

## Effect of oral supplements of vitamin A on the plasma retinol levels in calves and their immunological unresponsiveness \*

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**Summary** — Forty calves were used to evaluate the immune response effects induced by a wide range of dietary vitamin A intake levels. The immune response was not affected by the tested doses of vitamin A administered orally during a 56 day period. There was no change in plasma immunoglobulin concentrations and no difference in specific antibody titres following injection of keyhole limpet haemocyanin (KLH) at 10, 20 and 40 days of age. Some small and temporary variations were recorded in the plasma vitamin A concentration following daily oral administration of various doses up to 10 000 IU. Only oral supplementation of 20 000 IU of vitamin A daily over 50 days significantly increased the plasma level of vitamin A.

**vitamin A / plasma retinol / immune status / calves**

**Résumé** — Effet de la supplémentation orale de vitamine A sur le taux de rétinol plasmatique chez le veau et leur absence de réponse immunologique. Quarante veaux ont été utilisés pour évaluer la réponse immunitaire induite par des apports alimentaires très variés de vitamine A. La réponse immunitaire n'était pas affectée par les doses de vitamine A administrées oralement pendant une période de 56 jours. Il n'y avait pas de modifications de concentrations plasmatiques en Ig, ni des titres spécifiques des anticorps après injection de KLH (Keyhole Limpet Haemocyanin) à 10, 30 et 40 jours d'âge. Quelques faibles variations temporaires de la vitamine A plasmatique ont été observées après l'administration orale de diverses doses jusqu'à 10 000 IU. Seule la supplémentation orale de 20 000 UI de vitamine A par jour pendant 50 jours a conduit à une augmentation significative de la concentration du plasma en vitamine A.

**vitamine A / rétinol plasmatique / immunité / veau**

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## INTRODUCTION

A number of investigations support the role of retinoids as an immune stimulant in animals whose vitamin A nutritional status is normal (Ross, 1992). Early studies have demonstrated the importance of vitamin A to calf health. Scouring was reduced in calves having a higher plasma vitamin A level (Spielman et al, 1949), supporting an earlier finding that feeding vitamin A to calves reduced the incidence of scours and associated mortality (Lundquist and Phillips, 1943). Hidiroglou et al (1995) previously reported that vitamin C and alpha-tocopherol behaved as antioxidants in calves, scavenging free radicals in the lipid phase. Foods containing vitamin A also protect against the formation of oxygen radicals and lipid peroxidation. Schrimshaw et al (1968) suggested that vitamin A deficiency facilitates bacterial and viral infections. Dennert (1984), Nauss (1986) and Tjoelker et al (1988) reported the protective effects of vitamin A against infections. Diarrhea and pneumonia in young calves are the major impediments to achieving sustainable economic development in red meat production. The objective of the present experiment was to obtain additional data on the efficacy of vitamin A supplements as immunomodulators in calves.

## MATERIALS AND METHODS

### *Management*

Calves were housed in a heated, insulated, forced-air ventilated room with individual pens (1.2 x 2 m), consisting of solid puckboard sides, open front, a sand base and straw bedding to pack manure. Drinking water was available for ad libitum consumption. The general health of all the calves was good throughout the experiment. Care was taken to avoid stressing the calves during blood sampling.

### *Animals*

Forty newborn calves from Holstein cows were used. All calves received at least 50 mL kg<sup>-1</sup> body weight (BW) of the colostrum within the first 8 to 12 h, and were subsequently fed whole milk at 5% of their BW in the morning and in the evening. The milk was collected from the CFAR dairy herd in Ottawa. Ten calves were assigned to each of the four treatment groups: i) 0 IU, ii) 5 000 IU, iii) 10 000 IU and iv) 20 000 IU of vitamin A given orally, dissolved in the daily milk. Vitamin A propionate in corn oil plus an emulsifying agent (Tween 80) was used as the vitamin A source. All calves were given intramuscular injections of 50 mg keyhole limpet haemocyanin (KLH Megathura Crenulata, Sigma Chem, St Louis, MO, USA) at 10, 20 and 40 days of age. Feeding and management procedures were the same for all calves.

### *Analytical methods*

Blood samples were collected in 10 mL heparinized vacuum tubes, at birth and at 3, 8, 12, 15, 19, 22, 25, 29, 33, 36, 40, 43, 46, 50, 53 and 56 days of age. The tubes were centrifuged at 4 °C for plasma in a refrigerated centrifuge for 15 min at 1 000 g. Plasma was stored at -20 °C until being analyzed for vitamin A. Immunoglobulins (IgG1, IgG2, IgM) were determined by the single radial immunodiffusion procedure using a specific SRID kit (VMRD Inc, Pullman, WA, USA), and the standards and guidelines provided by the manufacturer. The method for the determination of KLH antibody titre was similar to one reported previously (Nowotny, 1969). The haemagglutinations were performed using KLH-coated sheep red blood cells prepared by the tanned red blood cell technique. KLH was used at a concentration of 5 mg/mL<sup>-1</sup>. The titre for the KLH antibody was given as the reciprocal of the last dilution which gave a positive haemagglutination. The KLH titre values were log transformed (Base 2) before analysis. Quantitation of vitamin A in blood plasma (Driskell et al, 1982) and milk (Batra and Hidiroglou, 1995) was performed by using high-performance liquid chromatography (HPLC) using a fluorescence detector (Perkin-Elmer LS-4, Oak Brook, IL, USA). The HPLC system consisted of a M6000 pump and WK septumless injector (Waters Associates Inc, Milford,

MA, USA). A Perkin-Elmer 650-150 fluorescence spectrophotometer equipped with a microflow cell unit was used for quantitation. Wavelength settings for vitamin A were 326 and 480 for excitation and emission, respectively. The column was a  $\mu$  Bondapak C<sub>18</sub> (3.9 mm x 30 cm) of 10  $\mu$ m particle size obtained from Waters Association Inc. Elution was performed with methanol:water (98:2 v/v) at a flow rate of 2 m/min.

## STATISTICS

The statistical procedures included repeated measure ANOVA using SAS (1985). The model included the effects of treatment, calves within treatment, sampling time, interaction of sampling time within treatment and error. Calves within treatment were used to

test the treatment effect, and the error was used to test the sampling time and interaction of sampling time with treatment. Duncan's multiple range test was used to test differences between means once a significant effect of treatment was indicated by ANOVA. Significance was declared at  $P < 0.05$ .

## RESULTS AND DISCUSSION

During the early neonatal life of the calves (from birth to 72 h after) there was a significant increase ( $P < 0.001$ ) in plasma vitamin A concentration (table I). The calves at birth had a very low vitamin A concentration ( $< 0.120 \mu\text{g/mL}$  plasma) because of

**Table I.** Means  $\pm$  standard error (SE) of vitamin A concentrations in blood plasma of calves from birth to 56 days of age given 0, 5 000, 10 000 or 20 000 IU of daily vitamin A supplement orally.

Age (days)	Vitamin A in plasma ( $\mu\text{g/mL}$ ) of calves							
	Group 1 0 IU control		Group 2 5 000 IU		Group 3 10 000 IU		Group 4 20 000 IU	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0.106	0.012	0.108	0.010	0.117	0.010	0.110	0.010
3	0.289	0.035	0.274	0.027	0.291	0.027	0.290	0.027
8	0.235 <sup>a</sup>	0.029	0.281 <sup>a</sup>	0.023	0.282 <sup>a</sup>	0.023	0.347 <sup>b</sup>	0.023
12	0.202 <sup>b</sup>	0.028	0.252 <sup>a</sup>	0.022	0.297 <sup>a</sup>	0.022	0.309 <sup>b</sup>	0.022
15	0.269	0.032	0.260	0.025	0.297	0.025	0.298	0.025
19	0.262	0.026	0.273	0.020	0.296	0.020	0.321	0.020
22	0.264	0.029	0.259	0.023	0.324	0.023	0.310	0.023
25	0.195 <sup>a</sup>	0.025	0.246 <sup>a</sup>	0.019	0.299 <sup>b</sup>	0.019	0.305 <sup>b</sup>	0.019
29	0.212 <sup>a</sup>	0.024	0.238 <sup>a</sup>	0.018	0.244 <sup>a</sup>	0.018	0.346 <sup>b</sup>	0.018
33	0.207 <sup>a</sup>	0.022	0.224 <sup>a</sup>	0.017	0.260 <sup>a</sup>	0.017	0.352 <sup>b</sup>	0.017
36	0.220 <sup>a</sup>	0.030	0.246 <sup>a</sup>	0.023	0.301 <sup>ab</sup>	0.023	0.350 <sup>b</sup>	0.023
40	0.208 <sup>c</sup>	0.020	0.267 <sup>a</sup>	0.015	0.294 <sup>a</sup>	0.015	0.333 <sup>b</sup>	0.015
43	0.206 <sup>a</sup>	0.026	0.260 <sup>a</sup>	0.020	0.281 <sup>ac</sup>	0.020	0.341 <sup>b</sup>	0.020
46	0.235 <sup>a</sup>	0.023	0.245 <sup>a</sup>	0.018	0.273 <sup>a</sup>	0.018	0.340 <sup>b</sup>	0.018
50	0.217 <sup>a</sup>	0.030	0.258 <sup>a</sup>	0.023	0.304 <sup>ab</sup>	0.023	0.341 <sup>b</sup>	0.023
53	0.217 <sup>a</sup>	0.026	0.269 <sup>a</sup>	0.020	0.308 <sup>ac</sup>	0.020	0.377 <sup>b</sup>	0.020
56	0.212 <sup>a</sup>	0.024	0.268 <sup>a</sup>	0.018	0.335 <sup>b</sup>	0.018	0.380 <sup>b</sup>	0.018

<sup>abc</sup> Means in the row with different superscripts differ statistically ( $P < 0.05$ ).

restricted transplacental transport of this vitamin (Bouda et al, 1980; Bardos et al, 1991). The increase at 3 days ( $> 0.274 \mu\text{g}/\text{mL}$  plasma) was probably related to the high intake of vitamin A through the colostrum and intestinal absorption of this vitamin (Perthes et al, 1985; Özpınar et al, 1988).

Plasma vitamin A concentrations are generally refractory in nature. They are not always immediately responsive to dietary changes and have limitations in their usefulness as an estimator of vitamin A status. According to Flachowsky et al (1990), the level of plasma vitamin A which is not reflective of that in the liver is unsuitable for estimation of the vitamin A status of calves and this is because plasma retinol concentration is homeostatically regulated (Donoghue and Kronfeld, 1983). Friedman and Sklan (1989) reported that despite the fact that hepatic levels of vitamin A represent the major portion of total body vitamin A, the circulating vitamin A levels might influence immune responsiveness more directly than body stores.

During the 56 day experimental period, few changes (table I) were apparent between doses at 3 and 56 days in calves on control and 5 000 IU vitamin A supplementation. A significant increase in plasma vitamin A concentrations occurred only in the group receiving 20 000 IU daily.

In the calves receiving 20 000 IU of vitamin A, plasma vitamin A was significantly higher than in control calves or calves supplemented with 5 000 IU of vitamin A. The level of vitamin A in the plasma of calves in group 4 was also higher than that in group 3 (table I), but the difference was not always significant. The plasma vitamin A concentration in all calves was over  $0.200 \mu\text{g}/\text{mL}$ , indicating adequate vitamin A status (NRC, 1988). Surynek et al (1976) reported vitamin A plasma concentrations of  $0.150\text{--}0.320 \mu\text{g}/\text{mL}$  in normal calves during the first 4 months of life.

In the present experiment, the average concentration of vitamin A in bulk tank milk was  $220 \pm 35$  (SD)  $\text{IU}/100 \text{mL}^{-1}$  and this was close to  $254 \text{IU}/100 \text{mL}^{-1}$  reported in Quebec by Block and Farmer (1987). Paulicks et al (1991) observed, however, lower vitamin A milk values:  $159 \pm 36$  (SD)  $\text{IU}/100 \text{mL}^{-1}$ .

There was no difference in weight gain or feed intake due to the vitamin A treatments ( $P > 0.05$ ). The inclusion of various amounts of vitamin A in the dietary milk did not increase antibody production or anti-KLH antibody titres (figs 1 and 2). There were some nontreatment related temporal changes. The lack of effect of vitamin A supplementation on the Ig concentration in the serum, an indicator of B-cell function, may have been due to the status of the immune

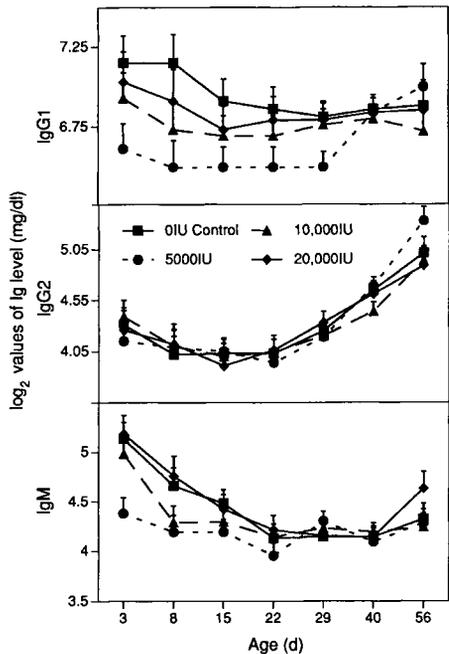
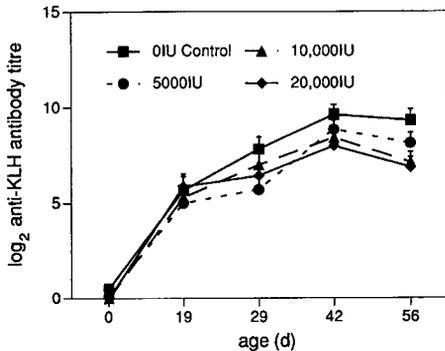


Fig 1. Effect of oral administration of various amounts of vitamin A daily to calves on immunoglobulin concentration.



**Fig 2.** Immune response to keyhole limpet haemocyanin (KLH) units are log<sub>2</sub> values of the reciprocal of the last dilution of plasma that gave a positive ELISA reading.

system during the experimental period. It is possible that the administered vitamin A dose in the present experiment was below the threshold amount necessary to produce an immunological response. It may also be, as was reported by Tomkins and Hussey (1989) in studies with humans, that the impact of vitamin A administration on the humoral system is very small. Ritacco et al (1986) observed in sheep as well that the administration of vitamin A (orally or by injection) did not affect the humoral response. Additional experiments with cattle are needed to evaluate the effects of megadosages of vitamin A on immune function.

All calves completed this study according to the protocol and none reported subjective adverse events. There were no clinically relevant changes of any vital signs. Thus, vitamin A toxicosis is not likely to become a practical problem in supplementing ruminant rations at either normal feeding or therapeutic levels (Mitchell, 1967). This may concern the human who can absorb high vitamin A-containing tissues, and namely the liver (Hidiroglou, 1996).

## CONCLUSION

It is apparent from the present study involving healthy calves that there were very few changes in the concentration of immunoglobulins and KLH antibody titres in the plasma following the oral administration of various amounts of vitamin A.

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