Seasonal Changes in Serum Vitamin A, Vitamin E and β -Carotene Concentrations in Japanese Black Breeding Cattle in Hyogo Prefecture

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(Received 29 July 2002/Accepted 6 May 2003)

ABSTRACT. The influence of the season on serum vitamin A, E and β -carotene concentrations was studied in Japanese Black breeding cattle. Blood samples were collected from 13 cows once a month for a year to determine the serum vitamin concentrations. Mean serum concentrations ($\pm$ SE) of retinol, α -tocopherol and β -carotene were 21.3 ± 0.5 $\mu\text{g/dl}$ (range 9.9 to 44.9), 215.9 ± 8.0 $\mu\text{g/dl}$ (range 59.7 to 551.6) and 67.3 ± 5.1 $\mu\text{g/dl}$ (range 10.6 to 425.2), respectively. All these concentrations were lower than the recommended levels. Serum retinol and α -tocopherol concentrations in summer were lower than those in winter. These results suggest that the vitamin A, E and β -carotene status of Japanese Black breeding cows is inadequate, and feeding high quality forages or dietary vitamin supplementation during summer should be recommended.

KEY WORDS: bovine, fat soluble vitamin, seasonal change.

J. Vet. Med. Sci. 65(9): 1001–1002, 2003

Fat-soluble vitamins, such as vitamin A, E and β -carotene, are important nutrients for maintaining health in dairy cattle. Dietary vitamin A and β -carotene enhanced host defence mechanisms in periparturient dairy cows [8]. Dietary supplementation with vitamin E enhanced bovine phagocytic cell function [6]. These nutrients were also related to reproductive performance in dairy cows [3]. The concentrations of vitamin E [5] and β -carotene [7] in the blood of cows were affected by the type of dietary forage. In Hyogo prefecture, Japanese Black breeding cattle are mainly raised indoors all year round, and little is known about the status of these vitamins. The purpose of this study was to evaluate the influence of the season on serum vitamin A, E and β -carotene concentrations in Japanese Black breeding cattle.

Thirteen Japanese Black breeding cows, 2 to 17 years old, were used. The average age of the cows was 6.1 years and they were raised indoors on 6 farms in Hyogo prefecture. All the cows were clinically healthy and 9 calved during the study period. The main roughage source was rice straw, rye grass straw, sudan hay, fescue grass straw, oats hay or cane top. All the cows were given a supplement of barley grain, wheat bran or commercial concentrates. Six cows on 3 farms were fed sun-cured oats and rye grass from winter to spring, and 4 of these cows were given wild grass in summer. The remaining 7 cows on 3 farms were fed the main roughage described above all the year round. Blood samples were taken from all cows once a month between July 1995 and June 1996 for the determination of serum vitamin concentrations. Concentrations of retinol, α -tocopherol and β -carotene were analyzed by high performance liquid chromatography (HPLC) using a modification of the method of Arnaud *et al.* [1]. In brief, 1 ml of serum was placed into a centrifuge tube together with 30 mg of sodium ascorbate and 1 ml of α -tocopherol acetate solution (4 $\mu\text{g/ml}$ ethanol) as an internal standard, and mixed. After 6 ml of

hexane was added to the tube, the samples were vigorously shaken for 10 min. The mixture was centrifuged at 1,000 rpm for 5 min and 4 ml of the hexane layer was removed and evaporated under nitrogen at 37°C. The residue was dissolved in 0.5 ml of methanol by sonication and filtered through a Cosmonice Filter S (pore size 0.5 μm , Nacalai Tesque, Inc., Kyoto, Japan), and 40 μl was injected into the HPLC column. The instruments used in this assay were as follows: the HPLC equipment was a Shimadzu LC-6A (Shimadzu Co., Ltd., Kyoto) with a Resolve C18 3.9×150 mm column (Nihon Millipore Ltd., Tokyo). The eluent was 100% methanol and the flow rate was 0.9 ml/min. Retinol, α -tocopherol and β -carotene were detected at wavelengths of 325, 292 and 450 nm with a Shimadzu SPD-10AV programmable multiwavelength detector. Serum concentrations of cholesterol and urea nitrogen were determined in 6 cows on 3 farms with COBAS READY (ARKRAY, Inc., Kyoto). Data were analyzed by one-way repeated measures analysis of variance (ANOVA). Differences between sampling months were tested by paired Student's *t*-test.

Mean serum concentrations ($\pm$ SE) of retinol, α -tocopherol and β -carotene were 21.3 ± 0.5 $\mu\text{g/dl}$ (range 9.9 to 44.9), 215.9 ± 8.0 $\mu\text{g/dl}$ (range 59.7 to 551.6) and 67.3 ± 5.1 $\mu\text{g/dl}$ (range 10.6 to 425.2), respectively. Serum retinol concentrations from November to April were significantly higher than those in July (Fig. 1). As observed in retinol, serum α -tocopherol concentrations from October to January and in May were significantly higher than those in July. Serum β -carotene concentrations were lowest in February and highest in April, but there was no significant change throughout study period. In both serum cholesterol and urea nitrogen concentrations, there was a tendency to decrease in summer and increase in winter (Fig. 2). The concentrations of serum retinol and α -tocopherol showed similar seasonal changes, *i.e.* low in summer and high in winter. A high ambient temperature results in decreased feed intake and

Fig. 1. Mean serum concentrations ($\pm$ SE) of retinol (●), α -tocopherol (■) and β -carotene (▲) in Japanese Black breeding cows ($n=13$) from July 1995 through June 1996. *, **Significantly differs from the value in July at $P<0.05$ and $P<0.01$, respectively.

subsequent deficient uptake of essential nutrients [9]. In this study, feed intake was not recorded but seasonal changes in both cholesterol and urea nitrogen were similar to those in retinol and α -tocopherol concentrations. Therefore, decreased serum concentrations of retinol and α -tocopherol may be related to decreased feed intake due to the high ambient temperature during summer. A tendency to higher serum β -carotene concentrations from April to June may be associated with the effects of forages, because 6 cows on 3 farms received sun-cured oats and rye grass during this season. Cows fed pasture grass had higher serum β -carotene concentrations than those fed hay [7]. Beta-carotene is converted to vitamin A in the intestinal mucosa. In this study, there was no correlation between the β -carotene and retinol concentrations. This may be attributed to the β -carotene concentrations, and which are too low to affect retinol concentrations. Normal serum retinol concentrations range from 25 to 60 $\mu\text{g/dl}$, and the serum α -tocopherol concentra-

Fig. 2. Mean serum concentrations ($\pm$ SE) of cholesterol (○) and urea nitrogen (□) in Japanese Black breeding cows ($n=6$) from July 1995 through June 1996.

tions $>400 \mu\text{g/dl}$ have been considered to indicate adequacy in adult cattle [2]. Desirable blood β -carotene concentrations are considered to be in excess of 300 $\mu\text{g/dl}$ in dairy cattle [4]. The mean serum concentrations of retinol, α -tocopherol and β -carotene measured in this study were lower than the recommended values for beef and dairy cattle. These results indicate that the vitamin A, E and β -carotene status of Japanese Black breeding cattle housed and fed mainly low quality forage or hay is inadequate. We should take into consideration feeding high quality forage or dietary vitamin supplementation during summer to provide possible benefits for enhancing immune function and reproductive performance.

REFERENCES

1. Arnaud, J., Fortis, I., Blachier, S., Kia, D. and Favier, A. 1991. *J. Chromatogr.* **572**: 103–116.
2. Frye, T. M., Williams, S. N. and Graham, T. W. 1991. *Vet. Clin. North Am. : Food Anim. Pract.* **7**: 217–275.
3. Harrison, J. H., Hancock, D. D. and Conrad, H. R. 1984. *J. Dairy Sci.* **67**: 123–132.
4. Herdt, T. H. and Stowe, H. D. 1991. *Vet. Clin. North Am.: Food Anim. Pract.* **7**: 391–415.
5. Hidiroglou, M. and Batra, T. R. 1994. *J. Dairy Sci.* **77**: 190–195.
6. Hogan, J. S., Smith, K. L., Weiss, W. P., Todhunter, D. A. and Schockey, W. L. 1990. *J. Dairy Sci.* **73**: 2372–2378.
7. Jukola, E., Hakkarainen, J., Saloniemi, H. and Sankari, S. 1996. *J. Dairy Sci.* **79**: 831–837.
8. Michal, J. J., Heirman, L. R., Wong, T. S. and Chew, B. P. 1994. *J. Dairy Sci.* **77**: 1408–1421.
9. Shearer, J. K. and Beede, D. K. 1990. *Agri-Pract.* **11**: 6–17.