Effects of small peptides or amino acids infused to a perfused area of the skin of Angora goats on mohair growth¹

R. Puchala², S. G. Pierzynowski³, T. Wuliji, A. L. Goetsch, T. Sahlu, M. Lachica, and S. A. Soto-Navarro

E (Kika) de la Garza Institute for Goat Research, Langston University, Langston, OK 73050

ABSTRACT: The effect of infusing dipeptides or their amino acids on mohair growth of Angora goats was investigated using a skin perfusion technique. Seven Angora wethers (average BW 24 ± 2.5 kg) were implanted bilaterally with silicon catheters into the superficial branches of the deep circumflex iliac artery and vein and carotid artery. The experiment consisted of three 28-d phases. In the first 14 d of Phases 1 and 3, saline was infused into deep circumflex iliac arteries supplying skin and in Phase 2 a mixture of dipeptides (methionine-leucine [Met-Leu], lysine-leucine [Lys-Leu]) was infused into the artery on one side, and free amino acids were administered on the other side. Infusion rates of peptides were 0.85 mg/h Met-Leu and 0.85 mg/h Lys-Leu in 2.4 mL saline. Infusion rates of amino acids were 0.474 mg/h Lys, 0.483 mg/h Met, and 0.743 mg/h Leu in 2.4 mL saline. A 100-cm² area within the perfused region was used to determine mohair growth. Two weeks after the cessation of infusions, perfused areas were shorn. Clean mohair production from the dipeptide- and amino acids-perfused regions were similar (4.21 vs 4.35 g/[100 cm² \pm 28 d], respectively; P >0.05). However, clean mohair production during dipeptides and amino acids infusions was greater (P < 0.01)than that observed during saline infusions (3.63 g/[100 $cm^2 \pm 28$ d]). There were no significant differences between dipeptides and free amino acids in concentrations of various hormones and metabolites in blood from deep circumflex iliac veins (P > 0.05). In conclusion, the studied small dipeptides and amino acids similarly increased mohair fiber growth, presumably through supplying limiting amino acids directly to the fiber follicle.

Key Words: Amino Acids, Angora, Dipeptides, Goats, Growth, Mohair

©2002 American Society of Animal Science. All rights reserved.

Introduction

Until recently, it was commonly believed that gastrointestinal digestion of proteins is complete and that only free amino acids enter circulation. However, a considerable body of evidence for absorption of peptides from the gastrointestinal tract has accumulated (Webb, 2000). There are also indications of peptide clearance from the blood (Druml et al., 1991). Therefore, many tissues appear to utilize peptides as donors of amino acids for protein synthesis. Intravenous infusion of peptides has been shown to support serum protein concentrations and nitrogen retention equivalent to free amino acids (Adibi et al., 1993). Intravenous infusion of small

Accepted December 11, 2001.

peptides can be used to deliver amino acids difficult to supply via infusion of free amino acids (Radmacher et al., 1993) that are relatively unstable or poorly soluble (Christensen, 1995).

J. Anim. Sci. 2002. 80:1097-1104

Pan et al. (1996) demonstrated in vitro that methionine (Met)-containing peptides could be used as Met sources for protein accretion. Methionine-containing peptides were superior to free Met in supporting protein accretion of cultured myogenic and mammary epithelial cells. However, the extent and efficiency of small peptide utilization in vivo has not been extensively studied.

Choung and Chamberlain (1995a,b) observed that abomasal infusions of the enzymatic hydrolysate of casein stimulated yield of milk fat to a much greater extent than did free casein or free amino acids. In skin perfusion experiments with Angora goats, infusion of both amino acids (Puchala et al., 1995) and small peptides (Pierzynowski et al., 1997) increased mohair production, although amino acids and small peptides have not been contrasted in the same experiment. Therefore, the objective of this experiment was to compare effects of infusing a defined area of skin of Angora goats with small quantities of a mixture of dipeptides, methionineleucine (**Met-Leu**) and lysine-leucine (**Lys-Leu**), and

 $^{^1\}mathrm{This}$ research was supported by USDA-CSREES grant no. 97-38814-4149.

²Correspondence: P.O. Box 730 (phone: 405-466-3836; fax: 405-466-3138; E-mail: rpuchala@yahoo.com).

³Dept. of Anim. Physiol., Lund Univ., Helgonavagen 3b, SE-223 62 Lund, Sweden.

Received August 17, 2001.

Puchala

Pha	se 1	Pha	se 2	Pha	se 3
Saline infusion		Amino acid infusion		Saline infusion	
14 d	14 d rest	14 d	14 d rest	14 d	14 d rest
Saline infusion		Dipeptide infusion		Saline infusion	

Figure 1. Description of activities in three 28-d experimental phases. Each phase consisted of 14-d infusion (shaded area) and 14-d rest (clear area) periods. Sides of infusion were selected randomly (shaded-dotted line and shaded-broken line). Perfused areas of the skin were shorn before and after each experimental phase (bold line). On d 7 and 14 in Phase 2, during the infusion period, blood was obtained from a carotid artery and a superficial branch of deep circumflex iliac veins. Blood flow was measured on d 14.

free amino acids on mohair growth and blood metabolite concentrations.

Materials and Methods

Animals

The experimental protocol was approved by the Langston University Animal Care Committee. Seven Angora wethers (BW 24 ± 2.5 kg, approximately 15 mo of age) were implanted bilaterally with Silastic catheters (Dow Corning, Midland, MI) in the superficial branches of the deep circumflex iliac artery and the deep circumflex iliac vein as described by Pierzynowski et al. (1994), and a Silastic catheter was placed in the carotid artery as well. The area supplied by the artery was identified using methylene blue (Sigma, St. Louis, MO) infusion, and an area $(10 \pm 10 \text{ cm})$ was marked by tattoo in the middle of the perfused region. Animals recovered from anesthesia within 5 to 6 h and were moved to individual cages, where they were allowed to rest for 1 wk. Animals received daily intramuscular injections of procaine penicillin (600,000 U, Pfizer, New York, NY) for 4 d. Catheters were flushed with heparin (100 U/mL) every week to maintain patency. Animals were fed a mixed concentrate-forage diet (37.8% bermudagrass hay, 7% alfalfa, 25% oats, 19% ground corn, 9% solvent-extracted soybean meal, 1% trace mineralized salt, 1% calcium carbonate, and 0.2% vitamin premix; DM basis), prepared according to NRC (1981) recommendations, once daily at 0800 at approximately 110% of consumption on the preceeding few days and had free access to water.

Experimental Design

Goats were shorn before the 84-d experiment, which consisted of three 28-d phases (Figure 1). The primary objective, to compare effects of amino acids and dipeptides, was addressed in the middle phase. A strength of the employed perfusion technique is that infusate treatments can be compared in the same animal at the same time, with one infusate administered to the right side and the second infusate to the left side. However, because the animal has only two sides, it is not possible to study two infusates and a control treatment simultaneously in the same animal. Therefore, our approach to gain insight regarding possible effects of infusions was to monitor mohair fiber growth both before (Phase 1) and after (Phase 3) infusions with dipeptides and amino acids (Phase 2). So that experimental conditions during Phases 1 and 3 would be as similar as possible to those in Phase 2, saline was infused for 14 d in Phases 1 and 3 just as amino acids and dipeptides were infused in Phase 2. Nonetheless, the comparison of saline infusion in Phases 1 and 3 with infusion of amino acids and dipeptides in Phase 2 was confounded with time. However, we believed that if measures were similar between Phases 1 and 3 it would be reasonable to assume that there were minimal differences due to time.

The tattooed region was shorn using a small Oster (Milwaukee, WI) clipper with a number 40 blade. For the first 14 d of each phase animals were infused either with saline (Phases 1 and 3) or a mixture of dipeptides or free amino acids (Phase 2) into the deep circumflex iliac arteries. Sides of infusion in Phase 2 of dipeptides and free amino acids were chosen randomly. For peptide infusions, 0.354 g Met-Leu, 0.354 g Lys-Leu, and 0.5 g BSA were dissolved in 1 L of saline, and pH was adjusted to 7.4 using NaOH. For amino acid infusions, 0.1975 g Lys, 0.2013 g Met, 0.3096 g Leu, and 0.5 g BSA were dissolved in 1 L of saline, and pH was adjusted to 7.4 using NaOH. Bovine serum albumin, used as a carrier for infused amino acids and dipeptides in Phase 2, was also added to saline infused in Phases 1 and 3. Solutions were infused with 60-mL syringe pumps (Harvard Apparatus, South Natick, MA). Infusion rates were 2.4 mL/h for saline or dipeptide or amino acid mixtures. The infusion provided 1.7 mg/h (6.10 µmol/ h) dipeptide mixture or 0.474 mg/h (3.24 µmol/h) Lys, 0.483 mg/h (3.24 µmol/h) Met, and 0.743 mg/h (5.66 µmol/h) Leu. Relatively small amounts of dipeptides

and amino acids were infused to limit effects to a defined area of skin, and so that the infusate of one side would not influence metabolism on the other side (Pierzynowski et al., 1994, 1997; Puchala et al., 1995, 1996).

Methionine and Lys are reported to be the most limiting AA for wool and mohair growth (Reis et al., 1990; Sahlu and Fernandez, 1992; Puchala et al., 1995). Leucine was also included in the mixture because it is known to serve as an energy-yielding substrate, is an important regulator of protein turnover, and modifies uptake of tyrosine, phenylalanine, and tryptophan (Bender, 1984). Leucine stimulates protein synthesis and its metabolite, α -oxoisocaproate, is responsible for inhibiting protein catabolism (Bender, 1984). The infusion rate of amino acids from free amino acids and dipeptides was designed to increase flow of amino acids in perfused areas by approximately 15% for Met, 3% for Lys, and 2.5% for Leu (calculated using average blood amino acid concentrations in deep circumflex iliac circulation and average blood flow in the perfused area). Assuming total blood volume to be 8% BW, the calculated contribution of infused dipeptides and free amino acids to whole-body amino acid metabolism (estimated as a fraction of daily amino acid blood flow) was negligible (Met = 0.1%, Lys = 0.03%, Leu = 0.03%). Therefore, the skin perfusion technique allowed for study of in vivo skin metabolism without altering metabolism in the rest of the body, and likewise facilitated simultaneous within-animal comparison of treatments.

On d 7 and 14 of peptide or amino acid infusions, blood was collected at 0800 from both deep circumflex iliac veins and the carotid artery. Blood was collected into three 7-mL tubes containing K₃ EDTA for hormone assays, sodium heparin for amino acid analysis, or potassium oxalate-sodium fluoride for quantification of other metabolites (Vacutainer, Becton Dickinson, Rutherford, NJ). Tubes were immediately chilled in an ice bath, transported to the laboratory, and centrifuged at $1,500 \pm g$ at 4°C for 20 min. Plasma aliquots were stored at -20°C until analysis. Also, on d 14 blood flow to perfused regions was measured through a primary dose of 10 mL of 0.5% (wt/vol) para-aminohippuric acid into iliac arteries followed by continuous infusion of the same solution with added dipeptides or amino acids at the rate of 12 mL/h. The amount of dipeptides or amino acids added to the para-aminohippuric acid solution was designed so that nutrient delivery rate was the same as described previously. After a 30-min equilibration period, six samples were taken at 20-min intervals from the carotid artery and iliac veins for analysis of para-aminohippuric acid, hemoglobin, oxygen saturation, and packed cell volume. At the end of each phase tattooed regions were shorn and mohair fiber was collected for analysis of yield, fiber diameter, and amino acids.

Analyses

Plasma hormones were analyzed using commercially available kits from ICN Biomedicals (Costa Mesa, CA)

(insulin: kit no. NK9910; total triiodothyronine: kit no. LN1305; and total thyroxine: kit no. LN1301). Analyses for the specific hormones were carried out in one run, and intraassay coefficients of variation were 5.9% for insulin, 4.8% for triiodothyronine, and 6.2% for thyroxine. Amino acid analyses were performed using an AminoQuant 1090 system (Hewlett-Packard, San Fernando, CA), utilizing precolumn derivatization with ophthalaldehyde and 9-fluorenylmethylchloroformate and UV detection. Plasma (0.45 mL) was deproteinized with 0.05 mL 50% (wt/vol) sulfosalicilic acid with internal standards (norvaline and sarcosine). Plasma glucose concentration was analyzed colorimetrically using a Sigma Diagnostic (St. Louis, MO) kit (catalog no. 315). Plasma urea N was determined as described by Chaney and Marbach (1962). Plasma was analyzed for paraaminohippuric acid by an automated procedure (Technicon Industrial Systems, 1972; No. 216-72T). Samples for oxygen and hemoglobin were drawn anaerobically. Samples were immediately analyzed for hemoglobin percentage and oxygen saturation of hemoglobin with an OSM 3 Hemoximeter (Radiometer, Westlake, OH). Remaining sample was then used to determine packed cell volume with heparinized tubes (Clay Adams, Parsippany, NJ). Fiber length and greasy and clean mohair yields were determined according to standards of the American Society of Testing and Materials (ASTM, 1988). Fiber diameter was determined using the Optical Fibre Diameter Analyzer (BSC Electronics, Myaree, Australia). To determine mohair amino acid profile, mohair samples were allowed to react with 3,3'-dithiodipropionic acid to convert cysteine (**Cys**) to stable Cys-3-mercaptopropionic acid and hydrolyzed with 6 N HCl using a MDS-2000 microwave system (CEM, Matthews, NC). The amino acid profile of digested mohair samples was determined using the AminoQuant system (Hewlett Packard) as noted earlier.

Calculations

Efficiency of utilization of each amino acid was calculated as the difference in quantities of mohair obtained between the peptide/amino acid phase and saline phases. This difference coupled with the mohair amino acid profile allowed for the determination of the amount of each amino acid in the mohair, which was used to estimate the percentage of infused amino acids retained.

Amino acid arterio-venous differences in Phase 2 were calculated from amino acid concentrations in the blood from carotid arteries and the superficial branch of iliac veins. The difference coupled with blood flow allowed the estimation of net flux of amino acids across the perfused skin area. Quantities of amino acids retained in mohair were calculated from average mohair fiber growth and the mohair amino acid profile for Phase 2. Uptake or release (positive values indicate uptake and negative values release) was divided by

1100

	I			
Mohair	Dipeptides	Free amino acids	SE	P <
Greasy mohair, g/100 cm ² Clean mohair, g/100 cm ²	$5.56 \\ 4.21$	$5.69 \\ 4.35$	$\begin{array}{c} 0.11 \\ 0.08 \end{array}$	$0.44 \\ 0.24$
Length, mm	23.3	23.0	0.26	0.49
Diameter, µm	30.8	30.6	0.21	0.3

Table 1. Mohair measurements after 14 d of infusions of dipeptides (Met-Leu and Lys-Leu) or free amino acids (Met, Lys, and Leu) and 14 d of regrowth (Phase 2)^a

an = 7. Least squares means.

^bDipeptides: 0.85 mg/h Met-Leu and 0.85 mg/h Lys-Leu in 2.4 mL saline. Free amino acids: 0.474 mg/h Lys, 0.483 mg/h Met, and 0.743 mg/h Leu in 2.4 mL saline.

retention of amino acids in mohair to yield a ratio of uptake or release.

growth was greater during Phase 2; however, fiber diameter was not different among phases.

Statistical Analyses

To compare effects of peptides and amino acids, Phase 2 data were analyzed as a randomized block design with a model consisting of treatment and animal (block) by GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Because type of infusate in Phase 2 (dipeptides and amino acids) did not affect measures, data for the two sides of each goat were averaged to compare measures among the different phases. These data, as well as feed intake in the different phases, were analyzed with a model consisting of phase. For blood measures on both d 7 and 14 of infusions, data were analyzed as a split plot with animal within treatment as the error term for testing the main plot of treatment. Residual error was used to test the subplot of sampling time and the interaction of time and treatment.

Results

Feed intake $(920 \pm 30 \text{ g/d})$ was not reduced by infusion and was similar among phases. Greasy and clean fiber mohair production, staple length, and fiber diameter were similar between infusions of dipeptides and free amino acids (Table 1). However, greasy and clean mohair production and staple length were greater (P < 0.05) during Phase 2 with infusions of dipeptides and amino acids than in Phases 1 and 3 when both sides were infused with saline (Table 2). Mohair length Assuming that in Phase 2 infused amino acids (free or dipeptides) were only used as building components of protein of mohair fiber produced above that observed in Phases 1 and 3, only 5% of Met was retained in protein of mohair fiber compared with 13% of infused Lys and 18% of infused Leu (Table 3). However, assuming that all Cys retained in protein of mohair fiber in Phase 2 was derived from transulfuration of infused Met, then total retention of infused Met in mohair fiber protein was 50%.

Infusions had no effect on blood flow (P > 0.05) in the perfused area and did not change oxygen saturation of hemoglobin, hemoglobin concentration, or packed cell volume (Table 4). There were no effects of sampling or interactions between time and treatment in blood metabolite concentrations. Plasma concentrations of urea N, glucose, insulin, triiodothyronine, and thyroxine were similar between dipeptides and amino acids. Arterio-venous differences, between blood from a carotid artery and superficial branches of iliac veins, were positive for all analyzed amino acids except alanine. Arterio-venous differences were similar between amino acid and dipeptide treatments (P > 0.05; Table 5). There were no differences in amino acid concentrations in mohair fiber between infusion treatments (data not shown) and phases (Table 6). The ratio of amino acid uptake to amino acids retained in mohair fiber among essential amino acids was highest for Met and lowest for Cys (Table 7).

Table 2. Mohair measurements for 28-d phases with infusion of saline (Phases 1 and 3) or amino acids (Met, Lys, and Leu) as peptides or free amino acids (Phase 2)^a

		Phase ^b		
Mohair	1 (Saline)	2 (Amino acids)	3 (Saline)	SE
Greasy mohair, g/100 cm ²	4.71^{d}	5.62°	4.52^{d}	0.19
Clean mohair, g/100 cm ²	3.68^{d}	4.28°	3.58^{d}	0.15
Length, mm	$19.2^{\rm d}$	23.1°	18.5^{d}	0.09
Diameter, µm	31.1	30.7	30.6	0.14

an = 7. Least squares means.

^bMean of values for infusion of dipeptides (0.85 mg/h Met-Leu and 0.85 mg/h Lys-Leu in 2.4 mL saline) and amino acids (0.474 mg/h Lys, 0.483 mg/h Met, and 0.743 mg/h Leu in 2.4 mL saline) or saline to both sides of the goat.

^{c,d}Within a row, means without a common superscript letter differ (P < 0.05).

Table 3. Utilization of amino acids infused as dipeptides or free amino acids for mohair production based on differences in mohair growth during infusion of amino acids and saline^a

Amino acid	Retained in mohair, mg	Infused, mg	Utilization, %
Methionine	8.3	162.3	5.1
Leucine	45.7	249.6	18.3
Lysine	21.2	159.3	13.3
Cysteine	72.4		44.6^{b}

^aEfficiency of utilization of each amino acid was calculated as the difference in quantities of mohair obtained between the peptide/ amino acid phase and saline phases. This difference coupled with the mohair amino acid profile allowed for the determination of the amount of each amino acid in the mohair, which was used to estimate the percentage of infused amino acids retained.

^bAssuming that infused Met was used as source of Cys.

Discussion

Utilization of Amino Acids and Dipeptides

Higher mohair fiber growth during infusion of dipeptides or free amino acid infusion (Phase 2) than during saline infusion (Phase 1 and 3) indicates a positive effect of amino acids, in the peptide or free form, on mohair fiber growth. Puchala et al. (1995) and Pierzynowski et al. (1997), using the skin perfusion technique, also demonstrated that infusion of Met, Lys, and Leu, either free or as peptides, increased mohair fiber growth. Likewise, Sahlu and Fernandez (1992) increased mohair fiber production by Angora goats by intraperitoneal infusion of Met and concluded that Met supply was limiting.

Infusions with dipeptides and free amino acids increased mohair fiber growth by 25% relative to growth in Phases 1 and 3 with saline. Smaller increases were noted by Puchala et al. (1995; 16%) with infusion of free amino acids (Met, Lys, Leu) and Pierzynowski et al. (1997; 17%) with dipeptides. A greater magnitude of change in the present experiment could relate to greater mohair growth rates. Sahlu and Fernandez (1992) with intraperitoneal infusion of Met observed only a 5% increase in mohair fiber growth, perhaps because of effects on whole-body amino acid metabolism relative to the local effects with the skin perfusion technique.

Results of this experiment indicate that amino acids in peptides can be used for mohair fiber production with similar efficiency as amino acids in the free form, suggesting efficient dipeptide hydrolysis. Morever, a number of human studies have indicated that infused peptides can serve as sources of amino acids (Furst and Stehle, 1993; Vazquez et al., 1993). The short half-life (about 3 min) of Ala-Cys, Gly-Cys, and Ala-Gln noted by Albers et al. (1988) and Furst et al. (1989) suggests hydrolysis by soluble and(or) plasma membrane-bound peptidases. Backwell et al. (1994) showed similar in vivo utilization of amino acids from Gly-[¹³C]Phe and Gly-[¹³C]Leu by lactating dairy goats and postulated hydrolysis by either the mammary cell surface or red blood cell hydrolases followed by uptake of liberated amino acids by the mammary gland.

The much lower retention of infused Met in mohair fiber protein than of Lys and Leu may be due its relatively low concentration in mohair fiber. However, it is known that Met is an important regulator of amino acid metabolism (Puchala et al., 1997), stimulating uptake of other amino acids (Puchala et al., 1995). Methionine after transulfuration can be also used as a source of Cys. Souri et al. (1998) reported that transulfuration occurred in isolated mohair follicles and that its rate was regulated by negative feedback mechanisms. In the present experiment, the presence of transulfuration in mohair follicles was indicated by high uptake of Met in the perfused area of the skin that exceeded the amount of Met retained in mohair fiber, and also by relatively low uptake of Cys that was lower than the amount retained in mohair fiber. It is also known that transport mechanisms in mammalian tissues across membranes are different for Cys and Met (Bender,

Table 4. Characteristics of blood from the superficial branches of the deep circumflex iliac veins during infusion of dipeptides or free amino acids^a

	Infusion ^b			
Item	Dipeptides	Free amino acids	SE	<i>P</i> <
Blood flow, mL/min	23.5	23.5	2.1	0.99
Oxygen saturation, %	70.0	70.3	0.8	0.82
Hemoglobin, g%	11.1	11.2	0.3	0.97
Packed cell volume, %	22.4	22.3	0.4	0.96
Glucose, mg/dL	60.8	60.9	1.2	0.94
Urea N, mg/dL	7.9	8.1	0.2	0.51
Insulin, µIU/mL	20.8	21.1	0.5	0.76
Triiodothyronine, ng/dL	241.6	242.9	13.3	0.94
Thyroxine, µg/dL	6.3	6.3	0.3	0.93

^an = 7. Least squares means.

^bDipeptides: 0.85 mg/h Met-Leu and 0.85 mg/h Lys-Leu in 2.4 mL saline. Free amino acids: 0.474 mg/h Lys, 0.483 mg/h Met, and 0.743 mg/h Leu in 2.4 mL saline.

Table 5. Differences in plasma amino acid concentration between the carotid artery and the superficial branches of the deep circumflex iliac vein during dipeptide or amino acid infusion^a

	I			
Amino acid	Dipeptides	Free amino acids	SE	<i>P</i> <
		— μmol\L ——		
Methionine	4.36	6.30	1.21	0.28
Cysteine	3.35	3.93	0.62	0.65
Lysine	3.92	3.38	1.07	0.73
Phenylalanine	1.69	1.93	0.46	0.82
Valine	4.75	5.57	1.93	0.77
Isoleucine	0.98	2.43	0.63	0.54
Leucine	3.38	3.74	1.24	0.84
Threonine	2.36	4.34	1.76	0.45
Arginine	8.97	16.30	2.80	0.11
Glutamic acid	6.35	9.58	1.22	0.13
Serine	6.18	3.40	1.24	0.16
Glycine	23.75	24.26	7.92	0.96
Alanine	-26.94	-17.59	8.75	0.48
Tyrosine	2.04	1.93	0.91	0.65

an = 7. Least squares means.

 $^{\rm b} Dipeptides: 0.85$ mg/h Met-Leu and 0.85 mg/h Lys-Leu in 2.4 mL saline. Free amino acids: 0.474 mg/h Lys, 0.483 mg/h Met, and 0.743 mg/h Leu in 2.4 mL saline.

1984); therefore, Cys and Met uptake can be individually controlled to allow adequate supplies of both amino acids for particular tissue needs. With approximately 10 times more Cys than Met in mohair fiber protein and a blood Cys concentration only twice that of Met, it appears that both processes, effective extraction of Cys from blood and Met transulfuration, may be used

Table 6. Amino acid profile of mohair fiber^a

	$Phase^{b}$			
Amino acid	1 (Saline)	2 (Amino acids)	3 (Saline)	SE
		— molar % —		
Methionine	1.11	1.08	1.12	0.07
Cysteine	11.60	11.67	11.74	0.34
Lysine	2.76	2.82	2.69	0.10
Phenylalanine	2.55	2.51	2.46	0.16
Valine	5.25	5.16	5.19	0.53
Isoleucine	2.92	2.84	2.88	0.09
Leucine	6.93	6.80	6.89	0.18
Threonine	6.20	6.25	6.22	0.16
Arginine	7.10	7.11	7.14	0.09
Histidine	0.94	0.94	0.94	0.03
Asparatic acid	7.16	7.17	7.14	0.21
Glutamic acid	12.66	12.56	12.51	0.24
Serine	10.38	10.53	10.49	0.17
Glycine	6.43	6.61	6.58	0.18
Alanine	5.72	5.62	5.68	0.11
Tyrosine	2.83	2.91	3.01	0.17
Proline	7.46	7.37	7.32	0.33

an = 7. Least squares means.

^bMean of values for infusion of dipeptides (0.85 mg/h Met-Leu and 0.85 mg/h Lys-Leu in 2.4 mL saline) and amino acids (0.474 mg/h Lys, 0.483 mg/h Met, and 0.743 mg/h Leu in 2.4 mL saline) or saline to both sides of the goat.

Table 7. Amino acid uptake/release during Phase 2and average amounts of amino acidsretained in mohair fiber^a

Amino Acid	Uptake/release ^b	Retained in mohair	Ratio
	μ	.mol\h	
Methionine	7.51	0.54	13.92
Cysteine	5.13	5.88	0.87
Lysine	5.15	1.42	3.62
Phenylalanine	2.55	1.26	2.03
Valine	7.28	2.60	2.79
Isoleucine	2.40	1.43	1.68
Leucine	5.02	3.42	1.46
Threonine	4.72	3.15	1.49
Arginine	17.81	3.58	4.97
Glutamic acid	11.23	6.40	1.75
Serine	6.75	5.22	1.29
Glycine	33.85	3.33	10.16
Alanine	-31.39	2.86	-10.97
Tyrosine	2.79	1.46	1.91

 $^{a}n = 7$. Least squares means.

^bNet flux was calculated using means of values for infusion of dipeptides (0.85 mg/h Met-Leu and 0.85 mg/h Lys-Leu in 2.4 mL saline) and amino acids (0.474 mg/h Lys, 0.483 mg/h Met, and 0.743 mg/h Leu in 2.4 mL saline). Quantities of amino acids retained in mohair were calculated from average mohair fiber growth and the mohair amino acid profile for Phase 2. Uptake or release (positive values indicate uptake and negative values release) was divided by retention of amino acids in mohair to yield a ratio of uptake or release.

to deliver a sufficient amount of sulfur amino acids for mohair fiber production.

In contrast to findings in the present experiment, Pan et al. (1996) found that many Met-containing peptides were superior to free Met in supporting protein accretion by in vitro cultured myogenic and mammary epithelial cells. One possible explanation for this disparity is that dipeptides with Met at the C-terminus were used in the present experiment. Pan et al. (1996) noted that dipeptides with Met at the N-terminus promoted greater protein accretion than dipeptides with Met at the C-terminus, which may be due a greater affinity for hydrolases to peptides with Met at the N-terminus. It is also known that there are special transport systems for di- and tripeptides (Payne et al., 2000), which may have contributed to the greater effect on protein accretion of small peptides in the Pan et al. (1996) in vitro study.

The similarity in effects on mohair fiber growth of dipeptides and free amino acids, along with similar blood metabolite concentrations, do not indicate regulatory effects of dipeptides as suggested by in vitro work of Marugg et al. (1995). This may be because the dipeptides used lacked biological activity of long peptides (Choung and Chamberlain 1995a,b).

Mohair Characteristics

Infusion with dipeptides and amino acids increased mohair fiber length by 20% compared with saline infusion. Similarly, local amino acid infusion increased mohair length growth by 6% (Puchala et al., 1995), and a 13% change was noted with local infusion of dipeptides (Pierzynowski et al., 1997). Sahlu and Fernandez (1992) also observed an increase in mohair length growth (9%) as result of intraperitoneal administration of Lys and Met. The lack of effect of amino acid or dipeptide infusion on fiber diameter in the present experiment may relate to an increased supply of amino acids for fiber growth (Met, Lys). Reis et al. (1990) found that omission of Met from dietary amino acids supplements reduced wool growth in Merino sheep by decreasing both fiber length growth rate and diameter. Increased mohair length growth without change in fiber diameter is desirable because the price for mohair fiber is negatively correlated with diameter.

Blood Metabolites

In accordance with the similar effect on mohair fiber growth of dipeptides and free amino acids, blood flow to the perfused region was comparable. Observed blood flows are similar to those reported by Harris et al. (1993) with Romney sheep but lower than those observed by Pierzynowski et al. (1994). The difference between our values and those of Pierzynowski et al. (1994) may be due to differences in conditions such as BW, mohair growth rate, season, level of feed intake, and diet composition. Webb et al. (1993) suggested that the energy cost of peptide incorporation into protein may be lower than with free amino acids; however, no differences in venous blood concentrations of glucose, insulin, thyroid hormones, or hemoglobin oxygen saturation suggest that energy utilization was not influenced by amino acid form. The absence of differences among phases and between infusion treatments in blood metabolite concentrations suggest that both small peptides and amino acids served as sources of amino acids in protein synthesis for mohair fiber growth.

Implications

Supplying small peptides (methionine-leucine and lysine-leucine) or their amino acids directly to the skin equally increased mohair production compared with saline. Similar blood concentrations of various hormones and metabolites suggest that small peptides were utilized by skin for mohair fiber growth via supplying free amino acids for protein synthesis; however, the exact mechanism of stimulation is unclear. In this regard, significant amounts of cysteine used in mohair fiber production may have arisen from transulfuration of infused methionine. Future research should consider different types of peptides.

Literature Cited

Adibi, S. A., H. Lochs, N. N. Abumrad, H. Daniel, and J. A. Vazquez. 1993. Removal of glycylglutamine from plasma by individual tissues: Mechanism and impact on amino acid fluxes in postabsorption and starvation. J. Nutr. 123:325–331.

- Albers, S., L. Pollack, and P. Furst. 1988. In vivo utilization of cystinecontaining synthetic short-chain peptides after intravenous bolus injection in the rat. J. Nutr. 118:1470–1474.
- ASTM. 1988. Annual Book of ASTM Standards. Standard Test Method D2130. Wool content of raw wool laboratory scale. American Society for Testing and Materials, Philadelphia, PA.
- Backwell, F. R., B. J. Bequette, D. Wilson, A. G. Calder, J. A. Metcalf, D. Wray-Cahen, J. C. MacRae, D. E. Beever, and G. E. Lobley. 1994. Utilization of dipeptides by the caprine mammary gland for milk protein synthesis. Am. J. Physiol. 267:R1–R6.
- Bender, D.A. 1984. Amino Acid Metabolism. 2nd ed. pp 39–59. John Wiley & Sons, Chichester, U.K.
- Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130–136.
- Choung, J. J., and D. G. Chamberlain. 1995a. Effects of abomasal infusions of sodium caseinate, a hydrolysate of casein or a corresponding mixture of free amino acids on milk yield and composition in dairy cows. J. Dairy Res. 62:29–37.
- Choung, J. J., and D. G. Chamberlain. 1995b. Effects of abomasal infusions of sodium caseinate and of casein hydrolysates varying in the relative proportions of peptides and free amino acids on milk production in dairy cows. J. Dairy Res. 62:423–429.
- Christensen, H. N. 1995. New insights into intravenous peptide metabolism. Nutr. Rev. 53:100-102.
- Druml, W., H. Lochs, E. Roth, W. Hubl, P. Balcke, and K. Lenz. 1991. Utilization of tyrosine dipeptides and acetyltyrosine in normal and uremic humans. Am. J. Physiol. 260:E280–E285.
- Furst, P., S. Albers, and P. Stehle. 1989. Availability of glutamine supplied intravenously as alanylglutamine. Metabolism 38:67-72.
- Furst, P., and P. Stehle. 1993. The potential use of parenteral dipeptides in clinical nutrition. Nutr. Clin. Pract. 8:106–114.
- Harris, P. M., B. W. McBride, M. P. Gurnsey, B. R. Sinclar, and J. Lee. 1993. Direct utilization of variant of insulin-like growth factor-I into the skin of sheep and effects on local blood flow, amino acid utilization and cell replication. J. Endocrinol. 139:463-472.
- Marugg, J. D., W. Meijer, R. van Kranenburg, P. Laverman, P. G. Bruinenberg, and W. M. de Vos. 1995. Medium-dependent regulation of proteinase gene expression in Lactococcus lactis: Control of transcription initiation by specific dipeptides. J. Bacteriol. 177:2982–2989.
- NRC. 1981. Nutrient Requirements of Goats. 1st ed. National Academy Press, Washington, DC.
- Pan, Y. L., P. K. Bender, R. M. Akers, and K. M. Webb, Jr. 1996. Methionine-containing peptides can be used as methionine sources for protein accretion in cultured C2C12 and MAC-T cells. J. Nutr. 126:232–241.
- Payne, J. W., B. M. Grail, S. Gupta, J. E. Ladbury, N. J. Marshall, R. O'Brien, and G. M. Payne. 2000. Structural basis for recognition of dipeptides by peptide transporters. Arch. Biochem. Biophys. 384:9–23.
- Pierzynowski, S. G., R. Puchala, and T. Sahlu. 1997. Effects of dipeptides administered to a perfused area of the skin in Angora goats. J. Anim. Sci. 75:3052–3056.
- Pierzynowski, S. G, T. Sahlu, R. Puchala, S. P. Hart, and A. Al-Dehneh. 1994. Local infusion of glucose and insulin in isolated skin perfusion sites in Angora goats. Small Rumin. Res. 14:137–141.
- Puchala, R., T. Sahlu, S. G. Pierzynowski, and S. P. Hart. 1995. Effects of amino acids administered to a perfused area of the skin in Angora goats. J. Anim. Sci. 73:565–570.
- Puchala, R., S. G. Pierzynowski, T. Sahlu, and S. P. Hart. 1996. Effect of mimosine administered to a perfused area of the skin in Angora goats. Br. J. Nutr. 75:69–79.
- Puchala, R., R. Zabielski, V. Lesniewska, P. Kiela, and W. Barej. 1997. Influence of duodenal infusion of L-methionine and Lmethionine dl-sulfoxide on amino acid concentration and duodenal electrical activity in Fresian calves. Anim. Feed Sci. Technol. 66:75–84.

- Radmacher, P. G., M. A. Hilton, F. K. Hilton, S. D. Duncan, and D. H. Adamkin. 1993. Use of the soluble peptide gamma-Lglutamyl-L-tyrosine to provide tyrosine in total parenteral nutrition in rats. J. Parenter. Enteral. Nutr. 17:337–344.
- Reis, P. J., D. A. Tunks, and S. G. Munro. 1990. Effects of the infusion of amino acids into the abomasum of sheep, with emphasis on the relative value of methionine, cysteine and homocysteine for wool growth. J. Agric. Sci. 114:59–67.
- Sahlu, T., and J. M. Fernandez. 1992. Effect of intraperitoneal administration of lysine and methionine on mohair yield and quality in Angora goats. J. Anim. Sci. 70:3188–3194.
- Souri, M., H. Galbraith, and J. R. Scaife. 1998. Conversion of methionine to cysteine by transulphuration in isolated anagen second-

ary hair follicles of the Angora goat. In: Proc. Br. Soc. Anim. Sci., Scarborough, U.K. p 139.

- Vazquez, J. A., H. Daniel, and S. A. Adibi. 1993. Dipeptides in parenteral nutrition: from basic science to clinical applications. Nutr. Clin. Pract. 8:95–105.
- Webb, K. E., Jr. 2000. Tissue, cellular and molecular aspects of peptide absorption and utilization. In: P. B. Cronjé (ed.) Ruminant Physiology: Digestion, Metabolism, Growth, and Reproduction. p 117. CABI Publishing, Oxon, U.K.
- Webb, K. E., Jr., D. B. Dirienzo, and J. C. Matthews. 1993. Recent developments in gastrointestinal absorption and tissue utilization of peptides: A review. J. Dairy. Sci. 76:351–361.