

# השפעת רמת חלבון החלב אלפא-לקטאלבומין וקיפולו על תפוקת החלב בפרות

## Effects of the milk protein $\alpha$ -lactalbumin levels and folding on milk production in dairy cows

**Roni Rak, Ph.D.;** cattle and sheep research, animal sciences, Volcani Institute-  
Agriculture Research Organization.

**Uzi Moallem, Ph.D.;** cattle and sheep research, animal sciences, Volcani Institute-  
Agriculture Research Organization.

## Research Summary

### Background and Hypothesis

The epithelial cells in the mammary gland are responsible for milk secretion. Milk yield naturally decreases after the peak production period, which correlates with an increase in programmed cell death (apoptosis) in the epithelial cells. Since epithelial cells are in direct contact with the milk, a direct association between milk composition and the producing tissue, which may lead to apoptosis-dependent death of the glandular epithelium, is possible.

alpha-lactalbumin ( $\alpha$ -LA) is a major component of cow's milk and is primarily involved in regulating lactose synthase during lactose production. In its normal, or native, conformation,  $\alpha$ -LA promotes milk production. However,  $\alpha$ -LA can undergo a conformational change, forming a complex (termed HAMLET/BAMLET- Human/Bovine Alpha-Lactalbumin Made Lethal to Tumor cells) that is cytotoxic, inducing apoptosis in cancer cells. This non-native form has been shown to cause death in mammary gland epithelial cells in mice.

The study hypothesized that Milk from cows at the end of lactation might contain higher levels of the non-native form of  $\alpha$ -LA compared to milk from the start of lactation. These

proteins may induce apoptosis in mammary epithelial cells, leading to a decline in milk production late in the lactation period.

## Key findings

### **Protein Concentration Measurements:**

The overall total protein concentration in the milk was found to be significantly higher in samples from the end of lactation ( $43.98 \pm 4.8$  g/L) compared to the start ( $34.67 \pm 10.3$  g/L,  $P < 0.01$ ). Initially, when comparing pooled samples, the concentration of the native  $\alpha$ -LA (measured by ELISA) was statistically higher at the start of lactation ( $17.9 \pm 5.6$  mg/ml) compared to the end ( $13.9 \pm 4.6$  mg/ml,  $P = 0.047$ ). However, when measuring samples from the same individual cows at both the start and end of lactation, the average  $\alpha$ -LA concentration showed no significant difference ( $P = 0.57$ ) and exhibited large variability among the individuals. Some cows had more  $\alpha$ -LA at the start, some at the end, and some showed no change.

**Added Milk Effect on Cell Viability and apoptosis** The effect of milk on cell viability and apoptosis was tested using A549 cells and L1 cells. Initially, pooled milk from the end of lactation reduced the viability of A549 cells compared to start-of-lactation milk ( $P < 0.005$ ). However, subsequent experiments comparing different concentrations or using individual samples showed that there was generally no significant difference in viability between start and end milk in L1 cells. In A549 cells, one individual sample comparison showed lower viability with end-of-lactation milk after 6 days, but a later comparison showed no significant difference. High concentrations of milk consistently damaged cell viability. When quantifying apoptosis (Annexin V/PI FACS), there was no significant difference observed in the effect of start versus end milk on either cell type, but a higher concentration of milk overall caused a higher rate of apoptosis in both cell types. A549 cells were found to be more sensitive to the presence of milk than L1 cells.

## Discussion and Conclusion

The research successfully developed accurate methods for quantifying native  $\alpha$ -LA and measuring cell viability and apoptosis. However, the hypothesis that a change in  $\alpha$ -LA levels causes the decline in milk production was not proven. The study found no link between the concentration of native  $\alpha$ -LA in the milk and the amount of induced cell

death. The large variability among individual cows in both  $\alpha$ -LA concentration and cytotoxic effect was a key finding. The concentration of native  $\alpha$ -LA may not be an accurate measure for the amount of the pro-apoptotic form, or that the factor inducing apoptosis is a different component in the milk. The results consistently showed that higher concentrations of milk induce greater levels of apoptosis and that A549 cells are more sensitive to milk presence than mammary epithelial L1 cells.