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The effect of essential oil compounds on performance and gut health

The three studies presented in this paper by ALEXANDRE PERON* confirm that essential oils compounds can be one of the tools that nutritionists can consider when challenged with the task of reducing antibiotic use in pig production.

Essential oil compounds (EOC) are volatile and aromatic molecules derived from plants. They represent an interesting alternative to AGP in animal production because this group of compounds possesses antibacterial, antiviral, antifungal, anti-inflammatory and antioxidant properties. All these effects have been well documented in the literature. Studies with pigs suggest that EOC can improve performance, increase nutrient digestibility, and enhance gut health status through modulation of the intestinal microflora and immune system. However, the benefits of EOC for swine are reported to be variable.

Commercial phytogenic or essential oils-based additives can vary widely with respect to their origin, composition and manufacture process. Differences in the type of products have often been reported to be the cause of inconsistent results when studying the effects of EOC. Essential oil compounds are volatile and prolonged storage or high feed processing temperatures can affect their stability. They can also be absorbed and metabolised rapidly by the animal.

Therefore, characteristics such as high concentration and protection of active components represent important features to maximise product bio-efficacy. For this reason, the present paper will focus on one specific EOC solution, based on a carefully balanced and encapsulated blend of thymol and cinnamaldehyde (Enviva EO, Danisco Animal Nutrition; hereafter referred to as EO). The

Table 1: Effect of EO on performance, diarrhoea frequency and microbial counts in faeces of piglets fed diets without antibiotics

(Adapted from Li *et al*, 2012a)	Control (C)	C + EO (50g/tonne)	C + EO (100g/tonne)	C + EO (150g/tonne)
Final body weight, kg	23.49^{b}	25.36^{a}	26.58^{a}	26.76^{a}
Average Daily Gain, kg	0.37^{c}	0.41^{b}	0.45^{a}	0.45^{a}
Average Daily Feed Intake, kg	0.73^{c}	0.78^{bc}	0.87^{a}	0.84^{ab}
FCR	1.96^{a}	1.90^{ab}	1.93^{a}	1.87^{b}
Diarrhoea frequency, %	7.50^{a}	4.54^{b}	3.13^{b}	3.33^{b}
Lactobacillus, cfu/g of faeces x10^{7}	1.93^{c}	2.23^{c}	4.42^{a}	2.42^{b}
E. coli, cfu/g of faeces x10^{6}	4.78^{a}	2.72^{b}	2.27^{b}	2.15^{b}

^{a,b,c} ***P<0.05***

Table 2: Effect of EO on serum immunity parameters of piglets fed diets without antibiotics

(Adapted from Li *et al*, 2012a)	Control (C)	C + EO (50g/tonne)	C + EO (100g/tonne)	C + EO (150g/tonne)
Lymphocyte transformation rate[1], %	1.11[b]	1.26[a]	1.29[a]	1.34[A]
Phagocytosis rate[2], %	11.92[b]	21.37[a]	21.63[a]	20.98[a]
Immunoglobulin A, mg/ml	0.07[b]	0.08[ab]	0.09[a]	0.09[a]
Immunoglobulin G, mg/ml	0.61[b]	0.73[a]	0.60[b]	0.65[b]
Immunoglobulin M, mg/ml	0.19	0.19	0.20	0.21
Complement C3[3], µg/ml	64.65[b]	77.81[a]	75.71[a]	71.89[a]
Complement C4[3], µg/ml	6.62[b]	9.28[a]	9.85[a]	9.09[a]

[1]Changes in morphology accompanying lymphocyte activation, in which small, resting lymphocytes are transformed into large, active lymphocytes. [2]The rate at which phagocytes engulf and digest microorganisms. [3]Complement C3 and C4 are blood proteins that enhances the body's ability to destroy bacteria and foreign particles
[a,b,c] $P<0.05$

Table 3: Effect of antibiotics or EO on performance, faecal consistency and nutrient digestibility of piglets

(Adapted from Li *et al*, 2012b)	Positive control (with AGP)	Negative control (no AGP)	Negative control + EO (100g/tonne)
Average Daily Gain, g	505[a]	442[b]	493[a]
Average Daily Feed Intake, g	846	783	789
FCR	1.67	1.79	1.62
Faecal consistency[1]	1.22[b]	1.53[a]	1.30[b]
Dry matter digestibility, %	87.0[a]	84.3[b]	86.9[a]
Crude protein digestibility, %	83.5[a]	76.5[b]	81.3[a]

[1]Faecal score was assessed visually and fresh excreta were ranked as follows: 1=solid, 2=semi-solid, 3=semi-liquid and 4=liquid
[a,b] $P<0.05$

author will discuss the results obtained from a series of trials carried out in China during the past two years.

In a first study researchers investigated the effect of graded levels of EO on the performance, immune status and gut microbial populations of weaned pigs raised without any antibiotics. A basal corn/soy-based diet and without any AGP, was either unsupplemented or supplemented with three levels of EO (50, 100 and 150g/tonne of feed). Performance results (Table 1) showed that the final bodyweight of piglets fed the EO-supplemented diets was significantly higher than the control treatment.

The addition of EO improved average daily feed intake and weight gain of young pigs and, for the highest inclusion level of EO, it also resulted in better feed conversion. Moreover, animals fed with the EO diets exhibited a significantly lower incidence of diarrhoea and reduced E.coli counts in the faeces, suggesting a positive effect of the EO compounds on the overall gut health of piglets raised without the use of antibiotics.

Analysis for the serum immunity parameters (Table 2) revealed that dietary addition of EO positively modulated the animal immune status. Piglets from the EO-supplemented treatments exhibited higher lymphocyte transformation and phagocytosis rates, as well as increased immunoglobulin and complement protein levels. Improvement in immunocompetence may reduce the risk of pathogen proliferation, especially with young animals raised under stressful environmental conditions.

The aim of the second study was to assess the potential of EO to replace AGP in piglet feed. A total of 96 crossbred piglets average weight: 8.4kg were housed in 24 pens and randomly allocated to three dietary treatments. Animals were fed a corn/soy-based diet. The dietary treatments consisted of an unsupplemented basal diet (negative control, NC), or similar diets supplemented with either AGP (positive control, PC – containing 150ppm chloretetracycline, 80ppm colistin sulfate and 50ppm kitasamycin) or EO (included at 100g/tonne of feed).

Performance and digestibility results (Table 3) showed that AGP and EO led to improvement in daily weight gain and feed conversion (although the FCR effect was only numerical) when compared with the unsupplemented treatment. Pigs fed with AGP or EO also had higher nutrient digestibility values and exhibited greater faecal consistency. Overall, the magnitude of EO response was very similar to the effect of AGP.

Assessment of gut morphology and microbial populations in the intestine (Table 4) revealed that EO and AGP significantly reduced E.coli counts in the lower gut when compared ▷

Table 4: Effect of antibiotics or EO on intestinal morphology and gut microbial populations of piglets

(Adapted from Li *et al*, 2012b)	Positive control (with AGP)	Negative control (no AGP)	Negative control + EO (100g/tonne)
Villus height to crypt depth ratio			
Duodenum	2.78	3.25	3.10
Jejunum	3.41[a]	2.96[b]	3.38[a]
Ileum	3.78	3.13	3.73
E.coli count, $\log_{10}$ cfu/g of digesta			
Caecum	4.69[b]	5.33[a]	4.53[b]
Colon	4.52[b]	5.46[a]	4.64[b]
Rectum	5.06[b]	5.55[a]	4.93[b]
Lactobacilli : *E.coli* ratio			
Caecum	1.45	1.29	1.43
Colon	1.47[ab]	1.30[b]	1.56[a]
Rectum	1.45	1.42	1.46

[a,b] ***P<0.05***

to the negative control treatment. Differences in the structure of the intestinal mucosa were only observed at the jejunum level, where EO and AGP treatments led to an increase in the villus height to crypt depth ratio.

Analysis of blood parameters (Table 5) showed that AGP and EO treatments significantly improved lymphocyte transformation rate and total antioxidant capacity compared to the negative control. Trends for positive effects of AGP and/or EO on the immunoglobulin levels were also noted.

Overall, replacing antibiotics by EO produced very similar responses in terms of animal performance, nutrient utilization, gut health and immune status. Therefore, in the conditions of this study, EO proved to be an effective alternative solution to the use of AGP in young pig diets.

The last trial of the series was designed to investigate the possible interactions between EO and feed enzymes. Studies have shown that the addition of feed enzymes can contribute to better gut health by limiting the amount of undigested

Table 5: Effect of antibiotics or EO on serum parameters of piglets

(Adapted from Li *et al*, 2012b)	Positive control (with AGP)	Negative control (no AGP)	Negative control + EO (100 g/tonne)
Lymphocyte transformation rate, %	1.28[a]	1.16[b]	1.28[a]
Immunoglobulin A, mg/L	49.7	45.5	46.9
Immunoglobulin G, mg/L	250	249	268
Immunoglobulin M, mg/L	56.5	46.4	56.4
Total antioxidant capacity, U/ml	11.97[a]	10.46[b]	12.37[a]

[a,b] ***P<0.05***

Table 6: Effect of xylanase and EO on performance and nutrient digestibility of piglets

(Zhang *et al*, unpublished)	Positive control PC	Negative control NC	NC + Xylanase	NC + EO	NC + EO + Xylanase
Overall performance					
Average Daily Gain, g	382[a]	348[b]	382[a]	383[a]	389[a]
Average Daily Feed Intake, g	633	634	630	631	633
FCR	1.65[b]	1.82[a]	1.64[b]	1.64[b]	1.62[b]
Apparent faecal digestibility					
Dry matter, %	81.2[a]	79.2[b]	81.1[a]	81.2[a]	81.4[a]
Crude Protein, %	79.4[a]	73.4[b]	77.5[a]	79.3[a]	79.6[a]
Energy, %	79.9[a]	76.3[b]	80.2[a]	81.2[a]	81.4[a]

[a,b] ***P<0.05***

Table 7: Effect of xylanase and EO on serum parameters of piglets

(Zhang *et al*, unpublished)	Positive control PC	Negative control NC	NC + Xylanase	NC + EO	NC + EO + Xylanase
Lymphocyte proliferation rate, %	1.22[b]	1.23[b]	1.23[b]	1.27[a]	1.27[a]
Immunoglobulin A, mg/L	46.4[b]	46.8[b]	47.6[b]	51.0[a]	50.4[a]
Immunoglobulin G, mg/L	245[b]	236[b]	234[b]	273[a]	276[a]
Immunoglobulin M, mg/L	49.7	50.2	49.8	52.5	50.8
Total antioxidant capacity, U/ml	11.19	11.18	11.27	12.07	11.94

[a,b] ***P<0.05***

Table 8: Effect of xylanase and EO on intestinal morphology and gut microbial populations of piglets

(Zhang *et al*, unpublished)	**Positive control PC**	**Negative control NC**	**NC + Xylanase**	**NC + EO**	**NC + EO + Xylanase**
Villus height, µm					
Duodenum	593	595	598	610	600
Jejunum	513	508	510	521	529
Ileum	470	477	464	485	478
Crypt depth, µm					
Duodenum	184	192	188	195	192
Jejunum	161	159	164	165	168
Ileum	131	135	136	143	140
E.coli, $\log_{10}$ cfu/g of digesta					
Caecum	5.13[(a)]	5.00[(a)]	5.04[(a)]	4.73[(b)]	4.72[(b)]
Colon	5.06[a]	5.16[a]	5.05[a]	4.70[b]	4.67[b]
Rectum	4.88[(ab)]	5.00[(a)]	4.94[(a)]	4.67[(b)]	4.77[(b)]

[a,b] ***P<0.05,*** [(a),(b)] ***P<0.07***

nutrients in the digestive tract, therefore reducing the risk of microbial proliferation. A total of 240 crossbred piglets average weight: 6.3kg were housed in 60 pens and randomly allocated to 5 dietary treatments. The trial design included a positive control diet (PC), a negative control diet (NC) reduced in digestible energy (100-150 kcal reduction vs PC), and negative control diets supplemented with either a commercial xylanase (Danisco Xylanase, Danisco Animal Nutrition), EO (included at 100 g/tonne of feed) or the combination of xylanase and EO. Diets were based on wheat, corn and soybean meal. They were all supplemented with phytase (500 FTU/kg), but did not contain any antibiotics. Growth and digestibility results (Table 6) showed that both EO and xylanase successfully improved performance and nutrient utilisation compared to the NC diet and restored those parameters to the level of the PC treatment.

Results from blood analyses (Table 7) were relatively similar to those of the first two trials. Piglets fed diets supplemented with EO (either alone or in combination with xylanase) exhibited significantly higher lymphocyte proliferation rates and immunoglobulin levels, suggesting better immunocompetence. There was also a positive trend for EO to improve total antioxidant capacity, but this effect was only numerical.

Data from the gut histology work did not reveal any significant differences between treatments, but microbial counts analysis showed that EO treatments reduced E.coli levels in the lower gut. This latest observation is in line with results from the previous studies.

Overall, the study confirmed most of the EO effects observed in the first two trials of the series. With regard to the combination of EO and feed enzyme, the present study did not allow to conclude on a possible additive or synergy between the two products at the tested inclusion rates.

The three studies presented in this paper confirmed that essential oils compounds can be one of the tools that nutritionists can consider when challenged with the task of reducing antibiotic use in pig production. Even if their mode of action is not fully understood, the positive effects of EO on performance and nutrient utilisation observed in the trials were most likely related to their positive action on digestive health, as expressed by the reduction in diarrhea frequency / improvement of faecal consistency, as well as the reduction of E.coli levels in the hindgut and improvement in the animal immunocompetence. *Ap*

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