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Effect of dietary addition of thymol on growth, salivary and gastric function, immune response, and excretion of *Salmonella enterica* serovar Typhimurium, in weaning pigs challenged with this microbe strain

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ABSTRACT: 64 weaned pigs (24d-old), were fed a control diet or a control plus 1% thymol diet, with or without *Salmonella* typhimurium challenge. Thymol reduced feed intake but did not affect significantly the final body weight after 29d of trial. Salmonella did not affect the performances. Body temperature was lower in the thymol group before, but also 1d after challenge, when *Salmonella* increased the values more in unsupplemented pigs. Fecal excretion of *S. typhimurium* in challenged pigs was not changed by the diet. The thymol group had a higher concentration of immunoglobulin (Ig) in serum before the challenge, but after there was an interaction between diet and challenge. The diet and the challenge did not affect saliva parameters, and ATPase gene expression in the fundic gastric mucosa. Thymol increased the number of parietal cells for 100 µm depth of oxintic gland. In conclusion the important supplementation with thymol reduced the intake, but not performance in the medium period. Some factors of the gut barrier were positively influenced. Salmonella challenge did not reduce significantly the performance of the weaned piglet.

Key words: Piglet, Weaning, Thymol, Salmonella.

INTRODUCTION – From the 1st January 2006, the antimicrobial growth promoters (AGPs) are banned in the UE. Finding a valid alternative to the AGPs is a topic for the scientists. In addition, research is paid to strategies to contrast *Salmonella* typhimurium, that is largely diffused in the piggery and it is a threat to the pork industry and for the human health. The essential oils (EOs) are volatile components of plants and could be used as bactericidal. The ability of these compounds to kill bacteria strongly depends on their chemical structure (Si *et al.*, 2006). Thymol, a phenolic molecule from thyme, has a high activity against *S. typhimurium in vitro* (Si *et al.*, 2006), presumably by damaging the cytoplasmatic membrane integrity of the pathogen. However the results of the *in vivo* experiments are contradictory and often done with additions of various extracts from thyme and with doses very lower than the *in vitro* dose effective against some enteric bacteria. The aim of this trial was to test the effect of a high dose of thymol, added in a weaning diet, on the performance of the piglet, and to study some physiological responses of the piglet fed this aroma.

MATERIAL AND METHODS – In 4 consecutive batches, 64 pigs, weaned at 24d, were divided in 4 groups, 16 subjects each, balanced for litter and weight. Two groups were fed a standard diet and two groups received the

same diet added with 1 % thymol. From start to d5 the subjects were reared in box of 2 individuals, then were individually housed and one group for treatment was orally challenged with 1.5 ml broth containing 1×10^9 CFU *S. enterica* serovar typhimurium. The thymol dose was fixed considering the concentration bactericide *in vitro* for *S. typhimurium* and toxicity data found in U.S Environmental Protection Agency report 2003. Before the challenge and at the sacrifice, a sample of blood serum was taken. At d5 and d19 a sample of saliva was collected. On d5, the saliva produced for unit of time was also quantified. Half of the pigs were sacrificed on d7 (+2 post-challenge), and half on d25 (+20 post-challenge), always at the same time from the last meal, anaesthetized with sodium thiopental and euthanized by intracardiac injection of Tanax[®]. Stomach, small intestine, cecum and colon were dissected, the pH's measured, and tissues weighted and measured. Stomach tissue was also collected. Gastric morphology, gene expression, IgA and IgM in the serum and saliva were assessed as reported by Bosi *et al.* (2006) and Bosi *et al.* (2007). Fecal samples were tested for *S. typhimurium* by a standard bacteriological method including non selective pre-enrichment, selective enrichment, subculture on solid media and serotyping. The data were analysed by analysis of variance using the GLM procedure of SAS , including diet, challenge, and time distance from the challenge, their 1st level degree interactions, and the block. The results of eight pigs of the 1st batch were excluded from the statistical analysis, due to the very low feed intake before the challenge (feed intake in the total period lower than 1% of the starting body weight).

RESULTS AND CONCLUSIONS – Pigs were almost healthy for all the trial, but 2 pigs fed the control diet died (1 before, 1 post-challenge). The challenge with Salmonella did not affect the overall growth performance and did not interact with Thymol treatment. In accordance with the data of Jugl-Chizzola *et al.* (2006) on the palatability of *Thymus vulgaris* in unchallenged piglets, our subjects fed thymol had a reduced daily feed intake before and after the challenge (Table1).

Table1. Influence of diet and challenge on pig performance, tissue weight and body temperature.

	Diet	SEM	Challenge	SEM		
	Control	Thymol		NO	YES	
Body weight before challenge, kg	7.08	6.58		1.04	—	—
Body weight 20 d after challenge, kg	14.10	13.06	1.51	13.59	13.57	1.51
DLWG before challenge, g	19	-48	55.3**	—	—	
DLWG 0d to 20d after challenge, g ^a	342	316	46.4	328	330	46.4
Daily feed intake, g						
- before challenge	127	89	26.7**	—	—	
- 0 d to 20d after challenge a	474	413	63.7*	437	451	63.7
Feed to gain to 20 d after challenge a	1.40	1.31	0.10*	1.33	1.37	0.10
Tissue weight (%/BW)						
- Stomach	0.72	0.72	0.08	0.70	0.74	0.08 [†]
- Cecum	0.30	0.32	0.05 [†]	0.31	0.31	0.05
Body temperature, °C	<u>Control</u>	<u>Control+Ch</u>	<u>Thymol</u>	<u>Thymol+Ch</u>		<u>SEM</u>
- before challenge	39.22	—	38.97	—		0.06*
- 1 d later ^b	39.07A	39.52 B	39.00 A	39.07 A		0.12

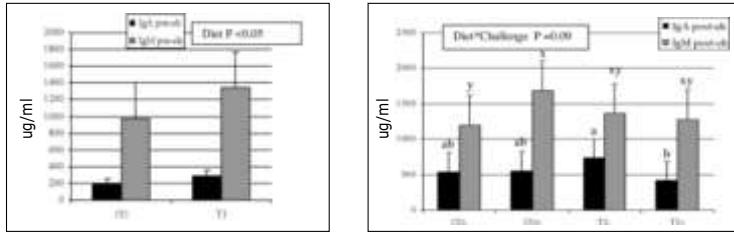
^a On 1/2 of the subjects. ^b Interaction, $P > 0.10$. Covariates for the temperature before the challenge * $P \leq 0.05$; ** $P < 0.01$; [†] $P \leq 0.10$; A,B: $P < 0.01$.

The final BW of the pigs was not significantly affected, but thymol reduced the daily live weight gain before the challenge. The favourable feed to gain in thymol group confirms that feed intake was the only one negative consequence of the addition. The performance data at the first sacrifice were not affected by the diet (data not shown). The challenged pigs were all negative for the excretion of *S. typhimurium* before the infection, and positive at 50% and 43 %, after 2 and 14 days respectively, with no effect of thymol addition. Thymol lowered the body temperature before the challenge ($P < 0.05$), presumably due to the low feed intake. After 24h, the Salmonella challenge increased

the body temperature of pigs fed the control diet ($P < 0.01$ vs unchallenged control pigs), instead thymol prevented this effect ($P < 0.01$ vs challenged control pigs). The challenge tended to increase the stomach tissue weight ($P = 0.10$). The diet did not affect the tissue weights and the pH of the GIT segments. Only the cecum tissue weight of the thymol group tended to be higher than that of the Control group ($P = 0.07$). Before the challenge, the thymol increased total IgA and IgM in the serum (Figure 1).

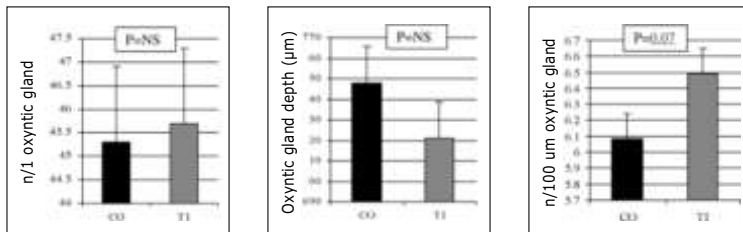
Figure. 1. Effect of diet and diet*challenge on the immunoglobulin concentration in the serum.

^{a,b,x,y} Mean values within a row with unlike superscript letters are different ($P < 0.05$).



Twenty days later there was an interaction between diet and challenge for these parameters: serum IgA did not differ in control pigs, while in thymol challenged subjects were reduced. The IgM were increased by the challenge in control pigs, but not affect in thymol treated. The diet and the challenge, did not affect the concentration of salivary IgA, the production of saliva per unit time, ATPase gene expression (data not show), the number of parietal cells for each oxyntic gland and the depth of this gland. Instead, the number of parietal cells measured per 100µm of the oxyntic gland tended to be higher for thymol than for control group (Figure.2).

Figure 2. Effect of the diet on the n°, depth and n°/100µm depth of parietal cells per oxyntic gland.



The TNF expression in stomach decreased with the thymol addition (-10%, $P = 0.08$) and with the increasing age (-15%, $P < 0.01$); the gastric pH also decreased with the age (-32%, $P < 0.01$) (data not show) in accordance with the higher parietal cells number (+30%). In conclusion a high addition thymol reduces the palatability of the diet but do not affect the performance. Furthermore there are some indications that the health is improved.

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