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Potential dietary feed additives with antibacterial effects and their impact on performance of weaned piglets: A meta-analysis

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ABSTRACT

This meta-analysis evaluated the use of potential dietary feed additives (pDFA) with antibacterial effects and their impact on the perfomance of weaned piglets. Twenty-three peer-reviewed in vivo studies, comprising 50 trials, were identified between January 2010 and January 2017. The pDFA in these studies could be grouped in 5 classes: antimicrobial peptides, chitosan, lysozyme, medium chain fatty acids/triglycerides and plant extracts. Mixed-effect meta-analyses with type of pDFA as fixed effect were performed for the growth parameters 'average daily gain' (ADG) and 'feed conversion ratio' (FCR), which are the two most important and used economic performance parameters for farmers.

For each class of pDFA, results of the meta-analysis showed significantly higher average daily gain in the group with pDFA compared to the negative control group, while no significant difference with the positive control group was observed. Furthermore, a positive effect on FCR was found, i.e. significantly less feed was needed to gain 1 kg of body weight in the group with pDFA compared to the negative control group. No significant differences with positive control groups were observed for each class of pDFA, except for plant extracts, where the FCR was also significantly reduced in the treatment group. These results suggest that pDFA could reduce the use of antimicrobials without significant negative effects on performance indicators.

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Introduction

In contrast to weaning in nature, domestic piglets are usually weaned abruptly between 3 and 4 weeks of age by separating them from the sow. The piglets face stressors such as changes in the diet, in the social environments and in physical environments (Weary et al., 2008). These stress factors and resulting histological changes in the small intestine may negatively affect the response of the immune system and lead to an intestinal gut dysfunction in pigs (McCracken et al., 1999; Lallès et al., 2004, 2007).

Pig production practices are often associated with regular use of antimicrobials (AMUs), such as colistin and amoxicillin to maintain health and productivity during these periods (Callens et al., 2012; Sjölund et al., 2016). AMUs contribute to an increasing selection pressure on bacteria to become resistant (Chantziaras et al., 2014). Both by under-dosing or by correct dosing, AMUs provoke the selection and spread of acquired resistances in bacteria. By co-selection (co-resistance or cross-resistance), bacteria can also become resistant to other (similar) AMUs. The development of antimicrobial resistance in veterinary medicine causes therapy failure in animals, resulting in a reduced productivity, loss of animals, decreased animal welfare and increased production costs.

In human medicine, problems may arise because resistant bacteria can be transferred from animals to humans (Da Costa et al., 2013; Tang et al., 2017). In humans, colistin is now considered as one of the last therapeutic options for the treatment of infections caused by multidrug-resistant Gram-negative bacteria (Walkty et al., 2009; Michalopoulos et al., 2011; Callens et al., 2016; Rhouma et al., 2017). Studies have reported the isolation of colistin-resistant *Escherichia coli* (*E. coli*) from pigs (Boyen et al., 2010; Morales et al., 2012), up to 35% in some countries (Harada et al., 2005). Food producing animals, particularly pigs, have been singled out as the most potential reservoirs for the spread and amplification of colistin resistance (Nordmann and Poirel, 2017).

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Thus, scientists and regulatory agencies such as the European Medicine Agency (EMA) have recommended reducing the use of colistin in animal production and to restrict its use to the treatment of sick animals as a last resort option (European Medicines Agency, 2016). From November first, 2016 colistin was banned to be used as a growth promotor in China (Walsh and Wu, 2016).

These problems call for initiatives to preserve antibiotic effectiveness. In general, it is considered that reduction of AMU use and responsible AMU use will result in a decrease of the occurrence of antimicrobial resistance. Reduction of AMU use mainly means that in general, the antibiotic use must be diminished, thinking in the first place of prophylactic group treatments. Prevention of illnesses by an ameliorated biosecurity, management, vaccination plans, but also nutrition will have to become more important (Postma et al., 2015). As the selection for resistance is linked to the level of AMU, the reversion to susceptibility is equally linked to the reduction in selection pressure. Several studies have shown that reducing AMU use is indeed translated in a reduced level of resistance (Dorado-García et al., 2016; Callens et al., 2018).

A number of alternatives/replacements for antibiotics have been proposed (Seal et al., 2013) such as dietary feed additives (pDFA) or zinc oxide (ZnO). However, a concern with feeding pharmacological levels of ZnO to pigs is that application of manure containing high levels of ZnO to agricultural lands has the potential to negatively impact the environment and therefore are highly debated (Jensen et al., 2018). As a result, the European commission issued a decision on June 26, 2017 to withdraw the marketing authorizations of ZnO derivatives at the latest five years from this date. Moreover, there are evidences on the role of ZnO in co-selecting resistant bacteria (Vahjen et al., 2015). Currently, a wide variety of pDFA such as immunity modulating agents, bacteriophages and their lysins, antimicrobial peptides, pro-, pre-, and synbiotics, plant extracts, inhibitors targeting pathogenicity (bacterial quorum sensing, biofilm, and virulence), enzymes and others show beneficial effects around weaning (Cheng et al., 2014). However, there exists scepticism on how effective these pDFA are and if they can replace antibiotics around weaning. From a wide range of pDFA, a possible antimicrobial mode of action was already evaluated in vitro. Yet, the in vivo effect cannot be automatically extrapolated from these in vitro studies due to possible degradation, inactivation and/or inhibition in the gastro-intestinal tract. Therefore, this meta-analysis combines the results of in vivo trials which tested a weaning diet containing antibiotics versus pDFA with an antimicrobial mode of action, based on the two most occurring performance indicators: ADG and FCR. It should be noted that other perfomance indicators exist, such as the frequency of diarrhea and medication, that are not included in this meta-analysis, due to the limited data available in the consulted literature.

Materials and methods

The literature database of Web Of Knowledge was used for this meta-analysis (Fig. 1). The search terms were initially: 'pig' + 'antimicrobial' or 'antibacterial', in addition there was a search on: 'pig' + 'essential oil', 'plant extracts', 'lysozyme', 'fatty acids', 'plasma', 'IgY', 'egg yolk'. The following boundaries were set: the trials had to be performed on weaned piglets, where the performance of a diet containing a pDFA, with shown antimicrobial action in vitro, is compared to a diet containing antibiotics, if applicable the negative control diet was also included. The database was consoled from January 2010 until January 2017 (1631 studies), since trials before 2010 were often designed to replace antimicrobial growth promoters (AGP's), while this review focusses more on the replacement of therapeutic antibiotics. Performance indicators are collected at start and end of the trial and significant differences were recorded. For each trial, the dose of pDFA and the antibiotic was noted, together with number of pigs per group, weaning age and weight, trial days and if the pigs were artificially challenged. The growth parameters (ADG and FCR) for the trials were noted and significant changes were indicated. For each pDFA, the relative differences were calculated for ADG and FCR.

DerSimonian-Laird mixed effects meta-analyses with type of pDFA as fixed effect were performed for the growth parameters ADG and FCR with the 'metafor' package in R version 3.3.1 (https://cran.r-project.org). For each growth parameter, pDFA was compared with the negative control group on the one hand and with the positive control group on the other hand. Forest plots were created to visualize the mean differences with corresponding 95% confidence intervals (95% CI).

The growth parameter FCR was defined as the amount of feed intake to gain 1 kg of body weight (ratio of feed intake and body weight gain). In some trials, feed efficiency was defined as the ratio of weight gain and feed intake. To obtain FCR as defined above, the inverse of the weight gain/feed intake ratio was taken and the variance was estimated with the delta method as follows:

$$\mathrm{Var}\left(\frac{\text{feed intake}}{\text{weight gain}}\right) = \frac{1}{\left(\text{weight gain}/\text{feed intake}\right)^4} \times \mathrm{Var}(\text{weight gain}/\text{feed intake})$$

Results

A total of 23 studies met the above criteria comprising a total of 50 trials (Table 1; Huang et al., 2010, 2011; Henn et al., 2010; Han et al., 2011; Hong et al., 2012; Li et al., 2012; May et al., 2012; Nyachoti et al., 2012; Tang et al., 2012, 2013, 2016; Wu et al., 2012; Yang et al., 2012; Ahmed et al., 2013; Oliver and Wells, 2013; Robbins et al., 2013; Oliver et al., 2014; Yoon et al., 2014; Gois et al., 2016; Long et al., 2016; Peng et al., 2016; Sbardella et al., 2016; Wan et al., 2016). Two trials were excluded, i.e. Tang et al. (2013) and Tang et al. (2016). These trials showed a very low daily growth for the control piglets, resulting from working with challenged piglets, which made the comparison with the other studies difficult.

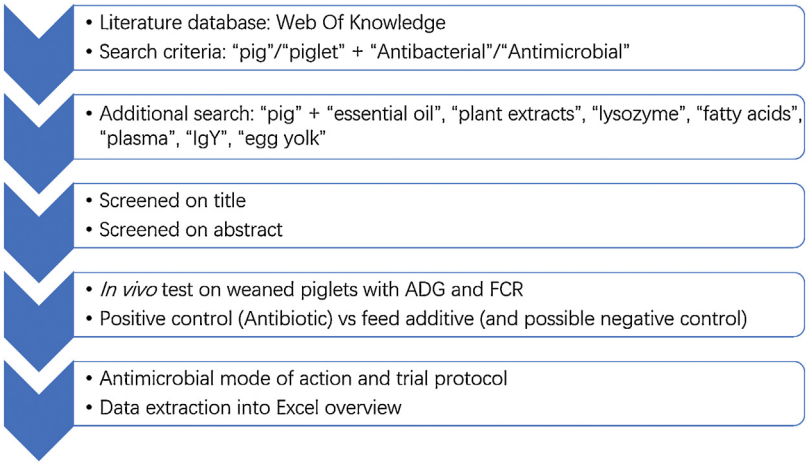

Fig. 1. Design of the literature review based on the database of Web Of Knowledge.

Table 1
The overview of the experiments included in this meta-analysis.

Reference	Type of pDFA	Antimicrobial Compound	Dosage (%)	Negative control	Positive control	Comments
Yoon et al. (2014)	AMP	AMP-A3	0.006		0.015% avilamycin	
Yoon et al. (2014)	AMP	AMP-P5	0.006		0.015% avilamycin	
Peng et al. (2016)	AMP	Beta-defensin 2	0.1	No	1.5% crude *P. pastoris* X-33 + 200 mg 10% colistin sulfate + 1.0 mg 10% zinc bacitracin/kg	
Peng et al. (2016)	AMP	Beta-defensin 2	0.5	No	1.5% crude *P. pastoris* X-33 + 200 mg 10% colistin sulfate + 1.0 mg 10% zinc bacitracin/kg	
Peng et al. (2016)	AMP	Beta-defensin 2	1.5	No	1.5% crude *P. pastoris* X-33 + 200 mg 10% colistin sulfate + 1.0 mg 10% zinc bacitracin/kg	
Tang et al. (2016)	AMP	Beta-defensin 2	0.01 (in water)		0.06% Neomycin (in water)	ETEC[2] 0149:K88
Tang et al. (2013)	AMP	Buferin	0.005 (in water)		0.005% colistin sulfate (in water)	ETEC 0149 0141 0164
Wu et al. (2012)	AMP	Cecropin AD	0.04		0.01% Kitasamycin + 0.08% colistin sulfate	
Wu et al. (2012)	AMP	Cecropin AD	0.04		0.01% Kitasamycin + 0.08% colistin sulfate	ETEC K88
Tang et al. (2012)	AMP	Lactoferricin	0.01		0.01% chlortetracycline	ETEC 0149 0141 0164
Wan et al. (2016)	AMP	Plectasin	0.006		0.006% colistin sulfate	
Yang et al. (2012)	Chitosan	Chito-oligosacharide	0.02		0.002% colistin sulfate	
Yang et al. (2012)	Chitosan	Chito-oligosacharide	0.04		0.002% colistin sulfate	
Yang et al. (2012)	Chitosan	Chito-oligosacharide	0.06		0.002% colistin sulfate	
Oliver and Wells (2013)	Lysozyme	Lysozyme	0.01		0.0055% carbadox + 0.025% Cu	
May et al. (2012)	Lysozyme	Lysozyme	0.01		0.0016% neomycin and oxytetracycline	
Nyachoti et al. (2012)	Lysozyme	Lysozyme	0.1[a]		0.25% chlortetracycline, sulfamethazine and penicillin	
Nyachoti et al. (2012)	Lysozyme	Lysozyme	0.1[a]		0.25% chlortetracycline, sulfamethazine and penicillin	ETEC
Nyachoti et al. (2012)	Lysozyme	Lysozyme	0.2[a]		0.25% chlortetracycline, sulfamethazine and penicillin/kg of feed	
Nyachoti et al. (2012)	Lysozyme	Lysozyme	0.2[a]		0.25% chlortetracycline, sulfamethazine and penicillin/kg of feed	ETEC
Long et al. (2016)	Lysozyme	Lysozyme	0.003		0.002% colistin sulphate + 0.005% kitasamycin	
Long et al. (2016)	Lysozyme	Lysozyme	0.006		0.002% colistin sulphate + 0.005% kitasamycin	
Long et al. (2016)	Lysozyme	Lysozyme	0.009		0.002% colistin sulphate + 0.005% kitasamycin	
Long et al. (2016)	Lysozyme	Lysozyme	0.012		0.002% colistin sulphate + 0.005% kitasamycin	
Oliver et al. (2014)	Lysozyme	Lysozyme	0.01		0.0055% Chlortetracylcine + 0.165% Denagard (Tiamulin hydrogen fumarate)	Clean nursery
Oliver et al. (2014)	Lysozyme	Lysozyme	0.01		0.0056% Chlortetracylcine + 0.165% Denagard (Tiamulin hydrogen fumarate)	Dirty nursery
Han et al. (2011)	MC FA/T	MCFA (Eucalyptus)	0.1		0.0033% tiamulin + 0.0044% lincomycin	
Han et al. (2011)	MC FA/T	MCFA (Eucalyptus)	0.1		0.0033% tiamulin + 0.0044% lincomycin	
Han et al. (2011)	MC FA/T	MCFA (Eucalyptus)	0.1	No	0.0033% tiamulin + 0.0044% lincomycin	
Hong et al. (2012)	MC FA/T	MCT	0.32		0.004% tiamulin + 110 ppm tylosin + 0.001% enramycin	
Hong et al. (2012)	MC FA/T	MCT	0.55		0.004% tiamulin + 110 ppm tylosin + 0.001% enramycin	
Hong et al. (2012)	MC FA/T	MCT	0.32		0.004% tiamulin + 110 ppm tylosin + 0.001% enramycin	
Hong et al. (2012)	MC FA/T	MCT	0.55		0.004% tiamulin + 110 ppm tylosin + 0.001% Enramycin	
Huang et al. (2011)	Plant ex.	Allicin	0.0025	No	0.0075% aureomycin + 0.01% arsanilic acid + 0.002% colistin sulphate	
Huang et al. (2011)	Plant ex.	Allicin	0.00375	No	0.0075% aureomycin + 0.01% arsanilic acid + 0.002% colistin sulphate	
Huang et al. (2011)	Plant ex.	Allicin	0.005	No	0.0075% aureomycin + 0.01% arsanilic acid + 0.002% colistin sulphate	
Huang et al. (2011)	Plant ex.	Allicin	0.00625	No	0.0075% aureomycin + 0.01% arsanilic acid + 0.002% colistin sulphate	
Huang et al. (2010)	Plant ex.	Cinnamaldehyde + carvacrol + eugenol + thymol + eugenol	0.1		44 ppm tylosin/kg	

Table 1 (*Continued*)

Reference	Type of pDFA	Antimicrobial Compound	Dosage (%)	Negative control	Positive control	Comments
Ahmed et al. (2013)	Plant ex.	Essential oil blend	0.0125		0.002% apramycin	KCTC 2571[b] and *Sal. Enterica srover Typhimurium*
Sbardella et al. (2016)	Plant ex.	Hops beta-acids	0.012		0.004% colistin	
Sbardella et al. (2016)	Plant ex.	Hops beta-acids	0.024		0.004% colistin	
Sbardella et al. (2016)	Plant ex.	Hops beta-acids	0.036		0.004% colistin	
Henn et al. (2010)	Plant ex.	Oregano oil	0.003		0.5% zinc oxide + 0.05% zinc bacitracin	
Robbins et al. (2013)	Plant ex.	Quaternary benzo(c) phenanthridine alkaloid	0.00015		0.00594% chlortetracycline	
Robbins et al. (2013)	Plant ex.	Quaternary benzo(c) phenanthridine alkaloid	0.000075		0.00594% chlortetracycline	
Gois et al. (2016)	Plant ex.	Red pepper oil	0.05		0.012% chlorohydroxyquinoline	
Gois et al. (2016)	Plant ex.	Red pepper oil	0.1		0.012% chlorohydroxyquinoline	
Gois et al. (2016)	Plant ex.	Red pepper oil	0.15		0.012% chlorohydroxyquinoline	
Ahmed et al. (2013)	Plant ex.	Resveratrol	0.2[c]		0.002% apramycin	KCTC 2571[b] and *Sal. Enterica srover Typhimurium*
Li et al. (2012)	Plant ex.	Thymol + Cinnamaldehyde	0.0018		0.015% Chlortetracycline + 0.008% Colistin sulfate + 0.005% Kitasamycin	

AMP, Antimicrobial peptides; ETEC, *Escherichia coli*; Plant ex, Plant extract.
[a] 0.1% based on the content of the product Entegard (4000 lysozyme units/mg) in experimental diet.
[b] KCTC 2571: *Escherichia coli* KCTC 2571.
[c] 0.2% based on the content of the product Respig (detailed composition not shown).

The pDFA used in the selected trials could be classified in 5 groups: anti-microbial peptides (AMP), chitosan, lysozyme, medium chain fatty acids or triglycerides (MC FA/T) and plant extracts (Table 1). Plant extracts and AMP are pDFA containing a wide variety of pDFA, while lysozyme is a single pDFA.

There exists a vast amount of other pDFA like porcine plasma, immunized egg yolk, etc. that are not included in this review due to the restrictions in selection. Some researchers are also very cautious to proclaim antimicrobial action as it is not easy to confirm bactericidal effect in vivo and often chose to address the growth promoting effect.

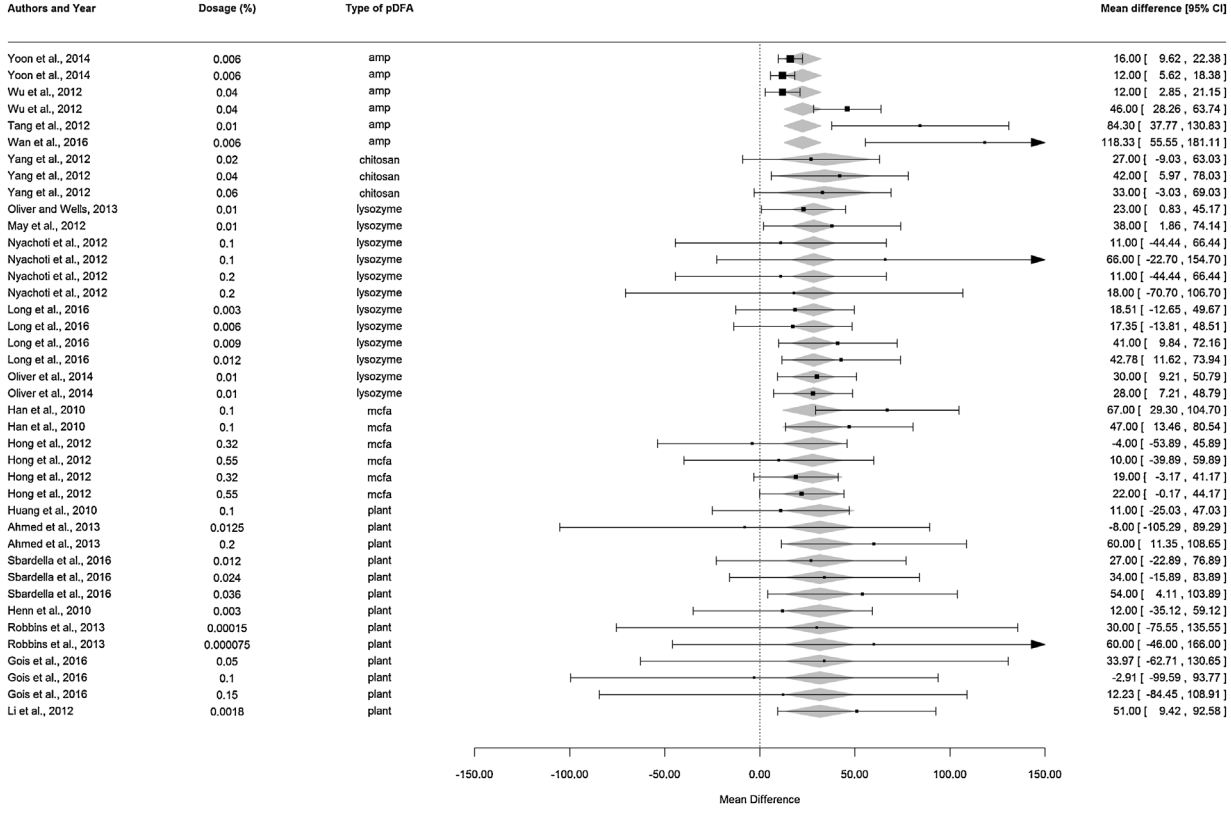

Fig. 2. This forest plot shows the results of the mixed effects meta-analysis with type of potential dietary feed additive (pDFA) as fixed effect. For the average daily weight gain (ADG, in g) the mean difference estimate (black square symbol) with corresponding 95% confidence interval (95% CI; black bar) between the treatment group with pDFA and the negative control group is shown. Whenever a 95% CI exceeded the plot limits of (−150; 150), this was indicated by an arrow. Per pDFA the summary estimate based on the model is shown in grey, with the outer edges of the polygon indicating the 95% CI limits.

Anti-microbial peptides

In six trials with AMPs, ADG was on average 22 g (95% CI 13, 32) higher compared to the negative control group (Fig. 2), while in 9 trials overall no significant difference with the positive control group was observed (95% CI −13, 13; Fig. 3). Regarding FCR, on average 96 g (95% CI 52, 140) less feed was needed in the treatment group compared to the negative control group to gain 1 kg of body weight (Fig. 4), while overall no significant difference with the positive control group was observed (95% CI −51, 48; Fig. 5).

Chitosan

There were only three trials from one study with chitosan. ADG was on average 34 g (95% CI 9, 59) higher compared to the negative control group (Fig. 2), while overall no significant difference with the positive control group was observed (95% CI −44, 20; Fig. 3). On average 142 g (95% CI 62, 221) less feed was needed in the treatment group compared to the negative control group to gain 1 kg of body weight (Fig. 4), while overall no significant difference with the positive control group was observed (95% CI −148, 47); Fig. 5).

Lysozyme

In 12 trials with lysozyme, ADG was on average 28 g (95% CI 17, 40) higher compared to the negative control group (Fig. 2), while overall no significant difference with the positive control group was observed (95% CI −28, 4; Fig. 3). On average 52 g (95% CI −7, −97) less feed was needed in the treatment group compared to the negative control group to gain 1 kg bodyweight (Fig. 4), while overall no significant difference with the positive control group was observed (95% CI −88, 25; Fig. 5).

Medium chain fatty acids or triglycerides

In 6 trials with MC FA/T, ADG was on average 28 g (95% CI 12, 43) higher compared to the negative control group (Fig. 2), while in 7 trials overall no significant difference with the positive control group was observed (95% CI −10, 32; Fig. 3). On average 79 g (95% CI 15, 143) less feed was needed in the treatment group compared to the negative control group to gain 1 kg of body weight (Fig. 4), while overall no significant difference with the positive control group was observed (95% CI −89, 68; Fig. 5).

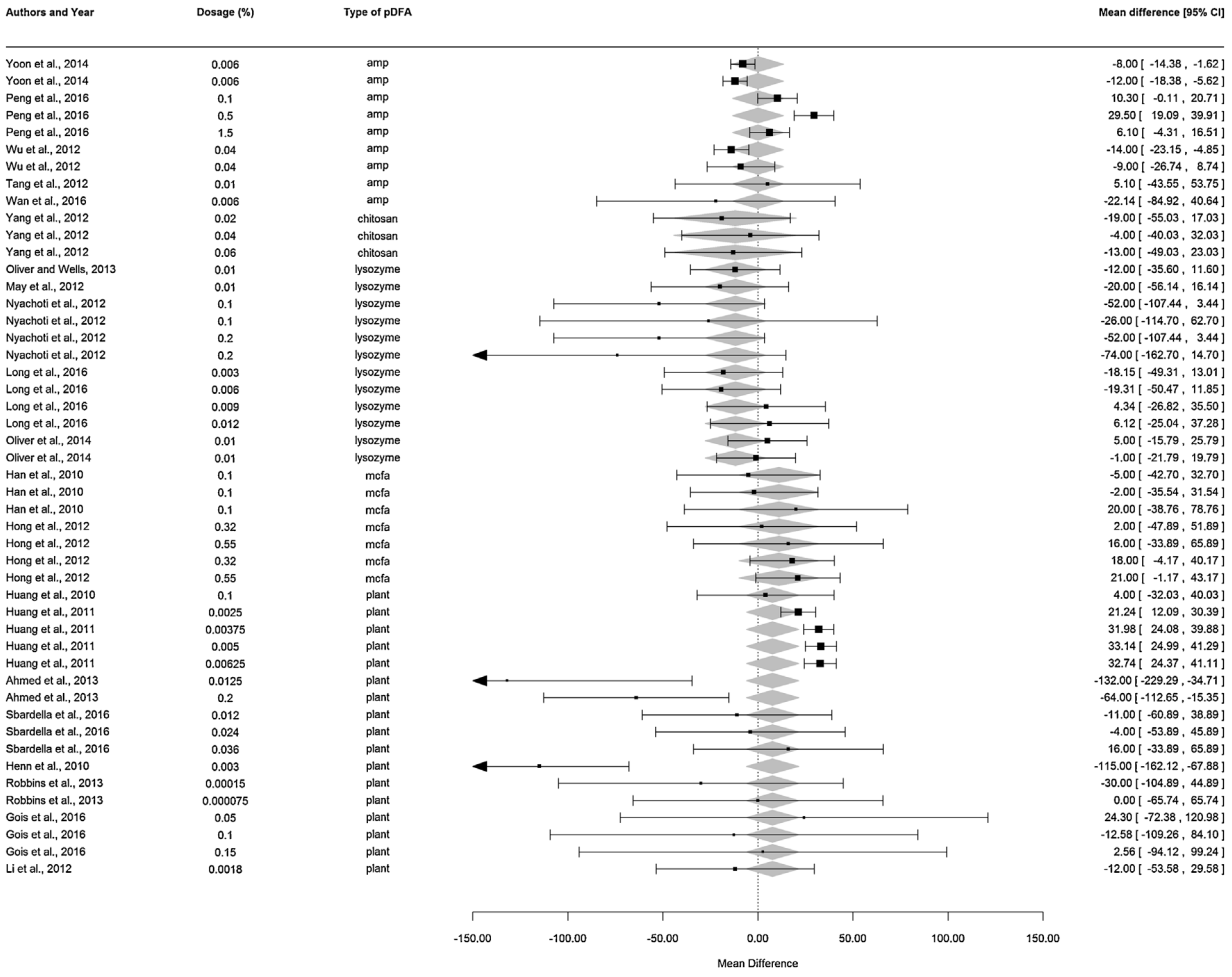

Fig. 3. This forest plot shows the results of the mixed effects meta-analysis with type of potential dietary feed additive (pDFA) as fixed effect. For the average daily weight gain (ADG, in g) the mean difference estimate (black square symbol) with corresponding 95% confidence interval (95% CI; black bar) between the treatment group with pDFA and the positive control group is shown. Whenever a 95% CI exceeded the plot limits of (−150; 150), this was indicated by an arrow. Per pDFA the summary estimate based on the model is shown in grey, with the outer edges of the polygon indicating the 95% CI limits.

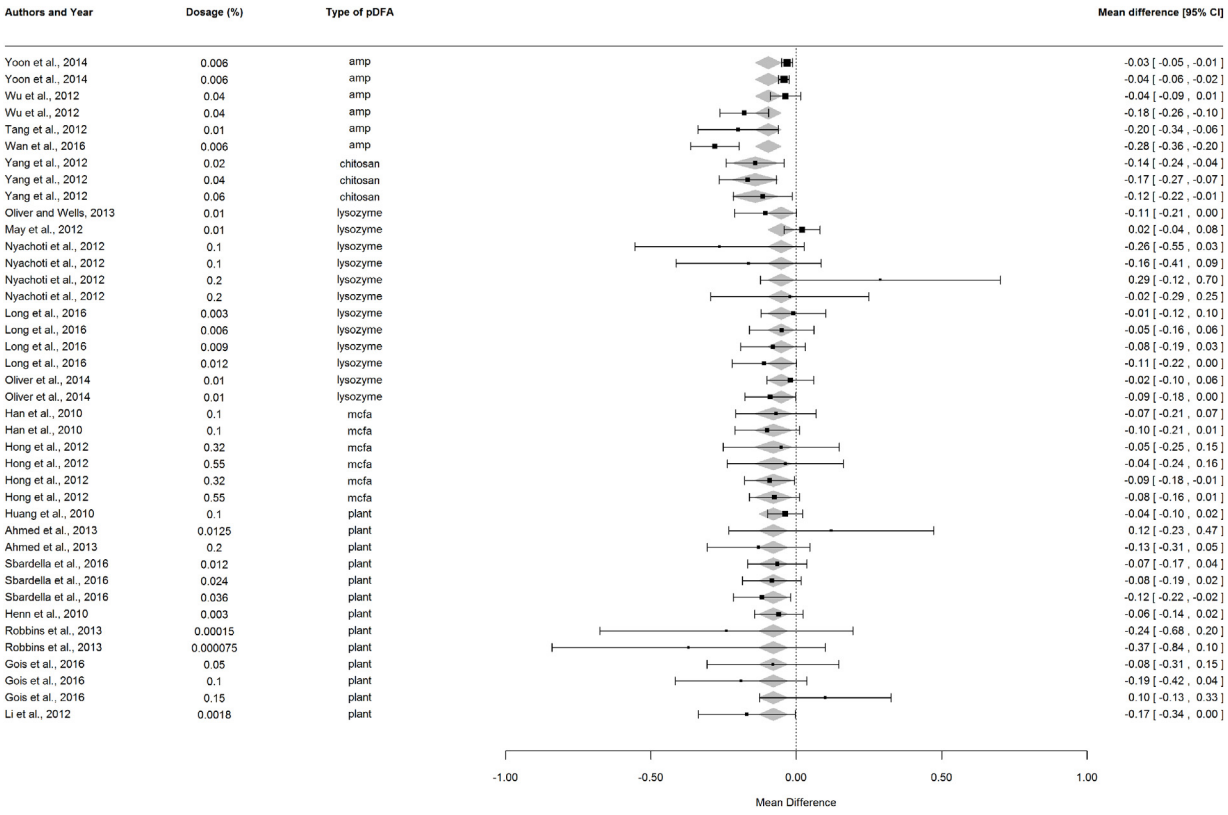

Fig. 4. This forest plot shows the results of the mixed effects meta-analysis with type of potential dietary feed additive (pDFA) as fixed effect. For the feed conversion ratio (FCR) the mean difference estimate (black square symbol) with corresponding 95% confidence interval (95% CI; black bar) between the treatment group with pDFA and the negative control group is shown. Per pDFA the summary estimate based on the model is shown in grey, with the outer edges of the polygon indicating the 95% CI limits.

Plant extracts and essential oils

In 13 trials with plant extracts and essential oils, ADG was on average 32 g (95% CI 14; 50) higher compared to the negative control group (Fig. 2), while in 17 trials overall no significant difference with the positive control group was observed (95% CI −6, 22; Fig. 3). On average 79 g (95% CI 30, 128) less feed was needed in the treatment group compared to the negative control group to gain 1 kg of body weight (Fig. 4). Compared to the positive control group, on average 75 g (95% CI 27, 123) less feed was needed in the treatment group to gain 1 kg of body weight (Fig. 5).

Discussion

This comprehensive meta-analysis provides a very valuable and balanced overview of the available literature on the effects of pDFA in weaned piglets on the performance parameters ADG and FCR. As mentioned before, the pDFA used in the selected trials could be classified in 5 groups, which will be discussed below.

Anti-microbial peptides are small biological molecules (<10 kDa) with a broad-spectrum activity against bacteria, fungi, protozoa and some viruses (Lai and Gallo, 2009). Anti-microbial peptides exert multiple antimicrobial activities that might provide a strategy to prevent bacteria from developing resistance (Peschel and Sahl, 2006). Anti-microbial peptides are potential alternatives to conventional antibiotics due to their broad-spectrum of activity, low level of induced resistance, and immunomodulatory properties (Peng et al., 2016). The literature review demonstrates that the AMPs have a beneficial effect on the ADG and FCR in comparison to non-treated groups. In comparison to AMU treatments, the results of the AMP groups are not significantly different. These results indicate that overall the AMPs may have potential to serve as substitutes to feed AMUs without jeopardising the production results.

Chitosan is obtained from the shell water of industries processing crab, shrimp, and crawfish, and can be used in animal diets due to its antimicrobial property (Singla and Chawla, 2001). Chito-oligosaccharides (COS) are the degraded products of chitosan or chitin prepared by enzymatic or chemical hydrolysis of chitosan. The solubility and low viscosity of COS have attracted the interest to utilize COS and their derivatives for various biomedical applications (Lodhi et al., 2014) and as alternative to feed-grade antibiotics (Yang et al., 2012). Although few trials were available using chitosan, it is clear from these trials that they have potential. In comparison to non-treated groups, chitosan positively effects the ADG and FCR, while no significant difference was observed in comparison to a treatment with AMUs.

Lysozyme is a naturally occurring enzyme, and functions as an anti-microbial by cleaving the glycosidic linkage of bacterial cell walls peptidoglycan (Ellison and Giehl, 1991). Lysozyme is an important defence mechanism and is considered a part of the innate immune system in most mammals. Lysozyme is rather ineffective against Gram-negative bacteria due to the outer membrane barrier that surrounds and protects the peptidoglycan layer (Varahan et al., 2013). Also in the case of lysozyme, our meta-analysis shows the promising capacity to improve ADG and FCR compared to a negative control. Compared to a positive control with AMUs, no significant effect could be observed.

Medium chain fatty acids or triglycerides are organic acids with 6 to 12 carbon atoms. The mechanism(s) underlying the bactericidal activity of MC FA/T is not fully understood; however, many studies suggest that they act as nonionic surfactants, which become incorporated into the bacterial cell membrane (Bergsson

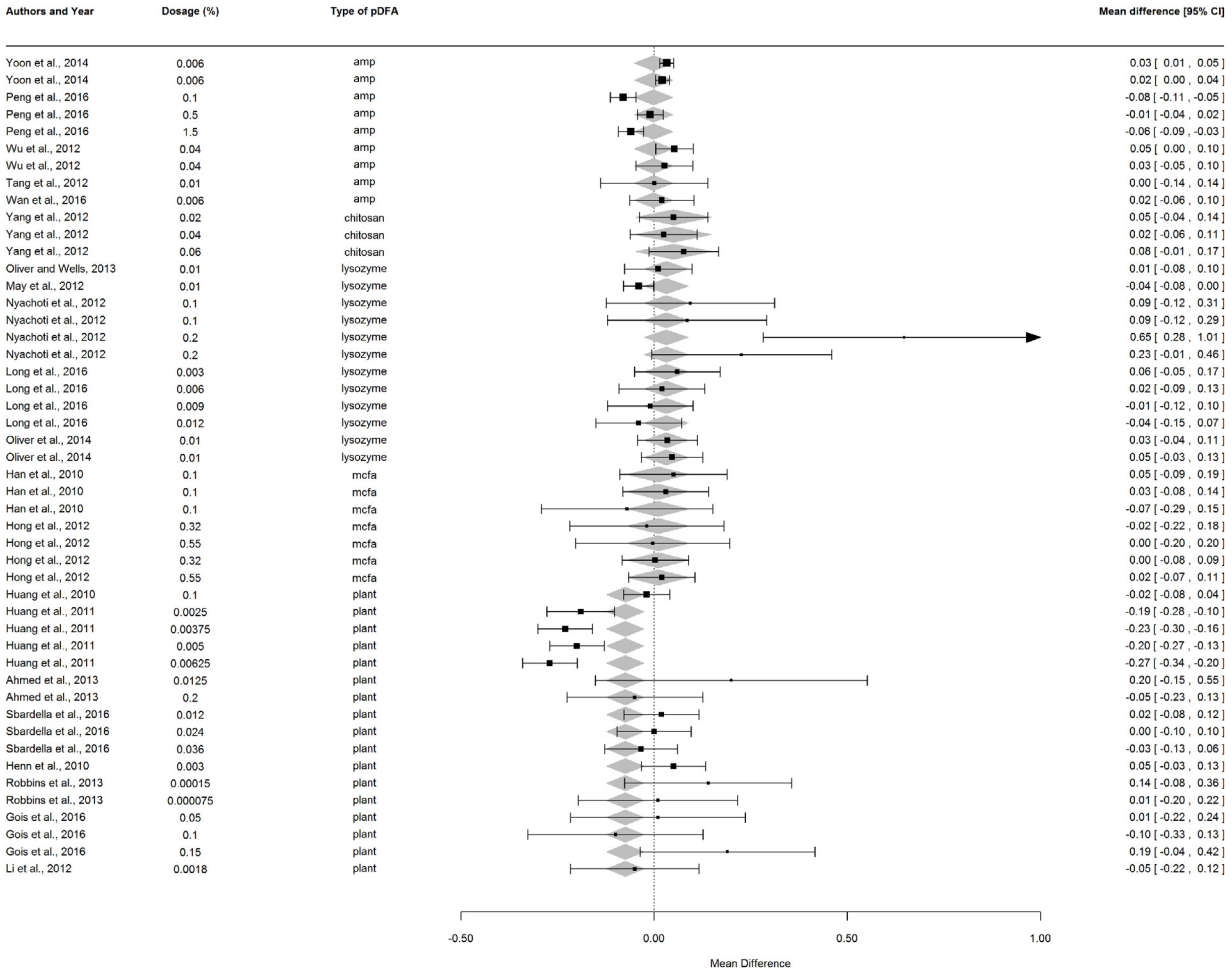

Fig. 5. This forest plot shows the results of the mixed effects meta-analysis with type of potential dietary feed additive (pDFA) as fixed effect. For the feed conversion ratio (FCR) the mean difference estimate (black square symbol) with corresponding 95% confidence interval (95% CI; black bar) between the treatment group with pDFA and the positive control group is shown. Whenever a 95% CI exceeded the plot limits of (−0.50; 1.00), this was indicated by an arrow. Per pDFA the summary estimate based on the model is shown in grey, with the outer edges of the polygon indicating the 95% CI limits.

et al., 2001; Altieri et al., 2009; Desbois and Smith, 2010). Studies also show that MC FA/T diffuse through the bacterial cell membrane and create transient or permanent pores, resulting in altered membrane permeability and cell death (Bergsson et al., 2001; Altieri et al., 2009; Desbois and Smith, 2010). The mechanisms underlying the bactericidal activity mediated by weak organic acids have been widely investigated. Un-dissociated organic acids penetrate the cell membrane and enter the cytoplasm, where they dissociate into charged anions and protons, thereby altering the hydrogen ion equilibrium inside the cell and raising the pH (Brul and Coote, 1999). From our meta-analysis, a similar conclusion could be drawn as compared to the previous pDFA. Medium chain fatty acids or triglycerides show the possibility to improve ADG and FCR compared to a negative control. In contrast, no significant effect could be observed compared to a positive treatment with AMUs.

Plant extracts and essential oils act along the animal digestive tract to improve appetite and modulate the microbiota, and are able to induce a number of other benefits (Franz et al., 2010). The antimicrobial properties of essential oils and extracts can be dose-dependently bacteriostatic and/or bactericidal. Several investigations have also shown their antioxidant effect, their effects on digestive physiology and digestion at weaning (Zabielski et al., 1999) and on the microbiology of the gut (Franz et al., 2010). From the amount of trials using plant extracts (17 out of 50), it is clear that they are most explored from all selected pDFA in this meta-analysis. Besides having a positive effect compared to a negative control, plant extracts also showed the capacity to improve FCR in comparison to a positive treatment with antimicrobials. However, ADG was not significantly different compared to a positive treatment.

Conclusions

Between January 2010 and January 2017, 23 in vivo studies (comprising 50 trials) were published that evaluated the use of pDFA against a positive control diet with antibiotics in weaned piglets, and reporting the performance parameters ADG and FCR. The results of the meta-analysis clearly show that adding a pDFA at weaning can improve performance indicators compared to an untreated group (negative control), suggesting that pDFA could increase growth and improve feed conversion. As such, pDFA could potentially enhance production without the negative side effect of AMUs.

Compared to the use of AMUs (positive control), the results of the meta-analysis show no overall significant difference. This is a beneficial result, as it suggests that the use of AMUs around weaning, a very commonly used practice, could be replaced by pDFA without significant negative effects on the performance indicators.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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References

Ahmed, S.T., Hossain, M.E., Kim, G.M., Hwang, J.A., Ji, H., Yang, C.J., 2013. Effects of resveratrol and essential oils on growth performance, immunity, digestibility and fecal microbial shedding in challenged piglets. Asian-Australasian Journal of Animal Sciences 26, 683–690.

Altieri, C., Bevilacqua, A., Cardillo, D., Sinigaglia, M., 2009. Effectiveness of fatty acids and their monoglycerides against gram-negative pathogens. International Journal of Food Science & Technology 44, 359–366.

Bergsson, G., Arnfinnsson, J., Steingrímsson, O., Thormar, H., 2001. Killing of Gram-positive cocci by fatty acids and monoglycerides. APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica 109, 670–678.

Boyen, F., Vangroenweghe, F., Butaye, P., De Graef, E., Castryck, F., Heylen, P., Vanrobaeys, M., Haesebrouck, F., 2010. Disk prediffusion is a reliable method for testing colistin susceptibility in porcine *E. Coli* strains. Veterinary Microbiology 144, 359–362.

Brul, S., Coote, P., 1999. Preservative agents in foods. Mode of action and microbial resistance mechanisms. International Journal of Food Microbiology 50, 1–17.

Callens, B., Persoons, D., Maes, D., Laanen, M., Postma, M., Boyen, F., Haesebrouck, F., Butaye, P., Catry, B., Dewulf, J., 2012. Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. Preventive Veterinary Medicine 106, 53–62.

Callens, B., Haesebrouck, F., Dewulf, J., Boyen, F., Butaye, P., Catry, B., Wattiau, P., De Graef, E., 2016. Risk of colistin resistance on the rise. Vlaams Diergeneeskundig Tijdschrift 85, 36–40.

Callens, B., Cargnel, M., Sarrazin, S., Dewulf, J., Hoet, B., Vermeersch, K., Wattiau, P., Welby, S., 2018. Associations between a decreased veterinary antimicrobial use and resistance in commensal *Escherichia coli* from Belgian livestock species (2011–2015). Preventive Veterinary Medicine 157, 50–58.

Chantziaras, I., Boyen, F., Callens, B., Dewulf, J., 2014. Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: A report on seven countries. Journal of Antimicrobial Chemotherapy 69, 827–834.

Cheng, G., Hao, H., Xie, S., Wang, X., Dai, M., Huang, L., 2014. Antibiotic alternatives : the substitution of antibiotics in animal husbandry? Frontiers in Microbiology 5, 1–15.

Da Costa, P., Loureiro, L., Matos, A., 2013. Transfer of multidrug-resistant Bacteria Between intermingled ecological niches: the interface between humans, animals and the environment. International Journal of Environmental Research and Public Health 10, 278–294.

Desbois, A.P., Smith, V.J., 2010. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Applied Microbiology and Biotechnology 85, 1629–1642.

Dorado-García, A., Mevius, D.J., Jacobs, J.J.H., Van Geijlswijk, I.M., Mouton, J.W., Wagenaar, J.A., Heederik, D.J., 2016. Quantitative assessment of antimicrobial resistance in livestock during the course of a nationwide antimicrobial use reduction in the Netherlands. Journal of Antimicrobial Chemotherapy 71, 3607–3619.

Ellison, R.T., Giehl, T.J., 1991. Killing of gram-negative bacteria by lactoferrin and lysozyme. Journal of Clinical Investigation 88, 1080–1091.

European Medicines Agency, 2016. European Medicines Agency to review guidance on colistin use in animals. Veterinary Record 178, 55.

Franz, C., Baser, K.H.C., Windisch, W., 2010. Essential oils and aromatic plants in animal feeding – a European perspective. A review. Flavour and Fragrance Journal 25, 327–340.

Gois, F.D., Cairo, P.L.G., Cantarelli, V., de, S., Costa, L.C., do, B., Fontana, R., Alaman, I.B., Sbardella, M., Junior, F.M., de, C., Costa, L.B., 2016. Effect of Brazilian red pepper (*Schinus terebinthifolius* Raddi) essential oil on performance, diarrhea and gut health of weanling pigs. Livestock Science 183, 24–27.

Han, Y.-K., Hwang, I.L.H., Thacker, P.A., 2011. Use of a micro-encapsulated eucalyptus-medium chain fatty acid product as an alternative to zinc oxide and antibiotics for weaned pigs. Journal of Swine Health and Production 19, 34–43.

Harada, K., Asai, T., Kojima, A., Oda, C., Ishihara, K., Takahashi, T., 2005. Antimicrobial susceptibility of pathogenic *Escherichia coli* isolated from sick cattle and pigs in Japan. The Journal of Veterinary Medical Science 67, 999–1003.

Henn, J.D., Bertol, T.M., De Moura, N.F., Antônio, P., De Brum, R., Casagrande, M., 2010. Oregano essential oils as food additive for piglets: antimicrobial and antioxidant potential. Revista Brasileira de Zootecnia 38, 1761–1767.

Hong, S.M., Hwang, J.H., Kim, I.H., 2012. Effect of medium-chain triglyceride (MCT) on growth performance, nutrient digestibility, blood characteristics in weanling pigs. Asian-Australasian Journal of Animal Sciences 25, 1003–1008.

Huang, Y., Yoo, J.S., Kim, H.J., Wang, Y., Chen, Y.J., Cho, J.H., Kim, I.H., 2010. Effects of dietary supplementation with blended essential oils on growth performance, nutrient digestibility, blood profiles and fecal characteristics in weanling pigs. Asian-Australasian Journal of Animal Sciences 23, 607–613.

Huang, R.H., Qiu, X.S., Shi, F.X., Hughes, C.L., Lu, Z.F., Zhu, W.Y., 2011. Effects of dietary allicin on health and growth performance of weanling piglets and reduction in attractiveness of faeces to flies. Animal 5, 304–311.

Jensen, J., Kyvsgaard, N.C., Battisti, A., Baptiste, K.E., 2018. Environmental and public health related risk of veterinary zinc in pig production — using Denmark as an example. Environment International 114, 181–190.

Lai, Y., Gallo, R.L., 2009. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends in Immunology 30, 131–141.

Lallès, J.-P., Boudry, G., Favier, C., Le Floc'h, N., Luron, I., Montagne, L., Oswald, I.P., Pié, S., Piel, C., Sève, B., 2004. Gut function and dysfunction in young pigs: physiology. Animal Research 53, 301–316.

Lallès, J.-P., Bosi, P., Smidt, H., Stokes, C.R., 2007. Weaning — a challenge to gut physiologists. Livestock Science 108, 82–93.

Li, P., Piao, X., Ru, Y., Han, X., Xue, L., Zhang, H., 2012. Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health. Asian-Australasian Journal of Animal Sciences 25, 1617–1626.

Lodhi, G., Kim, Y., Hwang, J., Kim, S., Jeon, Y., Je, J., Ahn, C., Moon, S., Jeon, B., Park, P., 2014. Chitooligosaccharide and its derivatives: preparation and biological applications. BioMed Research International 2014, 1–13.

Long, Y., Lin, S., Zhu, J., Pang, X., Fang, Z., Lin, Y., Che, L., Xu, S., Li, J., Huang, Y., Su, X., Wu, D., 2016. Effects of dietary lysozyme levels on growth performance, intestinal morphology, non-specific immunity and mRNA expression in weanling piglets. Animal Science Journal 87, 411–418.

May, K.D., Wells, J.E., Maxwell, C.V., Oliver, W.T., 2012. Granulated lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology of 10-day-old pigs. Journal of Animal Science 90, 1118–1125.

McCracken, B.A., Spurlock, M.E., Roos, M.A., Zuckermann, F.A., Gaskins, H.R., 1999. Weaning anorexia may contribute to local inflammation in the piglet small intestine. The Journal of Nutrition 129, 613–619.

Michalopoulos, A.S., Karatza, D.C., Gregorakos, L., 2011. Pharmacokinetic evaluation of colistin sodium. Expert Opinion on Drug Metabolism and Toxicology 7, 245–255.

Morales, A.S., Fragoso de Araújo, J., de Moura Gomes, V.T., Reis Costa, A.T., dos Prazeres Rodrigues, D., Porfida Ferreira, T.S., de Lima Filsner, P.H.N., Felizardo, M.R., Micke Moreno, A., 2012. Colistin resistance in *Escherichia coli* and Salmonella enterica strains isolated from swine in Brazil. The Scientific World Journal 2012, 1–4.

Nordmann, P., Poirel, L., 2017. Plasmid-mediated colistin resistance: an additional antibiotic resistance menace. Clinical Microbiology and Infection 22, 398–400.

Nyachoti, C.M., Kiarie, E., Bhandari, S.K., Zhang, G., Krause, D.O., 2012. Weaned pig responses to *Escherichia coli* K88 oral challenge when receiving a lysozyme supplement1,2. Journal of Animal Science 90, 252–260.

Oliver, W.T., Wells, J.E., 2013. Lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology in nursery pigs. American Society of Animal Science 91, 3129–3136.

Oliver, W.T., Wells, J.E., Maxwell, C.V., 2014. Lysozyme as an alternative to antibiotics improves performance in nursery pigs during an indirect immune challenge. Journal of Animal Science 92, 4927–4934.

Peng, Z., Wang, A., Xie, L., Song, W., Wang, J., Yin, Z., Zhou, D., Li, F., 2016. Use of recombinant porcine β-defensin 2 as a medicated feed additive for weaned piglets. Scientific Reports 6, 1–8.

Peschel, A., Sahl, H.-G., 2006. The co-evolution of host cationic antimicrobial peptides and microbial resistance. Nature Review Microbiology 4, 529–536.

Postma, M., Stärk, K.D.C., Sjölund, M., Backhans, A., Beilage, E.G., Lösken, S., Belloc, C., Collineau, L., Iten, D., Visschers, V., Nielsen, E.O., Dewulf, J., 2015. Alternatives to the use of antimicrobial agents in pig production: a multi-country expert-ranking of perceived effectiveness, feasibility and return on investment. Preventive Veterinary Medicine 118, 457–466.

Rhouma, M., Fairbrother, J.M., Beaudry, F., Letellier, A., 2017. Post weaning diarrhea in pigs: risk factors and non-colistin-based control strategies. Acta Veterinaria Scandinavica 59, 1–19.

Robbins, R.C., Artuso-ponte, V.C., Moeser, A.J., Morrow, W.E.M., Spears, J.W., Gebreyes, W.A., 2013. Effects of quaternary benzo(c)phenanthridine alkaloids on growth performance, shedding of organisms, and gastrointestinal tract integrity in pigs inoculated with multidrug-resistant Salmonella spp. American Journal of Veterinary Research 74, 1530–1535.

Sbardella, M., Perina, D.P., Andrade, C., Santos, C.B., Cairo, P.L.G., 2016. Effects of dietary hop β-acids or colistin on the performance, nutrient digestibility, and intestinal health of weanling pigs. Animal Feed Science and Technology 217, 67–75.

Seal, B.S., Lillehoj, H.S., Donovan, D.M., Gay, C.G., 2013. Alternatives to antibiotics: a symposium on the challenges and solutions for animal production. Animal Health Research Reviews 14, 78–87.

Singla, A.K., Chawla, M., 2001. Chitosan: some pharmaceutical and biological aspects — an update. Journal of Pharmacy and Pharmacology 53, 1047–1067.

Sjölund, M., Postma, M., Collineau, L., Lösken, S., Backhans, A., Belloc, C., Emanuelson, U., Beilage, E.G., Stärk, K., Dewulf, J., Beilage, E.G., GrosseLiesner, B., Körk, C.A., Lindberg, A., Seemer, H., Visschers, V., 2016. Quantitative and qualitative antimicrobial usage patterns in farrow-to-finish pig herds in Belgium, France, Germany and Sweden. Preventive Veterinary Medicine 130, 41–50.

Tang, X., Fatufe, A., Yin, A., Tang, Y., Wang, Z., Liu, S., Li, Z.X., T.-J, 2012. Dietary supplementation with recombinant lactoferrampin-lactoferricin improves growth performance and affects serum parameters in piglets. Journal of Animal and Veterinary Advances 11, 2548–2555.

Tang, Z.R., Deng, H., Zhang, X.L., Zen, Y., Xiao, D.F., Sun, W.Z., Zhang, Z., 2013. Effects of orally administering the antimicrobial peptide buforin II on small intestinal mucosal membrane integrity, the expression of tight junction proteins and protective factors in weaned piglets challenged by enterotoxigenic Escherichia coli. Animal Feed Science and Technology 186, 177–185.

Tang, Z., Xu, L., Shi, B., Deng, H., Lai, X., Liu, J., Sun, Z., 2016. Oral administration of synthetic porcine β-defensin-2 improves growth performance and cecal microbial flora and down-regulates the expression of intestinal TLR-4 and inflammatory cytokines in weaned piglets challenged with enterotoxigenic E. coli. Animal Science Journal 87, 1258–1266.

Tang, K.L., Caffrey, N.P., Nóbrega, D.B., Cork, S.C., Ronksley, P.E., Barkema, H.W., Polachek, A.J., Ganshorn, H., Sharma, N., Kellner, J.D., Ghali, W.A., 2017. Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. The Lancet Planetary Health 1, e316–e327.

Vahjen, W., Pietruszyńska, D., Starke, I.C., Zentek, J., 2015. High dietary zinc supplementation increases the occurrence of tetracycline and sulfonamide resistance genes in the intestine of weaned pigs. Gut Pathogens 7, 1–5.

Varahan, S., Iyer, V.S., Moore, W.T., Hancock, L.E., 2013. Eep confers lysozyme resistance to Enterococcus faecalis via the activation of the extracytoplasmic function sigma factor SigV. Journal of Bacteriology 195, 3125–3134.

Walkty, A., DeCorby, M., Nichol, K., Karlowsky, J.A., Hoban, D.J., Zhanel, G.G., 2009. In vitro activity of colistin (Polymyxin E) against 3,480 isolates of gram-negative bacilli obtained from patients in Canadian hospitals in the CANWARD study, 2007-2008. Antimicrobial Agents and Chemotherapy 53, 4924–4926.

Walsh, T.R., Wu, Y., 2016. China bans colistin as a feed additive for animals. The Lancet Infectious Diseases 16, 1102–1103.

Wan, J., Li, Y., Chen, D., Yu, B., Chen, G., Zheng, P., Mao, X., Yu, J., He, J., 2016. Recombinant plectasin elicits similar improvements in the performance and intestinal mucosa growth and activity in weaned pigs as an antibiotic. Animal Feed Science and Technology 211, 216–226.

Weary, D.M., Jasper, J., Hötzel, M.J., 2008. Understanding weaning distress. Applied Animal Behaviour Science 110, 24–41.

Wu, S., Zhang, F., Huang, Z., Liu, H., Xie, C., Zhang, J., Thacker, P.A., Qiao, S., 2012. Effects of the antimicrobial peptide cecropin AD on performance and intestinal health in weaned piglets challenged with *Escherichia coli*. Peptides 35, 225–230.

Yang, C.M., Ferket, P.R., Hong, Q.H., Zhou, J., Cao, G.T., Zhou, L., Chen, A.G., 2012. Effect of chito-oligosaccharide on growth performance, intestinal barrier function, intestinal morphology and cecal microflora in weaned pigs. American Society of Animal Science 90, 2671–2676.

Yoon, J.H., Ingale, S.L., Kim, J.S., Kim, K.H., Lee, S.H., Park, Y.K., Lee, S.C., Kwon, I.K., Chae, B.J., 2014. Effects of dietary supplementation of synthetic antimicrobial peptide-A3 and P5 on growth performance, apparent total tract digestibility of nutrients, fecal and intestinal microflora and intestinal morphology in weanling pigs. Livestock Science 159, 53–60.

Zabielski, R., Le Huerou-Luron, I., Guilloteau, P., 1999. Development of gastrointestinal and pancreatic functions in mammalians (mainly bovine and porcine species): influence of age and ingested food. Reproduction, Nutrition, Development 39, 5–26.