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Contra-lateral infusion of amino acids into the hind limb of lambs*

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ABSTRACT

A contra-lateral hind limb infusion of a mixture of six amino acids (MET, LYS, HIS, ARG, THR, CYS) had no significant effect on muscle growth or fat deposition but did increase wool growth. An infusion of casein or the six amino acids into the abomasum positively increased N retention (slope 0.34) It was concluded that the concentration of arterial amino acids *per se* did not control growth of muscles in the hind limb.

KEY WORDS: lamb, hind limb, amino acids, muscle, wool

INTRODUCTION

Fraser et al. (1990, 1991) have shown that sheep infused with amino acids or casein into the abomasum increased N retention or growth. Presumably infusion increases the amino acid supply to various tissue beds but whether these tissues respond simply to the supply (concentration and blood flow) or to a nutrient - endocrine interaction is less certain. Wolff et al. (1989) developed a contra-lateral hind limb infusion procedure to examine the effect of insulin and this approach appeared useful to examine *in vivo* responses of a tissue to changes in nutrient supply. Infusions of a mixture of amino acids were examined by this approach.

MATERIAL AND METHODS

In the first experiment amino acids and casein were infused into the abomasum of lambs. In the second and third experiments, amino acids were infused directly into each hind limb arterial system.

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Experiment 1. Abomasal infusion

This experiment used a 5×5 Latin Square design with five crossbred wether lambs (22.4±0.99 kg) fitted with abomasal catheters. Each period of the Latin Square involved two days of pre-infusion followed by measurement of digestibility and nitrogen balance over five days and results were analysed by ANOVA. Sheep consumed ryegrass hay (*Lolium multiflorum* cv. Aristocrat) at 25 g DM/kgW/day. The five treatments consisted of high and low amino acid infusions, high and low casein infusions, and a control (water) infusion (all 1500 ml/d). The amino acid mixture was made up of 50.8 mg methionine (Met), 50.8 mg cyst(e)ine (Cys), 227.6 mg lysine (Lys), 48.4 mg histidine (His), 66.8 mg arginine (Arg) and 126.4 mg threonine (Thr) (Fraser et al., 1991) with a low infusion level of this mixture (LAA, 600 mg AA/kg W/day) and a high level (HAA, 1200 mg AA/kg W/day). The low (3.4 g casein/kg W/d) and high casein (6.8 g casein/kg W/d) treatments, (Alanate 191, NZ, 148 g N/kg DM) were designed to supply the same amount of Met+Cys as in the corresponding amino acid treatments.

Experiments 2 and 3. Hind limb infusion

In the second and third experiments, 8 lambs were infused with amino acids, over a 7-8 week period, directly into the hind limb arterial system (left and right external iliac artery) using a contra-lateral infusion technique (Wolff et al., 1989). An additional 4 lambs were slaughtered at the start and 4 at the completion of the experiment. Wether lambs weighed 35.3±0.63 kg in Experiment 2 and consumed lucerne pellets at 50 g DM/kg W/day. In Experiment 3, ewe lambs weighed 26.6±0.57 kg and consumed ryegrass hay at 25 g DM/kg W/day.

In Experiment 2, lambs were continuously infused into the hind limb (0.1 ml/min) with an amino acid mixture (same ratio as above) at the rate 142 mg/kg W/day. In Experiment 3, eight sheep were continuously infused into the hind limb with four sheep at 140 mg/kg W/day and four at 255 mg/kg W/day. Plasma samples were taken from the jugular vein, weekly, to monitor plasma amino acid concentration. The animals were slaughtered after 7-8 weeks and the muscles and fat depots of the hind limb dissected and chemically analysed. Catheter placement was verified. Wool growth (greasy and clean weight) was monitored before and during the infusion through the clipping of patches on the lateral side of each hind limb.

Hind limb data was analysed with one-and two-tailed t-tests to test for differences in muscle or fat deposition or wool growth using the difference between infused and control for each component for each individual animal. The Wilcoxon matched pairs test was used to analyse means across treatments.

RESULTS

Experiment 1. Abomasal infusion

The ryegrass hay diet had an organic matter content (OM) of 908 g/kg DM, nitrogen content (N) of 20.9 g/kg DM and *in vivo* dry matter digestibility (DMD) of 64.4±0.32. The animals grew at 105±19.5 g/d over the experiment. Nitrogen balance was significantly increased ($P<0.05$) by all treatments compared to control (1.24±0.42, 2.62±0.33, 2.98±0.47, 5.10±0.87, 7.83±0.89 gN/d for control, LAA, HAA, low casein and high casein treatments, respectively). There was no significant difference ($P>0.05$) in nitrogen balance between the two amino acid treatments, but there was ($P<0.05$) between the two casein treatments, and between both casein treatments and the amino acid treatments. There was a significant positive relationship: N retained (g/d)=0.337 N intake (feed plus infused g/d)-2.633 ($r^2=0.79$).

Experiments 2 and 3. Hind limb infusion

In Experiment 2, lucerne had a N content of 23 g N/kg DM and an *in vivo* DMD of 54.1±0.23%. In Experiment 3, ryegrass hay had a N content of 27g N/kg DM and an *in vivo* DMD of 71.5±0.34%. Liveweight gain in experiment 2 was 244±15.1 g/d for the infused animals, and 274±26.2 g/d for those not infused, and in Experiment 3 was 38±6.8 g/d. Plasma amino acid concentration over the course of the experiment was variable with no time related changes.

No significant difference was seen in tissue weights between control and infused legs in either experiment. There was no significant difference in protein and lipid content of the dissected muscle groups in Experiment 2 but some differences in intra-muscular lipid in Experiment 3 (17% increase in lipid content of the semitendinosus muscle HAA level). There was no difference (0.75%) in muscle weight between both hind limbs dissected from initial and final slaughter control lambs.

In Experiment 2, wool production on infused legs was approximately 10% greater than on control legs or control sheep (not significant $P>0.05$). In Experiment 3 infused legs showed an average significant ($P<0.05$) 20% increase in clean wool production from the pre-infusion level, while the control legs showed no significant change from the pre-infusion level. The increase in wool production was 37% ($P<0.10$) for HAA and 8% ($P>0.10$) for LAA infused legs.

DISCUSSION

This study was undertaken to examine the hypothesis that increasing the arterial flow of amino acids to the hind limb, by increasing the concentration through infusion, would lead to increased hind limb protein synthesis and muscle growth. The hypothesis was examined in two different ways. Firstly, the nitrogen

balance response to a mixture of six essential amino acids or casein infused into the abomasum was measured in lambs. Secondly, hind limb muscle growth was compared after infusion of a mixture of six amino acids into the external iliac artery of one hind limb, while the other hindlimb was infused with a saline control. This contra-lateral infusion method enabled the arterial concentration of amino acids passing into the infused hind limb to be increased 44-87% above that in the control leg (Oddy et al., 1981).

Experiment 1. Abomasal infusion

Lambs increased N balance in response to abomasal infusion with a low efficiency of use of this extra nitrogen (0.34), which increased as expected for just amino acid N (0.48), and similar to that observed previously (Fraser et al., 1990).

Experiments 2 and 3. Hind limb infusion

No increase in muscle growth of the hind limbs with infusion was measured in either experiment suggesting that the route of infusion was important in eliciting a response. The experiments also indicated that long-term intra-arterial infusions may be sustained. Dissection of both legs of control lambs confirmed little variation between legs. Wool growth appeared to increase with infusion indicating that this dermal tissue bed is sensitive to concentration of amino acids. Jugular plasma amino acid concentrations showed no time trend and so infusion should have maintained a much higher concentration of the six amino acids in plasma reaching the infused hind limb compared to the control hind limb throughout the experiment.

Thus, on a ryegrass diet, lambs increased nitrogen balance in response to the abomasal infusion of a mixture of amino acids or protein. However, infusion of amino acids directly into the hind limb arterial supply did not elicit a significant muscle growth response. It was concluded that the concentration of arterial amino acids *per se* was not the controlling factor for growth of muscles in the hind limb of the sheep but did appear to control wool growth.

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