Thanh, V. H., Okubo, K., Shibasaki, K., *Tohoku. J. Agric. Res.* **26**, 41 (1975a).
Thanh, V. H., Okubo, K., Shibasaki, K., *Agric. Biol. Chem.* **39**, 1501 (1975b).
Weber, K., Osborn, M., *J. Biol. Chem.* **244**, 4406 (1969).
Wikman-Coffelt, J., Zelis, R., Fenner, C., Mason, D. T., *Biochem. Biophys. Res. Commun.* **51**, 1097 (1973).
Yamamoto, K., Fukazawa, T., Yasui, T., *Hokkaido Daigaku Nogakubu Hobun Kiyo* **9**, 116 (1973); *Chem. Abstr.* **81**, 10560u (1974).

Received for review May 12, 1976. Accepted October 5, 1976. This work was supported in part by the Australian Meat Research Committee.

Processing Damage to Lysine and Other Amino Acids in the Manufacture of Blood Meal

Paul E. Waibel,* Margita Cuperlovic,[1] Richard F. Hurrell, and Kenneth J. Carpenter

Four vat-dried and five ring-dried blood meals were analyzed for their total and reactive lysine contents, and for their potency in bioassays for lysine. The crude protein in the former contained, on the average, 9% less total lysine and 26% less fluorodinitrobenzene (FDNB)-reactive lysine than did the ring-dried blood meals. Using rats, chicks, and turkey poults for growth assays the potency of vat-dried blood meals as sources of lysine ranged from 0 to 43%, and that of ring-dried samples ranged from 80 to 97%, of the corresponding total lysine values. The vat drying resulted in an equally severe reduction in bioavailability of methionine, and the digestibility of the protein as a whole was greatly reduced. The ring-drying procedure, involving short-time, high-temperature heating, resulted in a product of reasonably good quality. Blood was not particularly sensitive to heat damage in controlled heating tests. Mixing blood with either corn meal, wheat bran, or starch prior to drying resulted in greater damage.

A considerable amount of blood is available as a by-product at abattoirs. Some goes to waste, but much is dried down to blood meal for animal feeding, and it has also been suggested that it could be recovered directly for the fortification of human diets (review by Happich, 1975). Its high protein and particularly high lysine content would make it a valuable supplementary protein in pig and poultry diets if the protein had a high digestibility and the lysine had a degree of availability equivalent to that in most other high-protein feedstuffs. In practice, however, it has been regarded as only poorly digestible and unpalatable (Morrison, 1956; Cullison, 1975). Winter (1929) reported that, while the nitrogen of specially prepared dried blood had a digestibility of 94% for pigs, the corresponding value for commercial blood meal was only 72%. Kratzer and Green (1957) reported that bioassays, using chicks and turkeys, gave values equivalent to only 60% of the total lysine content of commercial, vat-dried blood meals. In vat-drying, blood is dried by stirring in a steam-jacketed cylinder at a temperature of up to 165 °C for 10–12 h.

This investigation began with total and biologically available lysine assays of blood meal prepared by a newer process, ring drying, in which the blood is coagulated by steam and the sludge dried rapidly in a closed ring system in a current of very hot air (400–410 °C). Conventional (i.e., vat-dried) blood meals were used for comparison, and these gave such low values in the lysine assays that further tests were made of their digestibility and value in supplying the sulfur amino acids. Using fresh blood, the influences of controlled heating and carrier on fluorodinitrobenzene (FDNB)-reactive lysine were also studied.

Department of Applied Biology, University of Cambridge, Cambridge, England, CB2 3DX (M.C., R.F.H., K.J.C.), and the Department of Animal Science, University of Minnesota, St. Paul, Minnesota 55108 (P.E.W.).

[1]Present address: Institute for the Application of Nuclear Energy in Agriculture, Veterinary Medicine and Forestry, Zemun, Baranjska 15, Yugoslavia.

EXPERIMENTAL SECTION

Test Materials. The vat-dried and ring-dried blood meals were commercial samples of U.S. manufacture for which exact processing details were not obtainable. (Some analytical values for samples coded X949 and X953 have already been reported by Hurrell and Carpenter (1975)).

The fresh blood used was from steers. Portions were mixed with equal weights of either corn meal or corn starch, and a further portion with half its weight of wheat bran. These mixtures, and the blood itself, were divided up and either freeze-dried or dried in shallow dishes in air ovens set at 50 °C (for 48 h) or 100 °C (for 24 h). For a further experiment, portions of freeze-dried blood were adjusted to 18% moisture content, packed in glass ampules, sealed, and heated at controlled temperatures. Ampules containing crystalline hemoglobin (Sigma Chemical Co., St. Louis, Mo.) were heated at the same time.

Chemical Analyses. Analyses for total lysine were carried out essentially by the procedure of Spackman et al. (1958), using an ion-exchange column. Methionine and cystine were determined after preliminary oxidation with performic acid to methionine sulfone and cysteic acid, respectively (Moore, 1963). Fluorodinitrobenzene (FDNB)-reactive lysine was determined by the direct procedure of Carpenter (1960) as modified by Booth (1971).

Animal Assays. The turkeys used in the lysine assays were Large White males. Day-old poults were placed on a practical stock diet for 3 days, and the basal assay diet supplemented with 0.4% lysine for a further 4 days. They were then weighed and distributed on the basis of weight into groups of eight turkeys each. Three groups were assigned to each treatment. The experimental feeding period was 10 days. The basal diet contained (in percent) sesame meal 30, corn gluten meal 26, corn starch 34.55, corn oil 4, dicalcium phosphate 2.5, calcium carbonate 1.5, salt 0.5, fermentation residue product 0.25, taurine 0.025,

Table I. Response of Turkeys Fed a Lysine-Deficient Diet to Lysine or Blood Meal

Supplement	Level of suppl., %	Wt gain, g	Calcd potency of sample, % Lys (confidence limits $P = 0.05$)
Lysine[a]	0; 0.25	22.8; 50.6	
	0.50; 0.90	90.4; 151.6	
	1.20	186.0	
Ring-dried blood meals			
W5	2.84; 5.68	52.1; 72.8	7.6 (6.4-8.8)
W6	2.91; 5.81	47.0; 78.3	7.5 (7.0-8.0)
W19	2.81; 5.62	44.7; 74.3	7.3 (6.4-8.2)
Vat-dried blood meals			
X953	3.20; 6.41	23.9; 33.6	1.1 (0-2.2)
W8	3.20; 6.41	37.2; 44.0	3.3 (2.6-4.1)
W9	3.29; 6.58	33.6; 44.4	2.9 (2.1-3.7)
W10	3.42; 6.85	28.3; 40.4	2.0 (1.0-3.0)

[a] The actual supplement was L-lysine-HCl which was assumed to contain 80% lysine.

vitamin mix 0.5, and trace mineral mix 0.15. The vitamin mix provided the following per kg diet: vitamin A, 10 000 IU; vitamin D, 3600 ICU; vitamin E, 10 IU; vitamin K (as menadione dimethylpyrimidinol bisulfite), 4.4 mg; riboflavin, 8 mg; calcium pantothenate, 20 mg; niacin, 70 mg; choline chloride, 1200 mg; folic acid, 2 mg; vitamin B_{12}, 15 μg; biotin, 300 μg; thiamin·HCl, 10 mg; pyridoxine·HCl, 5 mg. The trace mineral mixture contained: 6% manganese, 0.12% iodine, 2% iron, 0.2% copper, 6% zinc, 0.2% cobalt, and 25% calcium. This diet contains 0.75% lysine by calculation. The standard lysine and test blood meals were added at the expense of corn starch.

For the rat lysine assays, female rats of the CFY strain (Carworth Europe Ltd., Huntingdon, U.K.) were obtained at 21 days of age weighing 45–50 g. They were fed the unsupplemented basal diet for 3 days, randomized and caged in pairs, and allotted to experimental diets for a further 7 days. There were four replicate cages per treatment. For the chick lysine assays, chicks were received at 1 day of age and fed the basal assay diet supplemented with 0.3% lysine (as 0.375% lysine·HCl) for 3 days. Those showing medial weight gains were randomized into cages of two chicks each. There were three replicate cages per treatment. The growth assay was conducted for the next 8 days. The basal diets used in the chick and rat assays have already been described (Waibel and Carpenter, 1972).

The chick assay for methionine was carried out by the procedure described by Carpenter et al. (1972), using diet 1 of that paper.

The relative biopotency of the samples and their confidence limits were calculated by the slope–ratio procedure (Finney, 1964). The assays for rats and chicks were calculated with "weight gain/food eaten" as the measure of response. The turkey assay used weight gains for the calculation of potencies.

For the digestibility determinations, rats similar to those used for the lysine assays were housed in pairs and fed the experimental diets ad libitum. After 3 days for adaptation to the diets, feces were collected for the subsequent 6 days. The test diets each contained 10% crude protein from a test material, and a N-free diet was fed as a negative control (Hurrell et al., 1976).

RESULTS

The results of the weight gain bioassays for lysine and methionine are summarized in Tables I and II. These all gave satisfactory data for calculation of potency of the samples, and the final estimates with their fiducial limits ($P = 0.05$) are set out in the same tables.

Table II. Results from the Amino Acid Bioassays with Rats and Chicks

	Suppl. to basal diet, %	Gain/food eaten	Calcd potency of suppl., % (confidence limits $P = 0.05$)
A. Rat Assay for Lysine			
L-Lysine[a]	0; 0.16	0.177; 0.311	
L-Lysine	0.32; 0.48	0.426; 0.494	
Blood meal, X949	2.5; 5.0	0.336; 0.477	8.6 (7.5-9.7)
Blood meal, X953	2.5; 5.0	0.210; 0.210	0.6 (−0.6-1.6)
(Pooled standard error of treatment means, 0.014)			
B. Chick Assay for Lysine			
L-Lysine[a]	0; 0.14	0.315; 0.437	
L-Lysine	0.28; 0.42	0.524; 0.578	
Blood meal, X949	2.2; 4.4	0.454; 0.553	8.2 (7.5-9.0)
Blood meal, X949	2.2 + 0.14 Lys	0.520	
Blood meal, X953	2.3; 4.6	0.299; 0.311	−0.9 (−1.9-0.1)
Blood meal, X953	2.3 + 0.14 Lys	0.433	
(Pooled standard error of treatment means, 0.0095)			
C. Chick Assay for Methionine			
L-Methionine	0; 0.02	0.328; 0.346	
L-Methionine	0.04; 0.06	0.479; 0.529	
Blood meal, X949	2.2; 4.4	0.433; 0.505	1.2 (1.0-1.5)
Blood meal, X949	2.2 + 0.02 L-Met	0.493	
Blood meal, X953	2.3; 4.6	0.342; 0.352	0.2 (0-0.4)
Blood meal, X953	2.3 + 0.02 L-Met	0.421	
(Pooled standard error of treatment means, 0.013)			

[a] The actual supplement was L-lysine·HCl which was assumed to contain 80% lysine.

Chicks showed a normal gain/feed response to added lysine in the presence of vat-dried meal X953, which by itself had produced no response (Table II). There was a similar finding in the chick assay for methionine. It appears, therefore, that the low response of chicks to meal X953 is not due to a growth or appetite inhibitor that prevents a normal response to the limiting amino acid in the assay.

Values for lysine analysis and assay by different methods have been assembled in Table III. The ring-dried samples have an average total lysine content of 9.7 g/16 g of N and the corresponding value for the vat-dried meals is 8.9. The difference in FDNB-reactive lysine values is proportionally greater, being approximately 8.4 g/16 g of N, or 86% of the corresponding total lysine value, for ring-dried meals and 6.2 g/16 g of N, or 70% of the total lysine value, for vat-dried meals. But the difference in bioassay values is greater still, with the ring-dried samples giving values equivalent to 80% or more of the total lysine values, while the corresponding values for the vat-dried meals are in the 0–50% range.

The two samples X949 and X953, as judged by the proportion of total lysine reactive with FDNB, were, respectively, the least and the most damaged. These were also the two samples assayed with chicks for available methionine and the results, given in Table II, were quite comparable to those for lysine. In X949 the methionine was apparently almost completely available while X953

Table III. Total and Reactive Lysine in the Blood Meals and Their Potency in Bioassays[a] for Lysine

Sample no.	Dry matter, %	Crude protein, N x 6.25%	Total Lys, % of sample	FDNB-reactive Lys, % of total Lys	Bioassay values, as % of total Lys values (confidence limits $P = 0.05$)		
					Turkeys	Chicks	Rats
Ring-dried							
X949	93.8	90.4	8.8	89		93 (85-102)	97 (86-110)
W5	93.1	91.0	8.8	84	86 (73-100)		
W6	93.5	90.2	8.6	86	87 (81-93)		
W19	89.7	89.1	8.9		82 (71-92)		
W21	90.1	88.5	8.6	87	80 (77-84)		
Vat-dried							
X953	96.5	87.3	6.8	62	14 (0-29)	0 (0-1)	8 (0-24)
W8	98.1	82.2	7.8	73	43 (38-52)		
W9	97.9	82.3	7.6	69	39 (28-49)		
W10	96.9	81.2	7.3	77	28 (14-42)		

[a] Bioassay data shown in Tables I and II, excepting W21 which was assayed in a similar experiment.

Table IV. Digestibility of the Crude Protein and Amino Acids of Test Materials by Rats

	Crude protein		Lysine		Methionine		Cystine	
	% of sample	% dig.	g/16 g of N	% dig.	g/16 g of N	% dig.	g/16 g of N	% dig.
Freeze-dried blood	(93.5)	87	(9.59)	99	(1.25)	99	(1.54)	99
Blood dried at 100 °C for 24 h	(94.3)	79	(9.15)	94	(1.20)	93	(1.46)	87
Ring-dried blood meal, X949	(90.4)	97	(9.66)	97	(1.44)	96	(1.42)	87
Vat-dried blood meal, X953	(87.3)	55	(7.75)	49	(1.04)	40	(0.69)	18

had little or no value as a source of this amino acid.

Further analytical and digestibility values for the lysine and sulfur-containing amino acids in samples X949 and X953 are given in Table IV, together with data for blood either freeze-dried or oven-dried at 100 °C in the laboratory. The cystine content of X953 is particularly low. This is a normal finding in severely heated material (cf. Bjarnason and Carpenter, 1970). The methionine content of the crude protein in X953 is also nearly 30% lower than the corresponding value for X949. This is not a normal accompaniment of heat damage and, by comparison with the blood samples dried in the laboratory, it appears to be the value for X949, i.e, 1.44 g/16 g of N, that is exceptionally high. However, the methionine value by chick bioassay for X949 was 1.2% of the sample, or 1.33 g/16 g of N, which is similar to the digestible methionine content, calculated as 96% of 1.44 g/16 g of N.

Turning to the digestibility values in Table IV, these are again lower for N and all three amino acids with X953 than with the other samples, though still not low enough to account for the near-zero bioassay values for the lysine and methionine in this sample. They are true digestibility values in the sense of being corrected for metabolic fecal losses as determined with a N-free diet. However, the particularly low value for cystine may be partly an artifact due to the indigestible protein stimulating extra secretion of pancreatic enzymes of high cystine content, and to these forming a considerable proportion of the total fecal protein (cf. Kwong and Barnes, 1975; Slump and van Beek, 1975).

The effects of controlled heating of hemoglobin and of freeze-dried blood on their FDNB-reactive lysine contents are set out in Table V. Storage at 37 °C for 7 days has resulted in a substantial, i.e. 10%, fall only with the freeze-dried blood. Falls under these conditions are associated with reducing sugar–lysine interactions. The relatively small changes (i.e., 2.5–5% per hour) in both materials held at 121 °C indicate their relative insensitivity to damage (cf. Carpenter, 1973, Figure 3).

Finally, Table VI summarizes the results from oven-drying blood in thin layers either by itself or mixed with vegetable materials as carriers. The use of these carriers resulted in a greater proportional loss of reactive lysine groups than when the blood was dried alone under the same conditions.

Table V. FDNB-Reactive Lysine (g/16 g of N) of Hemoglobin and Whole Dried Blood after Controlled Heating

	Hemoglobin (18% H_2O)	Freeze-dried blood (18% H_2O)
Unheated	8.77	9.24
1 h at 121 °C	8.83 (101)[a]	8.77 (95)
4 h at 121 °C	7.86 (90)	8.00 (87)
7 days at 37 °C	8.56 (98)	8.34 (90)

[a] Values in parentheses are expressed as percentages of the unheated controls.

Table VI. FDNB-Reactive Lysine in Blood Oven-Dried Alone or Mixed with Vegetable Materials

	Freeze-dried	Heated at 50 °C for 48 h	Heated at 100 °C for 24 h
Blood alone	(9.23)[a]	96[b]	92[b]
Blood:corn starch (2:1)	(8.50)	93	78
Blood:corn meal (2:1)	(7.80)	70	53
Blood:wheat bran (1:1)	(7.50)	81	64
Corn meal alone	(3.08)	88	70
Wheat bran alone	(3.52)	97	85

[a] Values for freeze-dried materials in g/16 g of N. [b] Values for oven-dried materials as percentages of the values for the corresponding freeze-dried samples.

DISCUSSION

These results are in agreement with those of other workers cited in the introductory statement, that conventional, vat-dried blood meals can have considerably lower protein quality than would be expected from their lysine content (Table III). This is not an inherent property of blood, as ring-dried material in our study, or spray-dried material (Kratzer and Green, 1957) can show a high digestibility and availability of lysine and methionine.

With the most severely damaged of the vat-dried samples, the overall digestibility of the nitrogen by rats was only approximately 50%. But the bioassays for available lysine and methionine gave even lower values,

little different from zero. The failure to stimulate weight gain or gain/feed response in the bioassays was not due apparently to positive growth inhibition by the heat-damaged material as the addition of pure lysine or methionine to the diets each gave a fully normal response in the presence of the vat-dried meal.

The considerable damage found in conventional blood meals is in contrast to experience with meat meals where the damage appears to be relatively small despite fairly drastic processing procedures (Atkinson and Carpenter, 1970; Herbert et al., 1974) and the generally low quality is explained by the poor amino acid composition of the raw materials. There was an early suggestion (Devlin and Zittle, 1944) that the major blood protein, hemoglobin, was itself particularly sensitive to heat damage, but we did not find this either in rat feeding tests (DeCombaz and Carpenter, 1973) or as indicated by changes in reactive lysine groups (Bjarnason and Carpenter, 1970).

"Is the damage to be explained by the presence of glucose in blood?" Taking a value of 50 mg/100 ml for ruminant blood (Annison and Armstrong, 1970), this could be equivalent, mole/mole, to less than 5% of the lysine present, and in another study it was found that a monosaccharide molecule would not bind more lysine than this, even under severe or prolonged conditions (Carpenter et al., 1962). Storage of samples of high moisture content at 37 °C for 10 days has been found adequate for reaction between reducing sugars and lysine groups. Blood stored under such conditions has shown approximately a 10% fall in reactive lysine content (Table V).

A small proportion of the lysine binding in severely damaged blood meals may therefore be explained by protein–sugar reactions, but there are several similarities between the damage in blood meal X953 and that in severely heated animal proteins of low carbohydrate content (cf. review by Carpenter, 1973). (1) There has been a considerable fall in overall protein digestibility and the fall in available methionine is similar to that in available lysine. (2) FDNB-reactive lysine, though a superior indicator of damage to total lysine, still does not fully reflect the fall in biologically available lysine. (3) As reported elsewhere (Hurrell and Carpenter, 1975) the arginine content of X953 is still high and the dye-binding value with Acid Orange 12 is much reduced as compared with X949.

On the other hand, the damaged meal has not shown an elevated level of lysine isopeptides or detectable lanthionine, and bovine plasma albumin, lactalbumin, casein, and ovalbumin have all, after severe heat treatment, shown one of these signs of the formation of cross-linking. Samples X949 and X953 have been found to contain, respectively, 0.35 and 0.37 g of lysine/16 g of N in the form of isopeptides (Hurrell et al., 1976). The quite high bioassay and digestibility results for X949 (Tables III and IV) indicate that the existence of isopeptides to the extent found in this sample cannot be regarded as necessarily leading to low protein quality in a test material. Some isopeptides are formed in fibrin during the clotting of blood (Pisano et al., 1969), but this would not explain the level found in X949. This should be investigated further.

It is possible that the procedure of Cole et al. (1971) used to determine lysine isopeptides may have given an underestimation for the severely damaged material X953, since the analysis depends on an initial in vitro digestion of the protein. There is no direct evidence so far for mechanisms which would fully explain the low quality of this sample. It may be that cross-linkages other than simple lysine–isopeptides were formed and that these were responsible for the greatly reduced N digestibility. But reduced digestibility only partly explains the low bioassay values for lysine and methionine. It has been suggested earlier, in reviewing similar findings, that lysine and methionine may be adsorbed from the gut in forms that are not utilizable for growth (cf. Ford and Shorrock, 1971). In another recent study with heated chicken muscle, changes in digestibility measured at the ileal level did appear to explain its fall in amino acid availability (Varnish and Carpenter, 1975). Studies of this general kind are difficult to interpret, since apparently contradictory findings may be due to factors such as the sugar content of the test materials or to the exact procedures used for the measurement of digestibility, etc.

The sticky nature of blood makes its rapid drying technically difficult, but our results indicate that drying on vegetable carriers to improve the physical properties may actually increase the susceptibility of the lysine in the blood to damage. Presumably this is the result of carbohydrate–protein interaction.

FDNB-reactive lysine, as discussed above, is an overestimate of nutritionally available lysine in severely heated material but it does appear to be a useful indirect indicator of blood meal quality. Values above 8 g/16 g of N have been associated with high quality while values of under 7 g/16 g of N were found for the vat-dried meals which all gave poor results in the bioassays.

The greater use of quality tests for the grading of blood meals by users will provide an incentive to manufacturers to improve their drying methods. Combs and Nott (1967) suggested that compounders assume a value of 65% for the availability of the amino acids in a blood meal of unknown history. This appears to be still a good average, but seriously overvalues some and undervalues others.

ACKNOWLEDGMENT

We are grateful to Sarah Hellorn, Ho Seung Yang, and Bruce R. Behrends for capable technical assistance and to Van Hoven Co., Inc. for providing the commercial blood meals.

LITERATURE CITED

Annison, E. F., Armstrong, D. G., "Physiology of Digestion and Metabolism in the Ruminant", Phillipson, A. T., Ed., Oriel Press, Newcastle, England, 1970, pp 422–437.
Atkinson, J., Carpenter, K. J., *J. Sci. Food Agric.* **21**, 366 (1970).
Bjarnason, J., Carpenter, K. J., *Br. J. Nutr.* **24**, 313 (1970).
Booth, V. H., *J. Sci. Food Agric.* **22**, 658 (1971).
Carpenter, K. J., *Biochem. J.* **77**, 604 (1960).
Carpenter, K. J., *Nutr. Abstr. Rev.* **43**, 424 (1973).
Carpenter, K. J., McDonald, I., Miller, W. S., *Br. J. Nutr.* **27**, 7 (1972).
Carpenter, K. J., Morgan, C. B., Lea, C. H., Parr, L. J., *Br. J. Nutr.* **16**, 451 (1962).
Cole, M., Fletcher, J. C., Gardner, K. L., Corfield, M. C., *Appl. Polym. Symp.* **18**, 147 (1971).
Combs, G. F., Nott, H., *Feedstuffs*, 36 (Oct 21, 1967).
Cullison, A., "Feeds and Feeding", Reston Publishing Co., Inc., Reston, Va., 1975.
DeCombaz, J. E., Carpenter, K. J., "Some Factors Influencing the Sensitivity of Proteins to Nutritional Damage during Processing", Department of Applied Biology, University of Cambridge, Cambridge, England, 1973, unpublished report.
Devlin, H. B., Zittle, C. A., *J. Biol. Chem.* **156**, 393 (1944).
Finney, D. J., "Statistical Methods in Biological Assay", 2nd ed, Hefner Publishing Co., New York, N.Y., 1964.
Ford, J. E., Shorrock, C., *Br. J. Nutr.* **26**, 311 (1971).
Happich, M. L., "Protein Nutritional Quality of Foods and Feeds", Part I, Friedman, M., Ed., Marcel Dekker, New York, N.Y., 1975, pp 161–185.
Herbert, L. S., Dillon, J. F., MacDonald, M. W., Skurray, G. R., *J. Sci. Food Agric.* **25**, 1083 (1974).
Hurrell, R. F., Carpenter, K. J., *Br. J. Nutr.* **33**, 101 (1975).

Hurrell, R. F., Carpenter, K. J., Sinclair, W. J., Otterburn, M. S., Asquith, R. S., *Br. J. Nutr.* **35**, 383 (1976).
Kratzer, F. H., Green, N., *Poultry Sci.* **36**, 562 (1957).
Kwong, E., Barnes, R. H., *J. Nutr.* **105**, 1457 (1975).
Moore, S., *J. Biol. Chem.* **238**, 235 (1963).
Morrison, F. B., "Feeds and Feeding", 22nd ed, Morrison Publishing Co., Ithaca, N.Y., 1956.
Pisano, J. J., Finlayson, J. S., Peyton, M. P., *Biochemistry* 8, 871 (1969).
Slump, P., van Beek, L., "Protein Nutritional Quality of Foods and Feeds", Part I, Friedman, M., Ed., Marcel Dekker, New York, N.Y., 1975, pp 67–78.
Spackman, D. H., Stein, W. H., Moore, S., *Anal. Chem.* **30**, 1190 (1958).
Varnish, S. A., Carpenter, K. J., *Br. J. Nutr.* **34**, 339 (1975).
Waibel, P. E., Carpenter, K. J., *Br. J. Nutr.* **27**, 509 (1972).
Winter, A. R., *Ohio Agric. Exp. Stn. Bull.* **No. 436** (1929).

Received for review May 7, 1976. Accepted September 14, 1976. We are grateful to the Fats and Proteins Research Foundation, Inc., Des Plaines, Ill., for a grant which in part supported this research. Scientific Journal Series Paper No. 9524, Minnesota Agricultural Experiment Station. A portion of this paper was presented at the 58th Annual Meeting of the Federation of American Societies for Experimental Biology, April, 1974, in Atlantic City, N.J. (*Fed. Proc., Fed. Am. Soc. Exp. Biol.* **33**(3), 657 (1974)).

Some Properties of Soluble Proteins from Alfalfa (*Medicago sativa*) Herbage and Their Possible Relationship to Ruminant Bloat

Ronald E. Howarth,* Subodh K. Sarkar,[1] Adrian C. Fesser, and G. Wayne Schnarr[2]

The soluble herbage proteins from alfalfa were analyzed by polyacrylamide gel electrophoresis and separate gels were stained with Coomassie Blue, Sudan Black, and periodic acid–Schiff reagent. Seventeen discrete protein bands were readily identified against heavy background staining. Four protein bands, in the fraction II group, were stained by Sudan Black. Possibly these proteins could make a greater than average contribution to the viscosity of rumen foams. Additional Sudan Black staining material was present in crude extracts but this material was removed by ion-exchange chromatography. Very faint staining was obtained with periodic acid–Schiff reagent. Fraction I protein stained most intensely with this reagent. The isoelectric point of fraction I protein was 5.6. Isoelectric points of fraction II proteins ranged from 3.3 to 6.2. Fraction II proteins present in highest concentrations had isoelectric points of 4.4, 4.8, 5.0, and 5.1. Both fraction I and the majority of fraction II proteins would be expected to produce viscous foams at the pH of rumen ingesta during bloat in cattle fed fresh alfalfa.

Several lines of evidence implicate soluble plant proteins as the foaming agents responsible for the frothy bloat which may occur when ruminant animals graze clover or alfalfa pastures (Clarke and Reid, 1974). For descriptive purposes the soluble herbage proteins are frequently classified into two fractions. Fraction I is a single protein, ribulosebiphosphate carboxylase, which comprises 30 to 50% of the total soluble protein. The other soluble proteins are known as fraction II proteins, a group which contains many proteins, each present in small amount.

Earlier studies on some foaming properties of alfalfa leaf proteins implicated fraction I protein as the primary foaming agent responsible for pasture bloat (McArthur et al., 1964; Stifel et al., 1968), but in these studies the fraction II proteins were apparently incorrectly identified and the experimental basis for excluding the fraction II proteins from consideration as foaming agents was invalid (Jones and Lyttleton, 1969; Howarth et al., 1973). We therefore wished to reexamine the question: do all soluble herbage proteins produce foams of equal persistence or do the various soluble herbage proteins differ in their abilities to produce persistent foams?

Jones and Lyttleton (1969, 1972) have compared some foaming properties of fraction I and II proteins from red and white clovers and found that both fractions gave persistent foams in the pH range which occurs in the rumen of bloated animals. On the basis of foam volume measurements, Rommann et al. (1971) suggested that alfalfa lipoproteins could play a role in the formation of persistent foam during ruminant bloat.

In our investigations we wished to compare the foaming properties of alfalfa fraction I and fraction II proteins and, in addition, to compare foaming properties of proteins within the fraction II group. However, the isolation of individual fraction II proteins in sufficient quantities for direct measurement of foaming properties would have been a difficult and time-consuming operation. Therefore, an indirect approach was selected. Since foaming and surface interfacial properties of proteins result from certain physical–chemical properties of proteins, we have characterized the soluble alfalfa proteins by measuring their isoelectric points and by their reactions with lipid and carbohydrate specific stains. From this information we have deduced probable foaming properties of specific alfalfa protein fractions.

EXPERIMENTAL SECTION

Extraction of Soluble Alfalfa Proteins. Alfalfa herbage was harvested during the prebud growth stage from the apical 10–15 cm of plants in a field plot of Beaver alfalfa. It was stored at −20 °C and ground in a plate grinder cooled with liquid nitrogen (Hikichi and Miltimore, 1970). Weighed samples were homogenized in a Duall, ground-glass tissue grinder (Kontes Glass Co., Vineland, N.J.) in 0.1 M Tris-glycine buffer (pH 8.9) and centrifuged at 70 000*g* for 30 min. The supernatant solution was used for electrophoretic characterization of the soluble alfalfa

Research Branch, Agriculture Canada, Saskatoon, Saskatchewan S7N 0X2, Canada.
[1]Present address: Environment Canada, Northern Forest Research Center, Edmonton, Alberta T6H 3S5, Canada.
[2]Present address: Department of Chemistry, Queen's University, Kingston, Ontario K7L 3N6, Canada.