Proc. Aust. Soc. Anim. Prod. 1994 Vol. 20

AMINO ACID REQUIREMENTS OF WOOL FOLLICLES IN CULTURE

M.J. NANCARROW and P.I. HYND

Dept of Animal Science, University of Adelaide, Waite Campus, Glen Osmond, S.A. 5064

It is widely accepted that the major nutritional factors influencing wool growth are the amounts and proportions of amino acids available to the wool follicles, particularly the sulfur-amino acids, methionine and cyst(e)ine (Reis 1979) and lysine, which are generally considered first- and possibly second-limiting respectively (Reis 1989). The relative importance of these, and other, amino acids for fibre growth in culture has not been ascertained. The aim of this study was to utilise recently developed wool follicle culture techniques (Hynd *et al.* 1992) to determine the dependence of wool follicles *in vitro* on various amino acids for maintenance of "normal" fibre production.

Individual wool follicles were liberated from strips of skin removed from the midside of a Tukidale sheep and placed into 500 μL of amino acid-deficient RPMI-1640 medium R7130 or R7634, supplemented as per Hynd *et al.* (1992). The amino acid-deficient medium was then supplemented with the various amino acids to create treatment groups with either a full complement of amino acids for RPMI-1640 or deficient in a single amino acid. Follicles were then maintained at 37°C in an atmosphere of 5% carbon dioxide. Follicle lengths were measured every 24 hours by image analysis (Bioquant IV) and the rate of fibre growth determined by linear regression of individual follicle lengths over time. Longevity was estimated from the fibre growth data by identifying the day on which maximum fibre length occurred for each follicle and then determining the first day on which fibre length exceeded the maximum length minus 100 μm (100 μm/day was considered the minimum growth rate of a viable follicle). Proliferative activity of the follicles was estimated by measurement of incorporation of [^{3}H]-thymidine by dividing cells within follicles. One μCi[^{3}H]-thymidine was added to the wells, the follicles incubated for 4 hours to allow incorporation; then the follicles were washed, solubilised, neutralised and counted in 5 mL scintillant.

Results were statistically analysed using Superanova™ and Duncan's New Multiple Range tests and all parameters were significantly reduced ($P < 0.05$) by omission of a single amino acid, with the exception of [^{3}H]-thymidine uptake when glutamine was omitted. Data are expressed as percentages of control values (Figure 1).

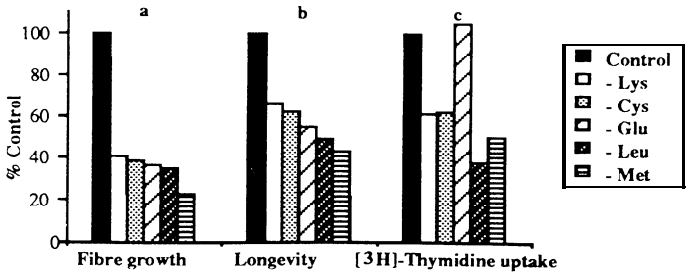

Figure. 1. Histograms of (a) fibre growth, (b) follicle longevity and (c) cell proliferation expressed as % of controls.

Clearly, omission of a single amino acid from RPMI-1640 medium reduces fibre growth and follicle **longevity,** probably through effects on cell division. Unpublished data (Nancarrow and Hynd) indicate that this is not a response to reduced energy supply. Thus we can conclude that there is no first or second-limiting amino acid for wool growth *in vitro* as lysine, methionine, cyst(e)ine, leucine and glutamine are essential for "normal" fibre production, and further that the follicle culture model utilised here provides a useful tool for studies on wool follicle nutrition and metabolism.

HYND, P.I., WINDER, L.M., JAHODA, C.A.B. and BICKERSTAFFE, R. (1992). *Wool Technol. and Sheep Breed. 92:* 102.

REIS, P.J. (1979). *In* "Physiological and Environmental Limitations to Wool Growth", (Eds J.L. Black and P.J. Reis) pp. 223-42 (University of New England Publishing Unit: Armidale).

REIS, P.J. (1989). *In* "The Biology of Wool and Hair", (Eds G.E. Rogers, P.J. Reis, K.A. Ward and R.C. Marshall) pp. 185-203 (Chapman and Hall: New York).