QUANTITATION OF BOVINE β LACTOGLOBULIN, β AND κ CASEIN IN MILK AND TISSUE CULTURE MEDIA BY ENZYME-LINKED IMMUNOSORBENT ASSAY

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The identification of factors that regulate the expression of bovine milk protein genes in vitro, either in mammary explants or epithelial cells in primary culture or in vivo requires specific, sensitive and rapid methodology for the measurement of the individual milk proteins. To date most studies have used tedious and expensive electrophoretic and chromatographic procedures to separate and quantify these proteins. Enzyme-linked immunosorbent assays (ELISA's) satisfy the above criteria and therefore in this study we have established specific ELISA's for β Lactoglobulin (BLG) and two major caseins of commercial importance, β casein and κ casein.

Specific monoclonal antibodies for β and κ casein were provided by Dr C.W. Beattie (USDA) while polyclonal antibodies for β and κ casein and BLG were produced in rabbits immunised with proteins in acrylamide excised from polyacrylamide gels in which a commercial source of these proteins (Sigma, St Louis MO) has been fractionated. The acrylamide containing the protein was lyophilised and rabbits received 500 ug of protein on each of seven occasions emulsified in Freunds complete (primary) and incomplete (boost) adjuvants. The maximum titres (l/dilution: ED 50 of colour reaction) achieved for BLG and β and κ casein were 1500, 1200 and 9000 respectively. For the BLG ELISA, plates are coated with polyclonal antibody and the concentration of BLG in the unknown sample determined by competitive binding with purified biotinylated BLG. Plates are then incubated with streptavidin-alkaline phosphatase and enzyme substrate and the colour reaction quantified. Crossreactivity with the other bovine milk proteins, BSA and culture medium components was below 1.4%.

β and κ casein are assayed by an 'antigen capture sandwich' ELISA using both monoclonal and polyclonal antibodies in which plates are coated with monoclonal antiserum and incubated with the unknown sample. The casein is then detected with a second polyclonal antibody and subsequently incubated with an alkaline phosphatase conjugated anti-rabbit IgG to which enzyme substrate is added to provide a colorimetric reaction. Although there is negligible crossreactivity with the whey and culture medium proteins, κ and αS casein crossreact in the β casein assay by 58% and 20% respectively while β and αS casein crossreact in the κ casein assay by 40% and 15% respectively.

Since there is no sequence homology between the caseins we suggest that the crossreactivity is due to clones being directed to the antigenic phosphate moieties on the molecules. In a preliminary study, treatment of both β and κ casein with alkaline phosphatase (20 units/mg for 2 hrs at 37°C) resulted in a significant reduction in the crossreactivity. This modification may then allow us to achieve our goal of establishing specific casein ELISA's.

ELISA	Working range	Sensitivity	Within assay SEM	Between assay SEM
β Lactoglobulin	300-20 ug/ml	100 ng/ml	8.23%	11.25%
β Casein	400-4 ug/ml	400 ng/ml	7.6%	8.7%
κ Casein	500-20 ug/ml	2 ug/ml	9.4%	7.5%

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