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Effect of supplementing sows' feed with α-tocopherol acetate and vitamin C on transfer of α-tocopherol to piglet tissues, colostrum, and milk: Aspects of immune status of piglets

A. Pinelli-Saavedra [a,b,*], A.M. Calderón de la Barca [a], J. Hernández [a], R. Valenzuela [a], J.R. Scaife [b]

[a] Centro de Investigación en Alimentación y Desarrollo, A.C., Carretera a la Victoria, Km 0.6, P.O. Box 1735, Hermosillo, Sonora 83000, Mexico
[b] Department of Agriculture and Forestry, University of Aberdeen, 581 King Street, Aberdeen AB24 5UA, Scotland, UK

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Abstract

The aim of this study was to investigate the effects of dietary supplementation of sows with α-tocopherol acetate (ATA) and vitamin C on deposition of α-tocopherol (AT) in piglet lymphoid organs, such as bone marrow, thymus, and spleen at birth and at weaning, as well as on indicators of immune response in piglets. Sows were given the following treatment diets: control, vitamin C 10 g/day, ATA 500 mg/kg feed, and combined vitamins (ATA 500 + Vit-C 10). Supplementation with vitamins started at the beginning of pregnancy and lasted until weaning at 21 ± 3 days of age. AT was determined in colostrum, milk, piglet plasma (cord blood) and tissues at birth and on day 21. Immunoglobulins were measured in piglet plasma, milk, and colostrum. Lymphocyte proliferation in response to PHA and ConA was determined in sow and piglet blood. ATA supplementation resulted in a significant increase ($P < 0.001$) in the AT content of colostrum, milk, piglet plasma, liver, thymus, bone marrow, and spleen at weaning. The AT content of colostrum and milk significantly ($P < 0.001$) influenced the AT content of piglet plasma and tissues at weaning (day 21). Total Ig and IgG concentrations in piglet plasma were significantly increased in piglets given the combined vitamin treatment. No effect of AT supplementation was observed on IgG and IgA in colostrum and milk. In sows, vitamin C given alone significantly increased lymphocyte response to ConA and PHA; whereas, in piglets, there was no significant effect of treatments on lymphocyte response to PHA and ConA.
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Keywords: Vitamin E; Vitamin C; Piglet tissues; Sow; Immune response

1. Introduction

Vitamin E is the most effective chain-breaking lipid soluble antioxidant present in cell membranes, and plays a major role in maintaining cell membrane integrity by limiting lipid peroxidation initiated by reactive oxygen species and free radicals in all cells, including those of the immune system. Vitamin C is important in limiting free radical damage in the aqueous phase of the cell, and plays a part in the regeneration of vitamin E by reducing vitamin E radicals formed when vitamin E scavenges the oxygen radicals (Combs, 1998). Due to limited placental transfer piglets are born with low vitamin E content even when the dietary intake of vitamin E by the gestating mother is high and her plasma vitamin E is elevated (Mahan and Vallet, 1997; Lauridsen et al., 2002; Pinelli-Saavedra and Scaife, 2005). Hidiroglou et al. (1993a) reported low levels of vitamin E in liver, brain, heart, kidney in piglets at birth.

In addition to its antioxidant function in biological processes, there is evidence that vitamin E enhances cellular

* Corresponding author. Address: Centro de Investigación en Alimentación y Desarrollo, A.C., Carretera a la Victoria, Km 0.6, P.O. Box 1735, Hermosillo, Sonora 83000, Mexico. Tel.: +52 662 2892400x293; fax +52 662 2800055.
E-mail address: pinelli@ciad.mx (A. Pinelli-Saavedra).

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and humoral immune responses in different species, including pigs (Pharazyn et al., 1990; Meydani and Tengerdy, 1993, Hidiroglou et al., 1995a; Brennan et al., 2000). Larsen and Tollersrud (1981) reported an increased phytohaemagglutinin (PHA) proliferation in lymphocytes from pigs (20–90 kg) supplemented with 40 mg/day of vitamin E and 0.5 and 0.1 ppm of selenium. Supplementing weaned pigs with 220 IU of vitamin E/kg of body weight significantly increased antibody titre in response to a challenge with sheep red blood cells (Peplowski et al., 1981). Supplementation of sows from day 56 of gestation until lactation with 110 or 220 IU/kg diet of vitamin E increased the proliferation of PBMC stimulated with PHA or ConA in 28-day-old piglets but not in their mothers (Nemec et al., 1994).

Vitamin C is present in high concentrations in leukocytes and there is evidence that it is involved in a number of neutrophil functions including chemotactic responses and phagocytosis (Siegel, 1993). Schwager and Schulze (1997, 1998) reported that PBMC from pigs with hereditary vitamin C deficiency and containing low concentrations of ascorbic acid were less responsive to mitogens and suggested that the sensitivity of IL-2 production and IL-2 receptor expression to cellular vitamin C status may be linked to the mechanisms involved in quenching cellular reactive oxygen species. In guinea pigs, vitamin C increases the response to T-cell mitogens only in conjunction with dietary vitamin E (Bendich et al., 1984). Lauridsen and Jensen (2005) reported that addition of 500 mg vitamin C/kg feed to the diet of weaned pigs born to sows previous supplemented with 150 or 250 IU α-tocopherol acetate (ATA)/kg feed increased the vitamin E (AT) accumulation in liver and muscle tissue during the first week after weaning and increased serum IgM concentration of weaned pigs. The aim of this study was to determine the effects of ATA and vitamin C supplementation in sow diets on AT deposition in piglet tissues and on indicators humoral and cellular immune response of sows and their piglets.

2. Materials and methods

2.1. Experimental design

The experiment was carried out in a pig farm in Sonora, Mexico, under commercial pig production conditions. The experiment started when the sows were artificially inseminated and lasted until weaning at 21 ± 3 days of lactation. At insemination 44 sows were allocated randomly to four dietary treatments. After one month, 13 sows that were not pregnant were removed and the study continued with those which were confirmed as pregnant. The final number of sows participating in this study in each of the treatment groups was as follows: (a) control ($n = 10$), fed with commercial diet containing 36 mg of ATA without vitamin C; (b) Vit-C ($n = 6$), fed with supplement of 10 g/day of vitamin C; (c) ATA ($n = 5$), fed with a supplement of 500 mg ATA/kg feed; (d) + C ($n = 10$), fed with a supplement of 500 mg ATA/kg of diet and 10 g vitamin C/day.

2.2. Animals

The sows used in this study were multiparous crossbred sows, mainly 90% Large White × Landrace and 10% Duroc with a mean parity of 4.06 ± 1.48, and were approximately 200 kg live weight and 2–2.5 years of age at the time of insemination.

2.3. Diet formulation

The diets were formulated according to the Nutrient Requirements of Swine (National Research Council, 1998). All the experimental diets were formulated to be adequate in vitamins, trace minerals, and major elements. During pregnancy sows were fed once daily with a gestation diet (Table 1) calculated to provide 26.6 MJ metabolizable energy (ME) per day (2.2 kg/feed per day per sow). From day 93 of gestation sows were given, once daily a prepartum diet (Table 1) calculated to provide 29 MJ ME (2.4 kg/feed per day per sow). After farrowing, sows were fed twice daily 2.5 kg of a lactation diet (Table 1) providing a daily ME intake of 66.5 MJ. Water was provided ad libitum during pregnancy and lactation. The ATA (ROVIMIX® E-50 Adsorbate, ROCHE; 50% activity) and vitamin C (vitamin C Type EC ROCHE, ascorbic acid, crystalline form, 97% activity) were added daily to the diets at the time at which animals were fed.

Table 1
Formulation and composition of gestation, prepartum, and lactation diets

	Gestation	Prepartum	Lactation
Ingredient			
Hard wheat 12%	694	655	532
Soybean meal 46%	66	75	238
Supplement Gesta-MIX*	60	60	60
Wheat bran	160	160	120
Brewers yeast	10	10	10
Fat (soybean oil)	10	40	40
Calculated composition ($g\ kg^{-1}$)			
Dry matter	713.2	716.2	683.3
Crude protein	140.6	140.1	194.4
Ether extract	29.8	58.5	55.4
Crude fibre	47.0	46.4	41.0
ME (MJ/kg)	12.1	13.2	13.3
Calcium	11.6	11.6	11.0
Total phosphorus	7.7	7.7	7.4
Available phosphorus	5.4	5.3	4.8
Methionine	2.2	2.2	2.9
Methionin + cysteine	5.0	4.9	6.4
Lysine	6.0	6.1	9.9
Threonine	4.6	4.6	7.0
Leucine	9.9	9.9	14.7
Isoleucine	6.3	6.3	9.6
Tryptophan	1.9	1.9	2.7

Composition of premix per kilogram diet: vitamin A 120,000 IU; vitamin D_3 18,000 IU; vitamin E 36 IU; vitamin B_{12} 0.24 mg; biotin 2.4 mg; riboflavin 72 mg; vitamin K_3 36 mg; niacin 360 mg; pantothenic acid 0.18 mg; thiamine 24 mg; pyridoxine 36 mg; choline chloride 3.6 g; zinc oxide 1.44 g; iron sulphate 1.44 g; magnesium oxide 360 mg; copper sulphate 144 mg; iodine 8.64 mg; selenium (selenite), 3.6 mg; BHT 360 mg.

2.4. Collection of samples

Sow blood samples were taken on day 0, 60, 103 of gestation and at 21 days post-farrowing (F+21) to quantify the AT content of plasma. To evaluate PBMC proliferation, blood samples were taken at 103 days and at F+21. Approximately 50 ml of colostrum and milk was collected from each sow from all functional nipples on days 0 and 21 ± 3, respectively. Blood samples were collected for immunoglobulin and AT analysis from all piglets in each litter immediately after birth (cord blood) before they were allowed to suckle and from the jugular vein of four piglets from each litter at 21 days of age. Samples were immediately placed on ice, transported to the laboratory, and kept at −70 °C until AT and immunoglobulin analyses was carried out. One piglet from each litter was killed with a barbiturate injection on days 0 and 21 and blood collected by cardiac puncture was used for lymphocyte proliferation assays. Samples of liver, spleen, thymus, and bone marrow were taken for determination of AT. The samples were immediately stored in a freezer at −20 °C. Fresh blood was used to measure lymphocyte proliferation.

2.5. Quantification of α-tocopherol from plasma, colostrum, milk, and tissues

AT was extracted from plasma as described by Hess et al. (1991). To 200 μl of plasma, 200 μl of distilled water and 400 μl ethanol were added and vortexed for 10 s. Then, 700 μl of hexane was added and shaken for 10 min, centrifuged at 14,000 rpm for 5 min, and 600 μl of the hexane layer was removed and dried at room temperature. The dried samples were dissolved in 200 μl of 1,4-dioxane: hexane 1:1 (v/v) and vortexed for 10 min. Approximately 120 μl placed in glass inserts suspended in 1.5 ml HPLC autosampler vials before injecting onto the high performance liquid chromatography (HPLC) column. From colostrum and milk, AT was extracted as described by Hidiroglou (1989), except that hexane was used instead of heptane. One millilitre of distilled water, 1 ml of colostrum or milk, 4 ml ethanol, and 2 ml of hexane were mixed, vortexed for 5 min, and centrifuged for 15 min at 3000 rpm at 4 °C. After centrifugation, 1 ml of the supernatant (hexane layer) was transferred into HPLC vials. AT from tissues was extracted by homogenisation of 2 g of tissue with 8 ml of 1.15% KCl. Then 1 ml of homogenate was saponified using 0.5 ml of 25% ascorbic acid solution, 1 ml of ethanol, and 1 ml of 10 M of KOH; and the mixture preheated in a water bath at 70 °C for 30 min. At the end of the saponification step, the tubes were cooled. Thereafter, 5 ml of *n*-hexane was added to the samples that were vortexed for 15 s, and then centrifuged at 1500 rpm at 10 °C for 7 min. Six hundred microliters of the upper hexane layer were transferred to an Eppendorf tube and the extract was washed by adding 200 μl of ethanol and 400 μl distilled water. The samples were then vortexed vigorously for 10 min using a multivortexer, and centrifuged for 5 min at 6000*g*. The upper hexane layer was withdrawn and placed into amber screw-cap HPLC vials (Onibi et al., 1998).

2.5.1. HPLC conditions

AT was measured by high-performance liquid chromatography (HPLC) with a Varian HPLC system (Palo Alto, CA) and a Shimadzu RF-535 fluorescence detector (Tokyo, Japan) set at an excitation wavelength of 296 nm and an emission wavelength of 326 nm. Separation was achieved using a Partisil Si (250 mm × 4.6 mm) column (Alltech, Carnforth, UK). The mobile phase was *n*-hexane and 1,4-dioxane, programmed to change linearly from 95:5 (vol/vol) to 75:25 (vol/vol) over a 7.5 min run time. Analyses were carried out in duplicate.

2.6. Piglet immunoglobulins

Total immunoglobulins and immunoglobulin G in piglet plasma at 21 days of age were determined by direct ELISA (González-Córdova et al., 1998). Briefly, antigen was diluted in coating buffer (35 mM $NaHCO_3$, 15 mM Na_2CO_3, 0.05% phenol red), pH 9.6, and incubated for 2 h in a 96-well plate. To remove non-coated antigen, the plate was washed three times with washing buffer (0.1 M Tris–HCl, 0.05% Tween 20, 0.05% Phenol Red, pH7.4), and then 1% milk powder was added and the plate was incubated for 20 min. The plate was then washed three times. The primary antibody (goat or rabbit anti-swine Igs) was incubated for 1 h at 25 °C, washed, incubated with the secondary antibody (peroxidase conjugated), for 30 min at 25 °C. After incubation with the substrate, the reaction stopped and the absorbance measured at 450 nm. A standard curve was prepared for each ELISA batch. The final values of IgG and total Ig were expressed in milligrams per millilitre (mg/ml). The plasma concentrations from two piglets per litter were analysed to obtain representative values of each litter. The inter-assays coefficient of variation was 4%.

2.7. IgG in colostrum and IgA in milk

Sample preparation was as follows: colostrum or milk (0.5 ml) was placed into an Eppendorf tube and centrifuged at 6000*g*, for 10 min at 4 °C to remove the fat fraction. After centrifugation, 50 μl of the middle fraction, colostral whey or milk whey was removed and was diluted with 450 μl of carbonate buffer, pH 9.6. For IgG analysis, colostrum samples were diluted serially to a maximum dilution of 1:10,000 in carbonate buffer, pH 9.6. The IgG content of colostrum was measured by direct ELISA using the same procedure for the determination of IgG in plasma but with two modifications. Firstly, 1% gelatine was used as washing buffer instead of 1% milk powder and, secondly, the first incubation was at 4 °C overnight. To measure IgA, milk samples were diluted serially to a maximum dilution of 1:25,000 in carbonate buffer. A standard curve using

IgA was prepared over a concentration range of 0.0025 to 0.16 μg/ml of purified porcine IgA. The IgA content of milk was measured by direct ELISA using the same procedure used for the determination of IgG and total Ig in piglet plasma, except that the first incubation was overnight at 4 °C, the washing buffer used was 1% gelatine instead of 1% milk powder, and the time of reaction after addition of substrate (TMB) was 4 min. The antibodies used were as follows: primary antibody, rabbit anti-swine IgA (prepared in our laboratory by repeated inoculations of rabbits with IgA), and second antibody, peroxidase conjugated swine anti-rabbit IgA.

2.8. PBMC proliferation assays

Blood samples were mixed with an equal volume Ficoll-Hypaque (Pharmacia, Co., USA) and centrifuged for 30 min at 500*g* at 15 °C. The PBMC were collected from the interface, washed twice with RPMI-1640 for 10 min at 400*g*. Then, PBMC were diluted in 4 ml of RPMI-1640 and their viability was determined by exclusion of trypan blue. Cells were adjusted to a concentrations of 2×10^6 viable cells (⩾95%) per millilitre of RPMI-1640 with 10% of foetal bovine serum (FBS). Proliferation assays were carried out in triplicate in 96-well sterile flat bottom microculture plates. Cells (2×10^5) were stimulated with PHA (20 μg/ml) or ConA (15 μg/ml) in RPMI-1640 supplemented with 10% of FBS. The cells were cultured at 37 °C in a humidified incubator under an atmosphere of 95% O_2/5% CO_2 for 72 h. During the last 18 h of culture, 37 kBq (1 μCi) of ^{3}H-methyl thymidine (74 GBq/mmol) was added to each well. Cells were harvested onto glass fiber filters using a multiple cell harvester (Titratek). The radioactivity incorporated into the DNA was counted using a Beckman LS 5000 counter (Beckman Fullerton, CA). The results were expressed as counts per minute (c.p.m.) according to Hernández et al. (1998).

2.9. Statistical analysis

Data were subjected to one way analysis of variance (ANOVA) using the Minitab Statistical Package (v. 12.0, Minitab, Inc, PA, USA) to determine the overall effect of dietary treatments. Significant differences among treatments were determined by Tukey's test ($P < 0.05$). Analysis of covariance (ANCOVA) was used to assess the effect of day 0 on the subsequent days of sampling on the analysis of AT in sow's plasma. For analysis of data on immunoglobulin concentrations and, lymphocyte proliferation in samples obtained from sows and piglets, 'repeated measures' ANOVA was employed. Four piglets per litter were analysed; sows and piglets were considered to be the experimental units.

3. Results

3.1. AT concentration in plasma, colostrum, and milk

Supplementation with 500 mg of ATA/kg of feed alone or in combination with 10 g of vitamin C/day (ATA/Vit-C) increased plasma concentrations of AT (Table 2) ($P < 0.001$), There were significant differences ($P < 0.05$) on day 0 in the concentration of AT in sows before starting the supplementation with vitamins. By day 60 both groups receiving AT supplementation (ATA and ATA/Vit-C) had significantly higher plasma AT concentration than the control group. As supplementation continued, those sows given ATA alone showed the greatest increase of AT in plasma, over the control and Vit-C groups. The group given the combined ATA/Vit-C treatment did not show such a large increase but still had significantly higher plasma AT concentrations compared to those not given any supplementary ATA. ANCOVA analysis showed an effect of day 0 on AT plasma of sows on D-60 and D-103 but not after farrowing (F+21).

The concentration of AT in colostrum and milk showed similar patterns to those in plasma (Fig. 1). Only sows fed with diets supplemented with ATA showed an increase in the concentration of AT ($P < 0.001$). AT content in colostrum and milk from sows fed on diets with vitamin C or control diet did not change. As previously reported the AT content in milk was lower ($P < 0.001$) than in colostrum for all treatments (Pinelli-Saavedra and Scaife, 2005).

3.2. IgG and IgA concentration in colostrum and milk and total Igs and Ig in piglet serum

Table 3 shows the IgG content in colostrum and the IgA concentration in milk 21 days after farrowing and the concentration of total immunolglobulins and IgG in serum of

Table 2
Effect of treatments on AT concentration (μg/ml) in sow's plasma during pregnancy and lactation

Treatments	D-0	D-60	D-103	D-F+21
Control	$1.4^{a} \pm 0.13$	$2.0^{a} \pm 0.15$	$2.5^{a} \pm 0.15$	$1.9^{a} \pm 0.28$
Vit-C	$1.8^{b} \pm 0.17$	$2.6^{ab} \pm 0.20$	$2.9^{a} \pm 0.19$	$2.6^{a} \pm 0.39$
ATA	$2.1^{b} \pm 0.18$	$3.4^{c} \pm 0.21$	$4.6^{c} \pm 0.23$	$5.6^{c} \pm 0.40$
ATA/Vit-C	$1.6^{ab} \pm 0.12$	$2.8^{bc} \pm 0.16$	$3.8^{b} \pm 0.14$	$4.1^{b} \pm 0.28$
P-Value	$P < 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Values given are means ± SE. Mean values within columns with different superscripts are significantly different. Control = commercial diet containing 36 mg/kg feed of ATA + 0 g of vitamin C; Vit-C = Control + Vitamin C (10 g/day); ATA = Control + ATA (500 mg/kg feed); (ATA/Vit-C) = Control + ATA (500 mg/kg feed) + Vitamin C (10 g/day).

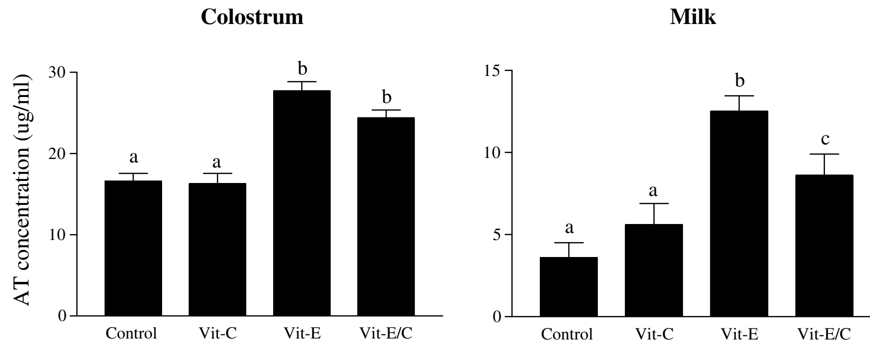

Fig. 1. Effects of treatments on α-tocopherol content in colostrum and milk. Different letters indicate values which are significantly different. $P < 0.001$. Control = commercial diet containing 36 mg/kg feed of ATA + 0 g of vitamin C; Vit-C = Control + Vitamin C (10 g/day); ATA = Control + ATA (500 mg/kg feed); (ATA/Vit-C) = Control + ATA (500 mg/kg feed) + Vitamin C (10 g/day).

Table 3
Effect of treatments on IgG and IgA concentrations (mg/ml) in colostrum and milk and on total Igs and Ig concentrations in piglets serum

Treatments	Sows		Piglets serum	
	IgG in colostrum	IgA in milk	Total Igs	IgG
Control	73.2 ± 6.05	4.1[b] ± 0.25	10.7[a] ± 1.10	10.3[a] ± 0.81
Vit-C	62.7 ± 7.67	3.2[bc] ± 0.32	11.5[a] ± 1.33	10.6[a] ± 0.96
ATA	60.7 ± 8.62	2.5[c] ± 0.16	10.6[a] ± 1.40	9.7[a] ± 1.05
ATA/Vit-C	77.6 ± 5.93	3.6[bc] ± 0.19	16.9[b] ± 0.99	13.5[b] ± 0.81
P-Value	$P > 0.05$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Values presented are means ± standard errors of the mean (SEM). Within a column means with different superscripts are significantly different. Control = commercial diet containing 36 mg/kg feed of ATA + 0 g of vitamin C; Vit-C = Control + Vitamin C (10 g/day); ATA = Control + ATA (500 mg/kg feed); (ATA/Vit-C) = Control + ATA (500 mg/kg feed) + Vitamin C (10 g/day).

suckling piglets. The IgG content of colostrum was similar in sows fed with control, Vit-C, and ATA diets ($P > 0.05$). The concentration of IgA in milk decreased in sows given the ATA diet ($P < 0.001$), but was unchanged in the other groups. The total immunoglobulins and IgG content of piglet serum was increased only in piglets born to sows fed diets with ATA/Vit-C ($P < 0.001$), no changes were observed in the other groups ($P > 0.05$).

3.3. Proliferation of PBMC from sows

Proliferation of PBMC stimulated with PHA or ConA was evaluated on day 103 and at 21 days after farrowing (Table 4). During gestation (D-103), proliferation was reduced in PMBC from all groups when cells were stimulated with either PHA or ConA ($P < 0.001$) compared to that after farrowing-F+21. Comparison between treatments revealed that proliferation of PBMC stimulated with PHA or ConA was not different in sows fed with ATA, ATA/Vit-C, or control diets ($P > 0.05$). An increment in proliferation was observed in sows supplemented with vitamin C ($P < 0.001$). After farrowing, proliferation in PBMC stimulated with PHA or ConA depicted a similar performance as that during gestation, only PBMC from sows fed on diets supplemented with vitamin C presented higher levels of proliferation ($P < 0.05$). PMBC

Table 4
Proliferation (c.p.m.) of PBMC from sows supplemented with α-tocopherol acetate (ATA) and vitamin C on day 103 of gestation (D-103) and 21 days after farrowing (F+21)

Treatments	PHA		ConA	
	D-103	F+21	D-103	F+21
Control	17,398[a] ± 1641	43,327[a] ± 6948	13,675[a] ± 1204	46,247[a] ± 5557
Vit-C	20,309[b] ± 2032	64,779[b] ± 7257	17,983[b] ± 1585	74,142[b] ± 6020
ATA	13,095[a] ± 2270	30,454[a] ± 10,263	10,424[c] ± 1851	54,364[a] ± 8700
ATA/Vit-C	14,507[a] ± 1487	44,300[a] ± 7611	12,167[a] ± 1160	40,030[a] ± 6624
P-Value	$P < 0.05$	$P < 0.05$	$P < 0.01$	$P < 0.001$

Values presented are means ± standard errors of the mean (SEM). Within a column means with different superscripts are significantly different. c.p.m. = counts per minute; Control = commercial diet containing 36 mg/kg feed of ATA + 0 g of vitamin C; Vit-C = Control + Vitamin C (10 g/day); ATA = Control + ATA (500 mg/kg feed); (ATA/Vit-C) = Control + ATA (500 mg/kg feed) + Vitamin C (10 g/day).

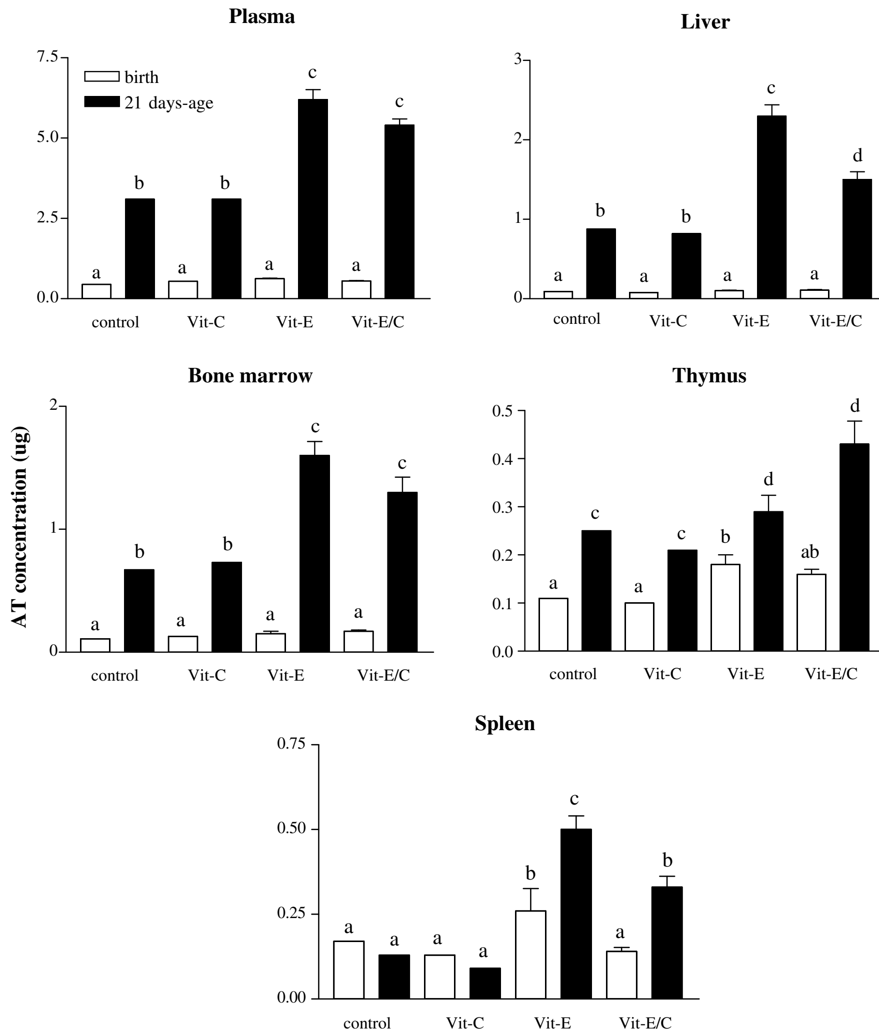

Fig. 2. Vitamin E distribution in piglet tissues (liver, bone marrow, thymus, spleen) and piglet plasma at birth and weaning, from sows supplemented with ATA and vitamin C. Different letters indicate values which are significantly different $P < 0.001$.

prepared from 21-day-old piglets showed a proliferative response to stimulation with PHA and ConA but there were no effects of treatment and the data have not been reported.

3.4. AT concentration in plasma and tissues of suckling pigs

With the exception of thymus and spleen, the tissue concentration of AT was low in all groups at birth (plasma < 0.170 μg/ml; liver < 0.150 μg/g; bone marrow < 0.170 μg/g). In thymus and spleen the AT content of tissues from piglets born to sows fed the ATA diet was increased significantly. At 21 days of age, the concentration of AT was significantly higher in all of the tissues measured for all of the treatment groups and with the exception of spleen, the tissue concentration of AT was significantly higher in piglets from sows fed ATA and ATA/Vit-C compared to those fed the control and Vit-C. In liver and spleen, AT concentration was higher ($P < 0.001$) in piglets from sows fed ATA than those from sows fed ATA/Vit-C. In comparison with other tissues, AT in the thymus was 2–3-fold lower than in bone marrow, 6–9-fold lower than in the liver, and similar to the spleen content ($P < 0.001$) (Fig. 2).

4. Discussion

In sows AT in plasma, colostrum, and milk increased with the inclusion of supplementary dietary ATA, and was significantly higher in colostrum than in milk. Similar effects of ATA supplementation have been reported by Malm et al. (1976), Loudenslager et al. (1986), Hidiroglou et al. (1993b), and Mahan (1991, 1994, 2000) although, in the present study AT concentration in colostrum and milk was higher than reported by these authors. These disparities are most likely due to differences in the composition of diets and the supplementation period. The increased content of AT in sows colostrum and milk was also reflected in the progeny at weaning, suggesting that α-tocopherol was successfully transferred via colostrum

and milk. AT concentration was significantly higher in newborn piglet plasma from sows supplemented with Vit-E as compared to those born from sows not supplemented. AT content was low in all tissues at birth analysed from non-supplemented and supplemented sows. In cord blood, plasma values were similar to those reported by Pinelli-Saavedra and Scaife (2005) and Mahan (1991) and increased in response to ATA supplementation. The data support the view that there is little placental transfer of AT and that at birth piglets can be considered AT-deficient prior to suckling.

Dietary supply of AT to piglets via colostrum and milk resulted in a marked increase in the AT content of piglet plasma (7–10-fold), liver (10–20-fold), and bone marrow (6–10-fold) (Fig. 2). The extent to which changes in bone marrow mirrored those in plasma may reflect the extensive vascularization of this tissue and, therefore, a large influence of plasma derived AT. Absolute concentrations of AT in thymus and spleen tissue were low compared to the other tissues. Thymus was a less responsive tissue (2–3-fold increases), and spleen AT content was only slightly increased by dietary supply. Thus the effects of sow dietary intake on colostrum and milk AT concentration were reflected to varying degrees in plasma and tissue levels at 21 days.

Mahan (1991), Mahan et al. (2000) and Hidiroglou et al. (1993b) previously reported that AT concentration in piglet plasma and liver was increased after intake of colostrum and milk from sows supplemented with ATA. Another study showed a variable tissue AT dose–response in a wide range of tissues in 3- and 7-day-old piglets given injections of 500, 1500, and 2500 IU ATA; the greatest dose–response being in liver and adrenal glands (Batra and Hidiroglou, 1994). The data in Fig. 2 show that maternal supplementation with ATA is effective in providing an enhanced supply of AT to the suckling piglet and can markedly influence tissue AT concentration by the time of weaning. The differential tissue responses to circulating AT levels may be related to a number of factors, such as the inherent requirement of specific tissues for AT, the presence of a tissue-specific tocopherol-binding protein, and tissue lipid content. It is interesting to note that from the immune tissues examined, bone marrow was the most responsive to dietary supply. However, it remains to be determined if this response has any significance for the early differentiation of immune cells in the neonate.

The level of ATA supplemented in the control diet is sufficient to meet the minimal requirements of swine. However, in this work we supplemented with higher levels of ATA, alone or in combination with vitamin C, in order to improve the immune response of sows and piglets. IgG is the major immunoglobulin fraction in piglet plasma. Previous studies (De Pasille et al., 1988; Mavromatis et al., 1999) reported IgG concentrations (9.5–11.3 mg/ml) in piglets from sows not given ATA supplementation that were similar to those presented in the control in the present study (10.3–10.6 mg/ml). The higher total Igs and IgG in plasma from piglets suckling from sows given ATA/Vit-C suggests a synergetic effect between the two vitamins. There were no differences between treatments in the concentration of IgG in the colostrum. At day 21, the immunoglobulin status of piglets reflects a combination of the effects of colostrum supply and endogenous synthesis, suggesting that the combined vitamin supplementation enhanced the latter. Hidiroglou et al. (1995b) reported a similar but not significant tendency towards higher IgG values in calves given a combined ATA and vitamin C supplement as compared to calves given a control diet or those supplemented with ATA alone. Vitamin C supplementation has been reported to affect Ig concentrations in humans. Vallance (1977) observed a significant positive correlation between leukocyte ascorbic acid levels and IgG when subjects were supplemented with 1 g of ascorbic acid/day and Prinz et al. (1977) reported that a dose of 1 g/day for a period of 11 weeks significantly raised the plasma levels of IgA and IgM of healthy men. However, there was no evidence from the present study that vitamin C supplementation of sows had any beneficial effects on plasma immunoglobulin levels in 21-day-old piglet.

The proliferation of PBMC during gestation (D-103) was low compared with that observed after farrowing. The differences in responses in sows before and after parturition may be explained by factors such as release of immunosuppression after farrowing. Immunosuppression in pregnancy is a well known phenomenon (Mangusson and Fossum, 1988; Deng and Chen, 1992; Nagahata et al., 1992; Bermas and Hill, 1997), most likely caused by local and systemic foetal factors that are known to regulate maternal immune responses (Sargent, 1993).

Among treatment groups, individual responses to mitogen stimulation was very variable, a common feature of this experimental approach also reported in other studies that used a similar technique (Nemec et al., 1994; Bonnette et al., 1990; Larsen and Tollersrud, 1981). Despite this variability, it is clear that, when fed alone, vitamin C increased the sows' lymphocyte response to ConA and PHA on day 103 and after farrowing (F+21). In piglets, although it is clear that neonatal PMBC can respond to mitogenic stimulation, there was no significant effect of treatments.

In conclusion, dietary supplementation of sows feed with ATA increased the AT concentration of colostrum and milk, as well as that of piglet tissues and plasma at weaning. This study is the first to provide data on the AT content of bone marrow thymus, and spleen. In piglets it was observed that the combined vitamin treatment significantly increased the total Igs and IgG concentrations, and vitamin C had an effect on lymphocyte response in sows on day-103 and F+21. Indicating differential effects of AT and vitamin C in sows and piglets. This may suggest that a combination of ATA and vitamin C supplementation could be beneficial for both sows and their progeny.

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