

## LARGE-SCALE GUANIDINATION OF PROTEIN FOR DIGESTIBILITY AND ENDOGENOUS AMINO ACID DETERMINATION

M. IMBEAH, K. ANGKANAPORN, C.J. MYLECHARANE and W.L. BRYDEN

Guanidinated proteins, in which lysine is converted to homoarginine, have been used in determining protein digestibilities and endogenous protein/amino acid secretions in miniature pigs (Schmitz et al. 1991) and in chickens (Siriwan et al. 1994) fed one meal containing the protein. These studies required small quantities (100g) of the guanidinated protein. Measurement of endogenous amino acids in pigs under steady state conditions, however, requires large quantities of the guanidinated protein. Therefore, a simple procedure was developed for large scale guanidination of casein and it is described below.

Fifty litres of 0.4M free o-methylisourea (OMIU) was prepared by dissolving o-methylisourea hydrogen sulphate in 50L tap water, then precipitating the sulphate with barium hydroxide. The clear supernatant was siphoned into a different container and the pH adjusted to 10.5. Five kilograms of casein was slowly added to the OMIU solution with stirring. The pH was adjusted again to 10.5 and the mixture was allowed to stand for 24h. After 24h, 30L of water was added to the mixture to reduce the viscosity for easy stirring. The pH was then gradually reduced to pH 3 to precipitate the protein using 4N HCl. The reaction mixture was allowed to stand for 2-3h, during which time the precipitate settled. The clear supernatant was discarded. The guanidinated protein (G-protein) was then washed three times with excess water at pH 3. After the last wash, excess water was removed from the G-protein by squeezing through calico cloth. The G-protein was dried in a forced draught oven at 35°C.

The percentage lysine conversion to homoarginine for three replicates of 5 kg preparations of casein were 98.3, 98.7 and 98.0. These conversion rates are the same as those obtained from laboratory-scale preparations, indicating that large-scale guanidination is feasible and efficient.

SCHMITZ, M., HAGEMEISTER, H. and ERBERSDOBLER, H. (1991). *J. Nutr.* 121: 1575.  
SIRIWAN, P., BRYDEN, W.L. and ANNISON, E.F. (1994). *Br. J. Nutr.* 71: 515.

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Department of Animal Science, University of Sydney, Camden, NSW 2570