Quantitative Determination of the Amino Acid Content of Rumen Fluid from Twin Steers Fed Soybean Oil Meal or Urea

D. Richardson and W. S. Tsien

J Anim Sci 1963. 22:230-231.

QUANTITATIVE DETERMINATION OF THE AMINO ACID CONTENT OF RUMEN FLUID FROM TWIN STEERS FED SOYBEAN OIL MEAL OR UREA[1]

D. RICHARDSON AND W. S. TSIEN
Kansas State University, Manhattan

ZUNTZ (1891) showed that microorganisms are capable of synthesizing microbial proteins from nonprotein nitrogen. Loosli *et al.* (1949) have shown that ruminants fed urea as the only dietary source of nitrogen synthesized 10 amino acids in the rumen. McDonald (1953) suggested that the conversion of dietary nitrogen to microbial proteins in the rumen of sheep would be higher with rations of natural feedstuffs. Duncan *et al.* (1952) used fistulated calves maintained on a purified ration to show that rumen microorganisms synthesize amino acids when urea is fed as the dietary source of nitrogen.

Although few amino acids seem to be dietetically essential to ruminants, probably all are physiologically essential. A comparatively complete analysis of amino acids in rumen liquor by the use of a chemical method has not been published. This work provides some information on 19 common amino acids in rumen fluid, and compares amino acid content in rumen fluid from steers fed soybean oil meal with those fed urea.

Experimental Procedure

Two pairs of fistulated identical twin steers were fed the same daily ration of 1 lb. alfalfa hay, 4 lb. prairie hay, and 5 lb. cracked corn. One of the steers in each pair was supplemented with 1 lb. of soybean oil meal; the other, with 60 gm. of urea and an additional pound of corn. One-half of the ration was fed at 7 a.m. and the other half, at 5 p.m. Samples of rumen contents were taken after the steers were maintained on these rations for 63 days.

Four 200 ml., strained samples were taken at 7 a.m. before feeding, 10 a.m., 1 p.m. and 4 p.m. The samples were immediately chilled and refrigerated. The 800 ml. combined collection was dried at about 90° C. and ground in a Wiley mill.

One gram of sample was hydrolyzed in 80 ml. of 6N HCl at 110° C., under vacuum for 24 hours inside an oven. The hydrolysate was concentrated to nearly dryness, 20 ml. of water were added and again the hydrolysate was concentrated to nearly dryness. The sample was made to a volume of 50 ml. with sodium citrate buffer at pH 2.2. One ml. was used for each Dowex-50 chromatogram by the method of Moore and Stein (1948).

Cystine and cysteine were determined as cysteic acid (Block and Weiss (1956)). According to previous determinations, the hydrolysate contained no other strongly acidic, ninhydrin-positive constituents and therefore cysteic acid was determined on Dowex-50, 100 cm. column. It emerged as a sharp peak in the 24th to 26th ml. of effluent. Five-tenth mg. of cystine was converted to 0.4 mg. of cysteic acid from the column. Therefore, the quantities of cysteic acid measured in the hydrolysates were routinely divided by 0.8 to give the final percent of cystine plus cysteine.

Tryptophan was determined microbiologically by the procedure of Miller and Ruttinger (1950). The sample was hydrolyzed by autoclave with 6N Ba $(OH)_2$ for 10 hours at 15 lb. of pressure.

Results and Discussion

The technique of sampling in this experiment should have eliminated the time factor in protein synthesis because the samples were withdrawn at selected intervals during the day. Hereditary differences were considered to have been eliminated from the comparisons by using identical twins. The adjustment period of 63 days should have reduced any carryover effect from the previous ration and allowed sufficient time for the microorganisms to adapt themselves to urea.

The crude protein level per liter of rumen liquor was 6.44 gm. and 6.23 gm., respectively, from the soybean oil meal- and urea- supplemented steers. The rumen liquor of steers supplemented with soybean oil meal contained substantially more amino acids than did rumen liquor from the steers fed urea. Lysine content was greater in rumen fluid from steers fed soybean oil meal than from those fed

[1] Contribution No. 288, Dept. of Animal Husbandry.

TABLE 1. AMINO ACID CONTENT OF RUMEN FLUID FROM TWIN STEERS FED SOYBEAN OIL MEAL OR UREA

Supplemental nitrogen	1 lb. soybean oil meal		60 gm. urea	
Steer				
	Mg. amino acid per liter			
Aspartic acid	346.03	382.19	305.76	242.01
Threonine	173.09	199.75	154.06	84.32
Serine	137.38	150.73	97.74	32.52
Glutamic acid	496.91	517.14	491.60	377.24
Proline	299.62	283.42	251.63	190.78
Glycine	168.93	168.02	148.88	112.84
Alanine	190.35	213.52	189.78	149.32
Methionine	89.75	93.73	79.52	71.89
Isoleucine	229.40	249.98	197.85	156.80
Valine	197.46	213.52	189.78	149.32
Leucine	291.69	303.31	254.42	220.90
Tryosine	151.38	156.91	99.54	79.55
Phenylalanine	253.13	277.98	150.92	126.69
Histidine	67.50	77.58	62.74	53.83
Lysine	300.13	305.91	273.54	227.42
Arginine	139.04	152.56	137.34	101.34
Tryptophan	78.20	129.23	55.99	45.78
Cystine and cysteine	52.22	54.34	37.59	29.11
Grams crude protein/liter	6.514	6.376	7.310	5.146
Total gm. amino acid/liter	3.666	3.903	3.168	2.471
% A.A. of total C.P./liter	56.3	61.2	43.3	48.0

urea. Methionine content was not large in rumen fluid from steers fed soybean oil meal and even smaller in that from steers fed urea. Perhaps this explains the benefits Gossett *et al.* (1960) found from adding lysine and methionine to a high urea fattening ration.

All amino acids were present in greater quantities from steers supplemented with soybean oil meal (table 1). This helps to explain Lewis's (1961) proposal that proteins synthesized in the rumen of lambs fed urea as the only source of nitrogen are not sufficient to support good growth. Total amino acids per liter of rumen fluid was 0.97 gm. greater from steers receiving soybean oil meal. Also, amino acids accounted for 13 percentage units more of the total crude protein per liter when soybean oil meal was used as the protein supplement (56 vs. 43 and 61 vs. 48).

Amino acids, such as cystine and cysteine, trytophan, histidine, and methionine, were present in smaller amounts than other amino acids in rumen liquor from all steers. This shows that the amino acid proportions in the rumen microbial proteins are similar to those in most feedstuffs.

Summary

Two pairs of fistulated identical twin steers were used to compare, by column chromatography, 19 amino acids in rumen fluid after feeding urea or soybean oil meal. Steers supplemented with soybean oil meal synthesized a greater amount of all amino acids than steers fed urea.

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