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Inoculation of weaned pigs with *E. coli* reduces depots of vitamin E

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ABSTRACT

This study was designed to investigate the effect of vitamin E supplementation on vitamin E depots and immune responses in weaned pigs after an *E. coli* inoculation. The design was a 2×2 factorial with vitamin E supplementation (150 mg/kg RRR-α-tocopheryl acetate versus a control diet containing 60 mg all-rac-α-tocopheryl acetate) and *E. coli* 0 149 inoculation (inoculation of 1×10^8 CFU on day 2 and 3 after weaning versus inoculation of vehicle). The pigs were housed individually during the experiment which lasted for 10 days from weaning at 7 weeks of age. Blood was sampled on day 1 (day of weaning) and 9 of the experiment, and serum was analyzed for α-tocopherol concentration. On day 10 of the experiment, pigs were killed and samples of liver, heart, muscle, adipose tissue and intestinal epithelium were obtained, and immune cells (alveolar macrophages) were harvested, and analyzed for α-tocopherol concentration. Immune cells were furthermore analyzed for PGE_2 synthesis after *in vitro* stimulation. The concentration of IgA and IgM was analyzed in samples obtained from the bile, and in mucosal and intestinal content from three sites of the intestine. The results showed that *E. coli* inoculation reduced the concentration of liver α-tocopherol with 30–37%, increased the concentration of IgA in bile, and reduced the concentration of IgM in intestinal content of pigs. The vitamin E supplementation increased the concentration of α-tocopherol in serum, organs and tissue samples except the adipose tissue. The stereoisomer composition of α-tocopherol in serum, liver and immune cells was highly influenced by the dietary provision of natural vitamin E. In conclusion, dietary natural vitamin E supplementation increased the α-tocopherol depots of the pigs, and notably the RRR-form of α-tocopherol, but had no influence on the measured immune responses. Irrespective of dietary supplementation with vitamin E, short-term inoculation of pigs with *E. coli* led to a decreased liver α-tocopherol status.

1. Introduction

Following weaning, many pigs are suffering from "growth check" characterised by low and variable feed intake, poor and variable growth rate and increased maintenance requirements (Williams, 2003). Furthermore, pigs have an increased susceptibility to enteric pathogens which may cause diseases among which "weaning diarrhoea" is the most common. Weaning diarrhoea usually occurs after a 3- to 4-day latency period, and shows its maximum around 1 week after weaning. Weaning diarrhoea is a multifactorial problem, and the clinical symptoms may be linked to a combination of different factors such as low feed intake during the first week after weaning, low hygiene, insufficient ventilation, low age at weaning, low piglet live weight at weaning, and a high number of pigs per pen (Madec et al, 1998).

Vitamin E deficiency has been found to predispose pigs to *E. coli* infection (Ellis and Vorhies, 1976) that may lead to weaning diarrhoea. This is in line with the finding that vitamin E supplementation has been shown to increase the

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cellular and humoral immunity in pigs (Peplowski et al., 1981; Jensen et al., 1988; Hayak et al., 1989; Barbinszky et al., 1991) and the level of vitamin in immune cells may drop during an immune or inflammatory response (Fritsche et al., 1992). It is, however, difficult to study factors such as vitamin E supplementation on spontaneous weaning diarrhoea because of low or variable incidences of this disease, and thus controlled *E. coli* inoculation models have been used (Madec et al., 2000; Melin et al., 2000) in order to simulate the outbreak of this condition. The experimental models of porcine post weaning colibacillosis have both used a combination of different strains for piglet inoculation (Madec et al., 2000), as well as a single pathogen strain (Melin et al., 2000). At our institute, a model has been established which uses a single strain of *E. coli* O149 in combination with a test for susceptibility of the experimental pigs to this bacterial strain (Sørensen et al., 2009).

The purpose of the present experiment was to study the effect of dietary natural vitamin E supplementation on vitamin E (α-tocopherol) depots, and on some cell-mediated and humoral immune responses in weaned pigs after an *E. coli* inoculation. Recently, we have published the effect on vitamin E supplementation on growth and diarrhoea in the *E. coli* inoculated pigs (Sørensen et al., 2009). In general, the *E. coli* challenge had no effect on growth and feed intake whereas faecal score and number of faecal haemolytic bacteria increased and faecal dry matter decreased. Furthermore, extra vitamin E did not affect weight gain while faecal dry matter decreased, and faecal score and number of haemolytic bacteria increased (Sørensen et al, 2009).

2. Materials and methods

2.1. Experimental design

The experiment was conducted as a two-factorial block design with dietary supplementation of vitamin E and *E. coli* inoculation as the two factors. The experiment used eight littermates from each of four sows. The control diet was supplemented with 60 mg of all-rac-α-tocopheryl acetate, and the vitamin E diet was additionally supplemented with 150 mg/kg RRR-α-tocopheryl acetate. Within a litter, four pigs received the control diet and four pigs received the vitamin E diet during the experimental period. Within diet, two pigs were inoculated with *E. coli O149* and two pigs received the vehicle. The experiment consisted of two blocks, and each block contained two sows with litters. While suckling, litters of the first block received the control diet as creep feed, whereas in the second block the vitamin E diet was used as creep feed.

2.2. Animals

The sows with litters were obtained randomly from the herd at the Institute with a specific-pathogen-free (SPF) health status according to the Danish SPF system (i.e., free from toxigenic *Pasteurella multocida*, *Sarcoptes scabei* var. *suis*, *Haematopinus suis*, *Brachyspira hyodysenteria*, and *Actinobacillus pleuropneumoniae* serotype 1,2,3,4,5,7,8,9,10, but reinfected with *Mycoplasma hyopneumoniae*). The sows were multiparous, and they were homozygotic carriers of the dominant gene encoding for intestinal F4 fimbria receptors (Jørgensen et al., 2003). All pigs from the sows therefore also expressed receptors for *E. coli* F4 adhesion. The experiment was part of a strategic research initiative regarding organic farming, thus the experimental pigs were weaned at 7 weeks of age to simulate the conditions in organic pig production. The piglets are identical with those used in the vitamin E sub-experiment of Sørensen et al. (2009).

2.3. Feed and feeding

The dams were fed *ad libitum* during lactation. The pigs had *ad libitum* access to feed from 2 weeks of age. The composition of the control diet was based mainly on organic farmed ingredients: barley 29.0%, oat 12.0%, wheat 15.0%, peas 15.0%, and toasted soya beans 13.0%, and in addition the control diet contained rape seed cake (double low) 7.5%, potato protein concentrate, 6.0%, vitamins and minerals, 2.40%. The experimental diet consisted of this dietary composition, but was added an extra vitamin E supplement (150 mg/kg provided as RRR-α-tocopheryl acetate). The diet contained 8.31 MJ NE and 167 g digestible protein per kilogram (Sørensen et al., 2009).

2.4. Pig housing and E. coli inoculation

The sows farrowed in loose housing farrowing pens with the dimensions of $2 \times 3.7\ m^2$, of which the dung area was $2 \times 1.4\ m^2$, and both areas had concrete floor with drain. At weaning, pigs were moved to individual concrete floor pens with the dimensions of $0.8 \times 1.3\ m^2$, where they had *ad libitum* access to feed and water. The pigs inoculated with *E. coli* were housed in one room, and the non-inoculated pigs were housed in an adjacent room in order to avoid cross contamination from the bacterial inoculation. The *E. coli* strain 9910045-1 (O149:F4) was originally isolated at the Danish Institute for Food and Veterinary Research from the intestinal content of a pig with post weaning diarrhoea. According to polymerase chain reaction (PCR) analysis of virulence factor genes, the bacterial strain harbours genes for enterotoxins STb, LT, EAST1 and fimbriae F4ac (Frydendahl et al., 2001). The bacteria cause beta-haemolysis when grown on blood agar (BA) (Colombia agar (Oxoid) supplemented with 5% calf blood).

E. coli O149 was stored at −80 °C in a Luria–Bertani (LB) medium (Merck) with glycerol (1:1 v/v). For each inoculation, a fresh inoculation culture was prepared. Frozen *E. coli* O149 was streaked on BA and grown at 37 °C for 18 hours. A sample of 10 μl of the BA colony material was suspended in 200 ml Veal Infusion broth (Merck) and grown for 5 hours at 37 °C in an incubator, constantly shaken at 200 rpm. After incubation the suspension was centrifuged at 12,170 rpm (17,696 G) at 4 °C for 20 minutes. The bacterial pellet was resuspended in sterile 0.9% sodium chloride (NaCl). This bacterial suspension was diluted in serial ten-fold dilutions with 0.9% NaCl as the diluent and plated on BA for quantitative determination of the *E. coli* CFU. Each piglet received a dose of 1×10^8 CFU in 20 ml 0.9% NaCl on days 2 and 3 after weaning (day 1 is the day of weaning) via an oro-gastric tube. The oro-gastric tube was inserted and flushed with 10% sodium bicarbonate ($NaHCO_3$) to ascertain that the tube was correctly placed. After inoculation, the tube was again flushed with 10% $NaHCO_3$ to ascertain that the total suspension of *E. coli* was given to the piglet. The control pigs received equivalent amounts (approx. 50 ml) of 10% $NaHCO_3$ via an oro-gastric tube.