Parenteral administration of vitamins A, D and E on the oxidative metabolism and function of polymorphonuclear leukocytes in swine[1]

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ABSTRACT.- Lima A.S., Weigel R.A., Morgado A.A., Nunes G.R., Souza F.N., Moreno A.M., Della Libera A.M.M.P. & Sucupira M.C.A. 2012. **Parenteral administration of vitamins A, D and E on the oxidative metabolism and function of polymorphonuclear leukocytes in swine.** *Pesquisa Veterinária Brasileira 32(8):727-734.* Departamento de Clínica Médica, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Av. Prof. Dr. Orlando M. de Paiva 87, São Paulo, SP 05508 270, Brazil. E-mail: msucupir@usp.br

The weaning period of piglets is characterized by physiological alterations, such as decreased weight gain, increased reactive oxygen species (ROS) and increased serum cortisol levels with possible effects on the immune response. The effect of parenteral administration of vitamins A, D and E on production performance, oxidative metabolism, and the function of polymorphonuclear leukocytes (PMNLs) was assessed in piglets during the weaning period. The sample was comprised of 20 male piglets that were given an injectable ADE vitamin combination (135,000 IU vitamin A, 40,000 IU vitamin D and 40mg vitamin E/animal) at 20 and 40 days of age. Weight gain, concentration of reduced glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD) and the microbicidal and phagocytic activity of PMNLs were assessed. No difference was observed in the average piglet weight during the study; however, a greater percentage of weight gain was observed after weaning in the treated group. The concentrations of GSH and SOD did not differ between groups, although lipid peroxidation was greater in the control group at 60 days of age. The investigated variables of oxidative metabolism were correlated as follows: -0.41 for GSH and MDA, -0.54 for GSH and SOD and 0.34 for MDA and SOD. The intensity of intracellular ROS production, the percentage of ROS-producing PMNLs and the intensity of phagocytosis by PMNLs did not differ between treatment groups. Administration of the injectable ADE combination improved the percentage of weight gain between 20 and 40 days of age, decreased oxidative stress at 60 days of age and did not influence the function of PMNLs in piglets.

INDEX TERMS: Immune response, weaning, piglets, GSH, MSD and SOD.

RESUMO.- [**Administração parenteral das vitaminas A, D e E no metabolismo oxidativo e sobre a função de leucócitos polimorfonucleares em suínos.**] O período de desmame nos leitões é caracterizado por alterações fisiológicas como menor ganho de peso, aumento na produção de espécies reativas de oxigênio (EROs) e aumento na concentração plasmática de cortisol com possíveis implicações para a resposta imune. Foi avaliado o efeito da administração parenteral das vitaminas A, D e E sobre o desempenho produtivo, o metabolismo oxidativo e a função de leucócitos polimorfonucleares (PMNLs) em suínos durante esta fase de crescimento. Foram utilizados 20 leitões, machos, com 20 dias de idade que receberam ADE injetável (135.000 UI vitamina A, 40.000 UI vitamina D e 40mg vitamina E/animal), aos 20 e 40 dias de idade. Foi determinado o ganho de peso e as concentrações de glutationa reduzida (GSH), malondialdeído (MDA) e superóxido dismutase (SOD) e a

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capacidade microbicida e fagocítica dos PMNLs. Não houve diferença entre o peso vivo médio durante o experimento, porém maior ganho de peso percentual foi observado 20 dias após o desmame para o grupo tratado. As concentrações de GSH e SOD não diferiram entre os grupos, porém a lipoperoxidação foi maior no grupo controle aos 60 dias de idade. As correlações entre as variáveis do metabolismo oxidativo foram -0,41 para GSH e o MDA, -0,54 para GSH e SOD e 0,34 para MDA e SOD. A intensidade da produção intracelular de EROs, a porcentagem de PMNLs que produziram EROs e a intensidade de fagocitose dos PMNLs não diferiram entre os tratamentos. A administração de ADE injetável melhorou o ganho de peso percentual no período de 20 a 40 dias de idade, diminuiu o estresse oxidativo aos 60 dias de idade e não influenciou função dos PMNLs dos leitões.

TERMOS DE INDEXAÇÃO: Resposta imune, desmame, leitões, GSH, MDA e SOD.

INTRODUCTION

Maternal separation, relocation, introduction to new social groups, the establishment of social dominance and a change in diet to a higher dry mass content induce physiological changes in recently weaned swine, with a consequent reduction in weight gain being observed (Sutherland et al. 2006, Kojima et al. 2008). These alterations lead to increased cortisol serum levels (Heo et al. 2003) and possible effects on the immune response of animals (Bonnette et al. 1990), such as a reduction in neutrophil microbicidal capacity and reduced function of natural killer cells and lymphocytes (Sutherland et al. 2006, Da Costa et al. 2008).

Metz and Gonyou (1990) observed that the immune system of swine weaned at four weeks of age is not yet fully developed. Therefore, these animals are more susceptible to disease. Animals weaned at four weeks began to eat food only after 2 or 3 days post-weaning. In response to stress and exposure to antigens, the immune system stimulates the production of proinflammatory cytokines in the brain that reduce the motivation to eat (Kent et al. 1996) and interact with growth hormone and insulin-like growth factor 1 (IGF-1). This interaction can decrease cellular growth (Kelly 2004).

During normal aerobic metabolism, cells maintain a balance between the production and elimination of reactive oxygen species (ROS). When the occurrence of oxidative events increases, the system tends toward the pro-oxidative side, resulting in "oxidative stress" (Lykkesfeldt & Svendsen 2007). An increase in oxidative stress might affect the concentration of antioxidants, such as glutathione and vitamin E, and finally leads to oxidative damage of lipids, proteins, carbohydrates and nucleic acids. This process might be so severe as to eventually cause cell death. The efficacy of the antioxidant system largely depends on the type of molecule triggering oxidative stress and its intra- or extracellular location. Damage to the cell membrane might be avoided more effectively by vitamin E, which reacts with peroxyl and hydroxyl radicals, compared to carotenoids, which act by reacting with singlet oxygen (Jordão et al. 1998).

ROS is the main oxidant substance. The physiological action of ROS is directly related to the antimicrobial protection mediated by polymorphonuclear leukocytes (PMNLs). PMNLs usually circulate in the bloodstream in an inactive state during which they may surround or phagocytize bacteria but do not cause damage (Winterbourn & Assman 1990). Upon exposure to bacteria coated by immunoglobulins, immunocomplexes, complement, or leukotrienes, the enzyme NAPDH-oxidase is activated in PMNLs, which triggers the production of ROS and facilitates the microbicidal action of PMNLs.

Antioxidants protect biological systems against the damage caused by processes that might lead to high levels of oxidation (Krinski 1992). Antioxidants are classified as endogenous, i.e., synthesized inside the organism, and exogenous, i.e., supplied by the diet. Vitamin E is one of the best-known exogenous antioxidants. Since the 1990s, vitamin E has been investigated as a treatment for minimizing the adverse effects associated with the transitional stage of development, which is represented in swine by weaning (Bonnette et al. 1990). Vitamin A is another exogenous antioxidant able to prevent the deleterious action of ROS released during inflammation, infection and stress or even during resting metabolism (Gomes et al. 2005). Vitamin D is comprised of two major groups: vitamin D3 (cholecalciferol, synthesized by animals) and D2 (ergocalciferol, synthesized by plants); the production of both groups requires sunlight. Animals synthesize approximately 10 different provitamins that form vitamin D upon being irradiated. Thus, special attention must be paid to supplemental vitamin D in animals raised in confinement. Lauridsen et al. (2010) concluded that independent of the dose or the form of vitamin D supplied to sows, only a small amount of vitamin D is transferred to the offspring. Thus, nursing pigs not exposed to sunlight might require supplemental vitamin D. Indeed, injected supplements of combined A, D and E vitamins are widely used in Brazilian systems of animal production.

Because animals kept in intensive production systems exhibit higher requirements of antioxidant agents, this study sought to investigate the effect of parenteral administration of vitamins A, D and E on the production performance, oxidative metabolism and PMNL function of swine during their growth phase.

MATERIALS AND METHODS

The sample group consisted of 20 male piglets at 20 days old with a 7.1 kg average live weight. The piglets were hybrids between commercial genetic lineages cb22 and ag337 Agroceres. Animals were allocated to a commercial farm in the town of Capivari, SP, Brazil. Weaning was performed at 21 days of age, and the piglets were subsequently housed in the nursery, where they remained until the end of the study at 60 days of age. Diet was based on corn, soy, bran, dairy products, vitamin and mineral supplements appropriately balanced to promote weight gain compatible with the growth curve of this lineage (Table 1).

The present study used a 'randomized blocks' design based on the farrowing rate of sows and the weight of piglets. Although kept in the same stall, piglets were distributed into two groups of 10 animals each. The treated group was given 0.5 mL of ADE vita-

Table 1. Diet Composition of pigs in the nursery. São Paulo, 2010

Diet Composition	
Sodium chloride	0,45
Limestone 39%	0,26
Corn Grain 8,0	62,2
Rice Bran	5,00
Soybean Meal 46,0	21,0
Yeast of sugar cane 37%	5,00
Meat and bone meal 44%	3,80
Fat lard	1,40
MineralSuplement[1]	0,10
Vitamin Suplement[2]	0,10
Phytase[3]	0,01
Copper Sulp Pent. 25%	0,08
Choline chloride 60%	0,04
DL- methionine 99%	0,08
Lysina HCl 78,8%	0,38
L-Threonina 98%	0,11
Total (%)	100

[1] Roligomix Suínos (DSM): Fe, 90g; Cu, 10g; Co, 2g; Mn, 40g; Zn, 2g e Excipient q.s.p. 500g.

[2] Premix Inicial (MCassab): Vit. A 8.00.000UI; Vit. D3 2.000.000UI; Vit E 10.000mg; Vit. K3 500mg; Vit. B1 1.500mg; Vit. B2 5.000mg; Vit. B6 2000mg; Vit. B12 20.000mcg; Niacin 25.000mg; Calcium Pantothenate 12.000mg; Folic Acid 800mg; Biotin 50mg, Selenium 280mg e Excipient q.s.p. 1000g.

[3] Ronozyme P5000G (DSM).

min combination (135,000 IU vitamin A, 40,000 IU vitamin D and 40mg vitamin E/animal/dose) via deep intramuscular injection at 20 and 40 days old. The control group was given the same volume (0.5mL/animal/dose) of sterile physiological saline solution at the same ages and by the same route to mimic the stress effects caused by handling and injection of the animals.

At 20 (M0), 40 (M1) and 60 (M2) days of age, animals were weighed to assess the production performance, and blood was collected to assess oxidative metabolism and PMNL function.

The oxidative metabolism was assessed by means of indirect markers, including a reduction in the endogenous antioxidant enzymes, glutathione (GSH) and superoxide dismutase (SOD), and a reduction in malondialdehyde (MDA), one of the 2-thiobarbituric acid reactive substances (TBARS), which are themselves products of oxidative reactions. The GSH content was measured in whole blood by means of the colorimetric method described by Beutler et al. (1963). The index of lipid peroxidation was indirectly established based on the MDA content in heparinized serum as measured by the thiobarbituric acid method described by Esterbauer & Cheeseman (1990). The activity of SOD was measured using the colorimetric method described by Woolliams & Wiener (1983), as adjusted for the LABMAX 240 automatic biochemical analyzer using the RANDOX SD 125 commercial kit (Ransel® Laboratories, Randox, Crumlin, UK).

Immune function was assessed in circulating PMNLs by measuring intracellular ROS produced *in vitro* following stimulation with phorbol 12-myristate 13-acetate (PMA), *Escherichia coli* lipopolysaccharides (LPS), and after phagocytosis of *Staphylococcus aureus* conjugated to propidium iodide (Sa-PI). PMNL function, as measured by phagocytosis of *S. aureus* (American Type Culture Collection (ATCC) 25923), as conjugated to propidium iodide and intracellular ROS production, were performed as suggested by Hasui et al. (1989). The samples were analyzed by flow cytometry on a FACSCalibur™ cytometer (Becton Dickinson Immunocytometry System™, San Diego, USA). Twenty thousand PMNLs were acquired, being identified by their size and granularity (Fig.1 and 2) as described by Busque et al. (1998).

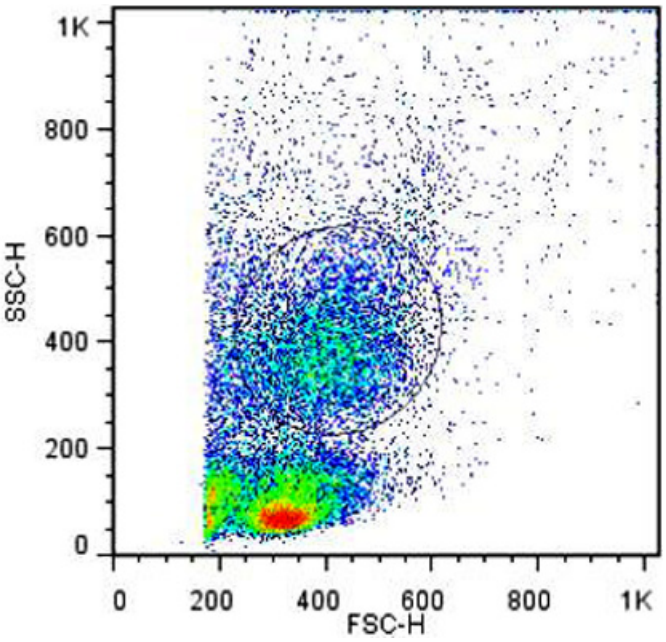

Fig.1. Cytogram of swine blood leukocytes by flow cytometry. Identification of the gate-delimited polymorphonuclear leukocytes (PMNLs) used to analyze PMNL function. São Paulo, 2010.

The normal distribution of data was verified by the Kolmogorov-Smirnov test. To assess the differences between the means of results, an analysis of variance (one-way ANOVA) and the Tukey's test were performed for data exhibiting parametric distribution. The Mann-Whitney test was applied to data exhibiting nonparametric distribution. Significance was established when $P<0.05$ in all tests. Data exhibiting a normal distribution are expressed as the mean (± standard deviation); data exhibiting nonparametric distribution are expressed as the median followed by the maximum and minimum values. The correlation among variables was investigated by means of the Pearson correlation coefficient. Correlation was rated high when $r > 0.6$, intermediate when r ranged from 0.3 to 0.6 and low when $r<0.3$. MINITAB® software version 14.1 (GlobalTech Informática™, Belo Horizonte, Minas Gerais, Brazil) was used for statistical analysis.

The present study was approved by the Bioethics Committee of the School of Veterinary Medicine and Zootechnics of the University of São Paulo (Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (FMVZ-USP) under protocol number 1703/2009.

RESULTS

At 60 days of age, the average live weight was 27.6 kg and 30.6 kg in piglets belonging to the control and treated groups, respectively. No difference was found in the average live weight throughout the study (Table 2). However, a greater percentage of weight gain was observed in vitamin treated animals by 20 days after weaning ($P=0.20$) (Fig.3).

Due to previous processing, the heparinized blood samples collected at M0 exhibited intense hemolysis. Thus, it was not possible to measure the concentration of SOD or the activity of PMNLs from these samples. Table 2 shows the results obtained for the variables of oxidative metabolism.

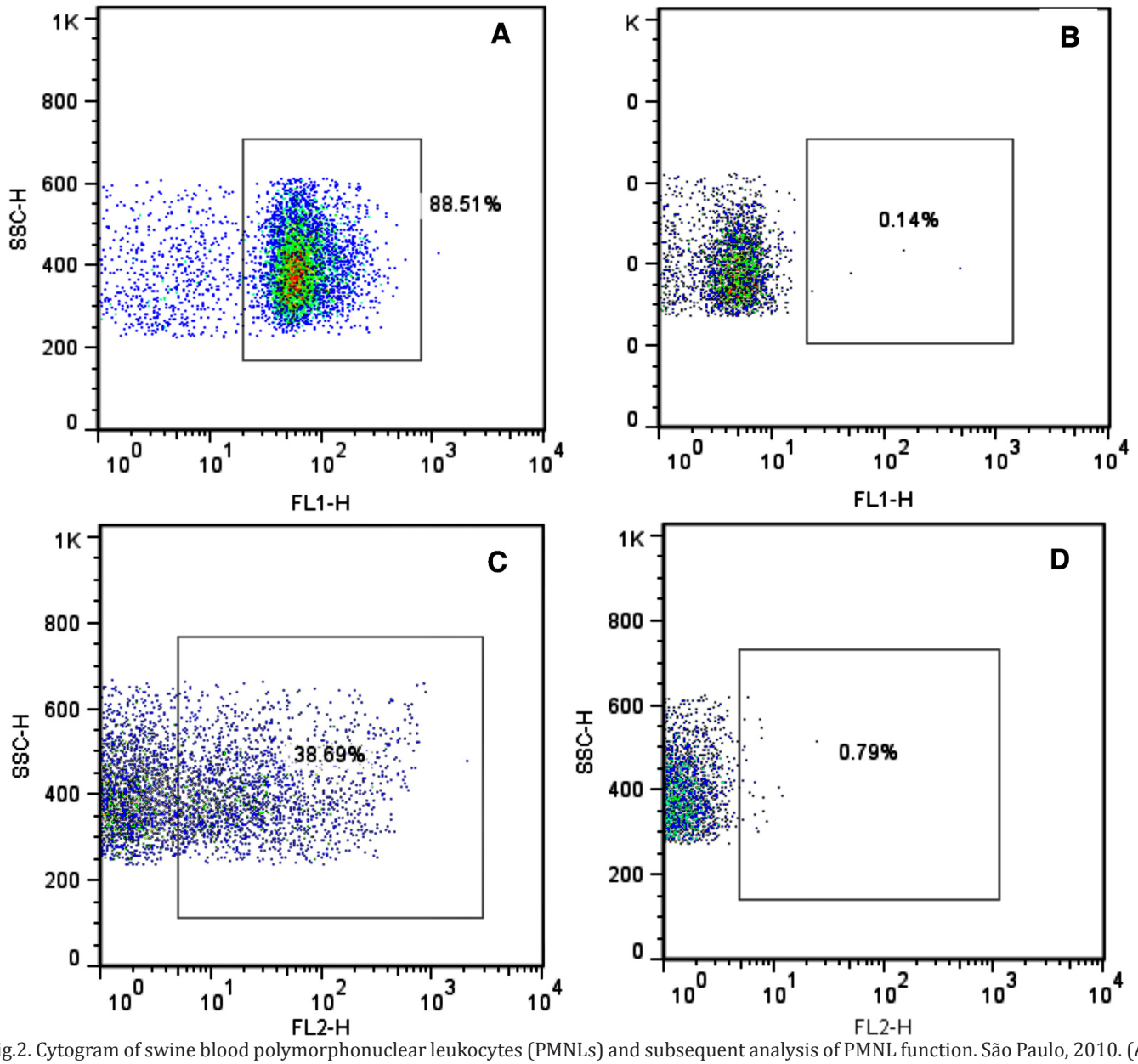

Fig.2. Cytogram of swine blood polymorphonuclear leukocytes (PMNLs) and subsequent analysis of PMNL function. São Paulo, 2010. (**A**) Population of PMNLs that produced reactive oxygen species (ROS). (**B**) Control sample of intracellular ROS production. (**C**) Population of PMNLs that performed phagocytosis. (**D**) Phagocytosis control samples.

Table 2. Mean and corresponding standard deviation of pig live weight following treatment with ADE vitamin combination or an equivalent volume of saline. São Paulo, 2010

	Live Weight (kg)	
	Treated	Control
M0	7.15 [a] (2.00)	7.1 [a] (1.35)
M1	15.4 [b] (4.05)	13.15 [b] (1.86)
M2	30.6 [c] (8.24)	27.6 [c] (2.91)

Note: M0 = weaning and first dose; M1 = 20 days after M0 and second dose; M2 = 40 days after M0. Different lowercase letters in columns indicate differences between time points (P<0.05)

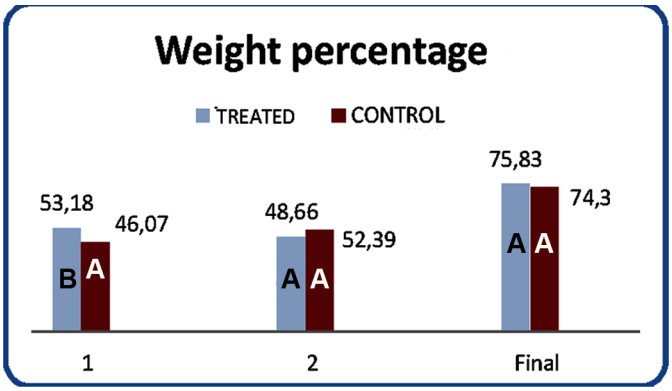

Fig.3. Mean percentage weight gain in control piglets and piglets treated with ADE vitamin combination. São Paulo, 2010.
Note: 1 = 20 days after weaning; 2 = 40 days after weaning.

The concentrations of GSH and the enzyme SOD did not differ between the control and treated groups throughout the study (Table 3). Lipid peroxidation was higher in the control group (P=0.012) at M0 (Fig.4).

The correlations between the variables of oxidative metabolism exhibited intermediate intensity. There was a negative correlation between GSH and MDA -0.41 (P=0.001) and between GSH and SOD -0.54 (P=0.001); the correlation between MDA and SOD was 0.34 (P=0.04).

The intensity of intracellular ROS production by PMNLs did not differ between treatments, but it was higher (P<0.01) at M2 compared to M1 (Table 4) in both groups. No difference was observed in the percentage of ROS producing PMNLs between groups, although higher percentages (P<0.02) were observed at 60 days of age compared to 40 days of age in both groups (Table 5). The intensity of phagocytosis by PMNLs did not differ between groups or between time-points. In spite of this lack of difference, the treated group exhibited a lower percentage (P=0.03) of phagocytosing PMNLs at M2 compared to M1 (Table 6).

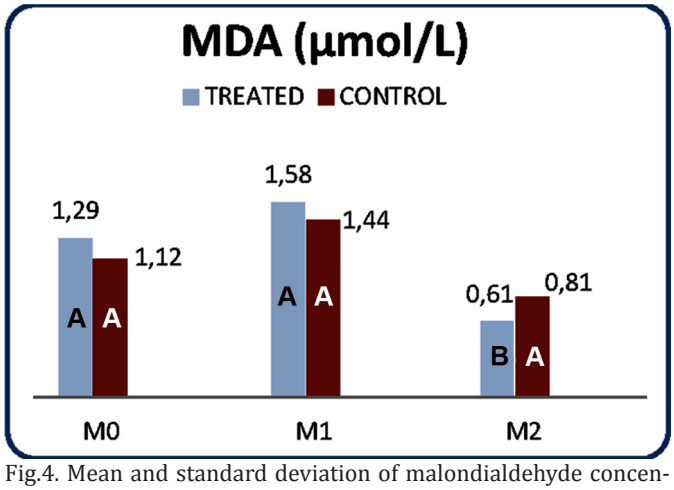

Fig.4. Mean and standard deviation of malondialdehyde concentration (µmol/L) in pigs treated with the ADE vitamin combination or saline only. São Paulo, 2010.

Note: M0 = weaning and first dose; M1 = 20 days after M0, and second dose M2 = 40 days after M0. Different uppercase letters indicate differences between treatments (P<0.05).

Table 3. Mean and standard deviation of the activity of reduced glutathione (mg/dL), superoxide dismutase (U/g Hb), and malondialdehyde (µmol/L) in pigs treated or not with ADE vitamin combination. São Paulo, 2010

	Reduced Glutathione (mg/dL)		Superoxide Dismutase (U/g Hb)		Malondialdehyde (µmol/L)	
	Treated	Control	Treated	Control	Treated	Control
M0	20.76 [a] (5.48)	19.29 [a] (7.92)	*	*	1.29 [a] (0.89)	1.12 [ab] (0.75)
M1	8.78 [b] (2.24)	11.50 [b] (5.79)	2041.5 [a] (1284.4/2102.5)	2111.2 [a] (1808.7/2188.1)	1.58 [a] (0.70)	1.44 [a] (0.65)
M2	21.56 [a] (5.44)	23.9 [a] (6.91)	1855.2 [b] (1359.9/2016.4)	1730.6 [b] (1618.1/ 2100.8)	0.61 [B b] (0.16)	0.81 [A b] (0.17)

Note: M0 = weaning and first dose; M1 = 20 days after M0 and second dose; M2 40 days after M0.

* Insufficient sample. Different uppercase letters in rows indicate differences between treatments (P<0.05). Different lowercase letters in columns indicate differences between time points (P<0.05).

Table 4. Mean staining intensity of intracellular reactive oxygen species in stimulated (i.e., cultured with *Staphylococcus aureus* and *Escherichia coli*-derived lipopolysaccharide) and non-stimulated (i.e., basal) blood polymorphonuclear leukocytes from piglets treated or not treated with the ADE vitamin combination. São Paulo, 2010

	Treated			Control		
	Basal	*Staphylococcus aureus*	LPS	Basal	*Staphylococcus aureus*	LPS
M1	73.76 [a] (± 42.24)	90.39 [a] (± 35.54)	65.11 [a] (± 41.93)	55.36 [a] (±25.79)	79.98 [a] (± 37.26)	62.66 [a] (± 29.05)
M2	143.70 [b] (± 26.34)	264.80 [b] (± 317.3)	138.70 [b] (± 17.45)	164.60 [b] (± 71.15)	172 [b] (± 95.52)	169.10 [b] (± 67.44)

Different lowercase letters in columns indicate P<0.01. Basal = non-stimulated. LPS = lipopolysaccharide from *Escherichia coli* (strain 055:b5).

Table 5. The percentage (%) of stimulated (i.e., cultured with *Staphylococcus aureus* and *Escherichia coli*–derived lipopolysaccharide) and non-stimulated (i.e., basal) blood polymorphonuclear leukocytes (PMNLs) that stain positive for reactive oxygen species; PMNLs were obtained from piglets treated or not treated with the ADE vitamin combination. São Paulo, 2010

	Treated			Control		
	Basal	*Staphylococcus aureus*	LPS	Basal	*Staphylococcus aureus*	LPS
M1	83.92 [a] (± 12.33)	87.09 [a] (± 5.62)	86.38 [a] (±5.89)	84.21 [a] (± 10.34)	84.92 [a] (± 8.53)	87.39 [a] (± 9.46)
M2	97.09 [b] (± 1.46)	93.56 [b] (± 4.54)	97.5 [b] (±1.20)	96.70 [b] (± 1.82)	96.70 [b] (± 1.82)	96.71 [b] (± 2.60)

Different lowercase letters in columns indicate P<0.02. Basal = non-stimulated. LPS = lipopolysaccharides of *Escherichia coli* (strain 055:b5).

Table 6. In vitro phagocytosis of propidium iodide (PI)-conjugated *Staphylococcus aureus* was assessed by the percent of PI positive and mean staining intensity of blood polymorphonuclear leukocytes from pigs treated or not treated with ADE vitamin combination. São Paulo, 2010

	Treated		Control	
	Percentage	Intensity	Percentage	Intensity
M1	**63.18 [a]** (± 19.40)	16.99 (± 19.89)	68.14 (± 17.67)	13.36 (± 7.75)
M2	48.42 [b] (± 20.69)	9.55 (± 7.56)	44.98 (± 18.31)	8.48 (± 7.98)

Different lowercase letters in columns indicate differences between time points (P=0.03).

DISCUSSION

The percentage of weight gain in piglets shows that the animals in the treated group exhibited better performance at the first time-point assessed, specifically when animals were transferred from the mother to the nursery.

The conditions favoring damage of tissues by ROS increase after weaning. Weaned piglets exhibit fast growth rates, intense development of muscles and increased metabolic activity. Additionally, piglets are exposed to several sources of stress during this phase, including diet, disease, change in temperature, social interaction and handling. The nutritive and metabolic need for antioxidant vitamins is therefore higher in this phase.

Few studies have investigated the effects of an injectable combination of vitamins A, D and E in swine production. Conversely, many studies have assessed the effects of these vitamins separately.

Initially, the serum concentration of vitamin E is high in piglets, probably due to greater bioavailability in milk compared to a diet based on concentrates. Immediately after weaning, the serum concentration of vitamin E falls drastically (Sivertsen et al. 2007). The importance of vitamin E is related to its solubility in lipids and its localization in the lipid membrane. For these reasons, this vitamin is able to move across the membrane layers, where it performs its main function: to interrupt the chain reaction of lipid peroxidation, mainly by neutralizing the peroxyl lipid radicals to produce lipid hydroperoxides and tocopheroxyl radicals (Faustman et al. 2009).

The concentration of vitamin A is high in swine colostrum, and the concentration falls by 25% during the first five days of lactation (Heidebrecht et al. 1950). Few studies have investigated the action of vitamin A in piglets, and most of them focused on sows. The effect of vitamin A (450,000 IU of retinol palmitate injected on the day of weaning or mating) on the productive performance was assessed by Silveira et al. (1998): These researchers concluded that injectable vitamin A supplements improved the reproductive performance of sows independent of the productive phase, including an increased number of total and live born piglets, and increased litter weight at birth. Thompson (1994) suggested that injectable vitamin A is more effective than dietary supplements because injectable vitamin A might be taken up by cells directly, rather than being stored and released by the liver.

The assessment of oxidative metabolism showed that the concentration of SOD decreased with time during the post-weaning phase, regardless of treatment, which may indicate a higher requirement for antioxidants. SOD, an endogenous antioxidant, belongs to the class of metalloenzymes that counteract superoxide anion toxicity (Celi 2010). SOD is the most abundant enzymes and is the fifth most abundant protein in quantitative terms. SOD is present in all aerobic organisms and is necessary for the clearance of a wide variety of superoxide radicals.

The concentration of GSH and MDA changed in disparate ways in our study. GSH acts directly or indirectly on important biological processes, and it is needed to prevent the peroxidation of unsaturated fatty acids in the cell membrane. For instance, GSH acts to keep the iron atoms of hemoglobin in the ferrous form (Rover Junior et al. 2001). Variations in the glutathione content directly affect the synthesis of proteins and deoxyribonucleic acid. GSH oxidation or depletion can decrease protein synthesis. Under conditions of intense oxidative stress, GSH can be irreversibly oxidized and thus rendered nonfunctional (Uhlig & Wendel 1992). Dietary supplementation with vitamin E or vitamin D3 can increase the levels of GSH in the liver (Sardar et al. 1996). MDA, the main representative of the thiobarbituric acid-reactive substances that arises during the peroxidation of fatty acids by ROS, is considered to be a marker of cellular damage and oxidative stress because its concentration increases with excess ROS (Celi 2010). In the present study, the lowest post-weaning levels of GSH were found in the treated group. Together with the lower index of lipid peroxidation exhibited by this group at M2 in both series of comparisons (intra- and inter-group), our data indicate that the antioxidant system of the treated group was better prepared to react to the oxidative stress caused by weaning.

The two markers of the oxidative metabolism investigated in this study, specifically, the decrease in GSH concentration and the increase in MDA concentration, behave in such a way as to counteract oxidative stress. Similar behavior of these metabolites was described by Weigel et al. (2010). These researchers assessed the oxidative metabolism of sheep intoxicated with copper.

The assessment of the innate immune response did not reveal beneficial effects of vitamins A, D and E on PMNL function at the time-points and doses assessed in this study. However, measures of immune function were greatest at 60 days in both groups, including the percentage and intensity of ROS production by PMNLs, and phagocytosis of *S. aureus* conjugates either basally or post-stimulation. Lower microbicidal capacity was observed at 40 days of age compared to 60 days, although the percentage of PMNLs that phagocytized *Staphylococcus aureus* was lower in the control group at age 60 days compared to 40 days. Thus, the lower microbicidal capacity at the age of 40 days might be a function of age (Hoskinson et al. 1990, Butcher et al. 2005) and the consequences of weaning (Sutherland et al. 2006, Kojima et al. 2008). Weaning increases the concentration of cortisol (Heo et al. 2003) and consequently reduces the microbicidal capacity of neutrophils (Hoskinson et al. 1990, Butcher et al. 2005). In agreement with this interpretation, Hoskinson et al. (1990) found lower microbicidal capacity between the third and fifth weeks of life in piglets, and a subsequent increase in this function at sixth weeks and older. These data agree with studies performed in other species (Coignoul et al. 1984, Da Costa et al. 2008) and with the findings of the present study. However, the present study falls short of discerning whether the lower microbicidal capacity at 40 days is age-related or is a function of weaning because data from 20-day-old piglets is absent.

Curiously, the present study found higher PMNL phagocytic activity at 40 days of age with a concomitant increase in PMNL microbicidal capacity. Although some authors observe decreased phagocytic capacity from birth to the third week of life followed by a gradual increase (Hoskinson et al. 1990), others have not observed any effect of age on this function (Monis et al. 1987). Additionally, weaning is associated with an increase in somatotropin concentration and a decrease in the concentration of insulin-like growth factors 1 and 2 (IGF-1 and IGF-II) (Matteri et al. 2000, Kojima et al. 2008). Higher IGF-1 concentrations are correlated with greater microbicidal capacity (Balteskard et al. 1998). Thus, the increase of corticosteroid serum levels (Liu et al. 1999) is related to an inhibition of apoptosis and a more efficient elimination of apoptotic PMNLs by macrophages. This finding might explain the higher percentage of viable, functional PMNLs found in the blood of 40-day-old piglets, which may, in turn, contribute to the higher frequency of

S. aureus-phagocytizing PMNLs exhibited by animals in the treated group but not the rate of phagocytosis on a per cell basis (as measured by staining intensity).

Dietary vitamin E supplements can potentially increase the resistance of sows and piglets to enteric diseases, such as those caused by *E. coli*. Indeed, *E. coli* infection is one of the most common illnesses among newborns and contributes to high mortality during the pre-weaning phase (Pinelli-Saavedra 2003). Vitamin A is also involved in the immune response of swine. As such, several studies report an increased concentration of antibodies against *Escherichia coli* and *Salmonella Dublin* lipopolysaccharides, as well as total gamma globulins, in piglets receiving vitamin A supplements compared to controls (Lüdke et al. 1985). The efficiency of vitamin A in the immune response is related with the maintenance of epithelium integrity, the function of the adrenal gland, which releases corticosteroids, and the antibody response to T-cell dependent antigens. Vitamin A plays an important role in maintaining appropriate numbers of natural killer cells (Zhao & Ross 1995). In addition, Katz et al. (1987) state that retinoic acid contributes to increased macrophage phagocytic capacity and a possible role in leukocyte differentiation. Published results suggest that retinoic acid is involved in increased pro-inflammatory cytokine production, including interleukin 1 (Trechsel et al. 1985).

CONCLUSIONS

The present study shows that administration of an injectable ADE combination (135,000 IU vitamin A, 40,000 IU vitamin D and 40mg vitamin E/animal) via deep intramuscular injection at 20 and 40 days of age can increase the percentage of weight gained at these time points.

Our data also show that reduced oxidative stress and MDA production is observed in treated animals at 60 days of age but that no effect on PMNL function was observed in treated piglets.

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