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Short note

Effects of vitamin E injection on body temperature and plasma α-tocopherol concentrations in pigs, lambs and calves*

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Summary — Fifteen pigs, 15 lambs, and 15 calves were used to study the effect of supplementation of vitamin E on body temperature and plasma α-tocopherol concentrations following 1 intramuscular injection in 3 doses (1x, 3x, 5x). The 1x doses for pig, lamb, and calf were 500 , 1 000, and 1 500 IU, respectively. Data on body temperature and plasma α-tocopherol concentration were analyzed by least-squares procedures separately for each species. In each species plasma α-tocopherol concentration increased with increase in the dose of vitamin E injection, highest on d 1 postinjection and declined slowly throughout the remaining observation period. There was a transient elevation of body temperature on d 1 and 2 after injection in all species.

vitamin E / pig / lamb / calf

Résumé — Effet de l'injection de vitamine E sur la température corporelle et la concentration plasmatique en α-tocophérol chez le porc, l'agneau et le veau. *Quinze porcs, 15 agneaux et 15 veaux ont été utilisés pour étudier l'effet de la supplémentation en vitamine E sur la température corporelle et la concentration plasmatique en α-tocophérol, après l'injection intramusculaire de 3 doses (1x, 3x, 5x). Les doses 1x pour le porc, l'agneau et le veau étaient respectivement 500 UI, 1 000 UI et 1 500 UI. Les données sur la température corporelle et la concentration en α-tocophérol ont été analysées par la méthode des moindres carrés, séparément pour chaque espèce. Dans chacune des espèces, la concentration en α-tocophérol a augmenté lorsque la dose de vitamine E injectée augmentait, étant maximale j1 post-injection, et diminuant lentement au cours du reste de la période d'observation. Il y a eu une élévation transitoire de la température corporelle j1 et j2 post-injection dans toutes les espèces.*

vitamine E / porc / agneau / veau

INTRODUCTION

Adequate vitamin E is important for the prevention of deficiency diseases, such as muscular dystrophy (Hidiroglou *et al*, 1972). Supplementary vitamin E is usually given orally or intramuscularly for the prevention or correction of vitamin E deficiency. Intramuscular injection of vitamin E resulted in a much greater absorption into the blood than did oral administration (Caravaggi *et al*, 1968). Vitamin E sufficiency is usually determined from lev-

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els in blood plasma or serum (Horwitt, 1980). The body does not store appreciable amounts of vitamin E and circulating levels are considered to reflect the current vitamin E intake of the animal (Pehrson and Hakkarainen, 1986). Higher levels of injection of vitamin E (7 500 IU) led to more than 2-fold increases in plasma vitamin E content compared with 4 500 IU injected during the 3 weeks post-treatment in non-lactating dairy cows (Charmley *et al,* 1992). The rate of vitamin E absorption was proportional to the amount injected (Hidiroglou and Karpinski, 1991).

Local swellings associated with disturbances of the general state of health have been observed in animals following injections of vitamin E preparations. Body temperature is one of the indicators of the general state of health of the animal and was therefore used as one of the parameters in this study.

The objectives of the present study were to determine the effects of different doses of vitamin E injection on body temperature and plasma α-tocopherol concentration in pigs, lambs and calves, because such data are limited.

MATERIALS AND METHODS

Animals and experimental design

Fifteen weaned Large White female pigs, 15 finishing male lambs of Arcott breed, and 15 Holstein female calves were used to study the effect of 3 levels of vitamin E injection, denoted as 1x, 3x, and 5x on body temperature and plasma α-tocopherol concentration. The 1x dose for pigs, lambs, and calves was 1 ml, 2 ml and 3 ml, respectively, as recommended by the manufacturer (Stuart Products, Bedford, TX, USA) based on age and body weight of each species. Doses of 3x and 5x were 3 times and 5 times the 1x dose for each species. Each millilitre of vitamin E preparation contained 500 IU of vitamin E (as D-α-tocopherol, a natural source of vitamin E) compounded with 20% ethyl alcohol and 1% benzyl alcohol (preservative) in an emulsified base. Fifteen animals of each species were randomly divided into 3 groups (1x, 3x, 5x) of 5 animals each. Vitamin E was injected intramuscularly in the brachiocephalicus muscle of the neck. The average age and body weight of pigs, lambs, and calves at the start of the experiment were 1 month nd 9.5 kg, 3 months and 40 kg, and 2 months and 52 kg, respectively. All experimental procedures were carried out according to the guidelines of the *Guide to the Care and Use of Experimental Animals.*

Rations

The pig ration was composed of corn (44.1%), barley (15%), soybean meal (25%), limestone (1.3%), mono-dicalcium phosphate (2%), lignin (1.5%), cobalt-iodized salt (0.4%), dry whey (7%), lysine (0.2%), tallow (2%), vitamin premix (0.75%), and mineral premix (0.75%). The vitamin premix contained wheat middlings (22.5%), choline chloride (16.67%), biotin (22.73%), calcium pantothenate (1.47%), vitamin A, 30 000 IU/g (5.5%), vitamin D3, 25 000 IU/g (0.44%), vitamin E, 50 IU/g (21.6%), vitamin K (1.6%), riboflavin (3.2%), niacin (0.89%), thiamine (0.6%), and vitamin B12 (2.8%). The mineral premix contained wheat middlings (71.45%), zinc oxide, 72% Zn (2.08%), ferrous sulphate monohydrate (5.16%), copper sulphate (0.64%), manganous oxide (0.67%), and selenium premix, 0.02% Se (20%). The lamb ration was composed of corn (62%), soybean meal (22%), dehydrated alfalfa (8%), limestone (1.5%), mono-dicalcium phosphate (0.4%), cobalt-iodized salt (0.8%), wet molasses (5%), mineral premix (0.2%), and vitamin premix (0.1%). The mineral premix contained cobalt-iodized salt (87%), zinc sulphate (6.9%) and manganese sulphate (6.1%). The vitamin premix contained wheat middlings (76.1%), vitamin E, 50 IU/g (8.42%), vitamin A, 30 000 IU/g (14.03%), and vitamin D3, 25 000 IU/g (1.45%). The calf ration was composed of soybean meal (20%), corn (24.65%), barley (38.5%), dehydrated alfalfa (5%), wheat bran (5%), wet molasses (3.75%), mono-dicalcium phosphate (0.5%), limestone (0.7%), mineral premix (0.9%), and vitamin premix (1%). The mineral premix contained cobalt-iodized salt (76.67%), sulphate potash magnesia (21.11%), zinc oxide (0.74%), manganous oxide (0.74%), and anhydrous cupric oxide (0.74%). The vitamin premix contained wheat middlings (89.39%), bovatec (2.66%), vitamin E, 50 IU/g (6.84%), vitamin A, 30 000 IU/g (0.95%), and vitamin D3, 25 000 IU/g (0.16%). Water was available *ad libitum* during the whole experiment.

Sample preparation and analytical methods

Blood samples (10 ml) from each animal were collected from jugular vein on d 0 (before injection), 1, 2, 3, 4, 5 and 8 d after the injection, into vacutainers containing EDTA. Each day the rectal body temperature was taken by digital thermometer at 10 am. The blood samples were centrifuged immediately after collection, and the plasma was removed and stored at –20°C until assay. High performance liquid chromatography (HPLC) with a fluorescence detector was used for analysis of α-tocopherol in plasma (McMurray and Blanchflower, 1979).

Statistical analysis

Data on body temperature and plasma α-tocopherol concentrations were analyzed separately for each species by least-squares analysis of variance using GLM procedure (Statistical Analysis System Institute Inc, 1986). The model included the effects of vitamin E level, sampling day and interaction between vitamin E level and sampling day.

RESULTS

Pigs

All pigs receiving 500 IU of vitamin E appeared to be normal with no swelling at the site of injection. Severe edema was observed around the neck in the ventral cervical region 2 d after injection in all the pigs injected with 1 500 IU and 2 500 IU vitamin E. The edema was firm and slowly disappeared during the next 3–4 d. Least squares analysis of variance revealed significant ($P < 0.01$) effects of vitamin E level and sampling day on body temperature and plasma α-tocopherol concentration. The body temperature of the pigs injected with either 1 500 IU or 2 500 IU of vitamin E was significantly ($P < 0.01$) higher than the body temperature of pigs injected with 500 IU (table I). The body temperature increased with increase in the time after injection and was highest on d 2 after the injection. The plasma α-tocopherol concentration increased with increase in the dose of vitamin E injection. The plasma α-tocopherol concentration was highest on d 1 after injection and then declined slowly throughout the remaining observation period.

Lambs

All lambs given 1 000 IU of vitamin E appeared to be normal with no swelling at the site of injection. A swelling in the brisket area was observed on d 3 after injection in all the lambs injected with 3 000 or 5 000 IU of vitamin E. The swollen area was firm to the touch and moved as a unit when pressed. The swelling gradually disappeared over a period of 3 to 4 d. Least-squares analysis of variance revealed significant ($P < 0.01$) effects of vitamin E level and sampling day on body temperature and plasma α-tocopherol concentration. The body temperature was significantly ($P < 0.05$) higher for the lambs injected with either 3 000 or 5 000 IU of vitamin E than those injected with 1 000 IU (table II). The plasma α-tocopherol concentration increased with increase in the dose of vitamin E injection. The higher level of vitamin E injection (3 000 IU) led to more than a 2-fold increase ($P < 0.05$) in plasma α-tocopherol concentration compared with the lambs injected with 1 000 IU of vitamin E during the first week after injection. The plasma α-tocopherol concentration was highest on d 1 post-injection and declined slowly throughout the remaining observation period.

Calves

All calves given 1 500 IU of vitamin E appeared to be normal with no swelling at the site of injection. Two out of the 5 calves injected with 4 500 IU of vitamin E and 3 out of the 5 calves injected with 7 500 IU of vitamin E developed a small swelling of about 2 cm in diameter in the brisket area 1 d after

Table I. Least-squares means and standard errors for body temperature and plasma α-tocopherol in pigs injected with various doses of vitamin E.

Variable	*Body temperature (°C)*	*α-tocopherol (μg/ml)*
Vitamin E injected (IU)		
0	39.6 ± 0.12	1.27 ± 0.18
500	39.7 ± 0.06	3.54 ± 0.41
1 500	40.0 ± 0.06	13.12 ± 0.41
2 500	40.0 ± 0.06	24.63 ± 0.41
Day of sampling		
0	39.6 ± 0.09	1.40 ± 0.62
1	39.8 ± 0.09	53.53 ± 0.62
2	40.5 ± 0.09	30.09 ± 0.62
3	40.4 ± 0.09	4.34 ± 0.62
4	39.8 ± 0.09	2.86 ± 0.62
5	39.6 ± 0.09	2.35 ± 0.62
8	39.4 ± 0.09	1.76 ± 0.62

Table II. Least-squares means and standard errors for body temperature and plasma α-tocopherol in lambs injected with various doses of vitamin E.

Variable	*Body temperature (°C)*	*α-tocopherol (μg/ml)*
Vitamin E injected (IU)		
0	40.2 ± 0.15	0.25 ± 0.13
1 000	40.3 ± 0.08	2.04 ± 0.62
3 000	40.5 ± 0.08	5.80 ± 0.62
5 000	40.6 ± 0.08	17.43 ± 0.62
Day of sampling		
0	40.5 ± 0.13	0.13 ± 0.95
1	40.5 ± 0.13	25.34 ± 0.95
2	40.9 ± 0.13	19.93 ± 0.95
3	40.8 ± 0.13	7.69 ± 0.95
4	40.3 ± 0.13	3.90 ± 0.95
5	40.2 ± 0.13	1.30 ± 0.95
8	39.9 ± 0.13	0.66 ± 0.95

injection. This swelling disappeared gradually over a period of 2–3 d in both groups. Least squares analysis of variance revealed significant ($P < 0.05$) effects of vitamin E level on plasma α-tocopherol concentrations, and sampling day on body temperature and plasma α-tocopherol concentrations. On d 2 after injection, the body temperature of the calves was highest (40°C) and then slowly declined to the initial body temperature at d 0 by d 5 (table III). The plasma α-tocopherol concentration increased with increase in the dose of vitamin E injection. The plasma α-tocopherol concentration was highest on d 1 after injection and declined slowly thereafter (table III).

Table III. Least-squares means and standard errors for body temperature and plasma α-tocopherol in calves injected with various doses of vitamin E.

Variable	*Body temperature (°C)*	*α-tocopherol (μg/ml)*
Vitamin E injected (IU)		
0	39.0 ± 0.13	0.54 ± 0.22
1 500	39.2 ± 0.09	8.36 ± 0.49
4 500	39.2 ± 0.09	10.81 ± 0.49
7 500	39.3 ± 0.09	16.73 ± 0.49
Day of sampling		
0	39.1 ± 0.13	0.46 ± 0.75
1	39.2 ± 0.13	36.31 ± 0.75
2	40.0 ± 0.13	23.63 ± 0.75
3	39.4 ± 0.13	12.12 ± 0.75
4	39.2 ± 0.13	5.20 ± 0.75
5	39.1 ± 0.13	3.70 ± 0.75
8	38.8 ± 0.13	2.36 ± 0.75

DISCUSSION

Animals of all 3 species receiving the manufacturer's recommended dose (1x) of vitamin E injection appeared to be normal with no swelling at the site of injection. Hidiroglou

and Karpinksi (1991) observed no local swelling in the area of intramuscular injection of vitamin E in sheep and no deviation in the body temperature. The body temperature of the animals injected with higher than recommended doses (3x, 5x) of vitamin E was higher than body temperature of animals injected with the 1x dose. Edema was also observed around the site of vitamin E injection in most of the animals injected with either 3x or 5x dose of vitamin E. Behrens *et al* (1975) reported some disturbances of health in sheep (local swelling, edema, lymphadenitis) by intramuscular injection of vitamin E in oily carrier substances. Caravaggi *et al* (1968) and Hidiroglou *et al* (1970) reported that a peak in plasma α-tocopherol concentraton occurs within 24 h of intramuscular administration, and in most cases, declines over the next 4 d. Similar results were observed in the present study; plasma α-tocopherol concentration increased with increase in the dose of vitamin E injection and was highest on d 1 after injection in animals of all 3 species. Charmley *et al* (1992) reported that the higher level of vitamin E injection (7 500 IU) led to a more than 2-fold increase in plasma α-tocopherol concentration compared with the lower level (4 500 IU) of vitamin E injection in non-lactating dairy cows. Similar results were observed in the present study when 3 000 IU of vitamin E injection led to more than a 2-fold increase in plasma α-tocopherol concentration compared to lambs injected with 1 000 IU of vitamin E. Hidiroglou and Atwal (1989) showed a positive relationship between plasma α-tocopherol concentration and vitamin E supplementation up to approximately 5 000 IU of supplemental vitamin E following a single intraperitoneal injection in dairy cows.

Injections of vitamin E in higher than manufacturer recommended doses (3x, 5x) may result in higher body temperature and edema around the site of injection for a few days after injection. This may be the result of necrosis in the tissues associated with edematization, which can be attributed to the large volume of injection, carrier substances, the vitamins, or the combination of these. The plasma α-tocopherol concentration increased with increase in the dose of vitamin E injection and was the highest on d 1 after injection.

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