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

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Analytical conditions for determination of bovine serum (or plasma) retinol, retinyl palmitate and α -tocopherol by high-performance liquid chromatography (HPLC) were determined. By these methods, Radial PAK C₁₈ (5 μ 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 α -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 μ 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 α -tocopherol was determined. The serum sample (300 or 500 μ 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 α -tocopherol. The within-day C. V. of this method were 1.10% (for extracts from 300 μ l of serum) and 1.01% (500 μ l) for retinol, and 1.95% (300 μ l) and 1.08% (500 μ l) for α -tocopherol. The day-to-day C. V. were 3.18% (300 μ l) and 3.48% (500 μ l) for retinol, and 2.5% (300 μ l) and 3.4% (500 μ l) for α -tocopherol.

(3) In a herd of beef cattle, the feeder cattle under 4 months of age showed a low plasma retinol (95.9 ± 34.5 IU/dl) and low level of α -tocopherol (91.8 ± 76.8 μ g/dl). The plasma retinol and α -tocopherol levels for the feeding cattle over 5 months of age were 159.0 ± 24.8 IU/dl and 148.1 ± 50.6 μ 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 α -tocopherol levels for dairy cows were 165.5 ± 37.0 IU/dl and 547.4 ± 230.3 μ g/dl, respectively. These values varied widely with the farms, seasons and diets.