Cellular Immune Response of Weaned Pigs Fed Diet Supplemented with an Essential Oil

Veronika HALAS [1] (✉)
Imre NOCHTA [2]
Zsuzsanna PÁSTI [1]
Csaba SZABÓ [1]
Róbert TÓTHI [1]
János TOSSENBERGER [1]
László BABINSZKY [3]

Summary

The objective of the present study was to investigate the effect of an essential oil product on growth performance and cellular immune response of 28-day-old weaned piglets. A total of 348 piglets (50% gilts, 50% barrows) were assigned to three dietary treatments (6 pens/trt). The basal diet was a commercial feed that was supplemented without any growth promoter (NC), with antibiotic growth promoter of 40 ppm avilamycin (PC), or with 0.25 g of an essential oil product (EO) per kg of feed. All pigs were immunized by inactivated Aujeszky's disease virus vaccine at week one and three of the experiment (28- and 44-days-age, respectively). Blood samples were taken four times (on day one, 16, 24, 32 of the experiment) for lymphocyte stimulation (LST) tests with ConA, PWM, PHA used as non-specific and Aujeszky virus used as specific mitogens from 2 pigs/pen. All piglets were individually weighed on day 0, 8, 16, 24 and 32 of the trial.

There was no significant difference among average daily gain, feed intake and feed conversion ratio of piglets fed different dietary treatments. The non-specific LST test at the 4th blood sampling showed higher values in pigs received feeds with essential oil supplementation (EO) than that of the positive (PC) and negative control (NC) groups ($P<0.05$). However, no significant difference in specific immune response of pigs in different dietary treatments was found. It can be concluded that essential oil supplementation may enhance the non-specific immunocompetence of 28-day-old weaning pigs without compromising their growth performance.

Key words

essential oil, weaned piglets, non-specific immunity, specific immunity

[1] Kaposvár University, Department of Animal Nutrition, Kaposvár, Hungary
✉ e-mail: halas.veronika@ke.hu
[2] Agrokomplex C.S. ZRT, Zichyújfalu, Hungary
[3] University of Debrecen, Department of Feed- and Food Biotechnology, Debrecen, Hungary

Received: May 30, 2011 | Accepted: July 10, 2011

ACKNOWLEDGEMENTS
The authors (V. Halas, Cs. Szabó, R. Tóthi) were supported by TÁMOP-4.2.1.B-10/2/KONV-2010-0002 that is gratefully acknowledged.

Introduction

Weaning is a stressor for the piglets and the following few days is a critical period in pig production. This time piglets are very sensitive to any infection and the mortality and morbidity of the herd is higher in comparison to other periods. Therefore, feed supplements that boost the immune responses of weaned piglets are highly recommended. Essential oils of herbal extracts (from oregano, rosemary, cinnamon, garlic, anise, peppermint) are often referred as one of the alternatives for growth promoter antibiotics (Wenk, 2003). Several studies failed to prove significant improvement in average daily gain and/or feed conversion ratio of pigs and poultry (Neil et al., 2006; Hernandez et al., 2004). Although the growth promoter effect is rather inconsistent it is often reported that essential oils enhance the secretion of digestive juices and promote the immune system of animals as well as they may have antibacterial, coccidiostatic, anthelmintic, antiviral or anti-inflammatory activity and particularly antioxidant properties (Wenk, 2003).

The aim of the present study was to investigate the effect of an essential oil product on growth performance, feed intake and feed conversion ratio as well as on specific and non-specific cellular immune response of 28-day-old weaned piglets.

Material and methods

Animals, dietary treatments and housing

A total of 348 Hungarian Large White x Danish Landrace (F1) 28-day-old weaned piglets were used in the trial with an initial mean body weight of 7.9 kg. Three dietary treatments were achieved as follows: the basal diet was a commercial feed for weaned piglets that was supplemented without any growth promoter (NC), with antibiotic growth promoter of 40 ppm avilamycin (PC; 0.2 g/kg Maxus-G-200, Eli Lilly and Co. Ltd., Liverpool, United Kingdom), or with 0.25 g of an essential oil product (EO; Cinergy, Provimi, Rotterdam, the Netherlands) per kg of feed. The main components of Cinergy are essential oil compounds from oregano, clove and cinnamon; the product is a unique association of four stabilized essential oils' natural identical compounds in a specific solvent and a specific carrier. Diet composition and nutrient content of the basal feed are shown in Table 1. The animals were kept in groups (19-20 pigs/group) and six groups were assigned to each of three dietary treatments. The trial was conducted in the intensive growth phase of the rearing period and took 32 days. The animals were kept in flat deck pens; feed and water were offered *ad libitum* during the trial.

Table 1. Composition and analyzed nutrient content of the basal diet (g/kg)

Components	g/kg	Nutrient content	g/kg
Corn	93.8	Dry matter	915.0
Fish meal (70% CP)	70.0	DEs (MJ/kg) [b]	15.7
Whey powder (11% CP)	100.0	Crude protein	229
Full fat soya (33% CP)	140.0	Ether extract	112
Flaked corn	140.0	Crude fiber	18
Milk product blend (31.4% CP)	220.0	Crude ash	60
Flaked wheat	160.0	N-free extract	497
Vegetable oil	45.0	Calcium	6.8
Vitamin and mineral premix[a]	5.0	Phosphorus	6.3
Monocalcium-phosphat	4.0	Lysine	16.5
Acid Lac dry (acidifier)	8.0	Methionine	5.1
L-Lysine	4.1	Cystine	3.2
DL-Methionine	0.5	Met+Cys	8.3
L-Tryptophan	0.4	Threonine	8.8
Threonine	0.4		
Choline cloride 60%	0.5		
Mould inhibitor	0.5		
Flavor	0.4		
Antioxidant	2.0		
Sweetener	0.4		
Total	1000.0		

[a] - 1 kg premix contained: Ca 94.1 g/kg, K 0.7 g/kg, P 1.5 g/kg, available P 1.3 g/kg, Fe 40014 mg/kg, Mn 12028 mg/kg, I 402 mg/kg, Co 100 mg/kg, Se 80 mg/kg, Zn 30022 mg/kg, Mg 1905 mg/kg, Cu 33000 mg/kg, vitamin A 2400 IU/g, Vitamin D_3 400 IU/g, Vitamin E 30000 mg/kg, Vitamin K_3 1223.6 mg/kg, Vitamin B_1 703 mg/kg, Vitamin B_2 1040 mg/kg, Pantothenic acid 3589 mg/kg, Vitamin B_6 1010 mg/kg, Vitamin B_{12} 7 mg/kg, Niacin 7110 mg/kg, Folacin 201 mg/kg, Biotin 26 mg/kg ; [b] - DEs - calculated data

Experimental procedure

All pigs were immunized with inactivated Aujeszky's disease virus (AyV) vaccine (Auphyl Plus, CEVA-Phylaxia) on the first day of the experiment and after two weeks of the experiment (28- and 44-days-age). Blood samples, for cellular tests were taken four times during the trial on the days one, 16, 24 and 32 from 2 pigs (a gilt and a barrow; at 29, 44, 52 and 60 days of age, respectively) per each pen (12 data/trt). Blood samples were collected from the anterior *vena cava* at the same time (10.00 a.m.) on each day of sampling. Blood samples for analyzing cellular immune parameters were kept in heparinized vacuum tubes.

All piglets were weighed individually on day 0, 8, 16, 24 and 32 of the trial. Feed intake was recorded for each pen and the feed conversion was computed as the total feed intake (kg) per the total gain (kg) for each pen. Mortality and morbidity of the animals were recorded.

Analytical procedures

Immunological tests. The cellular immunity was monitored by lymphocyte stimulation assays (LST, Iwata and Inoue, 1993). Peripheral blood lymphocytes (PBL) were isolated by density gradient centrifugation (400 g for 15 minutes) through Ficoll-Paque (Pharmacia) according to standard protocols, and the immune function was tested by lymphocyte blastogenesis to the mitogens phytohemagglutinin (PHA), concanavalin A (Con A) and poke weed mitogen (PWM) as non-specific, and AyV as a specific stimulant. The number of viable PBLs was determined by trypan blue exclusion using a haemocytometer. The cells were diluted in DMEM (Dulbecco's Minimum Essential Medium, Sigma) supplemented with antibiotics and 10% fetal bovine serum. They were plated at a density of 1x105 cells/well into 96 well plates, four wells (100 µl each) for each mitogen. The cultures were incubated for four days at 37^0C. Blastogenesis was measured by a colorimetric assay (Hussain et al., 1993) using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium (MTT) as reagent (Sigma-Aldrich). 20 µl of MTT (5 mg/ml) was added to each well and incubated for four hours. The microtiter plates were centrifuged (1400 g, 10 minutes at room temperature) and the untransformed MTT was removed by pipetting. The optical density was measured by an ELISA reader at 570 nm and a reference wavelength of 630 nm after dissolving the crystalline formazan product with 100 µl of DMSO containing 0.01 N HCl.

Absorbance of the product at 630 nm was subtracted from the absorbance at 570 nm to calculate total conversion of dye. The cell stimulation index was calculated as the optical density (OD) of stimulated cells divided by the OD of unstimulated, control cells.

Chemical analysis. The chemical composition of the basal diet, i.e. dry matter, crude protein, crude fat, crude fiber, crude ash and Ca and P was determined according to AOAC procedures (1989). Amino acid content of the feed was analyzed according to Bech-Andersen et al. (1990).

Statistical analysis

The effects of dietary treatments on the growth performance and the studied immune parameters were tested using ANOVA (SAS, 1996) with the following general linear model:

$Y_{ij} = \mu + T_i + e_{ij}$,

where Y_{ij} = dependent variable; μ = overall mean; T_i = treatment effect; i = 3 (NC, PC, EO); e_{ij} = residual error. Tukey test was applied when the model was significant at the level of $P< 0.05$ (SAS, 1996).

Results and discussion

The effect of dietary treatments on the performance of rearing piglets is presented in Table 2. Our data show that neither essential oil nor antibiotic growth promoter had statistically significant impact on the average daily feed intake, average daily gain and feed conversion of pigs ($P>0.05$). The lack of performance response in piglets receiving different dietary treatments could be explained by the high standard of environmental conditions and the general hygiene with a very low bacterial challenge. Recent studies on any of growth enhancers are in accordance with earlier antibiotic growth promoter studies: under higher environmental pressure the treatment may result in a better improvement of the zootechnical parameters (such as the average daily gain, feed conversion) of pigs, however, their growth promoting effect is low if the animal performance is close to the genetic potential.

Table 2. The effect of dietary treatment on the average daily feed intake, average daily gain and feed conversion ratio of the weaned pigs

	Periods				
	0-8 d	8-16 d	16-24 d	24-32 d	Total
Average daily feed intake per pen (kg/d) [1]					
Negative control*	30.6	45.6	70.8	110.4	257.3
Positive control*	31.8	42.2	78.2	106.6	258.7
Essential oil	32.0	40.0	68.0	106.1	246.1
RMSE	4.91	8.81	19.61	15.89	28.33
P-value	0.88	0.55	0.66	0.88	0.70
Average daily gain (g/d) [2]					
Negative control*	138	331	414	343	307
Positive control*	146	318	401	376	311
Essential oil	138	294	381	372	300
RMSE	102.2	131.5	161.6	173.3	96.4
P-value	0.83	0.10	0.30	0.29	0.69
Feed conversion ration per pen (kg/kg) [1]					
Negative control*	1.56	1.02	1.24	2.08	1.43
Positive control*	1.52	0.93	1.53	1.71	1.42
Essential oil	1.58	1.06	1.40	1.81	1.45
RMSE	0.292	0.272	0.324	0.414	0.142
P-value	0.94	0.74	0.34	0.31	0.94

* negative contol diet contained no growth promoter, positive control diet contained 40 ppm Avilamycin/kg; [1] each treatment mean represents the average of data from six pens; [2] each treatment mean represents the average of individual data; RMSE: root mean square error

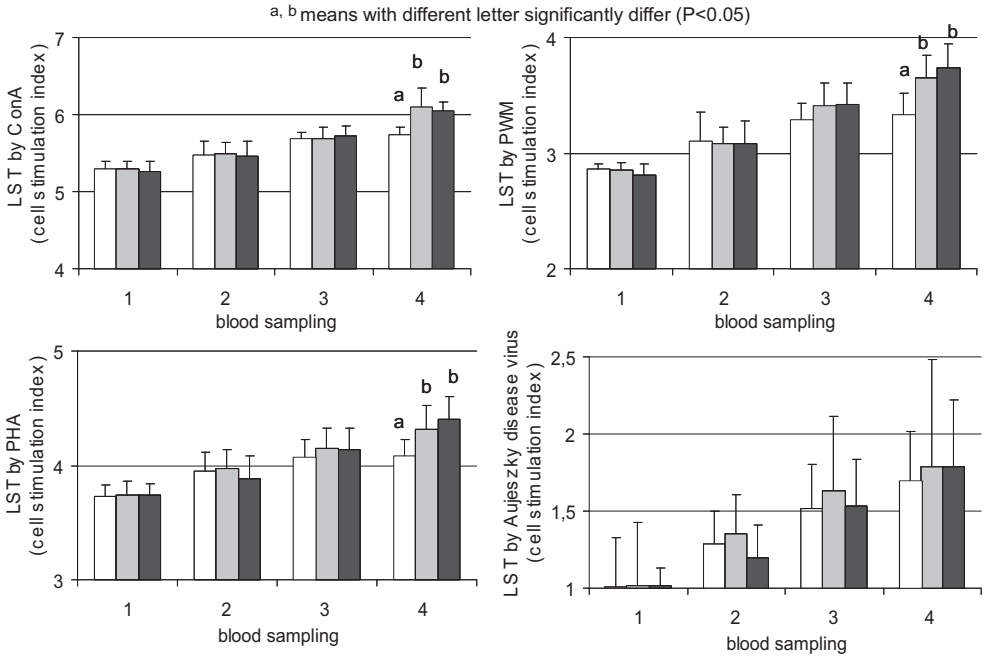

Figure 1. The effect of dietary treatments on lymphocyte stimulation with non-specific and specific mitogens (means ± s.d., □ negative control, ■ positive control, ■ essential oil product; blood samples were taken on days 1, 16, 24 and 32 of the experiment at 29, 44, 52 and 60 days of age, respectively)

There was no treatment effect on average daily gain of piglets that were used for blood sampling, and also data did not differ statistically from the identical treatment mean. ADG in 1st, 2nd, 3rd and 4th periods of the experiment was 127, 291, 347 and 350 g/d, respectively. The effect of dietary treatments on cellular immune variables is shown in Figure 1. It can be seen that the investigated cellular immunity did not show significant differences among groups assigned to dietary treatments during the first three blood sampling. On day 32, the LST measured as response to all of the non-specific mitogens (conA, PHA, PWM) was higher in EO and PC pigs than in NC pigs ($P<0.05$). The values of specific LST increased during the trial, but not statistically significant differences were found among treatments at each blood sampling.

Our data are in accordance with other studies showing that boost of non-specific immune response by any of dietary supplementation needs 21-28 days; however, the specific immune response usually appears earlier (Zukkermann, 2000). Therefore it is likely that the dietary treatments did not affect the specific cellular immunity either in the examined period or later. Considering that PHA and ConA mitogens stimulate the non-specific proliferation of primary T-cells, whereas PWM stimulates the non-specific proliferation of T and B cells. Therefore our results regarding the non-specific LST indicate that likely both the non-specific cellular and humoral immune responses were enhanced when the essential oil product was fed to the weaned piglets.

In general, better immune response requires extra nutrient supply. The magnitude of the energy and amino acid requirement of the defense mechanisms are difficult to define, however, higher values in immune parameters are accompanied with lower performance data in lots of studies. The fact that a better cellular immune response did not compromise the performance data in our study suggests that probably a better nutrient supply was both in EO and PC treatments. It is frequently reported that antibiotic growth promoters improve the digestibility of nutrients. Some studies also prove evidence that dietary supplementation of essential oils enhance the secretion of digestive enzymes (Kamel, 2001) that may lead to a higher rate of nutrient absorption. Although we did not carry out digestibility study to confirm this hypothesis, a better nutrient supply in EO and PC group would certainly explain our data.

Conclusions

It can be concluded from our results that essential oil supplementation may enhance the non-specific immunocompetence of 28-day-old weaning pigs without compromising their growth performance in the intensive growth phase of rearing period (from 0 to 32 days after weaning).

References

AOAC (1989). Official methods of analysis. Association of Official Analytical Chemist 13th ed. Washington, D.C.

Bech-Andersen S., Mason V.C., Dhanoa M. S. (1990). Hydrolysata preparation for amino acid determination in feed constituents. Modification to oxidation and hydrolysis conditions for streamlined procedures. J Anim Physiol Anim Nutr, 63: 188-197.

Hernandez F, Madrid J., García V., Orengo J., Megías M. D. (2004). Influence of two plant extracts on broilers performance, digestibility, and digestive organ size, Poult Sci 83:169–174.

Hussain R.F., Nouri A.M., Oliver R.T. (1993). A new approach for measurement of cytotoxicity using colorimetric assay. J Immunol Methods. 160 (1):89-96.

Iwata H., Inoue T. (1993). The colorimetric assay for swine lymphocyte blastogenesis. J Vet Med Sci 55 (4):697-698.

Kamel, C. (2001). Tracing modes of action and the roles of plant extracts in non-ruminants. In:. P.C. Garnsworthy, J. Wiseman (eds) Recent Advances in Animal Nutrition. Nottingham University Press, Nottingham, UK, pp. 135–150.

Neil C.R., Nelssen J.L., Tokach M.D. (2006) Effects of oregano oil on growth performance of nursery pigs. J Swine Health Prod. 14 (6): 312–316.

SAS (1996) SAS User's Guide: Statistics Inst., Inc. Cary NC.

Wenk, C. (2003) Growth promoter alternatives after the ban on antibiotics. Pig News and Information, 24 (1): 11N-16N.

Zukkermann F.A. (2000). Aujeszky's disease virus: opportunities and challenges. Veterinary Research 31, 121-131.

acs76_53