



Short communication

Effects of phytogenic feed additives on growth performance and on ammonia and greenhouse gases emissions in growing-finishing pigs

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ABSTRACT

The present study was conducted to evaluate the effects of two commercial phytogenic feed additives (PFAs) on growth performance and emissions of ammonia, methane, nitrous oxide and carbon dioxide compared to an unsupplemented control. The two tested commercial PFAs contained either essential oils or a mixture of essential oils and dried herbs and spices. In addition, both PFAs delivered the same amount of *Quillaja saponaria* saponins to the final feed, although dosage of the products differed. The PFAs were included in cereal-based grower and finisher diets via premixes. For negative control diet a placebo premix was used. The diets were fed to a total of 81 castrated male growing-finishing pigs (45–114 kg body weight, 27 per treatment) that were kept in nine gas-tight sealed chambers, each with three pens with fully slatted floors (three pigs per pen). The whole experiment lasted 72 days. Pigs were individually weighted and the feed consumption of pigs was recorded per pen at day 0, 24 and 72 of the experiment. From day 24 to day 72 (48 days) emission measurement took place.

The inclusion of the PFAs significantly improved average daily feed intake ($P=0.010$) and average daily gain ($P=0.018$) of pigs over the whole trial period of 72 days compared to the negative control. Feed conversion ratio was not affected by the treatments ($P>0.05$). Pigs that were fed with the PFAs had 3.6% higher final body weight ($P=0.017$) compared to the negative control. The inclusion of the PFAs reduced ammonia emissions per animal per day ($P=0.003$) as well as per kg body weight gain of pigs ($P<0.001$) on average by 21% and 26%, respectively, and tended to reduce carbon dioxide emissions per kg body weight gain ($P=0.092$) on average by 9% compared to the negative control. Methane and nitrous gas emissions were not affected by the inclusion of the PFAs ($P>0.05$).

In summary, it might be speculated that the observed increased intake and consequently higher growth was mediated via flavoring properties of the PFAs, irrespective of differences in composition. The reduction of ammonia emissions most probably was due to the inclusion of the quillaja saponins in the PFAs. It can be concluded that the tested PFAs have a potential as performance enhancers and are useful tools for the reduction of ammonia emissions from pig barns. Further research is warranted to identify the exact modes of action of PFAs.

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1. Introduction

In pig production, the reduction of nitrogen (N) input into soil via application of manure and the mitigation of ammonia (NH_3) and greenhouse gases emission into the atmosphere drew the attention of governmental regulations and of customers. In general, the N excretion of pigs can be divided in an inevitable part that occurs due to normal turnover processes of protein in the animals (Tamminga, 2003), and another evitable part that occurs due to overconsumption of crude protein (CP) or consumption of unbalanced ratios of amino acids (AA) in the diet (Aarnink and Verstegen, 2007; Rademacher, 2000). Hence, strategies to reduce environmental impact of animal husbandry include reduction of N supply via feed by phase feeding (Rademacher, 2000), diet formulation based on ideal digestibility of CP and AA (Stein et al., 2007; Moughan and Fuller, 2003) and the inclusion of synthetic AA (e.g. Canh et al., 1998) into pig diets. Almost half of the N excreted by a growing-finishing pig via urine and feces can emit during storage of the manure in the manure pit and during surface application of the manure. The underlying transformation of urea into NH_3 is catalyzed by the enzyme urease that is present in bacteria of the digestive tract or soil. In Europe, the emission of polluting gases is covered by the Industrial Emissions Directive (2010/75/EU). Farmers with more than 2000 growing finishing pigs, 750 sows or 40,000 chickens should use the best available techniques (BAT), such as air-washers or phase feeding, to reduce emissions into the environment. Unfortunately, housing systems with high consumer acceptance, such as straw based deep litter, have been shown to produce more pollutant gases as keeping pigs on slatted floors (Philippe et al., 2007).

During the past decade phytogetic feed additives (PFAs) are discussed as performance enhancers for animal production (Windisch et al., 2008). Moreover, phytogetic feed additives (PFAs) became an additional tool for the reduction of NH_3 emissions from pig production. Especially saponins, e.g. from *Yucca schidigera* and *Quillaja saponaria* were reported to reduce the NH_3 emissions from animal husbandry (e.g. Colina et al., 2001; Makkar et al., 1998). Suggested modes of action to reduce NH_3 emission include a direct binding of NH_3 and the inhibition of urease activity (e.g. Makkar et al., 1998). The aim of the present study was to evaluate the effect of two commercial PFAs, varying in their composition and contents of essential oils, but both containing same amounts of quillaja (*Q. saponaria*) saponins, on growth performance and emissions of NH_3 , methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) in grower-finisher pigs.

2. Materials and methods

2.1. Experimental design

The experiment was comprised three treatments. Treatments were randomly assigned to nine chambers with three pens of three animals each. For emission evaluation, the individual chamber was the experimental unit, resulting in three repetitions per treatment. For parameters of growth performance the individual pen was the experimental unit, resulting in nine (three chambers \times three pens) replicates per treatment.

The whole experiment lasted for 72 days. Pigs were individually weighted and the feed consumption of pigs was recorded per pen at day 0, 24 and 72 of the experiment. From day 24 to day 72 (48 days) emission measurements took place. Average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR; feed per gain) was calculated for the first phase of 24 days, for the second phase of 48 days as well as for the total 72-day experimental period.

2.2. Animals and housing

81 surgically castrated male growing finishing pigs (PIC genetic) with an average initial body weight of 45.4 ± 2.5 kg were used until the slaughter weight of 114.4 ± 4.2 kg. Pigs were housed in nine identically constructed gas-tight sealed chambers. Each chamber contained three pens with a surface area of 3.55 m^2 ($2.43 \text{ m} \times 1.46 \text{ m}$). Pigs were allocated to pens in order to achieve similar average body weights in all pens. Pens were equipped with a feeder with three feeding places and a nipple drinker. The floor of the pens was fully slatted and each chamber had a separate manure pit and an individual air outlet. Fresh air was supplied via a trickle ceiling that supplied air from the roof to the chambers. All chambers were connected to a central corridor. The doors connecting the chambers to the corridor were equipped with sensors to assure doors were tightly closed. Furthermore, a window in the wall of each chamber enabled animal inspection from the central corridor to avoid the opening of chamber doors as much as possible. The house was lit by artificial light of 45 lx (light: 12 h, dark: 12 h). Ambient temperature was maintained at 20.8°C .

2.3. Diet and feeding

Pigs were fed cereal-based grower (Phase 1: day 0–24) and finisher (Phase 2: day 25–72) diets (Table 1). Diets were formulated to meet or exceed nutrient requirements of grower and finisher pigs (Table 2; NRC, 1998). Feed was fed as mash (dry). Pigs had ad libitum access to feed and water.

In the phytogetic groups, the basal diets were supplemented with the commercial feed additives Fresta F Plus and Aromex ME Plus (Delacon Biotechnik GmbH, Steyregg, Austria), respectively. Fresta F Plus consisted of a blend of essential oils ($\geq 1.5\%$ of the pure product), with caraway and lemon oil as the main components, dried herbs and spices and quillaja saponins. Aromex ME Plus contained essential oils of rosemary, thyme ($\geq 12.8\%$ of pure product) and quillaja saponins. The

Table 1
Formulation of grower and finisher basal diets.

Ingredients (g/kg as-fed)	Grower	Finisher
Wheat	380.0	410.0
Wheat bran	30.0	86.0
Barley	385.5	365.0
Extracted rapeseed meal	80.0	60.0
Extracted soya meal	50.0	40.0
Extracted sunflower meal	30.0	–
Fat	15.0	10.0
NaCl	4.0	4.5
Aminovitan P1 Plus ^a	3.0	3.0
L-Lysine (10%)	4.0	4.0
L-Threonine (10%)	1.0	1.0
Monocalcium phosphate	5.5	4.5
Limestone	12.0	12.0

^a Aminovitan P1 Plus, Aminovitan Biofaktory Praha s.r.o., Praha, Czech Republic. Providing the following quantities per kg diet: 7200 IU vitamin A (E672), 1260 IU vitamin D3 (E671), 61.3 IU vitamin E (3a700), 1.5 mg vitamin K3, 1.5 mg vitamin B1, 4.2 mg vitamin B2, 2.0 mg vitamin B6, 24.9 µg vitamin B12, 15 mg niacinamide, 9.9 mg calcium pantothenate, 201 µg folic acid, 51 µg biotin, 100.5 mg cholin chloride, 17.0 mg copper, 80.1 mg iron, 0.75 mg iodine, 30 mg manganese (E5), 130 mg zinc (E6), 0.3 mg selenium (E8), 501 FTU 3-phytase (EC1.3.8; E1604), 100.2 U endo-1,3(4)-beta-glucanase (EC3.2.1.6; E1604), 70.1 U endo-1,4-beta-xylanase (EC3.2.1.8; E1604), 1.2 mg butylhydroxytoluene (E321), 0.24 mg butylhydroxyanisole (E320).

phytogenic products were included into the basal diet via a premix. The respective treatment premix was included in the diet via Spotmix multiphase feeding system (Schauer GmbH, Prambachkirchen, Austria) at the experimental farm. Dosage of premix was 10 g/kg diet, so that the dosage of the PFAs in the final feed was 150 mg Fresta F Plus and 100 mg Aromex ME Plus per kg diet, respectively. Below, the diets including the placebo premix, Aromex ME Plus or Fresta F Plus are referred to as negative control (NC) or treatment A or F, respectively.

2.4. Chemical analysis of feed

For chemical analysis the basal diet was ground to a particle size of 0.25 mm. Analyses of contents of dry matter, N, crude fat, starch, total sugars, crude fiber, crude ash, calcium and phosphorus in the basal diets were performed according to methods outlined by VDLUFA (1993; dry matter: VDLUFA III 3.1; crude protein: VDLUFA III 4.1.1 modified according to macro-N determination (vario Max CN); crude fiber: VDLUFA III 6.1.4; crude ash: VDLUFA III 8.1; crude fat: VDLUFA III 5.1.1; starch: VDLUFA III 7.2.1; total sugars: VDLUFA III 7.1.1; calcium: VDLUFA VII 2.2.2.6; phosphorus: VDLUFA VII 2.2.2.6).

2.5. Gas measurement and calculation of emissions

Each air outlet in the emission chambers was equipped with an anemometer (Flow sensor type SS 20.250, Schmidt, St. Georgen, Germany) for measurement of air speed and a Teflon tube for transporting air samples to the Multipoint Sampler INNOVA 1409 (LumaSense, Frankfurt, Germany). Gas concentration in the air samples was measured by the photoacoustic gas monitor INNOVA 1412i (LumaSense, Frankfurt, Germany). In brief, in photoacoustic spectroscopy the gas to be measured is irradiated by modulated light of a pre-selected wavelength. If the frequency of the light coincides with an absorption band

Table 2
Analyzed nutrient (g/kg, as-fed) and calculated metabolizable energy content (MJ/kg, as-fed) in the grower and finisher diet.

	Grower	Finisher
Dry matter	885	870
Crude protein	160	151
Crude fiber	51.2	47.9
Ash	52.1	50.5
Crude fat	41.6	37.4
Starch	456	472
Sugar	42.3	41.8
P	6.3	6.0
Ca	9.0	8.7
Metabolizable energy (MJ/kg) ^a	13.5	13.4

^a Calculated according to the Czech regulation 451/2001 with the following equation: ME = CP (g/kg) × 0.0223 + crude fat (g/kg) × 0.0341 + starch (g/kg) × 0.017 + sugar (g/kg) × 0.0168 + organic rest (g/kg) × 0.0062 – fiber (g/kg) × 0.0109.

Organic rest = dry matter (g) – CP (g) – crude fat (g) – starch (g) – sugar (g) – fiber (g).

of the gas in the cell, the gas molecule will absorb part of the light. The higher the concentration of gas in the cell, the more light will be absorbed. As the gas absorbs energy, it is heated and therefore expands and causes a pressure rise. As the light is chopped, the pressure will alternately increase and decrease – generating an acoustic signal, which is detected by two microphones. The acoustic signal is proportional to the concentration of the gas in the cell. Detection limits of the photoacoustic gas monitor used in this study were 0.2 ppm for NH_3 , 1.5 ppm for CO_2 , 0.4 ppm for CH_4 and 0.03 ppm for N_2O .

Each air sample measurement took approximately 1 minute. All chambers were sampled consecutively; so one full cycle of all 12 chambers took about 12 minutes. This results in a measurement interval of the gas concentrations in the air sample of about 12 minutes per chamber. Air speed in the air outlet of all chambers was recorded every minute. Ventilation rate (in m^3/h) was calculated by multiplying the air speed (in m/s) by the surface area (m^2) of the air outlet (diameter 0.554 m) and 3600 s/h. The measured gas concentration (in ppm) in the air sample was transferred to mg/m^3 by multiplying by the molecular mass of the respective gas divided by the molar volume (24.465 l/mol) of gas at normal temperature and pressure. For calculation of gas emissions per chamber (in g/h), the ventilation rates (in m^3/h) in between two gas measurements (12 minutes) were averaged and multiplied by the gas concentration (in mg/m^3). For calculation of emissions per animal per day, gas emissions per chamber were multiplied by 24 h/d and divided by number of animals per chamber. To account for differences in growth rate of pigs, gas emissions per chamber were divided by the body weight gain of all pigs per chamber during the period of measurement.

2.6. Statistical analyses

Performance data were analyzed using Proc GLM of SAS (2013; Version 9.4). Treatment was the fixed factor and the average initial body weight of pigs per pen was used as a covariate.

For each individual chamber and type of gas, least square means (LSmeans) for the average gas emission per hour (in g/h) were calculated using mixed model of SAS (2013) for repeated measurements (“observation” in REPEATED statement). From average emissions per hour (g/h) of the individual chambers, emissions per animal per day or per kg body weight gain were calculated under consideration of number of animals per chamber and hours per day or body weight gain of pigs per chamber, respectively. These data were analyzed for diet effects by Proc GLM of SAS (2013). Tukey test was used for post hoc analysis. All results are reported as LSmeans including the maximum standard error of the mean (SEM). The significance level of all Wald-type *F*-tests was set at $\alpha = 0.05$ and $0.05 < \alpha \leq 0.10$ was considered as near significant trend.

3. Results and discussion

3.1. General observations

All animals were considered healthy throughout the experiment by daily visual inspection, except for one pig of Treatment F that was removed at day 24 of trial due to an injured leg. As a negative impact of this injured pig on the recorded feed consumption of the pen in Phase 1 could not be ruled out, and therefore this pen was excluded for performance analysis and the number of pigs in chamber 9 was reduced to 8 for emission calculation per animal per day during the last phase of the trial.

3.2. Growth performance

Production performance parameters of pigs fed with NC or Treatment A or F are shown in Table 3. During the first 24 days of the trial (Phase 1) no differences in production performance parameters could be observed between the different treatments (all $P > 0.05$). However, in Phase 2 (day 24–72) pigs fed with Treatments A and F consumed 5.1% and 6.1% more feed ($P = 0.003$) and showed a 7.6% and 8.6% higher ADG ($P = 0.014$) compared to NC, respectively. For the whole experimental period, this resulted in 4.5% and 5.2% higher ADFI ($P = 0.010$) and 6.2% and 6.1% higher ADG for Treatments A and F compared to NC, respectively. Accordingly, pigs of Treatments A and F showed significantly higher final body weights compared to pigs of NC ($P = 0.017$). No differences were observed in FCR among treatments ($P > 0.1$). So under practical conditions dietary supplementation with the addition of these PFAs would lead to less days to market and would therefore be of economic relevance, although FCR was not affected by treatments in this study.

Due to the high variability, e.g. in composition, botanical origin and processing of the additives, as well as genetic, physiological status and housing conditions of pigs, performance results of in vivo studies conducted with different PFAs are hardly comparable among studies. In the present study, two PFAs were tested in one batch of pigs kept under similar conditions. The tested PFAs varied in their contents of essential oils, amounting to 14 ppm essential oil in diets of Treatment A and 3 ppm in diets of Treatment F. Nevertheless, the use of both additives led to a similar increase in feed intake, which might be due to an altered digestive physiology, such as gastric emptying characteristics (Juca et al., 2011) or relaxation of intestinal muscles (Souza et al., 2013), or due to their ability to improve sensory characteristics and hence, palatability of pig feed, which is discussed for different essential oil components (Windisch et al., 2008). The results of the present study confirm that PFAs have a potential to be used as performance enhancers in growing-finishing pigs. The lack of a response during the first 24 days of the experiment suggests that the PFAs tested in this study exert rather long term than immediate effects on production performance of grower-finisher pigs. In the second experimental period as well as over the whole trial,

Table 3
Growth performance of pigs from different treatments.

Treatment	NC	A	F	SEM	P trt
<i>N</i>	9	9	8		
Initial body weight (kg) (day 0)	45.7	45.9	44.6	0.905	0.534
Intermediate body weight (kg) (day 24)	68.3	69.0	68.5	0.787	0.796
Final body weight (kg) (day 72)	111.8 ^b	115.8 ^a	115.8 ^a	1.123	0.017
Phase 1 (day 0–24)					
Average daily gain (kg)	0.952	0.982	0.963	0.033	0.785
Average daily feed intake (kg)	2.303	2.380	2.372	0.038	0.255
Feed conversion ratio	2.457	2.433	2.468	0.077	0.946
Phase 2 (day 24–72)					
Average daily gain (kg)	0.906 ^b	0.975 ^a	0.984 ^a	0.020	0.014
Average daily feed intake (kg)	2.945 ^b	3.090 ^a	3.124 ^a	0.036	0.003
Feed conversion ratio	3.266	3.171	3.181	0.066	0.504
Whole trial (day 0–72)					
Average daily gain (kg)	0.921 ^b	0.978 ^a	0.977 ^a	0.016	0.018
Average daily feed intake (kg)	2.731 ^b	2.854 ^a	2.874 ^a	0.034	0.010
Feed conversion ratio	2.968	2.922	2.948	0.046	0.752

NC, negative control; A, Aromex ME Plus at 100 mg/kg feed; F, Fresta F Plus at 150 mg/kg feed; SEM, maximum standard error of the mean; P trt, P-value for treatment effect; values are presented as LSmeans; values with different superscripts differ significantly ($P < 0.05$).

the results of this study show that even in high performing animals ($ADG > 0.9$ kg) which were kept under good hygienic and management conditions the additives tested in this study could achieve a significant improvement of performance. It might be speculated that under commercial conditions, often associated with a lower hygienic status compared to experimental facilities, the performance enhancing effects of the PFA might be even more pronounced.

3.3. Gas emissions

In the present study, ammonia emissions were 9.4, 7.4 and 7.4 g NH_3 per animal per day for NC and Treatments A and F, respectively (Table 4), and were within the range of NH_3 emissions of growing-finishing pigs kept on slatted floor reported by Philippe et al. (2007). Related to kg body weight gain of pigs, ammonia emissions were 10.4, 7.6 and 7.6 g NH_3 for NC and Treatments A and F, respectively (Table 4). The botanicals included in Treatments A and F significantly reduced NH_3 emissions per animal per day ($P = 0.003$) as well as per kg body weight gain ($P < 0.001$) compared to NC. The reduction averaged 21% per animal per day, which is in agreement with the study of Veit et al. (2011) who reported a 21% and 22% reduction of NH_3 emissions in the grower and finisher phase, respectively, from fattening pigs (30–110 kg body weight) fed with 150 g/t Fresta F Plus compared to the unsupplemented control. Due to the above mentioned differences in feed intake and thereby higher CP intake of pigs from Treatments A and F, it is speculated that the reduction would have been even more pronounced if feed intake had been restricted to the same level for all treatments. Related to body weight gain of pigs, the reduction of NH_3 was 26.8% and 27.2% for treatments A and F compared to NC ($P < 0.001$), respectively.

The methane emissions averaged 10.5 g CH_4 per animal per day and did not differ between treatments ($P > 0.893$). Nevertheless, CH_4 emissions per kg body weight gain for pigs were numerically lower (–6.4%) in Treatment F compared to NC. Compared to the CH_4 emissions reported for growing-finishing pigs kept on slatted floor in the study of Philippe et al. (2007), measured CH_4 emissions were 15–42% lower. In contrast, observed nitrous emissions averaged 2 g N_2O per animal per day and were thereby 35–84% higher as reported in the aforementioned study. N_2O emissions per kg body weight gain for pigs from Treatment A were 8.6% lower compared to NC ($P = 0.148$). Although not significant, these reductions might be considered relevant because of the high global warming potential of N_2O .

Table 4
Gas emissions of pigs fed with the different treatments.

	NC	A	F	SEM	P trt
<i>n</i>	3	3	3		
NH_3 per animal per day (g)	9.41 ^a	7.41 ^b	7.44 ^b	0.27	0.0027
NH_3 per kg body weight gain (g)	10.38 ^a	7.60 ^b	7.56 ^b	0.28	0.0005
CH_4 per animal per day (g)	10.43	10.53	10.61	0.25	0.8934
CH_4 per kg body weight gain (g)	11.52	10.80	10.78	0.27	0.1757
N_2O per animal per day (g)	1.95	2.12	1.93	0.11	0.4811
N_2O per kg body weight gain (g)	2.21	2.02	2.08	0.06	0.1478
CO_2 per animal per day (kg)	3.33	3.49	3.12	0.12	0.1921
CO_2 per kg body weight gain (kg)	3.62	3.33	3.29	0.09	0.0919

NC, negative control; A, Aromex ME Plus at 100 mg/kg feed; F, Fresta F Plus at 150 mg/kg feed; SEM, maximum standard error of the mean; P trt, P-value for treatment effect; values are presented as LSmeans; values with different superscripts differ significantly ($P < 0.05$).

CO₂ emissions measured in the present study averaged 3.3 kg per animal per day and were 39–53% higher as reported in the study of Philippe et al. (2007). As anaerobic fermentation or aerobic degradation of organic matter in the manure is one source of CO₂ (Philippe and Nicks, 2015), it might be speculated that the content of fermentable/degradable organic matter in the manure was higher in the present study compared to the study of Philippe et al. (2007). There was a trend for lower CO₂ emissions per kg body weight gain, being 9.1% for Treatment F compared to NC ($P=0.092$).

Between the two tested PFAs, there were small differences in terms of NH₃ reduction. This finding emphasizes the importance of the quillaja saponins, which were included at the same level in the diets of Treatments A and F. The NH₃ reducing effect of *Y. schidigera* and *Q. saponaria* are discussed to be either due to NH₃ binding properties of saponins or due to inhibitory effects of saponins on bacterial urease enzyme (e.g. Makkar et al., 1998). Both possible modes of action are in agreement with the observation that the quillaja saponin containing feed additives tested in this study exclusively affect NH₃ and no other gas emissions. The slight reduction of CO₂ per kg body weight gain observed in the present study favors the inhibition of urease, as urea is hydrolyzed in NH₃ and CO₂. But with regard to the varying CO₂ emissions per animal per day the reduction of CO₂ emissions per kg body weight gain should rather be attributed to the higher body weight gain in Treatment F than to a real reduction of CO₂ emissions.

4. Conclusion

The improvement of growth performance of fattening pigs observed in the present study confirms the potential of PFAs to be used as performance enhancers. Furthermore, the significant reduction of NH₃ emissions achieved by the tested quillaja saponin containing PFAs suggests their use as an additional tool for the reduction of NH₃ emissions from pig production. However, further research is warranted to identify the exact mode of actions of these additives in terms of growth promotion as well as NH₃ emission reduction.

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