MAINTENANCE OF BOVINE MILK PROTEIN GENE EXPRESSION *IN VITRO*

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Nutritional responses in milk protein production result from increases in milk yield with little change in protein concentration. A major challenge to the dairy industry is to boost milk protein concentration and, in particular, the relative proportions of casein components required for manufacturing into dairy products. Before this can be achieved it is essential to identify the hormones and growth factors responsible for casein gene expression in mammary tissue.

We have established *an in vitro* culture method in which tissue is obtained surgically from late pregnant cows (21 days pre-partum) and divided into small explants (each of lmg) which are floated on siliconised lens paper (20 explants per well) in a rich growth medium (M199 containing antibiotics and buffering agents). These explants remain viable for up to 10 days. Milk protein gene expression is assessed using specific complementary DNA probes that recognise the relevant messenger RNA transcripts.

In an initial experiment, explants were incubated with insulin (I, 5ug/ml), cortisol (F, 500ng/ml), and prolactin (P, 5ug/ml) for 2, 4 and 6 days. It was observed that β and κ casein and β lactoglobulin gene expression decreased by day 2 to 57%, 58% and 60% respectively of the initial level and at day 4 to 18%, 7% and 12% respectively, which was then maintained to day 6 (Figure 1). Conversely β actin gene expression increased to 125% of initial expression by day 2 and was maintained at this elevated level to day 6 in culture; this is indicative of normal functionality of the tissue.

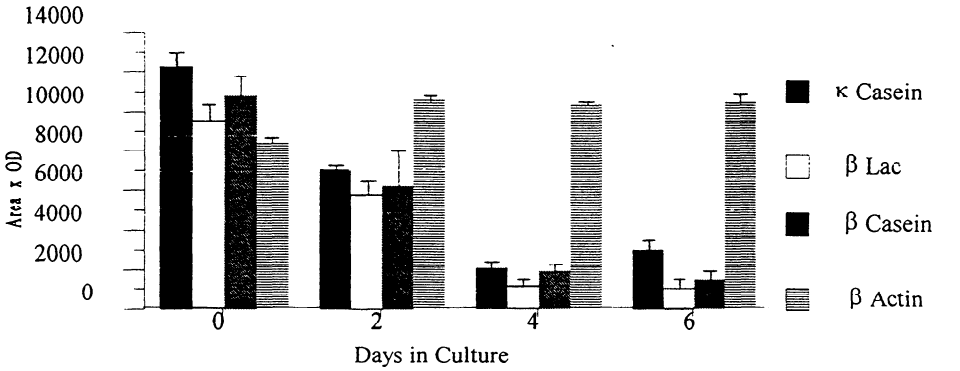

Figure 1. Milk protein gene expression in mammary explant culture maintained in I, F and P

Experiment 2 was designed to define the minimal concentration of P required to maintain gene expression. Explants were incubated with 5000, 500, 250,125, 62 and 0 ng/mL P in the presence of I and F at the same levels as above. β and κ casein gene expression was comparable to the levels of expression in Experiment 1 in all P Treatments from 5000 ng/mL to 62 ng/mL. However the absence of P resulted in a significant decrease in expression ($P<0.001$) which was similar to non-specific background. The expression of β lactoglobulin was controlled similarly although expression was increased significantly only when 500 ng/mL P was added. Again β actin gene expression was maintained regardless of P concentration.

We have established a culture system to examine the control of the expression of milk protein genes, albeit at a level approximating 15% of that in the lactating cow. This model has been used to identify a critical role for P in maintaining milk protein gene expression and is currently being used to identify other hormones and growth factors that regulate milk protein synthesis.