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\textsubscript{18} (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±34.5IU/dl) and low level of \( \alpha \)-tocopherol (91.8±76.8 \( \mu \)g/dl). The plasma retinol and \( \alpha \)-tocopherol levels for the feeding cattle over 5 months of age were 159.0±24.8IU/dl and 148.1±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±37.0IU/dl and 547.4±230.3 \( \mu \)g/dl, respectively. These values varied widely with the farms, seasons and diets.