiotics and pharmacological zinc oxide (**ZnO**). An important objective of our knowledge synthesis is to propose strategies, which could be considered relevant for further evaluation in a cost-effectiveness analysis. Our study of the literature revealed that few experiments have actually demonstrated the efficiency of a given intervention or its potential efficacy to reduce PWD; either because PWD was not a primary parameter of the study, the study was not designed to evaluate effect on PWD, or the given intervention was not available on a commercial basis. Our critical review therefore limited the available potential interventions for further cost-effectiveness analysis. In conclusion, this review pointed out that the following strategies: reduced dietary protein level, increased weaning age, and optimized hygienic status of weaning facilities for the pigs post-weaning would have an effect on the occurrence of PWD. Besides, there were a few more dietary factors which gained our special interest in terms of their potential in limiting PWD in pigs, i.e. organic acids and antibodies, but these alternatives require more development and research to pinpoint their efficacy in relation to prevention of PWD.

2. Review methodology

We performed a multiple database search to ensure comprehensive article retrieval in CABI and ISI Web of Science. The main key words were: pigs, piglets, weaning, diarrhoea, ETEC, *E. coli,* nutrition, feed, feed additives, management, genetics, environment, risk factors, post-weaning diarrhoea, diarrhoea, hygiene, age, antibodies, feeding strategy, organic acid and liquid feed. The following criteria were applied to the literature selection: 1) Peer-reviewed journal articles in English were included; 2) Articles or chapters in an edited book were selectively included; 3) Grey literature such as PhD theses and dissertations were selectively included; 4) Published reports from the official organisation such as The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) and European Food Safety Authority (EFSA) were selectively included;, 5) In vitro and in vivo investigations related to pigs were included; 6) In vitro and in vivo studies on humans, rodents and other animal species were selectively included, provided that the aims were to investigate the cell/host pathogen interactions and/or enteric infections; 7) Enteric diseases related to presence of *Brachyspira pilosicoli* and *Lawsonia intracellularis* were excluded. In addition, we used

the references from the articles obtained by this method to check for additional relevant material. Furthermore, we included Danish material available at SEGES Pig Research Centre (http://vsp.lf.dk/Publikationer.aspx) and DCA (National Centre for Food and Agriculture) (www.dca.au.dk).

3. Introduction

Diarrhoea is a common clinical condition in newborn and newly-weaned piglets (Lalles et al., 2007). At its core, diarrhoea is simply an altered movement of ions and water that allows an osmotic gradient. Under normal conditions, the gastrointestinal tract has tremendous capacity to absorb fluid. However, enteric pathogens can alter the balance towards net secretion, leading to diarrhoeal disease (Hodges and Gill, 2010). Diarrhoea is manifested as an increase in the water content of faeces and (or) as an increased daily passage of faeces. Diarrhoea may result from nutritional imbalance causing irritation and/or increased luminal osmotic forces; however, post-weaning diarrhoea (**PWD**) is primarily caused by enteric infection by Enterotoxigenic *Escherichia coli* (**ETEC**), also called enteric colibacillosis, appearing mostly within the first two weeks post-weaning (Fairbrother et al., 2005). The prevalence of PWD creates a major challenge not only for the pig farmer because of its economic impact but also for the society as diarrhoea results in decreased animal welfare and high use of antibiotics and zinc (**Zn**) with the consequent risk of developing bacterial antibiotic resistance (WHO, 2012). Antibiotic resistance causes a major threat to human health. The ongoing increase in multi-drug resistant bacterial infections (Blair et al., 2015) has emphasised an increased need for prudent use of antibiotics and has put major focus on the antibiotic consumption in production animals. According to the Danish Authorities (DANMAP, 2015), pigs consume 76% of the total antibiotic mass consumed by production animals, horses, pets and fish while only contributing with 43% to the live biomass. Pigs during the post-weaning period consume the largest amount of antibiotics compared with sows and suckling piglets, and finishers (DANMAP, 2015). In addition to treatment with antibiotics, dietary levels of zinc oxide (**ZnO**) at a high level can prevent PWD in weaner piglets (Owusu-Asiedu et al., 2003); (Pluske, 2013). In Denmark, the use of ZnO prescribed by veterinarians has increased over the last decade. From 2005-2011, there was a threefold increase in the use of Zn and ZnO reported to VetStat. Since 2011, the use of pharmacological zinc for pigs has continued to increase steadily (Erratum, 2015): from 471 tons (2010) to 525 tons (2015) of therapeutic zinc (as ZnO) to pigs in Denmark (Erratum, 2015). It should, however, be noted that the consumption of pharmacological zinc has decreased with approximately 4% from 2014 to 2015 (DANMAP, 2015).

Several reviews have been conducted on the treatment and control of ETEC infections in pigs (Hodgson and Barton, 2009)and the possible intervention strategies to control diarrhoea in pigs (Halas et al., 2007); (Amadori et al., 2012); (Heo et al., 2013); (Sugiharto et al., 2015a). However, little is known regarding the real efficacy of the proposed alternative strategies and interventions. The objective of the present review is to perform a knowledge synthesis based on existing literature, which could form the basis for the selection of relevant interventions to study in a cost-effectiveness analysis in order to identify economically feasible alternatives to in-feed antibiotics and zinc for preventing/reducing PWD.

4. Changes in the gastrointestinal system around weaning

During the last three decades, research and field experience have focused on stressors encountered by pigs around weaning in order to reduce them and thereby obtain good levels of production. A weaning age of 22-28 days is practiced in many pig-producing countries, including Denmark. Many scientists have considered the changes taking place around weaning concerning nutrition, housing, health, and behavioural and environmental requirements of the young pig (Pluske et al., 2003), and much recent research and commercial interests have focused on "gut health" and optimal immune function. One of the major changes at weaning is nutrition, i.e. from sow milk to solid feed. Creep feed is generally provided from around 14 days of age, but a significant creep feed intake is not observed until approximately four weeks of age (Lucas and Lodge, 1961). When milk supply ceases abruptly, the structure and function of the digestive tract begins to change within hours.

Alterations in the digestive tract may have long-term (one or more weeks) consequences and may lead to poor absorption of nutrients in the small intestine, which is often associated with proliferation of enterotoxigenic bacteria and/or increased microbial fermentation of nutrients. Many pigs experience a so-called "growth check" or a fall in growth rate post-weaning, e.g. due to reduced feed intake and infections. And the extent of the depression in growth depends on how rapidly the piglet can cope with the challenges and regain homeostasis; for example is gut health highly dynamic, and if the balance tips, the consequence is diarrhoea and in more severe cases probably enteric inflammation. In the following, we will briefly describe how the intestinal microbiota, intestinal barrier and immune function are affected by weaning.

Microbiota

Several studies have investigated the changes in the gut ecosystem in relation to weaning (Jensen, 1998). Pigs were weaned and followed for 11 days. A steady decrease in pH and dry matter in rectal samples was observed during the first week post-weaning. While lactic acid bacteria (LAB) were significantly suppressed, the number of coliform bacteria increased in the intestine after weaning, thus a reduced ratio lactobacilli:coliform bacteria was observed during the first week post-weaning (Jensen, 1998). These alterations may result in an increased susceptibility of the pigs to colonization by pathogenic bacteria such as ETEC, which typically colonize and proliferate in the distal jejunum and ileum. 16S sequencing analyses have revealed that the intestinal microbial community is very complex, and the majority of the bacteria colonizing the gastrointestinal tract in pigs have not been characterised in detail (Leser et al., 2002; Kim et al., 2011a, 2012; Looft et al., 2014a)

It is clear from many studies that the profile of the microbiota in the intestine of pigs is affected by the specific diet and nutrient content (Canibe and Jensen, 2003; Canibe et al., 2005); (Mikkelsen et al., 2007); (Liu et al., 2013); (Rist et al., 2013); (Heinritz et al., 2013). As expected, antibiotic treatments can also shift the intestinal microbiota (Kim et al., 2012); (Looft et al., 2012); (Looft et al., 2014b). Another important factor for the microbial composition is the environment. Surprisingly, it was demonstrated that exposure to a large variety of environmental microbes in early life (pigs housed outdoor) did not generate greater diversity in the adult gut than in pigs housed indoor (intensive) but rather led to a microbiota dominated by a limited number of phyla composed of lactobacilli (Mulder et al., 2009). Farm management may also have an impact on the amount of *E. coli* excreted, as conventional herds had higher number per gram colon content than organic farms (Kerouanton et al., 2014).

Jensen (Jensen, 1998)concluded that the microbiota is unstable during the first week post-weaning (from 28 to 35 days of age) and that it takes two to three weeks from weaning before the fermentative capacity in the hindgut is developed. (Kim et al., 2011b), on the other hand, reported continued changes in the faecal microbiota composition when studying pigs from 10 to 22 weeks of age.

Intestinal barrier function and nutrient absorption

In comparison to other compartments of the GIT and its accessory organs, it is probably the small intestine that is affected the most by weaning – largely irrespective of weaning age. Significant

changes occur in the structure and function of the small intestine during the immediate post-weaning period (Hopwood and Hampson, 2003), and it is generally accepted that there is a transient period of villous atrophy and crypt hyperplasia after weaning, as reviewed by e.g. Heo et al. (Heo et al., 2013), which could be ascribed to the reduction in feed intake. A reduced villous surface after weaning implicates a reduction in intestinal absorptive capacity as well (Wijtten et al., 2011) as the presence of villi helps to increase its surface area for digestion and absorption processes. In addition, the process of weaning affects the small intestinal barrier function in pigs (Wijtten et al., 2011). In healthy animals, antigen uptake is precisely regulated to train the immune system. The effect of weaning stress or reduced feed intake is less clear in relation to the intestinal barrier function (Pluske, 2016). Impaired intestinal barrier function or increased intestinal permeability may promote the translocation of bacteria and the entering of allergenic compounds from the gut into the body, which increase the immunological responses and the susceptibility to infections.

Immunity

The presence of commensal bacteria has a direct influence on the immune maturation, and the microbial exposure influences expression of a large number of immune-related genes (Mulder et al., 2011). Hence, host-independent factors in connection with weaning, such as nutrition and rearing environment, which influence the gut microbiota may also influence the immune activation. Weaning of pigs (between four and five weeks of age) suppressed a broad spectrum of adaptive immune components in blood (T-lymphocyte subsets, B-lymphocytes, monocytes and granulocytes, immunoglobulins, mannan-binding lectin) both immediately after weaning as well as after a lag period of about one week; on the contrary, the innate immune system seems to be stimulated immediately after weaning (Juul-Madsen et al., 2010). At the time considered to have highest infection pressure, T-cells and TLR4+ cells were markedly enhanced, whereas the expression of SLA 1 did not seem to be affected by weaning (Juul-Madsen et al., 2010). In general, immune parameters seem to be stabilised at an age of 8-10 weeks. Nutritional stress in terms of anorexia post-weaning coupled with its consequences on gut morphology have been reported to be major contributor to local intestinal inflammation during the immediate post-weaning period (McCracken et al., 1999). However, it still remains to be answered what the most important factors are (i.e. deprivation of milk intake and/or changes in nutrition, changes in microbial environment or natural age-dependent developmental changes) for the immune activation at weaning.

5. Pathogenesis and diagnosis of post-weaning diarrhoea

PWD is a multifactorial disease, and its pathogenesis is still unclear. Frequently described, this is a condition of weaned pigs characterised by frequent discharge of watery faeces during the first two weeks after weaning. This condition is typically associated with faecal shedding of haemolytic ETEC that proliferate in the ileum. However, ETEC is detected in both healthy and diarrhoeic pigs upon weaning. Pigs with *E. coli* PWD typically have watery diarrhoea that lasts from one to five days, but some pigs may die suddenly without observed diarrhoea. Those pigs may have an accumulation of fluid in the intestine and ETEC invasion in blood and tissues leading to sepsis. The pathophysiological mechanisms of infectious diarrhoea have been described by Hodges and Gill (Hodges and Gill, 2010)and involve alterations in ion transport and tight junctions. In brief, by the use of fimbriae, ETEC adheres to receptors of the small intestinal enterocytes where they produce one or more enterotoxins such as heat-labile toxins (LTs) or heat-stable (STs) toxins, which activate the cAMP and the cGMP systems. The LT toxins increase secretion of sodium, chloride and hydrogen carbonate ions into the lumen, whilst the ST toxins reduce the absorption of liquid and salts. This leads to hypersecretion of water and electrolytes to the small intestine that exceeds the ability of the colon to reabsorb, causing diarrhoea and dehydration, and results in reduced feed intake, reduced nutrient digestibility, reduced growth and even death. Another toxin, the shiga toxin Stx, is characteristic of shiga toxin-producing *E. coli* strains causing porcine oedema disease (ED) but may also be present in the ETEC strain (Hodgson and Barton, 2009).

To elicit diarrhoea in piglets, ETEC must first adhere to specific porcine enterocyte receptors for the respective fimbriae (Heo et al., 2013); (Fairbrother et al., 2005). This adhesion facilitates colonization of the distal jejunum and proximal ileum mucosa by ETEC and enables them to transmit enterotoxins. The fimbriae are thin and flexible structures with a diameter of about 2-4 nm whereas pili are more rigid structures with a diameter of about 7-8 nm and have an axial hole (Degraaf and Mooi, 1986), and both contain an adhesive lectin subunit. The biosynthesis of fimbria is influenced by several factors, and the pathogenic bacteria only fully express virulence factors like fimbria when the conditions are appropriate for adherence and subsequent colonization (Verdonck et al., 2004). Fimbriae designated F4 and F18 are the types that are commonly found on ETEC from PWD in pigs. In case of F4 fimbriae, expression is optimal at a temperature of 37 °C, a pH ranging from 6.5 to 8.0 and at the end of the exponential growth. F18 fimbria are typically associated with diarrhoea in nursing pigs whereas F4 fimbriae are associated with diarrhoea in nursing pigs as well as in weaned

pigs. Frydendahl (Frydendahl, 2002) identified genes for F4 and F18 in 92.7% of all ETEC from PWD. Recent results have shown that among diarrhoeic pools observed in 51% of the pens in batches of nursery pigs (10-66 days after weaning), *E. coli* F4 and F18 were detected in 13% and 11%, respectively (Weber et al., 2015)Adhesion of F18-fimbriated bacteria to the small intestine of susceptible pigs is mediated by the minor fimbrial subunit FedF (Coddens et al., 2008)Adhesion of F4-fimbriated bacteria to the small intestine is mediated by the fimbrial subunit FaeG adhesion (Verdonck et al., 2004).

Specific serotypes of ETEC with particular sets of virulence genes, i.e. ETEC expressing F4 or F18 fimbriae, have been associated with diarrhoea in pigs as well as in other mammals, including humans (Nagy and Fekete, 1999); (Fairbrother et al., 2005). The most frequent ETEC serotypes causing PWD in pigs are O serotypes (8, 138, 139, 141, 147, 149 and 157), and the predominant serotype of *E. coli* associated with PWD in pigs worldwide is O149 (Fairbrother et al., 2005); (Heo et al., 2013). This serotype was first detected in Denmark in 1966 and was still dominant in 2002 (Frydendahl, 2002). Dam and Knox (1974) were able to trace the spread of O149 ETEC from its first appearance on four farms in 1966 to its rapid spread over the entire country by 1969.
In the recent yearly report (Veterinærinstituttet, 2015), F4 and F18 appeared as the most predominant in faecal and intestinal samples from pigs from Danish herds, and O149 was the predominant hemolytic serotype of *E. coli* followed by O141, O139 and O138.

Diagnosis of post-weaning diarrhoea and enteric inflammation
Diarrhoea is the main cause of treatment with antibiotics in Danish pig production (Pedersen, 2014). Whereas the classical weaning diarrhoea associated with *E. coli* within the first two weeks post-weaning was a frequent diagnosis, the large consumption of antibiotics is not used for treatment of PWD but for treatment of diarrhoea among pigs appearing later than two weeks post-weaning (Pedersen, 2014). In Denmark, this may probably be ascribed to the use of pharmacological levels of ZnO as prevention against diarrhoea appearing during the first weeks post-weaning. It is generally accepted that *E. coli, Brachyspira pilosicoli* and *Lawsonia intracellularis* cause this diarrhoea and that they appear in combination during infections in around half of the Danish herds (Pedersen, 2014). By using qPCR on faecal and intestinal samples of pigs sent for diagnosis at the Veterinary Institute, Denmark, it was detected that 196 out of 840 samples were positive for *E. coli* F4; 379 out of 840 samples were positive for *E. coli* F18 (F107); 599 out of 833 were positive for Lawsonia

intracellularis; and 256 out of 834 were positive for Brachyspira pilosicoli (Veterinærinstituttet, 2015). A recent study (Weber et al., 2015) concluded that diarrhoeic status of pigs was a poor indicator of intestinal infection with *E. coli* and subclinical infections.

Many studies conducted in research or under commercial settings have reported the impact of the treatments under investigation on diarrhoea but often without being a primary response parameter.
It is often difficult to assess "diarrhoea" per se in commercial husbandry systems as it is associated with a high level of variability, and the studies use different measures as indicators of diarrhoea, e.g. frequency of treatment with antibiotics, faecal score, or number of days with diarrhoea. Diarrhoea index can be measured as proportion of days with diarrhoea in relation to total number of days, but information on whether or not the diarrhoea encompassed in the index required antibiotic treatment has not consistently been given (Madec et al., 1998). Trials performed in commercial settings, the frequency of diarrhoea may be given as "number of days with antibiotic treatments"; however, it may vary among the published studies how the decision was taken for antibiotic intervention.

In experimentally-induced diarrhoea, in which diarrhoea is induced by inoculation with *E. coli* (Sugiharto et al., 2014); (Sorensen et al., 2009), faecal samples are obtained frequently from each individual pig, and the faecal samples are scored according to the Bristol scale (1=hard, dry and cloddy, 2=firm, 3=soft with shape, 4=soft and liquid, 5=watery and dark, 6=watery and yellow, 7=foamy and yellow; a faecal consistency score >3 was defined as a clinical sign of diarrhoea). The aim of this experimental model is to induce a mild diarrhoea in pigs, which can be cleared by the pig itself. In parallel with diarrhoea score, faecal samples can be analysed for dry matter, and there is a good relationship between the diarrhoea score and the dry matter. ETEC diarrhoea is typically associated with faecal shedding of large numbers of β-haemolytic *E. coli* serotypes that proliferate in the small intestine of pigs (Fairbrother et al., 2005); (Heo et al., 2013). Quantitative analyses of the *E. coli* content in the faecal samples are therefore relevant as this is an indicator of the burden of the inoculum.

In many studies aiming to identify dietary and management tools to reduce diarrhoea in pigs, it is not indicated how diarrhoea was assessed, and the experiments have not been designed to study diarrhoea. It requires a large sample size to determine the prevalence of diarrhoea and to test the effect of a given intervention on the outcome of diarrhoea (Weber et al., 2015; Weber et al., 2017). However, many

studies have been performed to understand the mechanisms of the immune system and the function of the microbiota in order to prevent the enteric diseases and to develop tools to control it. In the following section, we will therefore describe how genetics, management and nutrition can influence the infection with ETEC.

6. Factors predisposing/influencing PWD caused by ETEC

It is clear from the above that many factors related to the gastrointestinal changes post-weaning can influence the presence of ETEC infection and diarrhoea. In order to identify alternatives to in-feed antibiotics and Zn for prevention of ETEC-associated diarrhoea, it is important to understand how genetic, management, environment and/or nutritional factors can influence the interactions between the host and the pathogen, and this starts with the harbouring of the ETEC to its infection of the host, which can subsequently lead to diarrhoea and intestinal inflammation. However, diarrhoea in pigs after weaning is a multifactorial disease (Pluske, 2013), and changes around weaning, i.e. the weaning-related stress and weakened immune competence, weaning age, feeding regimen and the presence of other infectious agents (such as rotavirus) are critical for the diarrhoea to develop (Fairbrother et al., 2005).

Genetics

To induce diarrhoea, fimbriae-designated F4 or F18 must bind to the specific receptor on the host intestinal epithelium (Heo et al., 2013). In pigs, only the ones with positive F4 or the specific receptor F18R on the intestinal enterocytes are susceptible to ETEC-diarrhoea, whereas pigs lacking these receptors are resistant (Nagy and Fekete, 1999); (Fairbrother et al., 2005). Knowledge regarding the distribution of the receptors in a herd or an experimental model for PWD is important to take into account when assessing the efficacy of a given treatment against ETEC PWD in pigs (Niewold et al., 2007). A DNA marker-based test has been employed to identify whether pigs lack or possess the F4 or F18R receptor (Fairbrother et al., 2005). The Mucin 4 gene on porcine chromosome 13 is proposed as a candidate gene for the production of the specific ETEC F4ab/ac receptor (Jørgensen et al., 2004) (Jensen et al., 2006) while the α-(1,2)-fucosyltransferase (FUT1) gene localised on the porcine chromosome 6 has been proposed as a key gene for controlling the expression of *E. coli* F18R (Bao et al., 2012). With the DNA-based marker test allowing genotyping for F4ab/ac ETEC resistance/susceptibility, three genotypes were observed and were called resistant (RR), susceptible heterozygote (SR) and susceptible homozygote (SS). It should be noted that, in other reviews,

(Hodgson and Barton, 2009) it was concluded that there is no definitive simple test for F4 resistance. In addition to the mucin 4 gene, the transmembrane mucin MUC13 appears to be significantly associated with susceptibility and resistance to F4ab/ac, as the MUC13 gene is closely linked to the genes encoding F4ab and F4ac receptors.

It seems obvious that there are host-specific factors that can influence the susceptibility to ETEC adhesion, and breeding programs with F4R negative pigs could result in prevention of F4ab/ac-induced ETEC diarrhoea in pig population. Such a screening has been applied in the Danish breeding program, and although the Danish pig population is considered genetically F4 ab/ac resistant, the incidence of diarrhoea has not been effectively reduced according to the authors' knowledge. Targeting a multifactorial production disease, such as PWD, by selection based on single-genes is probably not efficient, because several factors beyond the receptor on the surface of the intestinal epithelium of the pig are related to diarrhoea.

Management and environment

Weaning is probably one of the most stressful events in a pig's life. Besides the social challenge associated with separation from the sow, there are a number of stressors attributed to management and environment, which can enhance PWD, for example the different physiological environments (room, building, farm, water supply) and increased exposure to pathogens. In nature, pigs are weaned from the sow over a period of up to 12-18 weeks of age, where they experience a gradual reduction in the amount of contact with their dam, a reduction in suckling frequency, and a gradual change of diet from milk to solid or semisolid food(s) (Jensen and Stangel, 1992). In commercial production systems, pigs are abruptly separated from the sow at 4 weeks of age (conventional systems) or 7 weeks (organic systems). It seems clear from the literature, and in accordance with our own experiences, that age at weaning influence PWD, and that early weaning (< 21 days of age) is by far more challenging than weaning at 28 days of age (Kil and Stein, 2010).

The hygiene level, humidity, group size and stocking procedure were found to be important factors leading to risky or secure profiles (Madec et al., 1998). Weaning piglets into a dirty environment may expose them to pathogenic bacteria, thereby increasing the risk of PWD, and weaning pigs into a clean environment improves growth performance compared with weaning pigs into a dirty environment (Le Floc'h et al., 2006; Le Floc'h et al., 2009). Deterioration of sanitary conditions in post-weaning facilities has been shown to impair nutrient utilization and pig growth performance by

inducing a moderate inflammatory response (Le Floc'h et al., 2006). In his review, Pluske (Pluske, 2016)concluded that at weaning, and largely independent of weaning age, but dependent on diet type, diet form and the rearing environment, the piglet is subjected to myriads of changes (nutritional, psychological, environmental). These changes can further modify the overall structure and function of the gastrointestinal tract, such that the newly weaned pig endures a 'growth check' while exposing it to a greater disease and health risk.

The litter-size at birth may influence the individual pig susceptibility to PWD weaning. The use of hyperprolific sow lines has increased the number of piglets per litter, and has made the piglets within a litter more heterogeneous, and immunologically fragile (Lauridsen and Matte, 2017). In addition, the transfer of antibodies from the sow to the piglet influences the robustness of the suckling pig and probably also during the weaning period, and limited provision of colostrum because of large litter size per se may therefore influence the susceptibility to PWD. Although not of relevance for the modern pig lines of today, it should be noted that data from a Danish epidemiological study comprising 48,931 litters in 89 sow herds, conducted from 1976 to 1982, showed that the incidence of PWD increased with litter size at weaning, as a litter of 11-12 piglets at weaning had a 1.2 times higher risk of getting PWD than litters with 8-10 piglets (Svensmark et al., 1989), and this variation as well as the weight within a litter at weaning may also influence the susceptibility to PWD.

Feeding and Nutrition

According to the previous sections, the multifactorial nature of PWD is obvious and dependent on the complex interaction between the microbial population and the immune system including host factors for the ETEC infection. Many reviews have been published on nutritional approaches to control ETEC infections in pigs, and in this context, it is important to differentiate between dietary factors that may promote ETEC infections and those that may limit them. One of the major factors of importance is the level of feed intake, both before weaning and post-weaning, because of its influence on intestinal barrier function and maturity. This means that the low feed intake by the piglets after weaning is consistently associated with short intestinal villi, which reduces the absorptive area. Suckling pigs start eating feed ('creep feed') from about 14 days of age, but only few g per day until about 21 days of age. Subsequently the feed intake is significantly increased, and a Danish study showed that suckling piglets weaned at day 33 of age would triple the feed intake (0.2 to 0.6 kg/pig/day) during the last week in the farrowing stable (Callesen and Thorup, 2004). Pigs

characterised by being 'eaters' have better initial post-weaning performance than 'non-eaters' (Pluske, 2016). Sørensen et al. (Sorensen et al., 2009) revealed that a feed intake of less than 200 g during the first day after weaning seems to be associated with a relatively high incidence of a PWD in pigs weaned at week 7 and challenged with *E. coli* F4. Another study (van der Meulen et al., 2010) showed, however, that although pigs weaned at 7 weeks of age (compared to 4 weeks of age) had improved post-weaning feed intake and reduced weaning stress, no improvements in functional characteristics of the small intestine was observed. Hence, it is not obvious that increased feed intake would be reflected in more mature gut, i.e. enhanced intestinal morphology and digestive function. This is probably because such data are prone to a large variation, and according to Heo et al. (Heo et al., 2013) it is rather difficult to compare data on intestinal morphology from different experiments because of differences in the age, breed, diets and experimental conditions.

Furthermore, the critical role of feed intake is a focus point in relation to the feeding behaviour of the post-weaned pig. Following abrupt weaning, the piglet has to adapt its feeding and drinking behaviour very rapidly. However, in general, it fails to do this and, as a result, there is a dramatic reduction in dry matter intake that does not recover until the second week post-weaning (Brooks and Tsourgiannis, 2003). Besides the great variation in the feeding behaviour of pigs pre-weaning (in terms of creep feed intake), there is also a great variation immediately post-weaning. It is often difficult to assess the individual feed intake, but interesting data have been presented by Brooks and Tsourgiannis (Brooks and Tsourgiannis, 2003): The apparent smooth transition in feed intake as could be observed as an average for the population was actually a statistical artefact: Very large variations in the feeding pattern of individual pigs were observed, as half the pigs in the group had a peak feed intake on day 2 followed by a reduction that those pigs had not recovered from by the end of the first week. And almost half the piglets had erratic feed intakes with peak intakes occurring on days 4, 5 or 6 (Brooks and Tsourgiannis, 2003). Our own experience with pigs entering our *E. coli* challenge model confirms that not only is the variation between litters large, but also within a litter in terms of feed intake pattern. Generally speaking, however, pigs weaned from 3-4 weeks of age have lower feed intake than pigs weaned at 4-5 weeks of age. According to the authors' knowledge, there is a lack of knowledge regarding the importance of heterogeneous litters at birth and their treatment during the suckling period (such as cross-fostering and provision of milk replacer) in terms of feed intake for the individual pig post-weaning and the relationship to gut maturity. However, it should be noted that the gut epithelial tissue is the most rapidly growing tissue in the body, and that many nutrients

required for gut growth are absorbed directly from the gut lumen. Pluske et al. (Pluske et al., 1997) have demonstrated that a continuous supply of nutrients is essential in order to maintain the enteric villus structure post-weaning. However, suckling pigs must consume milk to satisfy their need for total volumetric fill, but they may fail to discriminate between the separate drives of hunger and thirst in the early post-weaning period, and may consequently be prone to drink water to get the gut fill (Brooks and Tsourgiannis, 2003). Overall, a sharp reduction in feed intake post-weaning may have tremendous consequences for the enteric development.

7. Prevention of weaning diarrhoea

As introduced earlier, a prominent objective of our present knowledge synthesis was to propose strategies that could be considered relevant for further study in a cost-effectiveness analysis. While our study of existing literature has revealed many attempts and research focusing on the alternatives to antimicrobial usage, less focus has been given to the identification of alternatives to the use of pharmacological levels of ZnO, which has actually been proposed, also recently, as an alternative strategy to in-feed antibiotics in the prevention of PWD (Heo et al., 2013).

7.1. Mode of action and use of antimicrobials and zinc

The most effective way to control PWD in piglets is still the use of antibiotics which function by attacking various essential processes in the bacterial cell. In 2012, 43% of the antimicrobials prescribed for pigs were used in nursery pigs (7-30 kg live weight), of which 75% were prescribed for oral treatment of gastrointestinal disease. With regard to treatment against *E. coli*, several antibiotics may function (Nielsen, 2012), but according to DANMAP (DANMAP, 2015), tetracyclines have been one of the most commonly used antimicrobials in the Danish pig production for a decade, and are almost entirely used for the treatment of gastrointestinal disease in weanling pigs and finishers. In 2014, the Danish pig producers committed themselves to reduce the consumption of tetracyclines by 50% by the end of 2015.Athough the use of tetracyclines has been reduced since 2014 it is, however, of some concern that the use of colistin in pigs has increased, especially from 2014 to 2015 (DANMAP, 2015), because colistin is of increasing importance as a last resort antimicrobial in human medicine.

Since the late 1980s, the practice of adding pharmacological amounts of ZnO to the pig feed has become widespread in commercial pig production, and the use of ZnO in the Danish pig production

has increased markedly over the last 10 years (DANMAP, 2015). Although the recommended dietary level of Zn for weaner pigs is 100 mg/kg (NRC 2012), pharmacological levels (up to 2,500 to 3,000 mg Zn/kg diet) have been used to ameliorate and (or) prevent PWD for 2 weeks post-weaning. This practice was initially stimulated by a report (Poulsen, 1989) showing that 3,000 mg Zn from ZnO resulted in a 60% reduction of the diarrhoea incidence when compared with lower dietary Zn levels (addition of 0, 100, 200, or 1,000 ppm Zn as ZnO). In a subsequent publication, (Poulsen and Larsen, 1995) showed in a study using 216 piglets from 36 litters that a supplement of 2,500 ppm Zn for two weeks after weaning (at 28 days) reduced the incidence of non-specific post-weaning diarrhoea by up to 50%, and had a growth promoting action: observations that have also been supported by more recent reports (Cho et al., 2015); (Kaevska et al., 2016); (Stensland et al., 2015); (Trckova et al., 2015). A large Danish trial with nursery pigs (approximately 3200) in a herd suffering from *E. coli* 0149 post-weaning also demonstrated a reduction in the number of diarrhoea treatments by supplementation of 2500 ppm Zn in combination with organics acids (Johansen, 2007).

Although ZnO is regarded as a potent prophylaxis against the development of PWD in piglets, zinc excretion in high amounts represents a hazard to the environment (Heo et al., 2013); (Sales, 2013). Moreover, Zn and other heavy metals are involved in antibiotic cross-resistance phenomena, which is why the practice of using ZnO for weaners is prohibited in some European countries, and is prone to be banned in the EU entirely. Nevertheless, in the quest for alternatives to antibiotics for preventing or treating PWD, there may be important lessons to learn from the ZnO-based mechanisms involved in stabilising the gut environment of piglets in connection with the weaning period.

The exact modes of action in relation to physiology of pigs for the effects of feeding pharmacological levels of Zn have not been fully elucidated. The effect has, however, been connected with improved feed intake by piglets (Broom et al., 2003); (Hahn and Baker, 1993), a crucial response parameter for all feed supplements aiming to improve the health and performance of weaners (Campbell et al., 2013), as well as with an improved physiological Zn status (plasma Zn level) of piglets. It has thus been suggested that Zn as well as copper additives may prevent the development of a physiological Zn deficiency (Carlson et al., 1999; Carlson, 2003); (Hill et al., 2001); (Poulsen and Larsen, 1995). Other reports have found changes in some pancreatic enzymes and hormonal status (Hedemann et al., 2006), (Li et al., 2006), and new studies show that Zn accumulation in the liver or pancreas of weaning pigs after feeding high dietary ZnO for 4 weeks changed the expression of genes and proteins related

to energy and amino acid metabolism, but also increased oxidative stress reactions in these organs (Bondzio et al., 2013); (Pieper et al., 2015).

Reported effects of, in particular, ZnO on the gastrointestinal microbiota point towards the involvement of non-physiological mechanisms. Højberg et al. (Hojberg et al., 2005), using 2,500 mg/kg Zn as ZnO, showed a reduced bacterial activity in the digesta from the gastrointestinal tract compared with that in animals receiving 100 mg/kg ZnO, most likely reflecting a reduced load of bacteria present in the gastrointestinal tract of animals receiving the high ZnO level. Data like these indicate a major influence of ZnO dose on the gastrointestinal tract microbiota and show that, besides a potential promotion of feed intake, high dietary ZnO doses may further render more energy available for the host animal by generally suppressing the commensal gut microbiota. This has actually also been suggested as one of the working mechanisms behind the effect of antibiotic growth promoters (Collier et al., 2003); (Gaskins et al., 2002). It should be emphasised, though, that it might not only be a question of energy availability for the host animal. Commensal bacteria, like lactic acid bacteria and certain clostridia, may also utilise essential feed components, such as amino acids, and may impair lipid/fat digestibility by hydrolysing bile salts (Knarreborg et al., 2002a; Knarreborg et al., 2002b).

A decrease in the total number of anaerobes and lactobacilli and an increase in the number of coliforms have been observed in ileum samples from animals fed a ZnO-amended diet (H›jberg et al., 2005); (Jensen, 1987). Thus, a direct inhibition of potential pathogens, as represented by enterobacteria or coliforms, has often not been reported in post-weaning piglets receiving high ZnO levels (Jensen, 1987); (Hojberg et al., 2005); (Broom et al., 2006); (Vahjen et al., 2010), even though ZnO has been shown to reduce the susceptibility of pigs to *E. coli* infection (Mores et al., 1998). High doses of dietary ZnO have, however, been shown to support a large diversity of coliforms/enterobacteria in weaned piglets (Katouli et al., 1999), (Vahjen et al., 2011). Thus, more specific modes of action than growth inhibition of an entire bacterial group may apply, and the impact of dietary ZnO on the diversity of the coliform community may prevent the blooming of, e.g., specific pathogenic ETEC strains. Indeed, *in vitro* studies have shown that members of different bacterial groups, commensals as well as pathogens, show a wide range of sensitivity to ZnO (Liedtke and Vahjen, 2012). Thus, the growth inhibiting effect in vivo may be governed by a more selective growth

inhibition, i.e., the presence of sensitive and resistant species or strains within the different microbial genera.

A recent study by Vahjen et al. (Vahjen et al., 2016)demonstrated that the level of specific *E. coli* toxin genes was actually decreased by a high ZnO dose (3000 ppm). However, this study also demonstrated a more detailed time lapse, where the *E. coli* was generally inhibited from 3-4 days after weaning, concomitantly with an increase in the faecal ZnO level, demonstrating that the piglets had started to eat. However, the *E. coli* numbers started to increase again 8-14 days after weaning: the period on which many published studies have focused. This scenario may indicate an adaptation of *E. coli* to ZnO.

Dietary doses of $CuSO_4$ (at 175 mg/kg) inhibited the coliforms, and thus potential pathogens as well, but overall the observed effect of $CuSO_4$ was limited compared to that of ZnO. In studies with both high levels of Zn and high levels of Cu, both were efficacious individually in terms of growth promotion, but they were not additive when they were added in combination in diets for weaning pigs. In the study by Poulsen (Poulsen and Larsen, 1995), the severity of diarrhoea seemed to be more pronounced among litters fed high copper (at high levels of zinc), although it should be noted that the results were confounded with the litter number. Hill et al. (Hill et al., 2001) reported that the improvements in performance with high Zn levels could be additive to antibiotics.

It may be concluded that it remains to be answered in detail how the growth-promoting and diarrhoea-reducing effects of excess dietary Zn are exerted. However, it seems that high dietary ZnO has a positive impact on the stability and diversity of the gastrointestinal microbiota, which contributes to an increased colonization resistance against pathogens, and thereby resistance to diarrhoeal infections, as well as better growth performance of the piglets (Katouli et al., 1999); (Vahjen et al., 2011); (Pieper et al., 2012).

7.2. Interventions to limit predisposition to PWD

Until 2006, in-feed antibiotics (such as Zincbacitracin, Virginiamycin and Salinomycin) have been frequently used in pig production to promote growth performance, and their mode of action has been reviewed by Jensen (Jensen, 1998). Several reviews have been conducted with the aim to present alternatives to in-feed antibiotics on health and growth, and these have included reports on feeding-

and management strategies, and the use of feed additives (see for instance (Pluske, 2013); (Halas et al., 2007); (Heo et al., 2013); (Papatsiros et al., 2013)).As a consequence of the ban on the use of growth-promoting antibiotics, as well as a general reduction of the prophylactic and therapeutic use of antibiotics in the swine production, high doses of dietary ZnO are used in pig nutrition. In the present section, it should be emphasised that, although we have initially focused the aim of our knowledge synthesis on identifying alternatives to antibiotics and high levels of zinc for prevention of diarrhoea in pigs post-weaning, we need to stress that the modes of action by which antibiotics and high doses of zinc are functioning are not exactly clear, and knowledge on this would form the basis for more efficient selection of alternative in future research.

7.2.1. Nutrition

Apart from pharmacological levels of ZnO, some feeds and feed ingredients have been suggested to possess immunomodulatory and antimicrobial properties. In a reviewed by Sugiharto et al. (Sugiharto et al., 2015a), dairy-based products gained increasing interest as a potential nutritional tool to prevent piglets from developing diarrhoea due to ETEC. Bovine colostrum, milk and milk fractions such as whey and casein contain several bioactive components with antimicrobial and immunomodulatory properties, but the knowledge related to the application of the dairy-based products to prevent ETEC infection and post-weaning diarrhoea is very limited. Recently, a review was conducted on the immunomodulating effects of probiotics, prebiotics and synbiotics for pig gut health, but so far only a limited number of studies are available in which the clinical end-point has been diarrhoea score (Roselli et al., 2017). Likewise, micronutrients, such as vitamins (E, D, and C) and some trace elements (selenium), confer gut epithelial protection due to their potent anti-inflammatory and anti-oxidant capacities (Lauridsen, 2010); (Dalgaard et al., 2017). Plant extracts and essential oils have been investigated for their use in animal nutrition, in particular for exploiting their antimicrobial, anti-inflammatory, anti-oxidative and anti-parasite properties (Namkung et al., 2004); (Brambilla and De Filippis, 2005); (Costa et al., 2007); (Liu et al., 2008); (Magi et al., 2006). The addition of plant-based bioactive additives to pig feed as a possible way to inhibit or reduce the growth of gastrointestinal pathogens has been reported both *in vivo* and *in vitro* in several studies (Pluske, 2013), (Roca et al., 2014), (Bontempo et al., 2014), (Michiels et al., 2012). However, in vivo effects of the additives are sparse, probably because the concentration of antibacterial compounds in the raw material is often too low to control bacteria *in vivo*. Further, sensory accept of feed additives by piglets may be a challenge if effective concentrations are too high. However, while widely researched in other animal

species and humans in relation to enteric diseases, further research is needed to elucidate the effectiveness of these feed ingredients and micronutrients (as well as dairy-based products, pro- and synbiotics, plant extracts, essential oils) to prevent ETEC infections and PWD in piglets before being considered as strategies to the prevention of PWD. Among the more promising strategies, we will consider the potential effect of reducing the dietary crude protein level; the supplementation of organic acids; the use of fermented liquid feed; and the use of dietary antibodies as described in the following.

7.2.1.1. Restriction of protein level

There is a considerable body of research dating back to the 1950s and 1960s which has implicated an association between protein level and diarrhoea after weaning (Pluske, 2013), and as also stated in their review (Kil and Stein, 2010), crude protein is the most important nutrient associated with digestive disorders in the pig. Upon consumption of a meal, pH in the stomach is increased whereby proliferating of *E. coli* is facilitated. In addition, feed ingredients such as soya bean meal, fishmeal and milk powder, which are typically used in feed for weaned piglets, also have a high buffering capacity which can also increase stomach pH and thereby limit pepsin activity (Manners, 1976). Hence, excessive crude protein intake by weaned pigs can lead to an increase in microbial fermentation of undigested protein, and this is a contributing factor to development of PWD. In **Table 1**, we have listed examples of studies in which reduction of the dietary protein level influenced the diarrhoea incidence in piglets post-weaning. Hence, one of our recommended alternative interventions to limit diarrhoea incidence is the use of low-protein diets, i.e. containing less than 18% crude protein. This strategy is not without challenges because reduction of the concentration of crude protein for weaned pigs may influence the growth performance because some of the indispensable amino acids may be present in concentrations below the requirement for maximum growth. However, the loss in growth of piglets on low-protein diets in comparison with piglets on diets with a normal protein content may be compensated on the longer term, as the lifetime performance in some studies did not differentiate (Wellock et al., 2009); (Kim et al., 2011b). The use of crystalline amino acids (lysine, methionine, threonine, tryptophan, isoleucine and valine) in low-protein diets to maintain the balance of the required amino acids for the weanling pigs and thereby pig performance (e.g. (Lordelo et al., 2008)). This approach is not a feasible approach in organic pig production because the use of synthetic amino acids is not allowed in organic feed for livestock (EC 889/2008), and one of the major problems in the formulation of feed for organic pigs is the lack of high quality protein sources to meet

the amino acid requirements to replace soya bean products, (EGTOP, 2015). Use of lysine, methionine and threonine is commonly used in the conventional pig feed production, but crystalline sources of tryptophan, isoleucine and valine are, however, very expensive. It is beyond the scope of this review to propose nutritional strategies for maximising growth performance while limiting crude protein, but recently published research is available (e.g. (Almeida et al., 2017). In this context, it should be mentioned that the study by (Kim et al., 2011b) showed no negative impact on lifetime performance or carcass characteristics of pigs provided low-protein diets without crystalline essential amino acids but it reduced the clinical expression of PWD. Hence, a strategy of using low-protein diets to contribute to a limitation of the development of PWD should encourage to further research and economic evaluation.

7.2.1.2. Use of dietary antibodies

Passive immunisation, i.e. the administration of antibodies (immunoglobulins) in order to protect against infection, is an old idea and seems to constitute a real and widely applicable alternative to antibiotics in modern animal production (Hedegaard and Heegaard, 2016)and also in prevention of PWD in pigs. A good example is the administration of colostrum feed supplements in which antibodies derived from the dam provide protection against infectious agents in the suckling offspring which underline the role of colostrum provision in the disease protection (Theil et al., 2014). Recently, passive immunisation using chicken egg yolk immunoglobulin (Igγ) has become an attractive approach with considerable attention, as it possesses a variety of advantages over mammalian IgG such as convenience, high yield and cost-effectiveness. Oral administration of specific chicken Igγ has been shown to be effective against a variety of intestinal pathogens, especially diarrhoeal pathogens, including ETEC. Based on a systematic review and meta-analysis, Diraviyam et al. (Diraviyam et al., 2014) demonstrated beneficial effects of Igγ in controlling and preventing the diarrhoea in domesticated animals, hence supporting the opinion that Igγ is useful for prophylaxis and treatment of gastrointestinal infection by oral immunisation as an alternative strategy to antibiotics.

Use of blood plasma for prevention of PWD has received interest, especially in countries using an early weaning age. Besides being an easy-digestible protein source with a good amino acid profile, the content of immunoglobulins is also considered to enhance the immunity of pigs. Use of 5% blood plasma (DAKA Porcine Plasma) in comparison with addition of 2500 ppm Zn to pigs weaned at a

weight below 9 kg in a Danish trial showed a positive influence on productivity and a reduction in diarrhoea treatments (Maribo, 2009).
However, Danish Pig Production, SEGES recommends not to use blood plasma (http://svineproduktion.dk/aktuelt/nyheder/2015/11/121115_kontrol-med-blodplasma-bliver-en-del-af-danish-ordningen) because of the risk for porcine epidemic virus diarrhoea. Results of a study with dietary specific antibodies in spray dried plasma powder showed protection against ETEC, and in F4 receptor positive piglets, the spray dried immune plasma powder reduced diarrhoea and decreased ETEC excretion (Niewold et al., 2007). In an experimental model for PWD, we recently investigated the oral administration of purified porcine immunoglobulin G from pooled natural pig plasma on enteric infection. We observed that the oral immunoglobulin product accelerated clearance of feacal hemolytic bacteria in pigs challenged with *E. coli* in comparison with pigs not receiving the immunoglobulin product (Hedegaard et al., 2017). The effect of this dietary intervention, which was observed during the seven days post-weaning, was equal to or better than that of dietary ZnO in reducing diarrhoea symptoms and in clearing faecal hemolytic bacteria for 14 days post-weaning.

Thus, provision of dietary antibodies based on blood plasma and eggs to pigs seem to be a promising alternative strategy to reduce the use of antibiotics and ZnO. The observations listed above warrant future experiments to investigate the efficacy in prevention of PWD. However, the products are not yet commercially available in Denmark, and will therefore not be suggested for economic evaluation..

7.2.1.3. Organic acids

A number of reviews can be found in the literature dealing with feeding strategies to control post-weaning diarrhoea in pigs, in which addition of organic acids to the diet is considered as a valid one (Mroz, 2001); (Metzler et al., 2005); (Heo et al., 2013); (Vondruskova et al., 2010)
Organic acids have been used for decades in feed preservation, protecting feed from microbial and fungal destruction or to increase the preservation effect of fermented feed, e.g. silages. In particular formic acid and propionic acid have been used extensively for this purpose.

A number of studies with piglets have shown that several organic acids have a positive influence on growth performance (reviewed by (Partanen and Mroz, 1999); (Maribo, 2014). Some studies have shown an impact on post-weaning diarrhoea/faecal score (e.g., (Maribo et al., 2000a); (Johansen, 2007); (Tsiloyiannis et al., 2001); (Callegari et al., 2016); (Partanen et al., 2007). As described above,

high diarrhoea incidence at weaning is triggered by a number of factors. An insufficient production of hydrochloric acid and digestive enzymes, and the feeding of pre-starter diets with high protein content and high acid binding capacity are some of the factors made responsible for problems at weaning. Organic acids are believed to have their beneficial impact of piglet diarrhoea through improving some of these aspects.

Single or mixtures of acids and/or their salts have been used in piglet diets, i.e., formic, acetic, propionic, butyric, lactic, sorbic, fumaric, malic, tartaric, citric, and benzoic acid. Organic acids together with other additives, like essential oils, herbs or phosphoric acid have also been used.

Mode of action

Although several hypotheses have been proposed, the exact mode of action behind the positive effects of organic acids on growth performance and diarrhoea frequency in piglets has not been established. The proposed mechanisms behind the positive effects of organic acids in weaners are related to the gastrointestinal microbiota, and nutritional aspects:

- Antimicrobial activity. The antimicrobial activity of organic acids can be due to a pH reduction and/or to a more direct antibacterial effect. Different aspects of the working mechanism of organic acids with respect to their antibacterial activity are given in the review articles of Cherrington *et al*. (Cherrington et al., 1991)and Russell (Russel, 1992). The antibacterial activity of organic acids is related to the reduction of pH, as well as their ability to dissociate, which is determined by the pK_a-value of the respective acid, and the pH of the surrounding milieu. The antibacterial activity increases with decreasing pH-value. Organic acids are lipid soluble in the undissociated form, in which they are able to enter the microbial cell. However, carrier-mediated transport mechanisms seem to be also involved in the membrane transport. Once in the cell, the acid releases the proton in the more alkaline environment, resulting in a decrease of intracellular pH. This influences microbial metabolism inhibiting the action of important microbial enzymes and forces the bacterial cell to use energy to release protons, leading to an intracellular accumulation of acid anions. This accumulation depends on the pH gradient across the membrane. The acid anion seems to be very important regarding the antibacterial effect of organic acids and their salts. Several investigations have shown a strong bactericidal effect of organic acid without significantly decreasing the pH-value in the GI-tract. Generally lactic acid bacteria are able to grow at relatively low pH, which means that they are more resistant to organic acids than other bacterial species, e. g. *E. coli*. An explanation for this may be that gram-positive bacteria have a high intracellular potassium

concentration, which provides a counteraction for the acid anions (Russell and Diez-Gonzalez, 1997).

A reduction in the bacteria numbers in the proximal GI-tract would both lower the risk of infections by inhibiting pathogenic bacteria proliferation, but also reduce the microbial competition with the pig for nutrients.

The in vitro antimicrobial activity of various organic acids used in piglet nutrition has been demonstrated (Knarreborg et al., 2002c); (Naughton and Jensen, 2001). (Knarreborg et al., 2002c) testing the impact of various organic acids on growth/death of coliform bacteria in stomach contents observed differences among the acids in the following order (from more to less antibacterial): benzoic acid > fumaric acid > lactic acid > butyric acid > formic acid > propionic acid. When testing the impact in small intestine content, only benzoic acid, formic acid and butyric acid showed a bactericidal effect. Benzoic acid was superior to the other acids tested in exhibiting a bactericidal effect on coliform as well as lactic acid bacteria in both stomach and small intestinal content. Potasium diformate reduced the number of coliforms in stomach and small intestine contents, too. In similar experiments carried out with *Salmonella typhimurium* in stomach content, it was found that the bactericidal effect of organic acids decreased in the following order: benzoic acid > sorbic acid > lactic acid > propionic acid > formic acid > acetic acid (Naughton and Jensen, 2001).

- Increased proteolytic activity. Dietary acidification (reduced buffering capacity) with organic acids lowers gastric pH, resulting in increased activity of proteolytic enzymes and gastric retention time. Pepsinogen is converted to pepsin, and pepsin is more active at low pH. Due to the lower rate of gastric emptying, large protein molecules may be better hydrolysed, which will have a beneficial effect on protein digestion.
- Increased pancreatic secretions. Dietary supplementation with organic acids can stimulate endocrine and exocrine pancreatic secretion. Intestinal acidification increases the contents of serum secretion, which stimulates exocrine pancreatic secretion and bile secretion (Partanen and Mroz, 1999). Thaela *et al.* (Thaela et al., 1998) showed that supplementation of 2.5% lactic acid in the diets of weaning pigs increased the volume and protein content of pancreatic juice, as well as the secretion of trypsin and chymotrypsin. However, the amount of bicarbonate produced in the animal intestine was not affected by the supplementation with lactic acid. Therefore, according to the authors, it is unlikely that the stimulation of pancreatic secretion was caused by decreased

dietary and gastric pH, but probably by the direct action of the acid (Thaela et al., 1998); (Costa et al., 2013).

- Improved physiology of the intestinal mucosa. Organic acids may act on the villi, maintaining their integrity, promoting an increase in the number of cells and preventing its flattening, as well as serving as a substrate in the intermediary metabolism of the citric acid-cycle (Partanen and Mroz, 1999). In (Costa et al., 2013), 54). This effect is more proved regarding SCFA, whereas little is known on the impact of dietary acidification on pig gut morphology (Partanen and Mroz, 1999)
- Organic acids can serve as substrates in the intermediary metabolism. Kirchgessner & Roth (Kirchgessner and Roth, 1988) have proposed that organic acids may stimulate intermediary metabolism resulting in improved energy or protein/amino acid utilization
- Organic acids may improve the absorption of minerals, particularly Ca and P. Further, organic acids also appear to influence the retention of minerals (Partanen and Mroz, 1999)

Where do organic acids have their action?

As mentioned above, organic acids exert their antimicrobial action both in the feed and in the GI-tract of the animal. Following dietary intake, organic acids are only recovered from the proximal part of the pigs GI-tract (stomach and small intestine). This is in agreement with observations that the strongest effect of organic acids with respect to digesta pH and antimicrobial activity are found in the stomach and the small intestine.

Impact on diarrhoea frequency in piglets

A compilation of studies conducted by SEGES, Denmark, in which the impact of dietary organic acid addition on diarrhoea frequency, or more specifically, on the treatment frequency against diarrhoea, were reported is shown in **Table 2**. A total of 35 studies are presented, in which several acids and mixtures of acids, or mixtures of acids and other additives were tested.

Only two studies showed a significant impact of the added organic acids on frequency of treatment for diarrhoea: a study in which feed added 2% benzoic acid was fed during two weeks from weaning at 4 weeks, followed by offering feed added 1% benzoic acid the next 4 weeks (Maribo et al., 2000a); and a study in which feed added 1% lactic+1% formic+0.5% benzoic was offered during six weeks from weaning at four weeks of age (Johansen, 2007). In the first study, there was a reduction in the treatment frequency (indicating the proportion of piglets treated) from 117 in the control group to 60

in the group fed with the diet containing benzoic acid. The number of *Enterobacteriaceae* and lactic acid bacteria in stomach, small intestine, caecum and mid-colon was also measured. The number of *Enterobacteriaceae* was not affected by adding benzoic acid, whereas that of lactic acid bacteria was significantly lower in all segments in the benzoic acid group. In the study of (Johansen, 2007), there was a reduction from a mean of 8.7 treatment days per piglet in the control group to 6.9 in the 2.5% organic acid group. Furthermore, one study showed tendency to reduction of treatment frequency ((Maribo, 1998). Adding 0.2% of a product containing a mixture of lactic, formic, citric, and propionic acid tended (P = 0.09) to reduce the treatment frequency from 44% in the control group to 3.2% in the acid-added group.

It has to be emphasized that in these studies, the impact of the organic acids on treatment against post-weaning diarrhoea was measured as a secondary response parameter, that is, the studies were not dimensioned to test this effect. A high number of animals would have been needed in order to appropriately test this hypothesis. Furthermore, it has also to be kept in mind that the dose of the acids reported in the table is that of the product used not of the pure acid, which means that the dose of the pure acid is lower and varies somewhat from product to product.
A review on data on the effect of organic acid addition to the feed on piglet growth performance obtained in the same studies shown in Table 1 reported that out of 58 studies, 19 showed significant positive results (Maribo, 2014). The author concluded that, in general, inclusion of minimum 1% pure acid in the feed is needed to give significant positive impact on growth performance.

A summary of results from studies testing the impact of dietary organic acids on faecal score or diarrhoea frequency from the international literature is shown in **Table 3**. Here 22 studies are presented, of which eight reported a significant beneficial impact of dietary organic acid addition. Moreover, one study testing the impact of benzoic acid and thymol addition showed a tendency (P = 0.08) to a faecal score compared to a control diet (Diao et al., 2015). Another study (Partanen et al., 2002) showed a tendency to a higher incidence of post-weaning diarrhoea in piglets fed 0.8% calcium formate. The studies showing significant effects tested both mixtures of acids or acids/salts alone, and included a number of different organic acids. The acid inclusion level tested in the studies showing significant reduction of diarrhoea varied too, from as low as 0.1% to 1.6%. A few studies reported the number of coliforms and lactic acid bacteria in the GI-tract or faeces, but these values did not always follow the results on faeces scores.

The relatively low number of studies showing significant impact on diarrhoea frequency/faecal score can be due to a real lack of effect of the products but also to the set-up of the studies. In order to see an effect of feeding organic acids (or any other additive or feeding strategy) on diarrhoea frequency, a high number of replicates is needed. Many studies in the literature testing the impact of organic acids on weaners have mainly focussed on effects on growth performance, digestibility of nutrients, and microbiota composition and their metabolites in the GI-tract, and not on diarrhoea as a main parameter (reviewed by (Partanen and Mroz, 1999) ; (Canibe et al., 2001); (Vondruskova et al., 2010); (Suiryanrayna and Ramana, 2015); (Costa et al., 2013); (Metzler et al., 2005); (Maribo, 2014)The impact of the acids on the number of coliform bacteria in the GI-tract or faeces has been used as an indicator of their potential to reduce the risk of post-weaning diarrhoea (the fewer coliform bacteria, the lower the risk for post-weaning diarrhoea). However, a direct relationship between these two parameters has not been proven. Therefore, in order to establish more solidly the impact of addition of organic acids to the feed on post-weaning diarrhoea, more studies designed to test the impact of organic acids on this parameter are needed.

7.2.1.4. Fermented liquid feed

Fermented liquid feed is prepared by mixing water or another liquid, e.g., whey, with feed and incubating the mixture for a certain period of time, at a certain temperature. During the initial hours of fermentation, a blooming of coliform bacteria is typically observed followed by an increase in the number of lactic acid bacteria, a reduced pH level, and, as a consequence, a reduced number of coliform bacteria (Canibe and Jensen, 2012).

Several studies have shown that feeding piglets with FLF of good microbial quality, that is, with high lactic acid bacteria counts (around 9 log cfu/g), low pH (around 4,5), and low counts of Enterobacteriaceae (around 3 log cfu/g), reduces the number of coliforms along the GI-tract or in faeces compared to feeding non-FLF or dry feed (Mikkelsen and Jensen, 2000); (Moran, 2001); (van Winsen et al., 2001a; van Winsen et al., 2002; Hong et al., 2009). On the other hand, feeding non-FLF (in practise, partially fermented) containing high numbers of coliforms, increases the number of coliforms along the GI-tract of growing pigs (Canibe and Jensen, 2003). Therefore, animals fed with partially FLF of suboptimal microbial quality will harbour higher numbers of coliforms in their GI-tract, what could increase the risk of diarrhoea incidence.

The main factors considered responsible for the bactericidal activity of FLF against Enterobacteriaceae are the low pH and high concentration of lactic acid observed in the FLF and in the stomach of the animals fed with FLF(van Winsen et al., 2001b); (Canibe and Jensen, 2003). Both factors are known to reduce the survival of *Enterobacteriaceae* to a great extent, greater than that of lactic acid bacteria (Knarreborg et al., 2002c).

Because diarrhoeic pigs harbour higher numbers of coliforms in the GI-tract than healthy subjects, a reduction in the number of coliforms along the GI-tract itself by feeding FLF is considered as indicator of a lower risk of post-weaning diarrhoea. However, to establish whether feeding FLF does reduce post-weaning diarrhoea in piglets, studies designed to test this parameter showing an effect are needed. Unfortunately, there are very few studies in the literature reporting this.

Six Danish studies (**Table 4**) have reported treatment for post-weaning diarrhoea in piglets fed with FLF as compared to those fed dry feed or non-FLF. None of the studies showed an impact of feeding FLF on this parameter. It is important to notice, though, that the incidence of diarrhoea in most of these studies was very low, what makes detection of an improvement very difficult to achieve. In two of the studies (Pedersen, 2011); (Maribo, 2012), the piglets received 2500 Zn in the feed the first two weeks post-weaning, what is considered to reduce diarrhoea frequency.

Data on the impact of FLF on post-weaning diarrhoea reported from studies published in the international peer-reviewed literature are shown in **Table 5**. All three studies are characterized by a very low number of replicates, and two of them are challenge studies, in which animals were challenged with *E. coli* O149:F4. Only one study showed some significant effects of feeding fermented liquid whey as compared to non-fermented whey, i.e., a reduced number of pigs with diarrhoea two days out of 12 days measured.

In summary, very few published studies have been designed to test the impact of FLF on post-weaning diarrhoea, therefore, there is no basis to conclude whether feeding FLF reduces post-weaning diarrhoea in piglets.

7.2.2. Management

A recent study had the hypothesis that well-managed 7-30 kg (weaners) productions had a set of common keyfactors characterizing their management practices (Fertner et al., 2015). There was a wide variation between the farmers regarding their perception concerning which management parameters were the reasons for success in terms of low mortality, high daily weight gain and limited

use of antimicrobials (Fertner et al., 2015). In this descriptive study, 10 out of the 11 Danish weaner producing farms used zinc the first 2 weeks post-weaning. Although this study may enhance our knowledge on the contribution of management parameters of importance for prevention of PWD, the results may not be extrapolated to farms not using pharmacological levels of ZnO post-weaning. While neonatal infection with *E. coli* can be prevented effectively by passive lactogenic immunity obtained by vaccination of the sow, vaccines for prevention of infection with ETEC post-weaning remain to be studied further (Melkebeek et al., 2013). This is not yet considered an efficient solution in Denmark, either because the vaccines are not available commercially or because the use of oral vaccines based on live bacteria for oro-gastric protection has several limitations as listed by Hedegaard and Heegaard (Hedegaard and Heegaard, 2016) – one of the reasons being the lactogenic protection at weaning. It should be noted, however, that oral immunisation of weaned piglets with purified F4 fimbriae induced a protective mucosal immune response (Verdonck et al., 2007), and that active immunizsation against the shiga-toxin producing *E. Coli* is possible (Ecoporc Shiga) thus preventing oedema disease. An orally provided F4 fimbria subunit vaccine was shown to be able to induce protection against F4 positive ETEC in an experimental model of PWD (Van den Broeck et al., 1999), and in Canada a commercial vaccine (Coliprotec®) for oral use against PWD has been marketed for some years (Melkebeek et al., 2013) and has also been approved for the European market. So far, efficacy data on this vaccine is not available (Hedegaard and Heegaard, 2016). Our focus in relation to management factors in the present paper has therefore been devoted to both 'host-specific' factors (i.e. the robustness of the pig at weaning in terms of the influence of age and weight), and to 'the hygienic level of the rearing environment' post-weaning (as limiting the presence of ETEC in the environment may limit the risk of ETEC related diarrhea).

7.2.2.1. Weaning age and weight

Our recommendation is to consider an increase in weaning age for further cost-effectiveness evaluation.

Weaning at a low age has been shown to be associated with the presence of weaning diarrhoea (Madec et al., 1998). In this cohort study, pigs above or below a weaning age of 26.5 days were compared, and odds for diarrhoea was 12 times higher in herds with the low weaning age. In addition, weaning age below 7.2 kg enhanced odds for diarrhoea 6.5 times compared with pigs weaned at age 8-9 kg but was almost unchanged compared with pigs weaned at age 7.2-8.1 kg (Madec et al., 1998). A Danish study compared weaning at 4 weeks (26 days of age) versus 5 weeks (33 days of age) and

observed more 'robust' piglets post-weaning (less mortality and less disease treatments, (Callesen and Thorup, 2004); 0.5 less diarrhoea treatment days, (Callesen and Thorup, 2005); and less diarrhoea treatments in two out of the three herds, (Callesen and Thorup, 2006)) (Callesen and Thorup, 2004, 2006);(Thorup et al., 2006).

It remains to be elucidated how weaning weight is related to the incidence of PWD, as most of the studies included in the previous sections have been performed with indication of weaning age but not indication of weaning weight. In practice, pigs of different litters are often mixed and moved around during the suckling period to obtain a homogenous litter at weaning with regard to weaning weight. It should be underlined that the weight of piglets at weaning does not reflect the age of piglets at weaning (Jensen and Thodberg, 2013). As example of the high variation, the following can be mentioned: the average weight in three different herds varied from 7.6 kg and 9.5 kg at age 24 and 33 days (Callesen and Thorup, 2004)6.6 kg and 7.9 at age 29 and 35 days (Callesen and Thorup, 2005)7.6 and 9.0 at age 28 and 33 days (Callesen and Thorup, 2006). In the study by Thompson (Thomsson, 2008)it was demonstrated that the mixing and moving procedure of pigs of different relative size (small, medium and large) resulted in lower BW gain compared to that of pigs weaned as whole litters, which was most pronounced in small pigs. Mixed and moved pigs also had lower feed intakes and higher diarrhoea scores (small and large pigs).

It should also be mentioned that although pigs in organic production systems are weaned at 7 weeks of age, at which age they are more mature and have a more stable microbiota, PWD is also seen in these herds (however, the infectious aetiology may differ from the aetiology in conventional productions). However, in organic production systems in which a weaning age of 10 or even 12 weeks is practiced (personal communication Charlotte Lauridsen) it seems like pigs are less prone to PWD. This information from practice may be partly supported by our preliminary results showing that pigs staying with the sow until 10 weeks seem to be more robust than pigs being weaned at 7 weeks of age, whereas microbiota of 7 and 10 weeks old pigs were rather similar.

7.2.2.2. Weaning environment

Practical recommendation regarding the weaning environment is to clean the rearing area with soap and to dry itout thoroughly between each batch of weaned pigs (personal communication Hanne Kongsted). The hygiene level is a factor in a lot of epidemiological studies, and there is a tendency to a reduced incidence of weaning diarrhoea (Laine et al., 2008)and diarrhoea between 15-40 kg (Chase-Topping et al., 2007)in herds with strict hygiene procedures. In addition, single studies have demonstrated a statistically significant relationship between hygiene status and diarrhoea incidence

(Madec et al., 1998); (Pearce, 1999). In a descriptive study including 11 Danish herds characterised by having a low antimicrobial usage, all herds had also a high hygiene level (Fertner et al., 2015). Although there is a lack of systematic research on which of the factors included in the weaning environment are the most important for predisposing pigs to PWD, our recommendation would be to ensure that pigs are weaned to an environment with a high hygiene level, because proper cleaning and drying would limit exposure to ETEC for the newly weaned pig.

8. Conclusion and Perspective

Several reviews have been conducted with the aim to present alternatives to in-feed antibiotics on health and on growth of pigs, and these have included reports on feeding, nutrition and management strategies and use of feed additives as well as breeding strategies. However, the intervention studies have focused mainly on proxy measurements, i.e. indicators of gut health and/or parameters indicating mode of action and the relation to gut health and/or the pathogenesis of ETEC. Attachment of pathogenic bacteria to host tissues is a critical early step in the development of disease, including ETEC associated post-weaning diarrhoea, but it may not necessarily lead to PWD. Likewise, it is a critical point that although PWD would often impair growth of the pigs, measurable reduction of growth or performance is not an indicator of PWD. The reason why several studies have included proxy measurements rather than the clinical endpoint diarrhoea is probably that it is a challenging disease to research. It requires either large trials, including many replicates, and/or inoculation experiments, which are very laborious and require a special permission. An objective of our present knowledge synthesis was to propose alternative strategies for prevention and treatment which could be considered relevant for further studies in a cost-effectiveness analysis. Since very few studies have actually demonstrated the efficacy of the given alternative intervention or their potential efficacy in relation to incidence PWD, our review limited the available potential interventions. In conclusion, this review pointed out that the following factors could limit the predisposition to PWD and as such could be relevant to consider in a cost-effectiveness analysis for reducing the usage of zinc and antimicrobials: restriction of dietary protein, increase in weaning age, and optimisation of the hygienic status of the environment for the pigs post-weaning. Besides, there were a few more dietary factors which gained our special interest in terms of their potential for limiting PWD in pigs, i.e. organic acids, fermented liquid feed and antibodies. More studies designed to test the impact of the dietary intervention on diarrhoea as primary parameters are needed.

Table 1. Examples of studies showing effect of dietary protein level on post-weaning diarrhoea

Diarrhoea	Protein lev	Measurement	Other parameters	Results	
72 female pigs weaned 21 d, infection/non-infection with E. coli 0149. No diet contained antimicrobials	High (256 g CP/kg for 14 d pw), low (175 g CP/kg for 7 d pw), low (175 g CP/kg for 14 d). LP diets were fortified with crystalline Ile and VAL to obtain ideal AA pattern. From 14-28 d all pigs were fed 213 g/kg	Diarrhoea incidence (3 point scale). Shedding of β-hemolytic *E.coli*	Protein fermentation Growth, feed intake	Reduction of diarrhea incidence (number of days with diarrhoea out of 14 days), and increase in feacal DM with LP diet compared with HP.	Heo et al. 2009
32 piglets, weaned at 7 weeks, infection/non-infection with E. coli 0149[1]. No diet contained antimicrobials.	Control (167 g (20.1% CP), and low (94 g (12.0%) digestible protein pr.kg	Diarrhoea incidence (6 point scale). DM. Shedding of β-hemolytic *E.coli*	Growth, feed intake	Protein restriction decreased feacal score and increased feacal DM, and weight gain tended to decrease.	Sørensen et al. 2009
150 pigs weaned at 3 weeks, 5 treatments, no antibiotics	Control diet of 17% CP (control); 2) 19% CP diets formulated with more soy protein concentrate (SPC19); 3) fish meal (FM19); 4) 23.7% CP diets formulated with more soy protein concentrate (SPC23); 5) fish meal (FM23).	Diarrhoea incidence (4 point scale): Fecal consistency was assessed visually and classified at 4 levels: 0, normal; 1, pasty; 2, semi-liquid; and 3, liquid.	Morphology, inflammation,	Significantly less diarrhoea incidence in pigs on control (Low protein). In conclusion, the 17% CP diet without in-feed antibiotics helped improve growth performance and relief of diarrhoea of 21 to 35 d-old weaned piglets. Dietary CP level, rather than its source	Wu et al., 2015

				(either fish meal or soy protein concentrate), has more significant impacts on the growth performance and intestinal health of 21 to 35 d-old weaned piglets when fed antibiotics-free diets	
Test in 2 herds (8272 pigs in total) with high presence of *E. coli* (0138 eller 0149). Piglets were weaned at 28 days	4 groups: 2 LP treatments (18% CP, 130 g digestible protein/FEs) 2 HP (21% CP, 155 % g digestible protein/Fes). Diets were based on 'expensive' and 'cheap' feed ingredients.	Diarrhea treatment	Performance, Mortality	Treatment for diarrhea was less in LP-pigs (1.4 days) corresponding to 25% reduction in treatment frequency. But daily gain was 28 g lower, and pigs weighed 0.6 kg less after 3 weeks.	Callesen and Johansen, 2006
200 individually fed pigs, experimentally infected with *E. coli* F4	High protein + antimicrobial compound diet (HP + AMC, 230 g crude protein (CP) with 2.5 g lincospectin and 3 g ZnO per kg feed), High protein diet (HP, 230 g CP/kg), Reduced protein + amino acid supplemented diet (RP + AA, 185 g CP/kg with added CEAA up to	Diarrhea incidence		Pigs fed the HP diet showed an increased faecal score ($P<0.05$–0.001), diarrhoea index ($P<0.001$), and mean number of therapeutic antibiotic treatments ($P<0.001$) compared with pigs fed other diets.	Kim et al 2011

	HP level), and Reduced protein diet (RP, 185 g CP/kg without CEAA supplementation).				

1) In total 4 dietary experiments were included in this study.

Table 2. Impact of organic acid addition to the feed on post-weaning diarrhoea in piglets (Danish studies).

Ref	Treatment	Dose (%)	Starting age (d)	Study length (d)	Number of pigs/treatment	Replicates	Diarrhoea, %[w]	Faecal coliform bacteria (cfu/g)	Faecal lactic acid bacteria (cfu/g)
1	control		27	14	479	4	16.70		
	fumaric acid	1.5	27	14	479	4	8.10		
2	control		27	40	116	12			
	tylosin	40 ppm	27	40	116	12			
	etheric oils	0.01	27	40	116	12			
	fumaric acid, lactic acid, propionic acid, formic acid, citric acid	0.5	27	40	116	12	NS (values not given)		
	formic acid+propionic acid	0.5	27	40	116	12			
	peppermint, juniper and garlic	0.05	27	40	116	12			
3[x]	control		27/28	34/41	250/163	8			
	Zn	2500 ppm	27/28	34/41	250/163	8	20.4/3.0 - 38.0/9.8 (without formic acid)		
	formic acid in water	0.2	27/28	34/41	250/163	8	17.5/2.2 - 28.7/27.8 (with formic acid)		
	zn+formic acid in water	2500ppm+0.2	27/28	34/41	250/163	8			
4[x]	control		23/26	41/37	240/178	8	164-25		
	orto-phosphoric acid, formic acid, acetic acid, propionic acid, citric acid	1.4/1.2	23/26	41/37	240/178	8	175-13		
	tylosin	40 ppm	23/26	41/37	240/178	8	176-13		
5	control		26	46	157	16	NS (values not given)		
	avilamycin	40 ppm	26	46	157	16			
	fumaric acid, malic acid, citric acid, ortho-phosphoric acid	0.20	26	46	157	16			
	herbs, spices, essential oils	0.02	26	46	157	16			
	herbs	0.5	26	46	157	16			
	formic acid, lactic acid, free fatty acids	0.04	26	46	157	16			
6	control		26	46	159	16	NS (values not given)		
	tylosin	40 ppm	26	46	159	16			
	3 diets P-712, P-140 og P-120		26	46	159	16			
	control +methionine analogue		26	46	159	16			
	control+ garlic, herbs	1.2/0.7	26	46	159	16			
	control+ ammonium formate, formic acid, lactic acid, fumaric acid, citric acid	0.70/0.5	26	46	159	16			
7	control		25	46	152	16	47.3		
	avilamycin	40 ppm	25	46	152	16	12.9		
	formic acid, acetic acid, sorbic acid, propionic acid, lactic	0.5	25	46	152	16	18.1		
	lactic acid, fumaric acid, citric acid, formic acid, propionic	0.5	25	46	152	16	30.4		
	plant extract	0.05	25	46	152	16	11.1		
	etheric oils	0.01	25	46	152	16	43.1		
8	control		26/24	48/35	755	17	8		
	lactic acid	5g/dl water; 6.4g/pig/d; 12.8g/pig/d	26/24	48/35	787	17	2.9		

Ref	Treatment	Dose (%)	Starting age (d)	Study length (d)	Number of pigs/treatment	Replicates	Diarrhoea, %[w]	Faecal coliform bacteria (cfu/g)	Faecal lactic acid bacteria (cfu/g)
9	control		25	46	157	16	44		
	avilamycin	40 ppm	25	46	157	16	5.3		
	citric acid, ortho-phosphoric acid	0.8	25	46	157	16	10.6		
	lactic acid, formic acid, citric acid, propionic acid	0.2	25	46	157	16	3.2*		
	formic acid	1.25	25	46	157	16	6		
	formic acid, sodium formate, ortho-phosphoric acid, lactic acid, citric acid	0.65	25	46	157	16	25.9		
10	control		27	47	420	16	0.6		
	lactic acid	2.00/1.00	27	47	420	16			
11	control		27	47		16	128.3		
	methionine hydroxy analogue		27	47		16			
	propionic acid, formic acid	0.6	27	47		16			
	propionic acid, formic acid, phytase	0.6	27	47		16			
	propionic acid, formic acid	0.6	27	47		16			
12	control		28	47	151	16	20		
	Enterococcus faecium	120 ppm	28	47	151	16			
	Enterococcus faecium	80 ppm	28	47	151	16			
	organic acid mix1	0.4	28	47	151	16			
	organic acid mix2	0.4	28	47	151	16			
	garlic and cinnamon oil	0.1/0.05	28	47	151	16			
13	control		28	42	148	19	49		
	calcium formate	1.25	28	42	148	19			
	sorbic acid	2.0	28	42	148	19			
14	control		ca 28	ca 42	112	14	35		
	formic acid, lactic acid, phosphoric acid	0.3	ca 28	ca 42	112	14			
	formic acid, lactic acid, phosphoric acid	0.7	ca 28	ca 42	112	14			
	yeast wall oligosaccharides	0.3/0.15	ca 28	ca 42	112	14			
15[y]	control		29	50	155	16	50	$4.09^{a}/8.15^{a}/8.44^{a}/8.53^{a}$	$8.31^{ab}/8.71^{a}/8.89^{a}/9.62^{b}$
	lactic acid	0.7	29	50	155	16		$3.97^{ab}/8.36^{a}/8.28^{a}/8.29^{a}$	$8.76^{a}/9.21^{b}/9.33^{b}/9.39^{ab}$
	lactic acid	1.4	29	50	155	16		$4.32^{a}/7.33^{b}/8.30^{a}/8.21^{a}$	$8.77^{a}/9.16^{bc}/9.03^{ab}/9.36^{ab}$
	lactic acid	2.8	29	50	155	16		$4.25^{a}/8.10^{a}/7.33^{b}/7.58^{b}$	$8.61^{ab}/8.91^{abc}/9.09^{ab}/9.20^{a}$
	formic acid	0.7	29	50	155	16		$3.28^{b}/8.01^{a}/8.00^{a}/8.15^{a}$	$8.73^{a}/9.13^{bc}/9.25^{b}/9.08^{a}$
	formic acid	1.4	29	50	155			$3.62^{ab}/7.91^{a}/8.29^{a}/8.39^{a}$	$8.08^{b}/8.80^{ac}/8.90^{a}/9.12^{a}$
16	control		ca. 28	ca. 42		20	71		
	phosphoric acid, citric acid, lactic acid, malic acid, tartaric	0.2	ca. 28	ca. 42		20			
	phosphoric acid, formic acid, lactic acid	0.3	ca. 28	ca. 42		20			
	ammonium formate, formic acid, lactic acid, fumaric acid, tartaric acid, malic acid	0.5	ca. 28	ca. 42		20			
17	control		ca. 28	ca. 42	168	22	26		
	formic acid, ammonium formate, potassium hydroxide	0.6/0.5	ca. 28	ca. 42	168	22			
	formic acid, ammonium formate, potassium hydroxide	1.2/1	ca. 28	ca. 42	168	22			
	lysine	4.3/3.9	ca. 28	ca. 42	168	22			
	fructoligosaccharides	1.25/0.5	ca. 28	ca. 42	168	22			
18[y]	control		ca. 28	ca. 42		20	117^{a}	3.12/6.29/6.23/6.56	$7.75^{a}/8.24^{a}/8.95^{a}/9.24^{a}$
	lactic acid+formic acid	0.7+0.7	ca. 28	ca. 42		20	113^{a}	3.20/5.81/6.05/6.16	$7.0^{b}/8.27^{a}/8.85^{a}/9.12^{a}$
	benzoic acid	2.0/1.0	ca. 28	ca. 42		20	60^{b}	3.15/5.13/5.83/5.93	$5.58^{c}/6.91^{b}/7.74^{b}/7.88^{b}$

Ref	Treatment	Dose (%)	Starting age (d)	Study length (d)	Number of pigs/treatment	Replicates	Diarrhoea, %[w]	Faecal coliform bacteria (cfu/g)	Faecal lactic acid bacteria (cfu/g)
19	control		ca. 28	ca. 42	450	18	113		
	formic acid, phosphoric acid, lactic acid	1.2/0.6	ca. 28	ca. 42	450	18			
	formic acid, phosphoric acid, lactic acid	1.20	ca. 28	ca. 42	450	18			
	formic acid, phosphoric acid, lactic acid	1.8/1.2	ca. 28	ca. 42	450	18			
20	control		ca. 28	ca. 42	165	22	9.6		
	flavours	0.02	ca. 28	ca. 42	165	22			
	flavours	0.04	ca. 28	ca. 42	165	22			
	formic acid	0.7	ca. 28	ca. 42	165	22			
	flavours+formic acid	0.02+0.35	ca. 28	ca. 42	165	22			
21[y]	control		ca. 28	ca. 42	400	16	1.3 d/pig	3.4/7.4[a]/6.7/7.3	7.3/8.4/8.8/9.1
	formic acid, propionic acid, special lignin	1.0	ca. 28	ca. 42	400	16		3.4/6.9[a]/6.8/6.9	7.1/8.1/8.7/9.2
	formic acid, propionic acid, special lignin	1.5	ca. 28	ca. 42	400	16		3.4/5.1[b]/6.3/7.0	7.2/8.0/8.7/8.9
	formic acid, propionic acid, special lignin	2.0	ca. 28	ca. 42	400	16		3.4/6.7[ab]/7.0/7.1	6.8/8.0/8.4/8.9
22	control		ca. 28	ca. 56	400	16	1.9 d/pig		
	lactic acid, phosphoric acid, salts of free fatty acids	0.3	ca. 28	ca. 56	400	16			
23	control		ca. 28	ca. 42	165	21	1.1 d/pig		
	etheric oils oregano+lactic acid, formic acid, butyric acid	0.12/0.07+0.4	ca. 28	ca. 42	165	20			
	etheric oils oregano	0.12/0.07	ca. 28	ca. 42	165	21			
	flavours	0.2	ca. 28	ca. 42	165	20			
	benzoic acid	0.5	ca. 28	ca. 42	165	20			
24	control		28	ca. 56	400	16	1 d/pig		
	citric acid	4.5/1	28	ca. 56	400	16			
25	control		ca.28	ca.42	144	22	0.8 d/pig		
	lactic acid+formic acid/lactic acid+formic acid	0.6+0.6/0.3+0.3	ca.28	ca.42	144	22			
26	control		ca.28	ca.42	144	22	0.9 d/pig		
	lactic+formic+mannan oligosaccharide/lactic+formic+mannan-oligosaccharide	0.3+0.3+0.2/0.15+0.15+0.05	ca.28	ca.42	144	22			
	mannan-oligosaccharide	0.4/0.1	ca.28	ca.42	144	22			
27[y]	control		ca.28	ca.42	144	22	0.5 d/pig	3.5/6.6/6.5/6.5	8.0/8.7/9.3/9.3[a]
	formic acid, sodium formate, phosphoric acid, lactic acid, citric acid	0.3	ca.28	ca.42	144	22			
	formic acid, sodium formate, phosphoric acid, lactic acid, citric acid	0.6	ca.28	ca.42	144	22		3.2/6.2/6.4/6.7	8.5/9.2/9.5/9.7[b]
	formic acid, sodium formate, phosphoric acid, lactic acid, citric acid	0.9	ca.28	ca.42	144	22			
	ammoniated formic acid, sodium formate, phosphoric acid, potassium sorbate, citric acid	0.6	ca.28	ca.42	144	22		3.3/6.7/7.1/7.2	8.4/9.1/9.3/9.2[a]
28	control		8.6-13 kg		226	37	0.14 d/pig	7.1[a]	
	lactic acid+formic acid	0.5+0.5					0.08 d/pig		
	benzoic acid	1.00					0.07 d/pig	6.5[b]	

Ref	Treatment	Dose (%)	Starting age (d)	Study length (d)	Number of pigs/treatment	Replicates	Diarrhoea, %[w]	Faecal coliform bacteria (cfu/g)	Faecal lactic acid bacteria (cfu/g)
29	control		8 kg		200	32	1.5 d/pig		
	potasium diformate+0.6% formic acid	0.6+0.6	8 kg		200	32			
30	control		ca.28	ca.42		40	8.7 d/pig[a]		
	lactic acid+formic acid+benzoic acid	1+1+0.5	ca.28	ca.42			6.9 d/pig[b]		
	lactic acid+formic acid+benzoic acid+Zn	1+1+0.5+2500 ppm	ca.28	ca.42			0.9 d/pig[c]		
31	control		ca. 35	ca. 38	684	65	2.4 d/pig		
	chicory	7.5							
	chicory	15.0							
	chicory+benzoic acid	7.5+0.5							
32	control		7.3 kg		180	18	4.1 d/pig		
	sodium butyrate	0.30					4.8 d/pig		

[w]Diarrhoea is in most cases reported as the frequency of antibiotic treatment against diarrhoea. Otherwise the units are stated.

[x]Diarrhoea values from two farms.

[y]Coliforms and lactic acid bacteria in stomach, small intestine, caecum and colon.

*p<0.10 with respect to the control treatment.

1) (Jakobsen, 1985); 2) (Jørgensen, 1995); 3) (Jørgensen, 1996); 4) (Callesen, 1997a); 5) (Callesen, 1997b); 6) (Callesen, 1997); 7) (Callesen, 1998); 8) (Jørgensen, 1998); 9) (Maribo, 1998); 10) (Maribo, 1999); 11) (Callesen, 1999); 12) (Maribo and Olsen, 1999a); 13) (Maribo and Olsen, 1999b); 14) (Maribo and L.E., 2000); 15) (Maribo et al., 2000b); 16) (Maribo et al., 2000c); 17) (Hansen, 2000); 18)(Maribo et al., 2000a); 19) (Maribo, 2000); 20) (Maribo, 2001); 21) (Maribo and Jensen, 2001); 22) (Maribo, 2002); 23) (Maribo, 2003a); 24) (Maribo, 2003); 25) (Maribo, 2003b); 26) (Maribo, 2003c); 27) (Maribo, 2004); 28) (Jørgensen, 2004); 29) (Maribo, 2007); 30) (Johansen, 2007); 31) (Maribo, 2010); 32) (Jørgensen, 2013).

[abc]Values with different superscript within study are different $P < 0.05$.

Table 3. Impact of organic acid addition to the feed on post-weaning diarrhoea in piglets.

Ref	Treatments	Dose (%)	Age start (d)	Length of study (d)	Number of pigs/treatment	Replicates	Diarrhoea	Faecal coliform bacteria (log cfu/g)	Faecal lactic acid bacteria (log cfu/g)
1	control		24	42	12	12	frequency 23.4/0.4%[a]		
	HCl	1.4					11.1/2.4[ab]		
	fumaric acid	1.8					24.6/4[a]		
	sodium formate	1.8					13.1/1.2[ab]		
	tylosin	40 ppm					7.1/0[b]		
	B. cereus Toyoi	$0.5x10^6$ cfu/g					18.7/0.8[ab]		
2	control		25	42	12	12	frequency 24.2/3.6%		
	sodium hydrogencarbonate	2					29.8/7.1		
	calcium formate	1.8					19.4/4.8		
	calcium formate+sodium hydrogencarbonate	1.8+2					22.1/4.8		
	formic acid	1.25					15.9/0		
	formic acid+sodium hydrogencarbonate	1.25+2					23/5.6		
3	control			38	12	12	frequency 5.3%		
	sorbic acid	1.2					6.6		
	sorbic acid	1.8					5		
	sorbic acid	2.4					4		
4	control			42	12	12	frequency 8.2%		
	formic acid	0.85					6.7		
	diformate product A	0.65					8.7		
	diformate product A	1.30					6.5		
	diformate product A	1.95					6.3		
	diformate product B	0.65					8.4		
	diformate product B	1.30					5.8		
	diformate product B	1.95					5.4		
5a	control_low energy		(7.25 kg)	45	48	12	20 days(4%)		
	potassium diformate_low energy	1.8					3 (0.6%)		
	control_high energy						28 (5.5%)		
	potassium diformate_high energy	1.8					10 (2%)		
5b	control_low energy		(9.08 kg)	45	72	6	330 days(87%)		
	potassium diformate_low energy	1.8					236 (31%)		
	control_high energy						362 (47.9)		
	potassium diformate_high energy	1.8					317 (42%)		
5c	control barley		(8.6 kg)	42	96	12	6 days (1.2%)		
	potassium diformate barley	1.8					2 (0.4%)		
	control wheat						4 (0.8%)		
	potassium diformate wheat	1.8					4 (0.8%)		
	control corn						16 (3.2%)		
	potassium diformate corn	1.8					2 (0.4%)		
	control mix						9 (1.8%)		
	potassium diformate mix	1.8					7 (1.4%)		
6	control		25	28	48	4	score 5.63[a]		
	lincomycin+spectinomycin	44					1.7[b]		
	propionic acid	1					4.41[c]		
	lactic acid	1.6					1.94[bd]		
	formic acid	1.2					2.5[de]		
	malic acid	1.2					3.49[cf]		
	citric acid	1.5					3.21[ef]		
	fumaric acid	1.5					3[ef]		

Ref	Treatments	Dose (%)	Age start (d)	Length of study (d)	Number of pigs/treatment	Replicates	Diarrhoea	Faecal coliform bacteria (log cfu/g)	Faecal lactic acid bacteria (log cfu/g)
7	control		28/38	30/20	12	6	index 4.1		
	lactic acid	0.8					4.5		
	formic acid	0.8					1.5		
	calcium formate	0.8					8.3		
	sodium benzoate	0.8					6.1		
8	spray-dried plasma prot		10	14	15	5	score 1.6^{a}	53^{ab}	
	pea protein isolate (PPI)						2.7^{b}	85^{a}	
	PPI+egg yolk antibody						1.3^{a}	27^{b}	
	PPI+ZnO	3000 ppm					1.4^{a}	62^{ab}	
	PPI+fumaric acid	0.2					1.3^{a}	64^{ab}	
	PPI+carbadox	55 ppm					1.1^{a}	29^{b}	
9	control		32	24	36	4	pens 2	5.9	7.3
	carbacol,cinnamaldehyde,capsicum oleoresin	150 ppm					0	6.2	7.9
	carbacol,cinnamaldehyde,capsicum oleoresin	300 ppm					0	5.6	8.7
	formic acid	0.5					2	5.8	7.9
	formic acid+ (carbacol,cinnamaldehyde,capsicum oleoresin)	0.5+150 ppm					1	5.9	7.6
	formic acid+(carbacol,cinnamaldehyde,capsicum oleoresin)	0.5+300 ppm					0	5.3	7.9
10	control		19	35	64	8	score 3.2^{a}		
	50 ppm carbadox	50 ppm					4^{b}		
	anis oil, citrus oil, oregano oil, flavours + phosphoric acid, lactic acid	0.4/0.2+0.4/0.					3.43^{ab}		
11	control (contains 3000 ppm ZnO)		21	28			score 2.6^{ab}		
	lactic acid,formic acid,phosphoric acid	1.2/0.9					2.5^{ab}		
	lactic acid,acetic acid,phosphoric acid	1.2/0.9					2.4^{a}		
	lactic acid,formic acid,phosphoric acid	1.5/1.1					2.7^{b}		
12	control		21	8	20	20	4.4 days^{a}	8.4^{a}	
	free calcium formate	1.2					2.9^{b}	7.7^{b}	
	fat-protected calcium formate	1.2					3.1^{b}	7.6^{b}	
13	control		26 or 36	34-24	40	20	score 9.6^{b}	8.3^{b}	
	avilamycin	40 ppm					5.4^{a}	8.0^{b}	
	formic acid	0.8					5.4^{a}	7.9^{ab}	
	formic acid,propionic acid,potassium sorbate	0.6					7.8^{ab}	7.8^{ab}	
	formic acid,propionic acid,sodium benzoate	0.6					9.5^{b}	7.9^{ab}	
	formic acid in diatomaceous earth	1.86					6.6^{ab}	7.4^{a}	
14	control		21	14	24	24	2.6 days	0.8	
	inulin	8					1.2	0.4	
	benzoic acid	0.5					2.9	0.4	
	benzoic+inulin	0.5+8					0.8	0.1	
15	control		28	35	80	8	score 0.322^{a}		
	B. Cereus Toyoi	10^{9} cfu/kg					0.096^{bc}		
	benzoic acid	0.5					0.132^{b}		
	B. Cereus Toyoi+benzoic acid	10^{9} cfu/kg+0.5					0.064^{c}		
16	control (water pH 8)		28	35	ca.289	12	frequency 2.1%		
	water pH 4 (lactic,formic,propionic,acetic (1%cu +0.01%zn))						2.1		
	water pH 5 (lactic,formic,propionic,acetic (1%cu +0.01%zn))						1.9		
	water pH 6 (lactic,formic,propionic,acetic (1%cu +0.01%zn))						1.5		

Ref	Treatments	Dose (%)	Age start (d)	Length of study (d)	Number of pigs/treatment	Replicates	Diarrhoea	Faecal coliform bacteria (log cfu/g)	Faecal lactic acid bacteria (log cfu/g)
17	control		21	17	12	6	score 3.1^{c}		
	challenged						2.5^{ab}		
	challenged+dried bovine colostrum	0.20					2.8^{bc}		
	challenged+pineapple stem with bromelain	0.20					2.3^{a}		
	challenged+yeast preparation from S. cerevisiae	0.10					2.9^{c}		
	challenged+(formic acid,ammonium formate,acetic acid)	0.70					2.3^{ab}		
	challenged+thyme essential oil	0.015					2.5^{ab}		
18	control		28	21	50	5	incidence 14.97%^{a}		
	sodium butyrate	0.1					10.73^{b}		
19^{x}	control		24	42	36	6	score 30$^{(a)}$	9.6/9.05/9.15^{a}/8.56^{a}	6.8^{b}/8.47/7.2/8.57
	benzoic +thymol	0.1+0.01					12.3$^{(bc)}$	8.7/9.07/7.71^{b}/7.77^{ab}	7.88^{ab}/8.75/7.88/8.92
	benzoic +thymol	0.1+0.02					17.2$^{(b)}$	8.62/9.3/7.56^{b}/7.55^{b}	7.54^{ab}/8.63/7.64/8.78
	benzoic + thymol	0.2+0.01					7.67$^{(c)}$	8.48/8.94/7.85^{ab}/7.91^{a}	8.53^{a}/8.74/7.89/8.53
20	control (with 40ppm colistin and 3680/3000 ppm ZnO)		23		24	8	frequency 62.5%^{a}	6.5	8.3
	fumaric acid, malic acid, lemon essential oil	0.07					20.83^{b}	6.4	8.1
	phosphoric acid, citric acid, malic acid, fumaric acid	0.02					33.33^{b}	6.9	8.1
	formic acid,phosphoric acid,propionic acid, its salts	0.85					25^{b}	6.5	8.2
21	control		21	7	48	6	frequency ca.27%^{a}		
	calcium formate,calcium lactate,citric acid,medium chain fatty acids	0.5			40	5	ca.28^{a}		
	calcium formate,calcium lactate,citric acid,medium chain fatty acids	0.3			40	5	ca.20^{b}		
22	control		(7.27 kg)	35	50	5	score 1.63^{a}	7.05^{a}	8.05
	ZnO	2500 ppm					1.24^{b}	6.93^{ab}	8.5
	lactic acid, formic acid, citric acid	0.2					1.35^{ab}	6.60^{b}	8.6
	bacteriophages	0.1					1.26^{b}	6.58^{b}	8.59

^{x}Coliform bacteria and lactic acid bacteria: ileum day 14, caecum day 14, ileum day 42, caecum day 42.

1) (Eidelsburger et al., 1992a); 2) (Eidelsburger et al., 1992b); 3) (Kirchgessner et al., 1995); 4) (Roth et al., 1996); 5) (Paulicks et al., 2000); 6) (Tsiloyiannis et al., 2001); 7) (Partanen et al., 2002); 8) (Owusu-Asiedu et al., 2003); 9) (Manzanilla et al., 2004); 10) (Kommera et al., 2006); 11) (de Freitas et al., 2006); 12) (Bosi et al., 2007); 13) (Partanen et al., 2007); 14)(Halas et al., 2009); 15) (Papatsiros et al., 2011); 16) (De Busser et al., 2011); 17) (Spitzer et al., 2014); 18) (Fang et al., 2014); 19) (Diao et al., 2015); 20) (Callegari et al., 2016); 21) (Wang et al., 2016); 22)(Hosseindoust et al., 2017).

^{abc}Values with a different superscript within study are different $P < 0.05$.

Table 4. Impact of feeding fermented liquid feed on post-weaning diarrhoea in piglets (Danish studies)

Ref	Treatment	Age start (d)	Weight start (kg)	Length of study, d	No. pigs/treatment	Replicates	Diarrhoea frequency[x]
1a	dry feed	ca.42	9	42	303	6	
	liquid feed				300		39
	fermented liquid feed				302		
1b	dry feed		9.8		291		
	partially fermented feed				288		79
	fermented liquid feed				291		
2	dry feed+20% maize	26	6.9	28	480	16	6
	dry feed+20% fermented maize		15				
3[y]	liquid feed with cereals/dry feed	ca.28-35	7.6	56	843	44	1.2
	liquid feed with ferm cereals/dry feed						1.1
4	liquid feed		8.4	ca. 63	3860	39	0.1
	liquid feed+50% fermented cereals		8.4		3868	39	
5[y]	fermented liquid feed/dry feed	ca. 35		49	387	28	
	fermented liquid feed+lactic acid bacteria[z]/dry feed				387	28	0.47
	fermented liquid feed+*Kazachstania exigua* /dry feed				387	28	
6	dry feed				546	43	
	dry feed+15% fermented rapeseed cake product				559	44	1
	dry feed+11% standard rapeseed cake				551	43	

[x]Frequency of antibiotic treatment against diarrhoea.

[y]Treatment describes the feed during the first three weeks of the study and the remaining four-five weeks.

[z]Lactobacillus rossiae/L. sp. CS1/L. siligionis +Lactobacillus sanfranciscensis/L. sanfrancisco/L. lindneri.

1) (Pedersen, 2001a); 2) (Pedersen, 2001b); 3) (Pedersen, 2006); 4) (Pedersen, 2009); 5) (Pedersen, 2011); 6) (Maribo, 2012).

Table 5. Impact of feeding fermented liquid feed on post-weaning diarrhoea in piglets

Ref.	Treatments	Age start (d)	Weight start (kg)	Length of study, d	No. pigs/treatment	Replicates	Diarrhoea	Coliform bacteria (cfu/g)	Lactic acid bacteria (log cfu/g)
1a[wx]	liquid feed+whey+dextrose		7.5	12		8	$7^{a}/5^{a}$ pigs		
	liquid feed+fermented whey+dextrose						$2^{b}/1^{b}$		
	dry+0.1% lincomycin						$7^{a}/2^{a}$		
1b	liquid feed+whey+dextrose		7.9	12		8			
	liquid feed+fermented whey+dextrose						NS		
	dry+0.1% lincomycin								
2[z]	dry feed	28	7.9	28	15	3		8/8.1/7.6	9.1/8.2/9
	fermented liquid feed+5.5x10^{10} cfu/ml *Pediococcus acidilactici*				15	3		7.8/7.6/7.3	9.2/8.1/9.3
	dry feed	27	6.3	28	15	3	NS (values not given)	7.5/6.5/7.5	8.8/9.2/9
	dry feed+5.5x10^{10} cfu/ml *Pediococcus*				15	3		7.2/6.6/6.7	9.1/9.4/8.8
	fermented liquid feed+5.5x10^{10} cfu/ml *Pediococcus acidilactici*				15	3		7/7.2/7.5	8.6/8.9/9
3[wy]	no challenged-no whey permeate	30	7.84	11	6	3	11 days	5.7/7.43/7.68/7.97	
	challenged-no whey permeate				8	4	18	6.01/7.75/7.48/7.53	
	challenged+ whey permeate				10	5	17	5.6/7.11/7.4/7.43	
	challenged+fermented whey permeate 1				8	4	11	5.15/6.99/7.52/7.1	
	challenged+fermented whey permeate 2				10	5	21	5.61/6.91/6.77/6.92	
	challenged+fermented whey permeate 3				10	5	18	5.34/6.87/7.41/6.99	

[w]Challenge study with E. coli.

[x]Diarrhoea values indicate number of pigs with diarrhoea on day 7 and 9 post-challenge.

[y]Coliform bacteria in stomach, small intestine, caecum and colon. $P_{diet} = 0.07$.

[z]Coliforms and lactobacilli in faeces on days 8, 15 and 22.

1)(Amezcua et al., 2007); (Missotten et al., 2015); 3) (Sugiharto et al., 2015b).

[ab]Values with a different superscript within study are different $P < 0.05$.

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