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APPLICATIONS OF RADIOIMMUNOASSAY AND RELATED METHODS IN ANIMAL SCIENCE

PAŃSTWOWE WYDAWNICTWO NAUKOWE

ENZYME IMMUNOASSAY FOR PROGESTERONE APPLIED TO MILK SAMPLES

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The enzyme immunoassay which we adapted for the progesterone determination in milk samples is a modification of the assay, described by

Joyce *et al.* The assay involves the use of horseradish peroxidase as the label which was coupled to 11α-hydroxyprogesterone-11-hemisuccinate by a mixed anhydride procedure. Antiserum to the bovine serum albumin derivative of 11α-hydroxyprogesterone-11-hemisuccinate was raised in rabbits. To facilitate separation of free from bound label, the antiserum was coupled to cellulose by the cyanogen bromide method. Under chosen conditions the extent of conjugate binding by the solid-phase antibody is proportional to the concentration of free progesterone in the incubation mixture. Separation of bound from free label is achieved by centrifugation and suitable washing of the cellulose pellets. The bound label is estimated by measuring the peroxidase activity of the pellets using an o-phenylene-diamine assay. The standard curve covers the range from 0.1 pmol to 10 pmol (0.03 to 3.1 ng).

The modified enzyme immunoassay is already being used to monitor the variation in milk progesterone levels during the oestrous cycle of the cow. Butteroil is prepared from milk and after purification by solvent extraction progesterone is determined in 5 μl aliquots. The results obtained by the enzyme immunoassay are in good agreement with those obtained by the radioimmunoassay used routinely in our laboratory, and show that for the present applications enzyme immunoassay is a suitable alternative to radioimmunoassay.