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Original article

Effect of vitamin E supplementation on immune status and α-tocopherol in plasma of piglets *

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Summary — Twelve (Yorkshire) gilts were assigned to 2 dietary fat supplement groups starting at 57 d of gestation. *Group 1* received no fat and *Group 2* was supplemented with 5% Canola oil. Each group was supplemented with 0.1 ppm Se and 22 IU of DL-α-tocopherol acetate/kg of feed. Colostrum (d 0) and milk (7, 14, 21 and 28 d post partum) were sampled from gilts. At farrowing 3 piglets from each gilt of both groups were injected with α-tocopherol at birth (500 IU) and at 7 and 14 d (1 000 IU) of age and 3 piglets were injected with saline and used as control. Blood samples were taken from the newborn piglets at birth and at 7, 14, 21, 28 and 35 d of age. α-Tocopherol concentration in the colostrum of gilts was significantly higher than in the milk. Plasma α-tocopherol concentrations and antibody titres to Keyhole limpet haemocyanin of piglets injected with vitamin E were significantly higher than the control piglets. Vitamin E injected piglets had significantly higher α-tocopherol concentrations in spleen, liver, kidney, heart, lung and hip muscle than the control piglets.

vitamin E / immune status / dietary fat / piglet

Résumé — Effet de la vitamine E sur l'état immunitaire et sur la concentration en vitamine E plasmatique chez le porcelet. *À 57 j de gestation, 12 truies (Yorshire) ont été partagées en 2 groupes.* Groupe I : *aucun supplément en matières grasses supplémentaires apporté à leur ration.* Groupe II : *supplément de 5% d'huile de colza. En plus, chaque truie a reçu 0,1 ppm de sélénium et 22 UI de DL-α-tocophérol acétate par kg de régime. Le colostrum (jour 0) et le lait (7, 14, 21 et 28 j) post-partum ont été échantillonnés. Lors de la mise bas, 3 porcelets de chaque truie ont reçu 500 UI d'α-tocophérol, puis 2 injections de 1 000 UI à 7 et 14 j. Le même nombre de porcelets a reçu une solution saline à leur naissance, puis à 7 et 14 j. Des échantillons de sang ont été prélevés chez les porcelets à leur naissance, puis respectivement à 7, 14, 21, 28 et 35 j. Le colostrum était plus riche en vitamine E que les laits. L'α-tocophérol plasmatique ainsi que les taux d'anticorps à l'hæmocyanine Keyhole étaient plus élevés chez*

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les porcelets injectés avec de la vitamine E que chez les témoins. Les porcelets traités à la vitamine E avaient également des concentrations plus fortes en vitamine E dans la rate, le foie, les reins, le cœur, les poumons et les muscles de la cuisse que les témoins.

vitamine E / état immunitaire / matière grasse alimentaire / porcelet

INTRODUCTION

One factor contributing to loss of neonatal pigs is their low body energy reserves. Addition of 10–15% fat to sow diets during the late gestation can increase the body fat of the newborn pig (Pond, 1991). Malm *et al* (1976) reported that gestating sows fed corn oil had lower plasma vitamin E than those fed lard, and related this to differences in dietary polyunsaturated fatty acids. They also stated that piglets had significantly higher serum α-tocopherol concentrations than their dam, illustrating efficient placental transfer. The major function of vitamin E in the body is considered to be as an antioxidant (Machlin, 1980). This is important in maintaining cell membrane integrity and immune status of the animal. Low vitamin E in piglets induces a variety of lesions such as skeletal muscle degeneration, mulberry heart disease (Van Vleet, 1980) and depression of immune function (Peplowski *et al*, 1981).

Ullrey (1981) also reported that vitamin E may also play a role in the immune response to infection. Supplementation of 30 IU of vitamin E/kg of feed should be adequate under most circumstances. When the diets contained considerable amounts of oxidized fat or the pigs were stressed by infection, even higher levels of vitamin E may be necessary. The administration of high levels of vitamin E has been suggested as one method that can be used to improve a depressed immune response of a stressed pig. Duncan *et al* (1960) reported that low-fat diet would adversely affect the absorption of α-tocopherol and that increasing the fat content would improve absorption. Supplementation of vitamin E to dams improve vitamin E status of their newborn. Intramuscular injection of vitamin E directly to the neonatal and young piglets could perhaps be more efficient.

The objectives of this study were to determine the effects of dietary fat on milk α-tocopherol concentration of gilts and the effect of injections of vitamin E in the piglets born from these gilts on their plasma α-tocopherol levels, serum immunoglobulin (IgG) levels, and antibody titres to Keyhole Limpet Haemocyanin (KLH).

MATERIALS AND METHODS

Experimental design and diets

Gilts from the minimum disease herd of the Centre for Food and Animal Research were fed a pelleted standard growing diet (Hidiroglou *et al,* 1993) containing 22 IU/kg α-tocopherol until they reached 95 kg body weight. At their next oestrus they were bred to randomly selected boars. Gilts were fed the diet (Hidiroglou *et al,* 1993) at the rate of 2 kg/d. Thirty days after mating, gilts were tested with a Prognosticator probe (Animark Co, Denver, CO, USA) to confirm pregnancy. On d 57 of gestation 12 gilts were assigned to 2 diet groups as follows: *Group 1*: 6 gilts fed no-fat gestation diet; *Group 2*: 6 gilts fed gestation diet with 5% Canola oil. Gilts were fed a gestation diet (table I) twice daily in individual feeding crates during pregnancy. At farrowing 3 of the newborn piglets from each gilt of both groups were administered 500 IU of D-α-tocopherol (Stuart Products, Bedford, TX, USA) by intramuscular injection and 3 piglets used as controls were injected with a saline solution in the crural muscle. The treated piglets were injected again at 7 and 14 d with 1 000 IU of vitamin E and 10 mg of KLH. Water

Table I. Percentage (DM basis) composition of gestation diets.

Ingredients	*No-fat gestation diet*	*Canola oil (5%) gestation diet*
Corn	50	50
Soybean meal	23	23
Corn starch	19	14
Wheat bran	2	2
Canola oil	–	5
Limestone	1.5	1.5
Dicalcium phosphate	1.5	1.5
Salt (iodized)	0.5	0.5
Lignosol [a]	1.5	1.5
Vitamin [b]	0.5	0.5
Mineral premix [c]	0.5	0.5

[a] Lignosol FG (Reed Ltd, Chem Div, Lignin products, Quebec); a binding agent. [b] Premix provides per kg of diet: 8 250 IU vitamin A; 550 IU vitamin D; 22 IU α-tocopherol; 4.4 mg vitamin K; 3 mg thiamine; 8 mg riboflavin; 44 mg niacin; 33 mg calcium pantothenate; 0.028 mg vitamin B_{12}; 500 mg choline chloride; 0.25 mg D-biotin; 1 mg folic acid; and 3 mg vitamin B_6. [c] Premix provides per kg of diet: 8 mg Cu; 80 mg Fe; 20 mg Mn; 75 mg Zn; and 0.2 mg Se.

was available *ad libitum* for sows and piglets during the whole experiment. After farrowing, the gilts were switched to a pelleted lactation diet (table II) containing the same levels of vitamin E and fat as during gestation. During lactation gilts received feed *ad libitum*.

Blood and milk sampling

Samples of colostrum (d 0) and milk on d 7, 14, 21 and 28 were collected by hand after intramuscular injection of 30–50 IU of oxytocin. Approximately 30 ml of colostrum or milk was obtained at each sampling. Blood samples were taken from newborn piglets 3 h after suckling and then from piglets at 7, 14, 21, 28 and 35 d of age. Blood samples of 1 ml were taken from vena cava. Plasma was removed from the blood after centrifugation at 1 000 *g* and stored at −70°C until analysis.

Table II. Percentage (DM basis) composition of lactation diets.

Ingredients	*No-fat lactation diet*	*Canola oil (5%) lactation diet*
Corn	46.9	46.9
Soybean meal	17	17
Barley	25	25
Canola oil	–	5
Corn starch	5	–
Limestone	1.3	1.3
Dicalcium phosphate	1.8	1.8
Salt (iodized)	0.5	0.5
Lignosol [a]	1.5	1.5
Vitamin premix [b]	0.5	0.5
Mineral premix [c]	0.5	0.5

[a,b,c] See table I.

Blood and milk analyses

α-Tocopherol concentrations in plasma, colostrum and milk were analyzed by HPLC, according to McMurray and Blanchflower (1979). Serum immunoglobulin (IgG) was determined at 7, 14, 28 and 35 d by single radial immunodiffusion procedure using a specific SRID kit (VMRD, Inc, Pullman, WA, USA). Tests were performed using the standards provided by the manufacturer and according to their guidelines. An enzyme immunoassay (ELISA) was used to measure specific antibody response to KLH. It was performed according to standard techniques (Coligan *et al*, 1992). One hundred microlitres of KLH at 10 μg/ml was adsorbed to polystyrene microlitre plates (Costar, BioRad Laboratories, Mississauga, ON, Canada) in 0.05 M bicarbonate buffer, pH 9.6. After addition and serial dilution of serum, specific antibody was detected by addition of peroxidase-labelled anti-swine immunoglobulin (Dako Laboratory, Denmark) and substrate (2,2'-azinobis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) and H_2O_2). Plates were read on an automated microlitre reader and OD490 0.1 was chosen as the endpoint for titration. The antibody titres for the KLH were determined at 7, 21, 28 and 35 d of age and are given as the reciprocal of the last dilution with a positive reading.

Tissue samples

Twenty-four pigs were killed by exsanguination at 28 d of age. One pig from each of the groups of 12 gilts was killed, one from the vitamin E-injected group and one from control (no vitamin E injection) group. Portions of spleen, liver, kidney, heart, lung and hip muscle (gluteus) were removed. All tissues were stored at –70°C until they were analyzed for α-tocopherol. Tissue samples were prepared for α-tocopherol determination according to the method of Ingold *et al* (1987). Quantization of α-tocopherol in tissues was performed by HPLC using a fluorescent detector (McMurray and Blanchflower, 1979).

Statistical analysis

The data on α-tocopherol concentrations in milk of gilts were analyzed by repeated measure analysis of variance using the SAS general linear model procedure (SAS, 1989). The following linear model was used:

$$Y_{ijk} = \mu + D_i + G_j(D_i) + T_k + (DT)_{ik} + e_{ijk}$$

Where Y is the concentration of α-tocopherol in the milk at a specific time; μ is the overall mean; D is the effect of dietary fat supplementation; $G(D)$ is the effect of gilt within dietary fat supplementation; T is the effect of time of sample collection; (DT) is the interaction of dietary fat with time; and e is the error term. Colostrum data (d 0) were not combined with the milk data (7, 14, 21, 28 d) as mean levels and variances were quite different.

Piglet mean values for α-tocopherol in the plasma, serum IgG levels and antibody titres to KLH for each gilt and time were analyzed rather than individual piglet values, as the among-litter error was significantly larger than the among-piglet error. Logarithms of serum IgG levels and antibody titres to KLH were taken to stabilize the variances which increased with increasing means. The following model was used for the analysis of plasma α-tocopherol concentration, serum IgG levels and antibody titres of KLH of piglets:

$$Y_{ijkl} = \mu + D_i + V_j + (DV)_{ij} + T_k + (DT)_{ik} + (VT)_{jk} + (DVT)_{ijk} + e_{ijkl}$$

Where Y is the concentration of α-tocopherol or serum IgG levels or antibody titres to KLH at a specific time; μ is the overall mean; D is the effect of dietary fat; V is the effect of vitamin E injection; (DV) is the interaction of dietary fat with vitamin E injection; T is the effect of time of sampling; (DT) is the interaction of dietary fat with time; (VT) is the interaction of vitamin E injection with time; (DVT) is the interaction among dietary fat, vitamin E injection and time; and e is the error term. ANOVA were performed on the logarithms of the tissue vitamin E concentrations (SAS, 1989). The model included the effects of dietary fat, vitamin E injection and interaction of the dietary fat with vitamin E injection. Logarithms of the tissue α-tocopherol concentrations were taken to stabilize the variances which increased with increasing concentrations (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Experimental diets and animal weights

The mean and standard error ($X \pm$ SE) of litter size was 10.2 ± 0.35 (9.7 ± 0.38 alive) with an average piglet live birth weight of 1.27 ± 0.04 kg. These parameters did not show significant effect due to dietary fat supplementation. This may be due to the small number of litters examined.

Colostrum and milk α-tocopherol concentration in gilts

The effect of dietary fat on colostrum and milk α-tocopherol concentration was not significant ($P > 0.05$). The effect of day of sampling on milk α-tocopherol concentration was significant ($P < 0.05$). Milk α-tocopherol concentrations decreased with advance in lactation in both no-fat and Canola oil supplemented groups (table III). This was similar to the trend observed by Malm *et al* (1976) in lactating sows. α-Tocopherol concentration in the colostrum was significantly ($P < 0.05$) higher than in the milk in both groups. Similar results were also reported by

Nielsen *et al* (1973). There was a slight decline in the α-tocopherol concentration in the milk of gilts from 7 to 28 d of lactation in both groups (table III).

Plasma α-tocopherol concentrations in piglets

At birth plasma α-tocopherol concentrations in the piglets of the control groups were very low and could not be measured (table IV). This observation is similar to that of Dvorak (1974) who found very low vitamin E concentrations in the plasma of piglets. This is mainly due to inefficient placental transfer of tocopherol (Young *et al*, 1977). Schlotke *et al* (1978) reported that α-tocopherol status of the newborn piglet before ingesting colostrum is not dependent on the vitamin E supply of the sows. The low plasma α-tocopherol concentration observed in newborn piglets born from gilts receiving 22 IU vitamin E in the diet either with no fat or 5% Canola oil did not manifest any clinical vitamin E deficiency symptoms. Chung *et al* (1992) reported that dietary D-α-tocopherol may be more effectively absorbed and retained by weanling swine than DL-α-tocopheryl acetate.

Table III. Least square means and standard errors of α-tocopherol (μg/ml) in the colostrum and milk of gilts.

Day	*Mean*		*SE*
	No-fat diet	*Canola oil (5%) diet*	
0	10.31	11.24	1.21
7	2.29	2.31	0.40
14	1.90	2.22	0.45
21	1.61	1.95	0.30
28	1.55	1.92	0.27

The intake of colostrum and its content of vitamin E played a decisive role on the plasma α-tocopherol concentrations of piglets. Dietary fat supplementation to the gilts had no significant ($P > 0.05$) effect on the plasma α-tocopherol concentration of the piglets. The effect of vitamin E injection to the piglets was significant ($P < 0.05$) on

Table IV. Least square means and standard errors of α-tocopherol (μg/ml) in the blood plasma of piglets.

Piglet age (d)	*Sow diet*					
	No-fat diet			*Canola oil (5%) diet*		
	Control (mean)	*Vitamin E-injected (mean)*	*SE*	*Control (mean)*	*Vitamin E-injected (mean)*	*SE*
0	ND	6.24	0.58	ND	6.21	0.76
7	2.74[a]	9.53[b]	0.58	3.14[a]	13.30[b]	0.76
14	2.69[a]	8.46[b]	0.58	2.81[a]	9.78[b]	0.76
21	2.52[a]	7.22[b]	0.58	2.72[a]	7.21[b]	0.76
28	2.47[a]	4.84[b]	0.58	2.57[a]	5.07[b]	0.76
35	1.43	2.51	0.58	1.66	2.57	0.76

ND = not detected. [a,b] Means with different letters within the same row are different ($P < 0.05$).

the plasma α-tocopherol concentration. Plasma α-tocopherol concentrations of piglets after ingestion of colostrum and injection of vitamin E (500 IU) was significantly ($P < 0.05$) higher than the control pigs before colostrum intake. Plasma α-tocopherol concentrations of piglets injected with vitamin E at birth (500 IU) and again at 7 and 14 d of age (1 000 IU) were significantly ($P < 0.05$) higher than the control piglets from 7 to 28 d of age (table IV). Plasma α-tocopherol concentrations on d 7 were highest in both the control and vitamin E-injected group, which decreased from 14 to 35 d of age (table IV).

Immunoglobulin (IgG) levels and antibody titres to KLH in the piglets

Effects of dietary fat and vitamin E injection were not significant for IgG concentration in the piglets, while the effect of time of sampling was significant ($P < 0.05$). Serum IgG levels averaged across days were similar for the vitamin E-injected and control group. Serum IgG concentration was highest on day 7 and then slowly declined with age (table V). Similar results were reported by Hidiroglou *et al* (1992). Effect of vitamin E supplementation was significant ($P < 0.05$) for titre to KLH. Titres to KLH were higher at 28 and 35 d than at 21 d of age (table V). This suggests that the peak for antibody titres to KLH was reached about 14 d after the second vaccination. Similar results were also reported by Hidiroglou *et al* (1992) in calves. The antibody titres to KLH in the vitamin E-injected piglets were significantly ($P < 0.05$) higher than the control piglets at 21 d (17 *vs* 2), 28 d (121 *vs* 261) and at 35 d (760 *vs* 175).

Tissue α-tocopherol concentrations

The effect of dietary fat was not significant ($P > 0.05$) while the effect of treatment was significant ($P < 0.05$) for the concentrations

Table V. Least square means and standard errors of serum immunoglobulin (IgG) level and titre to KLH in the piglets.

Age (d)	*Control*		*Vitamin E-injected*	
	Mean	*SE*	*Mean*	*SE*
Immunoglobulin (IgG) level (mg/100 ml)				
7	159	10	162	10
14	80	9	77	9
28	42	4	49	4
35	50	3	51	3
Titre to KLH[1]				
7	2	1	0	1
21	2[a]	5	17[b]	5
28	261[a]	186	1 216[b]	174
35	175[a]	142	760[b]	133

KLH = Keyhole Limpet Haemocyanin, units are the reciprocal of the last dilution which gave a positive haemagglutination. [a,b] Means with different letters within the same row are different ($P < 0.05$).

Table VI. Least square means and standard errors of α-tocopherol concentrations (μg/g) in tissues of control and vitamin E-injected piglets.

Tissue	*Control*		*Vitamin E-injected*	
	Mean	*SE*	*Mean*	*SE*
Spleen	2.83[a]	0.21	9.37[b]	0.57
Liver	3.79[a]	1.08	13.97[b]	1.13
Kidney	2.52[a]	0.92	13.48[b]	0.96
Heart	4.03[a]	0.92	16.19[b]	0.96
Lung	3.57[a]	0.74	13.32[b]	0.77
Hip muscle [c]	2.83[a]	3.89	40.44[b]	4.08

[a,b] Means with different letters within the same row are different ($P < 0.05$). [c] Hip muscle refers to the gluteus muscle.

of α-tocopherol in all the tissues. Vitamin E-injected piglets had significantly ($P < 0.05$) higher α-tocopherol concentrations in all the tissues than the piglets not injected with vitamin E (table VI). Various tissues responded differently to vitamin E injections. The concentrations of α-tocopherol in different tissues were similar to the ones reported by Batra and Hidiroglou (1994). The highest concentration of α-tocopherol in the vitamin E-injected piglets was in the hip muscle followed by heart, liver, kidney, and lung (table VI).

CONCLUSIONS

Tocopherol concentration in the colostrum was significantly higher than in the milk. Dietary fat supplementation to the gilts had no significant effect on the α-tocopherol concentration in the milk of gilts and plasma α-tocopherol concentration in the piglets. Vitamin E injection to the piglets significantly increased the α-tocopherol concentration in the plasma and tissues as well as antibody titres to KLH in the piglets.

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