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Author(s)	SENNA, Kazuhiro
Citation	Japanese Journal of Veterinary Research, 33(1-2), 93-93
Issue Date	1985-04-30
Doc URL	<a href="https://hdl.handle.net/2115/2348">https://hdl.handle.net/2115/2348</a>
Type	departmental bulletin paper
File Information	KJ00002374320.pdf



DETERMINATION OF BOVINE SERUM OR PLASMA RETINOL, RETINYL  
PALMITATE AND  $\alpha$ -TOCOPHEROL BY HIGH-PERFORMANCE  
LIQUID CHROMATOGRAPHY

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Analytical conditions for determination of bovine serum (or plasma) retinol, retinyl palmitate and  $\alpha$ -tocopherol by high-performance liquid chromatography (HPLC) were determined. By these methods, Radial PAK C<sub>18</sub> (5  $\mu$ m particle size) was used as an HPLC column, and retinyl acetate was used as an internal standard (I. S.). Then the serum (or plasma) retinol, retinyl palmitate and  $\alpha$ -tocopherol levels in several herds of beef and dairy cattle in Hokkaido prefecture were determined.

(1) A condition for simultaneous determination of serum (or plasma) retinol and retinyl palmitate was determined. The serum sample (200  $\mu$ l) was deproteinated with ethanol containing I. S., extracted with n-hexane and injected on an HPLC column. Elution was performed by the methanol/tetrahydrofuran/water gradient solvent system (from 51 : 35 : 14 to 46 : 50 : 4 for 10 min) at 3.0ml/min for 12min. Detection was performed with a fluorometer (exi. 330nm, emi. 480nm). The within-day coefficients of variation (C. V.) of this method were 1.79% for retinol and 7.08% for retinyl palmitate.

(2) A condition for simultaneous determination of serum (or plasma) retinol and  $\alpha$ -tocopherol was determined. The serum sample (300 or 500  $\mu$ l) was treated by a similar procedure to (1). Elution was performed by methanol/water (98 : 2) at 4.0ml/min for 6.5min. Detection was performed with a UV spectrophotometer (325nm) for retinol and I. S., and a fluorometer (exi. 295nm, emi. 34nm) for  $\alpha$ -tocopherol. The within-day C. V. of this method were 1.10% (for extracts from 300  $\mu$ l of serum) and 1.01% (500  $\mu$ l) for retinol, and 1.95% (300  $\mu$ l) and 1.08% (500  $\mu$ l) for  $\alpha$ -tocopherol. The day-to-day C. V. were 3.18% (300  $\mu$ l) and 3.48% (500  $\mu$ l) for retinol, and 2.5% (300  $\mu$ l) and 3.4% (500  $\mu$ l) for  $\alpha$ -tocopherol.

(3) In a herd of beef cattle, the feeder cattle under 4 months of age showed a low plasma retinol ( $95.9 \pm 34.5$  IU/dl) and low level of  $\alpha$ -tocopherol ( $91.8 \pm 76.8$   $\mu$ g/dl). The plasma retinol and  $\alpha$ -tocopherol levels for the feeding cattle over 5 months of age were  $159.0 \pm 24.8$  IU/dl and  $148.1 \pm 50.6$   $\mu$ g/dl, respectively. The plasma retinyl palmitate level for most of the cattle in this herd was under 4IU/dl.

(4) The serum retinol and  $\alpha$ -tocopherol levels for dairy cows were  $165.5 \pm 37.0$  IU/dl and  $547.4 \pm 230.3$   $\mu$ g/dl, respectively. These values varied widely with the farms, seasons and diets.