Research into the pathobiome composition of fattening pigs

The following research introduces the in vitro development of a herbal feed additive that modulates the proliferation of the swine enteral pathobiome responsible for causing diarrhoea, such as Clostridium perfringens or the spirochetes, like Brachyspira sp. and Lawsonia intracellularis and therefore results in a healthy, diarrhoea-free fattening period. In the development process multiple farm isolates from all over the world were included, indicating the worldwide incidence of the pathogens.

The efficacy of the herbal feed additive is demonstrated in four different in vivo trials.

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Diarrhoea is a very common symptom of enteric diseases in fattening pigs, caused by a wide variety of micro-organisms. Control strategies in managing the challenges involves the application of multiple wide-spectrum antibiotics. Upon banning the application of antibiotics as growth promoters for preventing swine enteric diseases, new alternatives are being introduced to the market.

The most common enteric diseases – although in general believed to be associated with Clostridium perfringens – are in fact porcine proliferative enteritis, swine dysentery and porcine intestinal spirochetosis, caused by different Brachyspira species or Lawsonia intracellularis. Both Gram negative micro-organisms are difficult to detect, isolate or grow in in vitro conditions, skilled expert research institutes include for example the Foshan University, China.

The above-mentioned micro-organisms are either facultative pathogens or obligate pathogens, and – altogether, with other enteric pathogenic agents that may cause enteric diseases – are part of the so called 'pathobiome'. Manifestation or expression of the disease requires the presence of more than a single anaerobic pathogen.

The development of the pathobiome results in a shift of the normal microbiome and therefore manifestation of diseases, such as swine dysentery or porcine proliferative enteropathy.

Swine dysentery is a disease caused by the well-characterised beta-haemolysing B. hyodysenteriae, or B. hampsonii and the 'freshly' evolved B. suanatina, while in case of porcine proliferative enteropathy, the aetiological agent is an obligate intracellular single species, Lawsonia intracellularis.

Next generation sequencing allows us to have an insight into the microbial population, either in the excreted faeces or the lumen, which serves as important information for enteric disease states.

The majority (>90%) of the bacteria of the intestinal microbiome are from two Phyla: Firmicutes and Bacteroidetes, while the ileum has a high percentage of bacteria in the phylum Proteobacterium (up to 40%).

Table 1. In vivo trial conditions.

Parameter	Trial	Control	Trial	Control	Trial	Control	Trial	Control
Trial location	Europe 1		Europe 2		Europe 3		Europe 4	
No. of animals	128	128	127	124	72	24	100	100
No. of pens	4	4	4	4	36	12	10	10
No. of days of the trial	113		70		70		79	
Feed additive suppl. (kg/t)	1	–	1	–	1	–	1	–

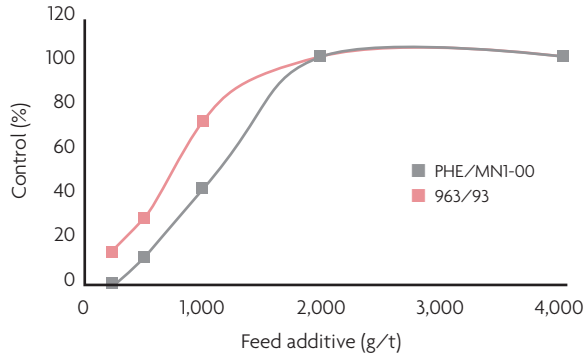

Fig. 1. Inhibitory efficacy of the herbal feed additive on the growth of different Brachyspira spp. and C. perfringens isolated from farm conditions.

Perturbations in the microbiome occur in response to many factors, such as stress (temperature variations, transportation, mixing pigs), antibiotic treatment or feed composition.

The aim of our research was to develop herbal feed additives that help in preventing the diarrhoea with suppressing the pathobiome of fattening pigs and therefore supporting the growth of normal microbiome with a multiple mode of action: either influencing the growth of the pathobiome in general or with providing nutrients that can only be digested by the good gut bacteria.

The research focused on influencing the growth of the following agents of the pathobiome:

- Brachyspira sp. in general (with the inclusion of type strains of B. hyodysenteriae, B. hampsonii, B. murdochii and B. suanatina).
- Clostridium perfringens.
- Lawsonia intracellularis.

The developed herbal feed additives were first evaluated under in vitro conditions with using multiple isolates of Brachyspira species and C. perfringens, and two isolates of L. intracellularis as the most common pathogenic agents of diarrhoea in fattening pigs.

The tested isolates are originating worldwide from swine farms, emphasising the pathobiome occurrence in all countries from all over the world.

Upon determining the appropriate dosage for controlling the particular pathobiome constituent and establishing the correlation between in vitro results and field dosages, in vivo trials were performed at different locations under different farm conditions to evaluate the universal applicability of the novel diarrhoea prevention and pathobiome control strategy.

Materials and methods

In vitro trials:

For developing feed additives, several herbal components were extracted with Soxlet extraction: 10g of raw material was extracted with 90ml of ethanol for three hours at room temperature and the extracts were tested in serial twofold dilutions in a 48-well containing microtiter plates against the pathogens (data not shown).

Optimal combinations of the extracts were established using statistical experimental designs and different initial compositions of feed additives were developed with drying the herbal components on carriers. The feed additives were extracted with water for four hours at room temperature and tested in

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Parameter	Control	Trial	P value	Control	Trial	P value
Trial location		Europe 1			Europe 2	
No. of animals	128	128	–	127	124	–
No. of pens	4	4	–	4	4	–
Finishing body weight (kg)	115.8	117.6	0.037	119.7	119.9	0.645
BW (kg)	88.6	90.8	0.017	83.8	85.1	0.013
ADG (g)	784.0	803.7	0.017	741.2	753.0	0.013
Feed intake per animal, avg. (kg)	255.8	243.0	0.140	254.5	241.4	0.075
FCR	2.94	2.67	<0.001	3.03	2.84	<0.001

Table 2. Results of European Trial 1 and 2.

Parameter	Control	Trial	P value	Control	Trial	P value
Trial location		Europe 3			Europe 4	
No. of animals	72	24	–	100	100	–
No. of pens	36	12	–	10	10	–
Finishing body weight (kg)	106.0	110.1	0.001	105.6	107.4	0.006
BW (kg)	70.2	74.5	<0.001	70.2	72.6	<0.001
ADG (g)	1002.7	1064.9	<0.001	889.0	919.1	<0.001
Feed intake per animal, avg. (kg)	201.0	199.5	0.703	192.1	193.4	0.215
FCR	2.86	2.68	<0.001	2.83	2.70	0.033

Table 3. Results of European Trial 3 and 4.

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dosages ranging from 0.5-2.5kg/t feed concentration against different target pathogens.

In vitro dosage dependence of the herbal feed additive against Brachyspira species and Clostridium perfringens isolates.

The screening procedure included approximaely 50 different Brachyspira sp. (including B. hyodysenteriae, B. murdochii, B. hampsonii, B. pilosicoli and further unidentified farm isolates of swine dysentery), with B. hyodysenteriae ATCC 27164 as the reference strain.

Isolates originating from all over the world from farms with dysentery issues, indicating the worldwide occurrence of the pathogen. In case of Clostridium perfringens, 35 different farm isolates were included in the screening, with C. perfringens ATCC 13124 as the reference strain.

In vitro dosage dependence of the feed additive against L. intracellularis isolates

Cultivation and maintenance of L. intracellularis is extremely complicated and therefore only few laboratories and research institutes are capable of working with the pathogen. Altogether two type strains, PHE/MN1-00 and 963/93 were tested.

Microtiter plates were inoculated with McCoy cells and incubated for 24 hours. The next day, approximately 10^7 L. intracellularis cells were suspended in separate solutions that contained the extract of the herbal feed additive in different concentrations.

The suspensions were placed onto the 24h McCoy host cells, the L. intracellularis viability and growth was evaluated by estimating the percent of heavily infected cells (HIC) in each well using an inverted microscope.

In vivo trials:
The aim of the in vivo trials was to test the developed herbal feed additive under in vivo conditions too, and to verify the diarrhoea preventing results of the laboratory screenings. Altogether, the results of four European trials are presented in this article.

Trials were performed in farms with different numbers of fattening pigs, under normal hygienic conditions. Diets of the trial groups involved no antibiotic supplementations, only the developed herbal feed additive was applied in 1kg/t feed concentration. Table 1 details the trial conditions.

Results of the in vitro trials

Figs. 1-2 detail the obtained results of the pathobiome-specific controlling rates of the developed herbal feed additive, tested against multiple isolates of the pathobiome constituents, such as Brachyspira species, C. perfringens and Lawsonia intracellularis isolates.

The obtained results suggest that although there is an isolate specific variation in the efficacy of the developed herbal feed additive, 76% of the tested Brachyspira species and 73% of the tested C. perfringens isolates can be inhibited successfully using 1kg/t or less than 1kg/t dosage, while in case of Lawsonia intracellularis, the optimal concentration of the herbal feed additive is 2kg/t.

Results of the in vivo trials

Results of the four European farm trials are summarised in Table 2. Implementation of the herbal feed additive significantly improved the production parameters of the trial animals. By the end of the trial, significantly higher final body weights, body weight gains and average daily gains were achieved. The feed conversion ratios also improved significantly in favour of the trial group.

Final body weights improved with 1.6, 3.9 and 1.7% in the trials of Europe 1, Europe 3 and Europe 4, respectively, while body weight gains and average daily gains improved with 2.5, 1.6, 6.1 and 3.4% in the trials of Europe 1, Europe 2, Europe 3 and Europe 4, respectively.

Feed conversion ratios improved with 9.2, 6.3, 6.3 and 4.6% in the trials of Europe 1, Europe 2, Europe 3 and Europe 4, respectively.

Economic calculations indicated that in all four trials, the herbal feed additive containing groups realised increased profits of €8.2-5.7, compared to the control groups (€8.2, 5.7, 7.9 and 6.4/pig margin over operating costs in Trial 1, Trial 2, Trial 3 and Trial 4, respectively, in the trial groups; while the margin over operating costs in the control groups were €7.3, 5.3, 7.2 and 6.1/pig, respectively representing an improvement with 12.3, 7.6, 9.7 and 4.9% in favour of the trial groups, respectively).

Conclusions

The present study investigated the efficacy of a herbal feed additive to prevent diarrhoea in fattening pigs with selectively controlling the proliferation of the pathobiome and its effect on the production parameters. In vitro studies showed that 76% of the farm specific Brachyspira species and 73% of C. perfringens isolates can be inhibited with 1kg/t concentration of the herbal feed additive, and 2kg/t concentration successfully limited the growth of two L. intracellularis isolates.

The performed in vivo trials resulted in diarrhoea-free fattening without any antibiotic implementation and additionally, significantly better production parameters, most likely due to the control of subclinical pathobiome proliferation.

The studies indicated that the developed herbal feed additive represents an effective natural solution for maintaining the normal microbiome composition and eliminating the pathobiome constituents of the intestinal microbiome.

Continuous feeding of the developed herbal feed additive therefore helps in preventing diarrhoea during the fattening period, maintaining the microbial balance of the gut system, and enable routine antibiotic free healthy animal production. With the use of the herbal feed additive, antibiotics can be reserved strictly for disease treatment. ■

References are available from the author on request

Fig. 2. Inhibitory efficacy of the herbal feed additive on the growth of two L. intracellularis type-strains, PHE/MN1-00 and 963/93.