Effects of a phytogenic feed additive on the growth performance of weaned piglets from 25 to 69 days of age

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Abstract: Since the ban on antibiotic growth promoters in the EU, the industry is looking for alternatives such as phytogenic feed additives (PFA) to further improve the technical performances of weaned piglets. PFAs consist of herbs, plants and plant-derived products such as essential oils, but it is not a very well-defined term. It has been claimed that certain PFAs have promising results on gastrointestinal health, due to their antimicrobial and antioxidative properties. Piglets experience many events in their life in which their immune system is challenged or compromised, a system that costs tremendous amounts of energy. A product that supports gastrointestinal health is therefore promising, as this can have a favourable effect on the piglets' growth performance. Many in-vitro studies support this claim. However, in-vivo studies show varying results. This study was conducted to determine the efficacy of a specific commercial PFA on the gastrointestinal health and growth performance of weaned piglets. In a 42-day study, 25-day-old weaned piglets (n=240; 7.30 ± 0.99 kg BW) were assigned within sex and body weight (BW) blocks to 1 of 2 treatments, using 12 pens (20 pigs per pen; 6 replications per treatment). Dietary treatments included a positive control (basal diet) and a treatment group (basal diet + PFA 150 g/tonne) supplemented with a commercial PFA, containing an encapsulated blend of herbs and essential oils including thymol, oregano and licorice. Due to logistical errors that were made during the trial, by which both the trial and control group received the same feed without PFA, the growth performance was found to be identical between the two treatment groups. Consequently, solid conclusions on the efficacy of PFA to promote the growth performance in weaned piglets cannot be drawn.

Keywords: phytogenic feed additive (PFA), growth performance, weaned piglets, gastrointestinal health

INTRODUCTION

In the pig industry, a high growth rate and an efficient conversion of feed into meat are required for an economically viable porcine sector. Therefore, growth promotors are of great interest and for many years antibiotic growth promotors (AGP) were used to enhance the growth performance of fattening pigs. Due to an increase of antibiotic resistance however, the EU has banned the use of APG and the industry is now looking for alternatives including phytogenic feed additives (PFA) (1). Phytogenic feed additives consist of a range of herbs, spices and plant-derived products like essential oils that have several antimicrobial (2)(3) and antioxidative (4)(5) properties ascribed to them. Besides these properties, one of the main reasons PFA could be an alternative for AGP are the promising in vitro results of their anti-inflammatory characteristics (6) and inhibition of pathogenic microbes in the gastrointestinal tract (7)(8). Feeding an additive that has the potential to support gastrointestinal health by e.g. reducing inflammation, lowering free radical and pathogenic microbial load could be of great benefit to piglets who undergo many different stress-inducing circumstances at this stage of life (1). The immune system requires a lot of energy. Energy saved on the immune system could therefore result in increased growth performances in weaned piglets. However, not all published studies show consistent results on the efficacy of PFA on growth performances (9)(10).

In-vitro studies have been conducted to show the potential of PFA and have tried to dissect the reason for their efficacy (9) (11). The mode of action seems to be divided into several effects the constituents of these PFAs have on an intra- and extracellular level.

Reduction of oxidative stress is one of the effects that is at the core of the immune enhancing properties of PFAs. The oxidants that are formed during metabolism are normally combatted by the antioxidant system. Oxidative stress is the state an organism is in when there is an imbalance in the formation of free radicals and their detoxification (12). This can cause harm to cell structures and lead to tissue damage. Oxidants are activators of nuclear factor of kappa-light-chain-enhancer of activated B-cells (Nf-kB), which is the pivotal activator of the immune system (12)(13). Polyphenols are constituents of PFAs that have been shown to reduce the activation of Nf-kB (6)(11)(14), by being able to block the phosphorylation necessary for activation (15). In addition to that, polyphenols themselves serve as an antioxidant, actively scavenge for ROS and indirectly activate various antioxidant enzymes (12). For piglets that go through many circumstances that can trigger oxidative stress, a feed high in polyphenols (such as the current PFA studied) could therefore be beneficial according to these studies.

Nf-kB is also activated through bacterial and viral stimuli. Polyphenols are secondary plant metabolites that protect the plant from pathogens like fungi, bacteria and viruses (12). In-vitro studies have found many bacteria sensitive to essential oils (generally high in polyphenols) (16)(17)(18)(19). Beneficial gut bacteria like Lactobacilli and Bifidobacteria were found to be less sensitive than potential pathogenic bacteria like E. coli and C. perfringens, resulting in a positive modification of the intestinal microbiota (17)(20)(21).

To determine gastrointestinal inflammation calprotectin is widely used in humans as a diagnostic biomarker (23)(24)(25). Calprotectin is a non-invasive marker of neutrophil activity in the intestines (22), and was chosen as the diagnostic method for the intestinal inflammation status of the piglets in this study.

The objective of this study was to evaluate the efficacy of a new commercially available phytogenic blend of essential oils, herbs and active components on growth performance and gastrointestinal health of weaned piglets. The current study was conducted in a commercial setting because it was anticipated that

this would enhance the extrapolation of the results to the practical field conditions.

MATERIALS AND METHODS

Animals, experimental design and housing

This randomized controlled non-blinded trial was conducted on a commercial pig farm located in Vinkel, the Netherlands. A total of 240 weaned SPF piglets (PIC x PIC 408) with an average age of 25 days (SD 3 days) were used in this 42-day trial. Upon arrival, all piglets were allocated to 12 pens according to initial bodyweight (BW) and sex. There were 6 replicates per treatment, divided into three weight categories according to starting weight (light, medium, heavy), and 20 pigs per replicate (50% barrow; 50% gilt). The pigs were kept in two identical units, with the same number of control and treatment replicates per unit. Pens were randomly allocated to the dietary treatments. The pigs were housed in pens (4.6 x 1.7 m) with a partially concrete (74% of the surface) and partially slatted (26%) floor. The first 48 hours of the trial the pens were artificially lit. After that the lighting regime was provided by natural light (in the Netherlands, during December-January is about 8 hours/day) through one small window (60x80 cm) in each unit. The temperature was kept at 28° for the first 14 days and lowered gradually to 21° until d42. Each pen was provided with a VEWI Feeder (VEWI Techniek B.V. Heesch, The Netherlands) with eight water nipples that allowed for ad libitum access to feed and water throughout the trial, and was designed to minimise spillage and feed waste. Caretakers were blinded, they were not made aware of which pen received which feed.

Animal welfare and clinical observations

Only individual diseased pigs were treated with antibiotics when deemed necessary by a veterinarian. This was the case for a handful of piglets in both groups (5 cases in the trial group vs. 10 cases in the control group). The farm where the pigs were kept was diagnosed with Porcine Reproductive and Respiratory Syndrome (PRRS), which might have contributed to the coughing and sneezing in both units. Overall, group treatment was not deemed necessary and diseased piglets seemed to improve overtime.

Prevalence of stragglers was deemed higher than usual. For the sake of these piglets' health and welfare it was decided to take them (N= 17) out of the experiment. Individuals were weighed and documented and put in an infirmary pen together.

Experimental diets

The dietary treatments consisted of a control and a test feed. The test consisted of the control feed supplemented with 150 g/tonne of a commercial phytogenic compound which consisted of a blend of encapsulated phytogenic substances (herbs and essential oils including thymol, oregano and licorice*). A 3-phase feeding program was adopted in the current experiment (Table 1). During the transition between feeds 50% of the new feed was mixed in with 50% of the old feed for 2 days.

* The exact ingredient composition is not provided for commercial reasons.

Table 1. Ingredient and chemical composition of the control feed.

Unless otherwise noted, all values are expressed as g/kg.

Ingredient	Starter (0-10 days)	Grower Feed I (10-30 days)	Grower Feed II (30-42 days)
Constant components (1)	985	985	985
Premix (2)	15	15	15
Analysed composition			
Dry matter	882	882	883
Crude protein	166	168	167
Crude fiber	42	42	42
N-free extract	559	558	565
Starch	371	380	384
Crude fat	62	57	50
Crude ash	53	57	59
Calculated composition as fed			
Lysine (%)	1.19	1.23	1.23
Digestible lysine (g/kg)	11.0	11.0	11.0
Methionine (%)	0.44	0.46	0.45
Metabolizable energy (MJ ME)	13.52	13.40	13.24

(1) Constant components consisted of 100%: barley, corn, extruded soybean meal, toasted soybeans, wheat, bakery by products, potato protein, sugar, sugar beet pulp, dairy products, palm kernel oil, beet molasses, oats, monocalcium phosphate, salmon oil, salt, soylecithine, soy fatty acids

(2) Premix (per kg): vitamin A, 13500-16000 IU; vitamin D3, 2000 IU vitamin E, 65-140 lU; Fe(II), 100-200 mg; I, 2.0-4.5 mg; Cu, 80-130 mg; Mn(II), 40-50 mg; Mn, 30 mg; Zn(II), 81 mg; Zn,19 mg; Se, 0.30 mg; sepiolate 0.6-10.6 g; 4a16 6-fytase 275-300 OTU; 4a1606 Endo-1,4-beta-xylanase 10 U; benzoic acid, 2.5 g; Bacillus licheniformis (DSM5749)/Bacillus subtilis (DSM5750) (1:1) 1280 MCFU

Recording of body weight and feed consumption

During the trial, each pen was provided with a one- to twice daily portion of feed, that would ensure ad-lib feed consumption. Feed disappearance which we assume to have been consumed was recorded on a pen basis with the use of an automated feeding computer (Fancom cu71, Fancom B.V, Panningen, the Netherlands). Piglets were weighted individually at days 0, 14 and 42, i.e. the last day of the experimental period.

Collection of samples

To determine the level of intestinal inflammation, faecal samples were collected via rectal swabs from 6 piglets (50% male; 50% female) per pen on d8 of the trial. The samples were analysed individually two weeks after sampling and stored in the freezer (-20º C) until analysis.

Feed samples of all three different feeds were collected for analysis. A sample of 160 gram was taken out of the trough of every pen on day 0, 14 and 35 and sent to determine the PFA inclusion and nutrient composition. Every week a sample of 80 gram was taken out of the trough of every pen to keep record of which feed was given to which pen.

Chemical analysis

Faecal Samples

Faecal calprotectin was measured using a commercially available test kit (GD Deventer, the Netherlands). Special faecal collection tubes are used to add a standardize amount of faeces into the medium which, after being thoroughly vortexed, is used to analyse calprotectin. Minimum detection level is 30 mg/kg.

Feed samples

A Weende analysis was performed on the samples taken on d0, d14 and d35. Inclusion of the PFA was determined by gas chromatography-mass spectrometry (GC-MS) calibrated method on the samples taken on d0, 14 and 35 for both control and test feed.

Calculations and statistical analyses

Pen was considered as statistical unit. All data were statistically evaluated by subjecting the data to ANOVA (SPSS v26) using the following model;

$$Y = \mu + BLOCK_i + TREATMENT_j + e_{ij},$$

where Y = response variable (Body weight, growth etc), μ = overall mean, $BLOCK_i$ = housing unit (i = 11 and 12), $TREATMENT_j$ = experimental compound feed (j= 1 to 2; control or test feed), and e_{ijk} = residual error term. Throughout, the level of statistical significance was declared at $p < 0.05$.

RESULTS

Performance parameters

Initial BW of the piglets was similar (P = 0.880) between treatment (Table 2). Both treatments show a growth rate of 480 g/day (P = 0.929). Feed intake was similar (P=0.687) between dietary treatments. Consequently, the feed conversion ratio (FCR) was found to be identical (P=0.821) between the two diets.

Table 2. Growth performance of the piglets after the feeding of the experimental diets.

[1] **Calculated as: kg feed / kg body weight gain**

	Experimental diets		SEM	P-value
	Control	Test		
Body weight (kg)				
Initial	7.3	7.4	0.39	0.880
Final	27.2	27.4	1.62	0.920
Gain	19.9	20.1	1.26	0.929
Growth rate (g/day)	480	480	30	0.929
Feed intake (g/day)	710	720	24	0.687
Feed conversion ratio[1]	1.53	1.53	0.060	0.821

Mortality and stragglers

Both the control (7.5%) and trial (6.6%) group had a few piglets that showed growth retardation and stayed behind in development compared to the rest of the group, with no significance between the two groups (P=0.9858). These stragglers (N=17) were documented and taken out of the experiment. Mortality rate was double in the trial group (3.31%) compared to the control group (1.67%), but the difference was not significant (P=0.6868).

Table 3. Mortality and straggler rate of the control and trial group

		Mortality		Stragglers	
	Total	N	%	N	%
Control	120	2	1.67%	9	7.50%
Trial	121	4	3.31%	8	6.61%

Faecal calprotectin

The results of all of the faecal samples tested on calprotectin were below the minimal detection level of 30 mg/kg of the test that was used.

DISCUSSION

Results show no significant difference in performance between the control group and the treatment group (daily BW gain p>0.05). The trial resulted in insignificant evidence for the effect of this phytogenic feed additive and its growth-promoting properties. The feed samples that were taken during the experiment account for these outcomes. When analyzed on inclusion of PFA it became clear there was a negligible amount of PFA detectable in the trial feed (Figure 1).

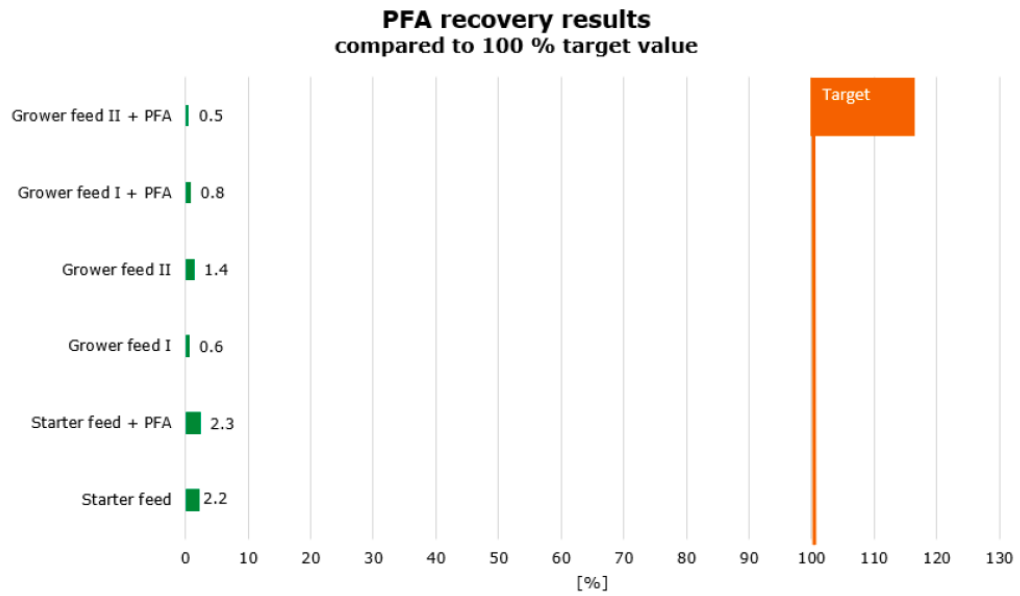

Figure 1. Inclusion of PFA in feed samples.

The feed samples taken on day 0, 14 and 35 were analyzed by GC-MS calibrated method after the conclusion of the experiment to determine sufficient amount of inclusion in the trial feed. The 100% target value would indicate an inclusion of 150gr/tonne feed supplement.

This explains the comparable results between the two treatment groups, given that it was now concluded that both groups received the same feed, neither of which was supplemented with a PFA.

In the current study, we attempted to show a difference in intestinal inflammation status of the piglets that were fed a PFA by determining calprotectin levels, a and non-invasive marker of neutrophil activity in the intestines (22). In piglets we found this to be an insufficiently sensitive method. All of the fecal samples, of both treatment groups, were found to be below the minimal detection level. Unfortunately, little can be concluded from these outcomes, besides the fact that the gastrointestinal inflammation present at the time of sampling was not evident enough to surpass the minimal detection level of the diagnostic method that was used.

The probability of finding an effect of the PFA in this study was to be considered low, regardless of the insufficient inclusion of the product. When compared to the Dutch national average of **320 g/day***, these piglets showed an 146% increase in daily growth compared to the national average. With an average daily growth of 480 g/day in the control group, it is therefore unlikely that this PFA, if properly administered, could have resulted in even better results in the trial group. Therefore it could be concluded that the population used in this study might have been too healthy to have shown a significant change in daily growth, feed conversion, and calprotectin levels.

There have been several in-vivo studies that have shown significant performance enhancement in farm-animals which they ascribe to the effects of PFA supplementation (7)(27)(19)(28). The first study mentioned looks at the same dosage (50, 100 and 150gr/tonne) attempted in this current study, and consists of similar components. The outcomes show an average daily growth of 450 gr/day for the piglets supplemented with 150gr/tonne PFA, compared to a 370gr/day growth in the control group. It is clear however, that extrapolation of in-vitro results to in-vivo situations has proven to be a challenge. When examining a feed additive, one of the reasons being the variable conditions of the digestive tract (10)(26). PFA constituents undergo many chemical modifications during digestion and absorption, which possibly alter their efficacy. Besides that, the essential oils incorporated in PFAs are very complex and volatile compounds that can vary in their concentrations and chemical compositions (7)(11). In-vitro results often rely on very high concentration which are not physiological in-vivo. Reasons like these, and given the fact that PFA is not a very well-defined term, have led to many different studies on various herbs, plants and their derivatives in various dosages, making it difficult to come to a general consensus on their efficacy.

* The national daily growth average of piglets in the Netherlands according to Agrovision from July 1st of 2019 – June 30th of 2020.

CONCLUSION

Despite the promising results that phytogenic feed additives showed in the studies mentioned, the current study unfortunately was unable to contribute to the better understanding of the efficacy of PFA in a commercial pig farm setting, because of logistical errors that occurred during the trial. Therefore, more research is needed to accurately show the effects of the PFA product that was used, in a commercial pig farm setting.

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