

## In Vitro Stability, in Vivo Hydrolysis, and Absorption of Lysine and Methionine from Polymerized Amino Acid Preparations

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The stability, hydrolysis, and absorption of L-lysine and L-methionine from polymers of L-lysine or L-lysine and L-methionine were determined in vitro and in vivo. <sup>13</sup>C NMR studies showed that a polymer of L-lysine hydrochloride, urea, and formaldehyde produced at a neutral pH was easily hydrolyzed by acid. Approximately 90% of the L-lysine hydrochloride in the neutralized polymer was protected for up to 5.5 months from microbial degradation in vitro. Rumen degradation of a nonneutralized polymer of L-lysine hydrochloride, urea, and formaldehyde increased as shelf life of the polymer increased. Methionine protection was 73% in a polymer of 3-day shelf life but was only 16% after 5.5 months. Lysine in the neutralized polymer escaping rumen degradation was hydrolyzed in the abomasum and duodenum-jejunum. Molar percentage lysine in portal and carotid plasma was higher ( $P < 0.05$ ) in animals fed polymerized L-lysine hydrochloride than in those fed free L-lysine hydrochloride.

Methionine and lysine are most likely to be deficient relative to tissue requirements in the mixture of amino acids absorbed from the small intestine of the ruminant. Postruminal administration of these amino acids either individually or together, depending on animal species and diet, has resulted in increased nitrogen retention (Ely et al., 1969; Devlin and Woods, 1964; Steinacker et al., 1970; Fenderson and Bergen, 1975; Schwab et al., 1976; Richardson and Hatfield, 1978). Direct supplementation of the ruminant diet with free amino acids is of little benefit because the amino acids are degraded by rumen microorganisms. Recently, progress has been made in protecting amino acids from microbial degradation by various methods (Linton et al., 1968; Komarek and Jandzinski, 1978; Amos and Evans, 1978a). The L-lysine hydrochloride-urea-formaldehyde polymer used by Amos and Evans (1978a) delivered ~56% of the lysine in the polymer to the abomasum. However, <sup>13</sup>C nuclear magnetic resonance (NMR) studies on the polymer indicated that it was subject to autohydrolysis and that, after several weeks of storage, most of the L-lysine was present as the free amino acid entrapped in a urea-formaldehyde matrix (Barton et al., 1979).

The objectives of this work were to evaluate (a) the hydrolysis characteristics of a lysine-urea-formaldehyde polymer produced at a neutral pH by <sup>13</sup>C nuclear magnetic resonance spectroscopy, (b) the in vitro rumen stability of neutralized polymers of lysine or lysine-methionine after 5.5 months of storage, and (c) the in vivo stability, hydrolysis, and absorption of lysine and methionine from polymers produced at a neutral pH.

### EXPERIMENTAL PROCEDURES

**Polymer Preparation.** The nonneutralized L-lysine hydrochloride-urea-formaldehyde polymer (polymer 2, molar ratios of reactants 0.55:1.67:2.29, respectively) was prepared as described previously (Amos and Evans, 1978b). A second nonneutralized polymer containing L-lysine hydrochloride and L-methionine (polymer 3) was prepared by dissolving 100 g of urea (1.67 M) in 186 mL of 37%

formaldehyde (2.29 M) and adding 5.0 g of L-lysine hydrochloride (0.27 M) and 5.0 g of L-methionine (0.34 M) with stirring after complete dissolution of the urea. The neutralized polymers (2c and 3c) were prepared by dissolving 21.9 g (0.55 M) and 10.9 g (0.27 M) of NaOH in 30 mL of H<sub>2</sub>O for polymers 2c and 3c, respectively, and mixing the dissolved NaOH with the L-lysine hydrochloride or L-lysine hydrochloride and L-methionine before the amino acids were added to the urea-formaldehyde solution. All reactions were conducted in 1000 mL of Virtis lyophilizer flasks. After the mixtures reacted for 16 h, the flasks were sealed and cooled in dry ice, and their contents were lyophilized. After the mixtures were dried, the polymers were removed from the flasks and ground through a 2-mm Wiley mill screen. Portions of each of the four polymers were hydrolyzed in 6 N HCl and analyzed for lysine or lysine and methionine on an automated Beckman amino acid analyzer as described previously (Amos and Evans, 1978a).

**Carbon-13 Nuclear Magnetic Resonance (NMR) Spectroscopy.** Carbon-13 spectra of the polymer were obtained on a JEOL <sup>13</sup>PS/PFT 100, pulsed NMR spectrometer. A Nicolet 1083 (20-bit word) computer system was interfaced to the spectrometer to collect the free induction decay (FID) and perform the Fourier transform (FET) and integration of peak areas. The observing lock and irradiating frequencies were calibrated with a Dana 8010B frequency counter. Line positions were referenced to sodium (trimethylsilyl)propionate (TSP) at -34.2 Hz with respect to tetramethylsilane (Me<sub>4</sub>Si) at 0 Hz. Frequency measurements (in hertz) were calibrated by the computer and were accurate to the line width described by one datum point (0.8 Hz). The instrument was tuned before each spectrum was taken and locked on the deuterium of deuterioacetone or deuterium oxide. Each spectrum was taken with a 600-Hz bandwidth and 8K data points were assigned to the FID unless otherwise noted. All spectra were taken with a 90° pulse angle of 25-μs pulse width. Pulses were repeated every 1.0 s (unless otherwise stated). The temperature in the magnetic cavity was 25 °C initially and 39 °C during hydrolysis.

Approximately 100 mg of polymer 2c (neutralized lysine polymer) was weighed into a 10-mm NMR tube and dissolved in 2.0 mL of 99.9% D<sub>2</sub>O, and the 0-h spectrum was taken.

The samples were adjusted to a pD of 0.85 (i.e., equivalent to a pH of 1.25) and brought to a temperature of 39 °C in the magnetic cavity for the remainder of the hy-

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hydrolysis period. Spectra were taken over 2-h blocks such that the average times were 0 (no hydrolysis), 1, 19, and 35 h.

**In Vitro Stability of Lysine and Methionine in the Polymers.** The stability of lysine and methionine to rumen microbial degradation was evaluated in two *in vitro* rumen fermentation studies. Preparation of the inoculum and the *in vitro* incubations were described previously (Amos and Evans, 1978b). Incubations were conducted in a 2-L Erlenmeyer flask containing 700 mL of inoculum and 9.33 g of ground Coastal bermudagrass hay as the substrate. Treatments were (1) control (substrate plus inoculum), (2) control plus L-lysine hydrochloride, (3) control plus polymer 2, (4) control plus polymer 2c, (5) control plus L-lysine hydrochloride plus methionine, (6) control plus polymer 3, and (7) control plus polymer 3c. Treatments 2, 3, and 4 had ~15.0 g of L-lysine added as free L-lysine hydrochloride or from the polymers per 30 mL of inoculum, and treatments 5, 6, and 7 had 7.50 mg of L-lysine and 11.71 mg of L-methionine added at 0 time per 30 mL of fermentation medium. The polymers were incubated in *in vitro* experiment 1 after 3 days of storage and were incubated in *in vitro* experiment 2 after 172 days of storage to ascertain the long-term stability of the bound lysine in the polymers. Three 30-mL aliquots were removed from each flask after 0, 4, 8, 24, and 48 h of fermentation in experiment 1 and after 0, 8, 24, and 48 h of fermentation in experiment 2. These aliquots from each treatment at individual fermentation times were combined in 150-mL lyophilizer flasks and immediately frozen in dry ice, and the contents were lyophilized. After the samples were dried, portions of each sample were hydrolyzed in 6 N HCl and analyzed for lysine and methionine.

**In Vivo Passage, Digestion, and Absorption of Lysine and Methionine from the Polymers.** Eight mature crossbred wethers were divided into four groups of two animals each and gradually adapted to a high-concentrate control diet containing 14% ground wheat straw as the roughage and sunflower meal as the supplemental protein (Amos and Evans, 1978a) and 0.625% poly(ethylene glycol) (PEG) as a marker. A 14-day adaptation and a 21-day experimental period were used in this study. The wethers received 400 g of the concentrate diet twice daily at 12-h intervals. Individual treatments were (1) control plus 5.0 g of L-lysine hydrochloride, (2) control plus 16.5 g of polymer 2, (3) control plus 17.0 g of polymer 2c, and (4) control plus 16.0 g of polymer 3c. All amino acid additions were divided into equal portions and fed twice daily during the last 14 days of the experimental period. Polymers used had been prepared 30 days before being fed; thus, the wethers were fed a 44-day-old polymer at the end of the experiment.

At the end of the experimental period, blood samples were taken in heparinized tubes from the carotid vein 1 h after feeding from one animal in each treatment and after 1.5 h from the remaining animal in each treatment. Immediately after the blood samples were taken, each animal was placed under a surgical level of anesthesia (sodium pentobarbital), the peritoneal cavity was opened, and an additional blood sample was taken from the portal vein. Animals were then sacrificed, and the gastrointestinal (GI) tract was ligated to permit collection of digesta samples from the (a) rumenoreticulum, (b) abomasum, (c) duodenum-jejunum, and (d) ileum. All digesta samples were taken within 15 min after the animal was sacrificed and were frozen for later analyses.

Lignin and PEG were used as markers to estimate the total quantity of particulate and liquid digesta present in

each section of the GI tract. Particulate and liquid phases of the digesta were separated by centrifugation at 10000g for 20 min. The liquid phase was analyzed for PEG (Ulyatt, 1957), total Kjeldahl nitrogen (Association of Official Analytical Chemists, 1970), and amino acids; the particulate digesta was analyzed for lignin (Van Soest, 1963), total Kjeldahl nitrogen, and amino acids. Total digesta (liquid and particulate) in each section of the GI tract was calculated from marker to protein ratios (PEG for liquid and lignin for particulate) by comparing the ratio in a respective GI tract sample to that in the diet.

Data were treated by a two-way classification of analysis of variance and differences among means determined by using Scheffe's multiple comparisons test (Kleinbaum and Kupper, 1978).

## RESULTS AND DISCUSSION

Amino acid analyses of the polymers used in the *in vitro* studies showed that polymers 2 and 2c contained 32.61 and 28.94% lysine, respectively. Polymer 3 contained 15.93% lysine and 19.91% methionine; polymer 3c contained 15.52% L-lysine and 19.40% L-methionine. There was a 22–25% increase in the lyophilized weight of the product due to the binding of formaldehyde and entrapment of water during formation of the polymers.

Four 6000-Hz spectra showing the hydrolysis of the neutralized lysine-urea-formaldehyde polymer with time (0, 1, 19, and 35 h) at 39 °C are shown in Figure 1. The pD of 6.4 in D<sub>2</sub>O is equivalent to a pH of 6.8 in H<sub>2</sub>O. The interpretation of this spectrum and the assignment of chemical shifts were made by Barton et al. (1979). Certain changes occurred during hydrolysis. The peak at 39.9 ppm (lysine  $\epsilon$ -carbon free) increased steadily during hydrolysis. The peaks at 44.6, 47.0, and 50.4 ppm, due initially to binding of the lysine  $\epsilon$ -carbon, decreased to the level of noise during hydrolysis. The peaks for the free lysine  $\delta$ -carbon (29.0 ppm at pD 6.4 and 27.2 ppm at pD 0.85) and bound lysine  $\delta$ -carbon (27.1 ppm at pD 6.4 and 25.9 ppm at pD 0.85) show the same trend as the lysine  $\epsilon$ -carbon; i.e., free lysine increases and bound lysine disappears during hydrolysis. The order of chemical shifts of the lysine  $\delta$ -carbon reverses when pD is lowered. The assignment of these chemical shifts were made from the pH studies of Saito and Smith (1973). At 1 h, the amount of free and bound lysine was essentially equal, such that hydrolysis would be complete in 5–6 h at pD 0.85 (pH 1.25). The spectra show no change after 19 h which meant hydrolysis was complete. The pD of 0.85 is lower than that found in the abomasum but *in vivo* data in the present study indicate that hydrolysis is completed within the time of digesta passage through the abomasum and that lysine would be available for absorption in the small intestine.

The quantities of lysine recovered from *in vitro* fermentations containing added lysine in the free or polymerized form are presented in Tables I and II. Lysine recovery (mg/30 mL) after 48 h of fermentation was less than the 0 h value for all treatments. However, for polymer 2 (neutralized) and polymer 2c (nonneutralized) lysine recovery was higher than the recoveries for treatments containing the free amino acid. Except for a few values that were higher at later rather than at earlier fermentation times, most likely due to sampling errors (e.g., polymer 2 at 8 and 24 h of fermentation compared to the 4 h value, Table I; polymer 2 at 8 h compared to 0 h of fermentation, Table II), lysine recovery decreased as the fermentation time increased.

The CBG plus inoculum treatment was used for a control value to calculate the percentage recovery of added lysine in each treatment at each specific fermentation time

Table I. Recovery of Lysine from in Vitro Fermentations Containing L-Lysine and L-Methionine in the Free or Polymerized Form (Experiment 1, 3-Day-Old Polymer)

treatment	recovery of Lys, mg/30 mL, at fermentation time					% recovery of added Lys after 48-h fermentation
	0 h	4 h	8 h	24 h	48-h	
CBG <sup>a</sup>	7.03	5.86	6.04	5.08	3.88	
CBG plus L-Lys	19.77	12.94	14.95	8.28	7.47	28.18
CBG plus polymer 2	21.73	14.16	16.33	18.66	15.03	75.85
CBG plus polymer 2c	24.73	20.31	18.87	21.11	19.22	86.67
CBG plus L-Lys plus L-Met	13.78	9.68	11.81	11.33	6.60	40.30
CBG plus polymer 3	15.17	14.78	10.20	11.65	12.67	107.98
CBG plus polymer 3c	19.05	15.10	13.72	13.45	12.38	70.71

<sup>a</sup> Coastal Bermuda grass; 0-time level 7.03 mg/30 mL represents total lysine in the inoculum and 0.4 g of CBG.

Table II. Recovery of Lysine from in Vitro Fermentations Containing L-Lysine and L-Methionine in the Free or Polymerized Form (Experiment 2, 172-Day-Old Polymer)

treatment	recovery of Lys, mg/30 mL, at fermentation time				% recovery of added Lys after 48-h fermentation
	0 h	8 h	24 h	48 h	
CBG <sup>a</sup>	7.73	7.50	4.46	4.02	
CBG plus L-Lys	19.43	12.95	9.37	6.94	24.96
CBG plus polymer 2	21.64	22.71	13.31	10.98	50.04
CBG plus polymer 2c	20.34	18.37	18.02	15.66	92.31
CBG plus L-Lys plus L-Met	17.24	13.59	9.53	8.65	48.68
CBG plus polymer 3	16.31	13.35	10.84	11.25	84.26
CBG plus polymer 3c	17.99	13.15	14.66	12.97	87.23

<sup>a</sup> Coastal Bermuda grass: 0-time level 7.73 mg/30 mL represents total lysine in the inoculum and 0.4 g of CBG.

(i.e., treatment lysine recovery minus control lysine recovery divided by lysine added in treatment). Thus, the recovery of lysine from the CBG plus L-lysine treatment was 28.18 and 24.96% and somewhat higher at 40.30 and 48.68% for the CBG plus L-lysine plus L-methionine treatment after 48-h fermentation in experiments 1 and 2, respectively. The percentage recovery of lysine in the CBG plus 3-day-old polymer 2 fermentation (experiment 1) was higher (75.85%) than the recovery for the 172-day-old polymer 2 (59.47%; experiment 2). The recovery

of lysine from the CBG plus polymer 2c treatment after 48 h was 86.67 and 92.31% in experiments 1 and 2, respectively. Lysine recovery in the CBG plus polymer 3 treatment (Table I) after 48 h appeared to be somewhat high at 107.98% of the 0-h value compared to 84.26% recovery in experiment 2 (Table II), perhaps again because of sampling error. After 48 h of fermentation, lysine recovery was essentially the same (84.26 and 87.23%) for CBG plus polymer 3 and CBG plus polymer 3c, respectively (Table II).

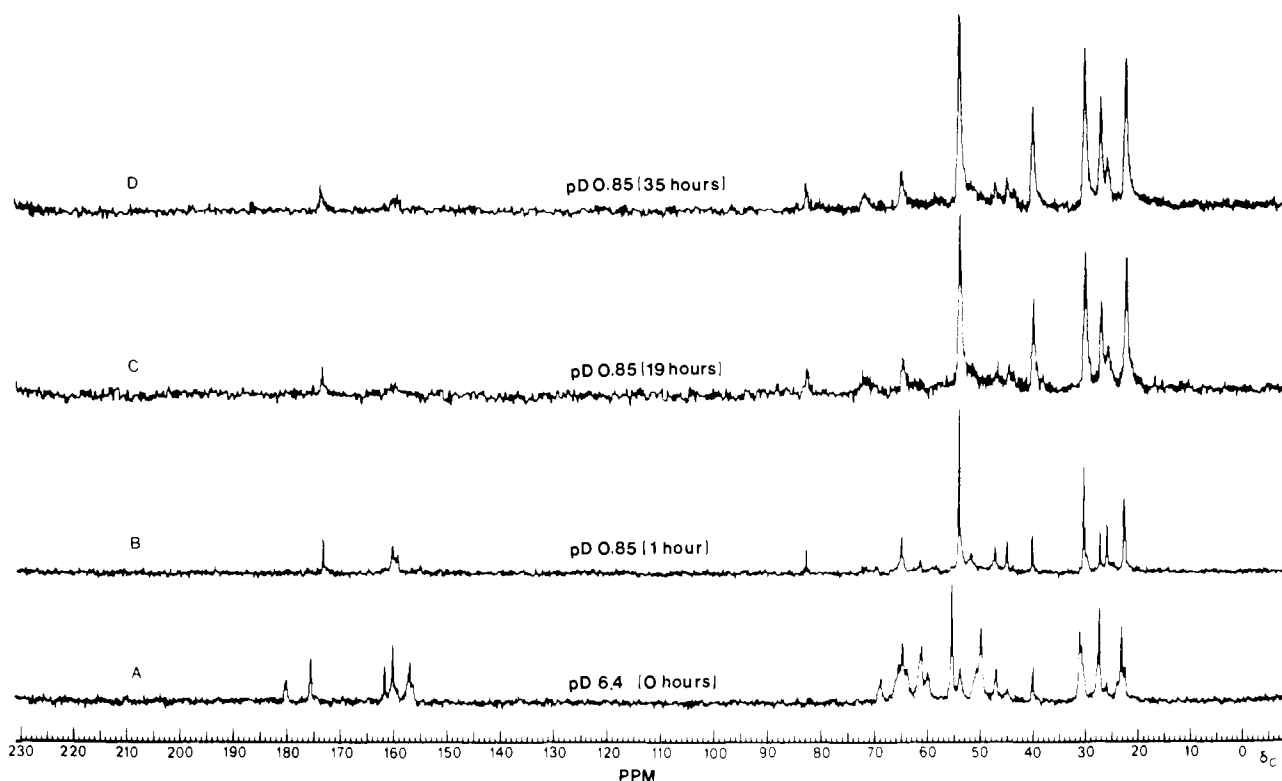
This increased protection of lysine in polymer 2c was expected, because Barton et al. (1979) showed with <sup>13</sup>C NMR spectroscopy that more of the  $\epsilon$ -carbon amino group of lysine is involved in formation of the polymer at a neutral pH than at an acid pH. In addition, the longer shelf life of polymer 2c compared to polymer 2 also agrees with results of the <sup>13</sup>C NMR studies of Barton et al. (1979) which showed that the neutral polymer was stable for at least 150 days and once the polymer was formed no further changes occurred. The reasons for the apparent increased stability of lysine in polymer 3 (nonneutralized) compared to polymer 2 are not clear at this time.

The recovery of methionine from fermentations containing free L-methionine or polymerized methionine is given in Table III. Age of the polymer at the beginning of the in vitro fermentation appeared to have a marked effect on the recovery of methionine; producing the polymer at a neutral pH compared to an acid pH appeared to only have a minor positive effect on the stability of methionine. Only 8.77 and 12.45% of the free L-methionine were recovered after 48 h of fermentation in experiments 1 and 2, respectively. The quantities of methionine recovered after 48-h fermentation for polymers 3 (10.37g) and 3c (10.73g) represented a recovery of 78.29 and 69.02% of the initial methionine in experiment 1. Only 15.37% (polymer 3) and 21.17% (polymer 3c) of the methionine added at 0 h were recovered after 48 h in experiment 2.

Table III. Recovery of Methionine from in Vitro Fermentations Containing L-Lysine Hydrochloride and L-Methionine in the Free or Polymerized Form

treatment	recovery of Met, mg/30 mL, at fermentation time					% recovery of Met after 48-h fermentation
	0 h	4 h	8 h	24 h	48 h	
	Experiment 1 <sup>a</sup>					
CBG <sup>b</sup>	4.89	3.02	3.87	3.05	2.04	
CBG plus L-Lys plus L-Met	15.04	9.90	12.33	6.77	2.93	8.77
CBG plus polymer 3	15.53	11.10	10.02	11.45	10.37	78.29
CBG plus polymer 3c	17.48	15.50	14.27	11.20	10.73	69.02
	Experiment 2 <sup>c</sup>					
CBG <sup>b</sup>	4.08	3.86		2.43	3.03	
CBG plus L-Lys plus L-Met	14.36	10.02		4.35	4.31	12.45
CBG plus polymer 3	13.06	9.76		4.43	4.41	15.36
CBG plus polymer 3c	13.67	9.99		5.30	5.06	21.17

<sup>a</sup> Polymers in experiment 1 were 3 days old. <sup>b</sup> Coastal Bermuda grass, 0-time values 4.89 and 4.08 represent total methionine in the inoculum and 0.4 g of CBG. <sup>c</sup> Polymers in experiment 2 were 172 days old.



**Figure 1.** 6000-Hz spectra of the neutralized lysine-urea-formaldehyde polymer (**2c**) hydrolyzed at pD 0.85 with sodium (trimethylsilyl)propionate as a reference. pD 6.4 = pH 6.8; pD 0.85 = pH 1.25. Spectrum A was taken at 0 h, B at 1 h, C at 19 h, and D at 35 h.

**Table IV.** Recovery of Lysine and Methionine from Various Gastrointestinal Tract Sections of Wethers Fed Polymerized L-Lysine Hydrochloride or L-Lysine Hydrochloride and Methionine

section	recovery, g/day			
	L-Lys-HCl	polymer 2	polymer 2c	polymer 3c
lysine				
in diet in	2.35	5.38	4.92	2.48
gastrointestinal tract				
rumen	3.11 <sup>a</sup>	4.53 <sup>a</sup>	5.88 <sup>b</sup>	4.48 <sup>a,b</sup>
abomasum	5.50 <sup>a</sup>	7.86 <sup>a</sup>	9.62 <sup>b</sup>	6.88 <sup>a</sup>
duodenum-jejunum	4.62 <sup>a</sup>	6.92 <sup>b,c</sup>	5.85 <sup>a,b,c</sup>	7.08 <sup>b</sup>
ileum	4.12	4.06	2.84	3.11
methionine				
in diet in	1.54	1.54	1.54	3.10
gastrointestinal tract				
rumen	1.21 <sup>a</sup>	1.50 <sup>a</sup>	1.29 <sup>a</sup>	2.35 <sup>b</sup>
abomasum	2.53 <sup>a</sup>	2.18 <sup>a</sup>	2.43 <sup>a</sup>	3.62 <sup>b</sup>
duodenum-jejunum	2.19 <sup>a,c</sup>	2.34 <sup>a,c</sup>	1.66 <sup>b,c</sup>	2.84 <sup>a</sup>
ileum	1.09 <sup>a,b</sup>	1.37 <sup>a,b</sup>	0.98 <sup>a</sup>	1.93 <sup>b</sup>

<sup>a-c</sup> Means in the same row with unlike superscripts are different ( $P < 0.05$ ).

Table IV gives the recovery of lysine and methionine from each GI tract section sampled. Lysine consumed in the basal diet was 2.35 g/day and lysine provided by the polymers was 5.38, 4.92, and 2.48 g/day for polymers 2, 2c, and 3c, respectively. Recovery of lysine was greater, except for polymer 2c, in the abomasum and duodenum-jejunum than in the rumen, indicating considerable amounts of endogenous proteins were secreted in these GI tract sections. Lysine recovery was lower in the ileum than in either the abomasum or duodenum-jejunum, indicating that lysine was absorbed during passage through the jejunum. Lysine recovery was higher ( $P < 0.05$ ) from the ruminal and abomasal digesta of wethers receiving polymer 2c (5.88 and 9.62 g/day) than in those receiving the L-lysine hydrochloride (3.11 and 5.50 g/day) or polymer 2 (4.53 and 7.86 g/day), respectively. Lysine recovery from the duodenum-jejunum digesta was greatest from wethers fed polymer 2 and polymer 3c. No difference was found

among treatments in lysine recovered from the ileal digesta; thus, lysine in the polymers escaping rumen degradation appeared to be hydrolyzed and absorbed during passage through the abomasum, duodenum, and jejunum. The 2.36, 4.12, and 1.38 g of lysine/day above control in the abomasal digesta of wethers fed polymers 2, 2c, and 3c, respectively, represents a 43.9, 83.7, and 54.1% recovery of the lysine fed initially.

Methionine consumed in the basal diet was 1.54 g/day for wethers receiving L-lysine hydrochloride, polymer 2, and polymer 2c, respectively; polymer 3c provided an additional 3.1 g of methionine/day. Methionine recovered from ruminal and abomasal digesta samples (Table IV) was higher ( $P < 0.05$ ) in wethers fed polymer 3c (lysine plus methionine) than in animals fed the other three treatments. Quantity of methionine decreased between the abomasum and ileum for all treatments. Quantity of methionine in the ileal digesta was somewhat higher for

Table V. Molar Percentage of Lysine and Methionine in the Plasma of Wethers Fed Free or Polymerized L-Lysine Hydrochloride or Polymerized L-Lysine Hydrochloride and Methionine

sample source	mol % with treatment			
	L-Lys-HCl	polymer 2	polymer 2c	polymer 3c
		Lysine		
portal	7.33 <sup>a</sup>	9.68 <sup>b</sup>	12.66 <sup>c</sup>	12.06 <sup>c</sup>
carotid	8.33 <sup>a</sup>	7.84 <sup>a</sup>	11.26 <sup>b</sup>	13.69 <sup>b</sup>
		Methionine		
portal	1.00 <sup>c</sup>	1.23 <sup>c</sup>	1.02 <sup>c</sup>	1.62 <sup>d</sup>
carotid	1.45 <sup>c</sup>	1.52 <sup>c</sup>	1.26 <sup>d</sup>	1.65 <sup>c</sup>

<sup>a-c</sup> Means in the same row with unlike superscripts are different ( $P < 0.05$ ). <sup>c,d</sup> Means in the same row with unlike superscripts are different ( $P < 0.01$ ).

polymer 3c, perhaps indicating some lack of hydrolysis and absorption. The mean quantities of methionine recovered from the rumen and abomasum for wethers fed L-lysine hydrochloride or polymers 2 and 2c (no supplemental methionine) were 1.33 and 2.38 g/day, respectively, and 2.35 (rumen) and 3.62 (abomasum) g/day for wethers fed polymer 3c. The 16.0 g of polymer 3c provided 3.10 g of methionine/day. Thus, the additional methionine provided by polymer 3c represented 32.9 and 40.0% recovery of supplemental methionine from the rumen and abomasum, respectively.

Feeding either of the three L-lysine polymers increased ( $P < 0.05$ ) the molar percentage of lysine in the portal blood plasma (Table V). Feeding polymers 2c and 3c also resulted in higher ( $P < 0.05$ ) percentages of lysine in the carotid blood plasma compared to feeding polymer 2 or L-lysine hydrochloride. The molar percentage of methionine in the portal blood plasma was elevated ( $P < 0.01$ ) in wethers fed polymer 3c compared to those fed the other three treatments. Methionine in the carotid vein plasma was less in wethers fed polymer 2c than for the other three treatments. These data confirm that significant quantities of the polymerized amino acids did reach the lower gastrointestinal tract as shown in Table IV and that these polymers are effective in increasing the amino acid supply available to the ruminant animal.

The results from this research indicate that lysine and methionine can be protected from rumen microbial degradation when reacted with urea and formaldehyde. Lysine can be protected approximately 90% when L-lysine hydrochloride is polymerized with urea and formaldehyde

at or near a neutral pH. The usefulness of the polymers in providing supplemental lysine and methionine was found to be closely related to age of the polymer. The recovery of both methionine and lysine from in vitro fermentations was lower for a 172-day-old polymer produced under acid conditions than for a 3-day-old polymer. Lysine recovery from a polymer produced at a neutral pH did not decline with age and is closely related to the percentage of the  $\epsilon$ -carbon amino group bound into the urea-formaldehyde matrix. Protection of methionine from ruminal degradation could not be increased by producing the polymer at or near neutrality. The neutralized lysine polymers would be an excellent oral lysine supplement for ruminants fed dietary regimes likely to produce a post-absorption lysine deficiency for tissue protein synthesis in that ~90% of the lysine fed reaches the abomasum and is hydrolyzed and absorbed before the digesta passes through the ileum.

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