IN VITRO PROTEIN SOLUBILITY AND SOLUBILIZATION OF SELECTED FEEDSTUFFS FOR RUMINANT FEEDING

By

A.B.I. UDEDIBIE,* and L.E. CHASE
Department of Animal Science,
Cornell University,
Ithaca, New York.

ABSTRACT

Protein solubility in mineral buffer, and protein degradability with bacterial protease, of condensed distillers solubles, distillers dried grains, brewers dried grains, raw, roasted and extruded soybeans, corn silage, haycrop silage, hay, corn gluten feed, maltlage solulac and lactosy-urea (Ewoplus) were investigated for use in ruminant feeding.

The proteins of distillers dried grains, brewers dried grains, roasted and extruded soybeans and corn gluten meal were highly insoluble in mineral buffer with average solubilities of 4.50%, 10.47%, 8.07%, 13.12% and 5.40% respectively. Lactosyl-urea (Ewoplus) was 100% soluble.

The protease of streptomyces griseus was used to determine protein degradability. The degradable proteins of distillers grains, brewers grains, raw and roasted soybeans, and corn gluten meal were resistant to protease action. Extruded soybeans, distillers solubles, forage feedstuffs and Ewoplus were readily degradable.

Present address: National Veterinary Research Institute, Vom — Nigeria.

INTRODUCTION

Nutritive value has been defined as a complex of parameters including digestibility, consumption and effieincy (Van Soest, 1979). Laboratory evaluation of feedstuffs is essentially the easiest and quickest means of obtaining analytical data that predict the extent of biological degradation under specified conditions, animals and time. A knowledge of the relationship between and within parameters of chemical composition and nutritive value is of primary importance in evaluating feedstuffs.

One of the feed constitutents most altered by the rumen fermentation is the proteins. The synthesis of microbial proteins from non-protein nitrogen sources and the alteration in amino acid composition of balanced and unbalanced feeds take place in the rumen. These two processes are in competition with the passage of the nitrogenous substances out of the rumen. As observed by Fauconneau and Michel (1970) and Orskov and Fraser (1973), the passage of these nitrogenous substances is dependent mostly on the extent of their solubility in the rumen. Proteins which are very soluble in the rumen fluid or proteins which are readily degraded by the rumen microbes stand very high chances of being lost by the animals as they are rapidly converted to ammonia and lost as urea. That is why Lewis and Mitchell (1974) emphasized that the unique aspects of nitrogen metabolism in ruminants impinge mainly on the supply of amino acids to the small intestine rather than the amino acid requirements of tissues, which appear to be similar to those of non-ruminants. It has therefore become necessary to have knowledge of the extent of solubility of proteins of feedstuffs, particularly those commonly used in ruminant nutrition.

MATERIALS AND METHODS

Samples of feedstuffs, condensed distillers solubles, distillers grains, raw, roasted and extruded soybeans, brewers grains, haylage, hay, corn silage, corn gluten meal, corn gluten feed, solulac, maltlage and lactosyl-urea (ewoplus), were obtained from various sources and analyzed for total protein, soluble protein, unavailable protein and protease solubilized protein.

Analytical Procedures:

(1) *Total and Unavailable Protein:*
Total crude protein content of samples was determined by macro-kjeldhal method. Unvailable protein was determined by the method of Goering and Van Soest (1970). Acid detergent fiber was run on 1gm of sample and the crude protein content of the ADF determined by macro-kjeldhal method. The crude protein of the ADF (ADF-N) was then expressed as percent of the total crude protein.

(2) *Soluble Protein:*
A mineral buffer solution (pH 6.8 — 7.0) based on Goering and Van Soest's (1970) medium for in vitro digestion was used as solvent to determine the soluble protein of samples. The procedure used was that developed by Pichard (1977)

(3) *Protein solubilization:*
The protease of streptomyces griseus was used to determine the extent of degradation of the insoluble but potentially available portion of the protein based on the method of Pichard (1977) as modified by Krishnamoorthy (1982). The protease of streptomyces griseus has been shown to have broad substrate specificity (Matsurbara and Feder, 1971).

RESULTS AND DISCUSSION

Data on protein solubility are in table 1. Those on protein degradability with bacterial protease are in table 2.

TABLE 1

Protein Solubility of Selected Feedstuffs in Mineral Buffer

Feedstuff[a]	*CP*	*Soluble CP*	*Insoluble CP*	*Insoluble Avail. CP*	*Unavailable CP*
	%DM		*% of CP*		
Condensed distillers solubles (7)[b]	27.56	35.13	64.87	58.69	6.18
Distillers dried grains (9)	40.73	4.50	95.50	58.12	36.38
Brewers dried grains (4)	29.00	10.47	89.53	73.11	16.42
Extruded soybeans (4)	43.37	13.12	86.88	84.21	2.67
Raw soybeans (5)	41.80	27.52	72.48	57.82	14.66
Roasted soybeans (4)	41.68	8.07	91.93	74.00	17.93
Haylage (leg/grass) (2)	18.51	60.78	39.22	31.50	7.72
Hay (mixed grass) (3)	17.66	29.24	70.76	62.67	8.09
Corn silage (5)	8.37	66.67	33.33	23.06	10.27
Corn gluten meal (5)	62.88	5.40	94.60	90.14	4.46
Corn gluten feed (4)	23.14	35.00	65.00	60.18	4.82
*Solulac (1)	24.06	28.71	71.29	55.74	15.54
**Maltlage (2)	23.06	19.17	80.83	72.12	8.71
***Ewoplus (1)	40.00	100.00	0	0	0

[a]All values expressed on dry matter basis.

[b]Figures in parenthesis were numbers of samples analyzed.

*Solulac is the byproduct of Grain Processing Corporation, Muscaline, Iowa.

**Maltlage is the byproduct of malt drinks from Murphy Products Co., Wisconsin.

***Ewoplus is lactosyl-urea produced by Ewos AB, Sweden.

TABLE 2

Protein Solubilization of Selected Feedstuffs with Bacterial Protease

Feedstuff	*Incubation Time (hrs.)*										
	CP	*½*	*4*	*8*	*16*	*24*	*36*	*48*	*60*	*72*	*PDP*[a]
	% Undegraded CP										
Condensed distillers solubles (7)	27.50	51.99	43.71	41.74	33.72	26.25	23.21	22.18	22.00	22.10	46.50
Distillers dried grains (9)	40.73	93.12	82.94	77.22	65.00	54.55	50.54	43.73	34.09	34.14	58.12
Brewers dried grains (4)	29.00	86.10	70.23	55.09	51.89	45.46	28.28	24.93	22.46	21.57	69.00
Extruded soybeans (4)	43.37	75.24	61.87	45.15	27.18	19.41	15.06	12.13	11.73	11.53	79.12
Raw soybeans (5)	41.80	68.95	65.66	60.60	59.36	57.08	55.57	51.78	50.98	50.88	21.32
Roasted soybeans (4)	41.68	31.05	66.48	58.96	56.66	52.03	48.56	47.20	46.78	46.80	38.14
Haylage (leg/grass) (2)	18.51	37.71	30.15	25.12	22.64	20.10	19.61	19.12	19.13	19.10	57.36
Corn silage (5)	8.37	33.33	30.59	27.84	24.97	23.30	22.22	21.15	21.15	21.09	33.33
Corn gluten meal (5)	62.88	91.89	86.33	81.00	75.68	67.56	63.25	60.81	60.66	60.36	38.11
Corn gluten feed (5)	23.14	72.31	61.45	51.37	43.88	30.97	24.02	23.13	23.63	23.64	60.00
Solulac (1)	24.06	70.34	62.95	53.71	42.96	38.51	32.60	27.76	27.51	27.38	59.86
Maltlage (2)	23.10	79.91	64.23	52.77	45.77	45.54	36.69	31.20	22.92	19.81	19.78
Ewoplus (1)	40.00	0	0	0	0	0	0	0	0	0	0

72.72

[a]Insoluble protein degraded by protease.

Distillers Feeds:

The solubility of condensed distillers solubles averaged 35.13% with a range of 22.75 to 55.20%. That of distillers grains averaged 4.50% with a range of 1.98 to 11.04%. This result shows, based on the finding of Krishnamoorthy *et al* (1982) that concentrate and roughage feeds stay for about 18 hours and 24 hours respectively in the rumen, that about 30% or less of the proteins of distillers solubles may be available for post-ruminal degradation. Since the amount of true protein in soluble fraction is minimal (Pichard and Van Soest, 1977) it would appear that a large quantity of nitrogen in distillers solubles are non-protein nitrogen (NPN). And considering the fact that the insoluble fraction was somehow rapidly degraded by the protease (table 2), it would appear that the usefulness of distillers solubles as protein supplement for ruminants lies mainly in the fact that it contains unidentified growth factors for rumen microbes (Beeson) and Chen, 1976). The high insolubility of distillers grains (95.50%) observed in this study confirms the earlier findings of Waldo and Goering (1979) and Crooker *et al.* (1978). Apart from being highly insoluble in mineral buffer, the proteins of distillers grains were very slowly degraded by bacterial protease, an indication that when it comes to the question of ruminal by-pass distillers grains are better than the solubles. The high proportion of unavailable protein was due most likely to heat damage during processing.

Brewers Grains:

Protein solubility of brewers grains averaged 10.47% with a range of 5.48 to 17.09%. Unavailable protein ranged from 12.89 to 18.10%. The data in table 2 show that about 50% of the proteins of brewers grains can by-pass rumen degradation. This may go to support why a combination of brewers grains and urea would provide rumen micro-organisms with sufficient nitrogen to meet their requirements while minimizing nitrogen losses as absorbed ammonia from the rumen. Krause (1973) and Klopfenstein (1974) have shown that a situation like that is favourable for enhancement of urea utilization by ruminants.

Soybeans:

The solubility of the proteins of soybeans appears to be highly influenced by processing procedures such as heating or extrusion. Raw soybeans had a solubility of 27.52%, roasted and extruded soybeans had significantly less soluble proteins, 8.07% and 13.12% respectively. Roasted soybeans had highest unavailable protein likely to have resulted from heat damage. Extruded soybeans, although insoluble, were rapidly degraded by the protease, although insoluble, were rapidly degraded by the protease, thereby creating doubts as to their potential as a ruminant protein supplement. Raw and roasted soybeans were both highly resistant to protease degradation. This raised question as to why roasting is necessary as a method of rendering soybeans digestible. Since trypsin inhibitor is just about 6% of the total proteins of soybeans (Rackis and Anderson, 1964) and actual feeding trials with ruminants have not shown any difference between roasted and raw soybeans (Ahrar and Schingoethe, 1979) it can be suggested that roasting of soybeans is unnecessary for ruminants.

Forages:

Protein solubility of corn silage, haylage, and hay averaged 66.67%, 60.78% and 29.24% respectively. Unavailable proteins of the three classes of preserved forage did not, however, differ, confirming Pichard's (1977) observation that the acid detergent insoluble nitrogen is resistant to fermentation by silage micro-organisms. These results show that with regard to ruminal by-pass, hay is a better choice.

Others:

The solubility of corn gluten meal averaged 5.4%. However, 58 to 60% of the degradable fraction remained undegraded after 72 hours. The resistance of raw or roasted soybeans to bacterial protease was similar to that of corn gluten meal. The fact that these feedstuffs are well utilized by ruminants shows that there must be some intrinsic factors that enable the ruminants to digest and utilize them.

Corn gluten feed was relatively high in solubility, 24 — 54%, low in bound proteins and the proteins were less resistant to protease degradation.

Protein solubilities of solulac and maltlage were 28.71% and 19.17% respectively. Both were slowly degraded by bacterial protease, and indication of high potential as protein sources for ruminants. Ewoplus or lactosyl-urea which was 40% crude protein was 100% soluble in mineral buffer. Interestingly, McAllen *et al* (1975) obtained good results when ewoplus was fed to ruminants as against feeding just a mechanical mixture of pure urea and lactose. They demonstrated that ewoplus was insoluble in rumen liquor of calf during an anaerobic incubation in vitro. Frank (1979) also reported equal performance of dairy cows fed soybean meal or ewoplus. A possible explanation for the perculiar behaviour of the ewoplus used in this study could be that the two compounds, lactose and urea, did not actually combine chemically and therefore not a chemical compound.

Data from this study to some extent question the credibility of the general assumption that degradation of proteins by rumen microbes is generally related to solubility of the proteins in rumen fluid or mineral buffer (Hendricks and Martin, 1963; Crooker *et al.*, 1978; Wohlt *et al.*, 1973). For instance, even though the proteins of silage were over 60% soluble, the solubilization of the insoluble fraction with bacterial protease did not differ from those of hay which were only 29% soluble. Also the proteins of extruded soybeans were highly insoluble in mineral buffer but very rapidly degraded by bacterial protease. This goes to support the observations of Mahadeven *et al* (1980) that insolubility may not automatically translate into rumen bypass. From the foregoing, it can be recommended that to reduce protein solubility of ruminant rations, particular-

ly when urea or ensiled materials are used, the following feedstuffs out of the lot studied should be incorporated in the rations: distillers grains, brewers grains, corn gluten meal, raw or roasted soybeans and if available, maltlage.

ACKNOWLEDGEMENT

The authors are deeply grateful to Mr. Samuel Ochai for his excellent work in typing this manuscript.

REFERENCES

AHRAR, M. and D.J. SCHINGOETHE. 1979. Heat teated soybean meal as protein supplement for lactating cows. J. Dairy Sci., **62**; 932—940.

BEESON, W.M. and M.C. CHEN. 1976. In vitro studies on the effect of screened and centrifuged processed condensed distillers solubles on cellulose digestion and microbial protein synthesis. Proc. 31st Distillers Feed Conf., p.58.

CROOKER, B.A., C.J. SNIFFEN, W.H. HOOVER and L.L. JOHNSON. 1978. Solvents for soluble nitrogen measurements in feedstuffs. J. Dairy Sci., **61**; 437.

FAÛCONNEAU, G. and M.C. MICHEL. 1970. The role of gastro-intestinal tract in the regulation of protein metabolism. Mammalian Prot. Met., **4**; 481.

FRANK, B. 1979. Ewoplus (lactosyl-urea) as a protein supplement for dairy cows. Unpublished data.

GOERING, H.K. and P.J. VANSOEST. 1970. Forage fibre analysis. ARS, USDA Agr. Handbook No. 379, p.20.

HENDRICKX, H. and J. MARTIN. 1963. In vitro study of the nitrogen metablism in the rumen. Compt. Rend. Rech. Sci. Ind. Agr., Bruxelles 31:1.

KLOPFESTEIN, T. 1974. New concepts in protein nutrition for ruminants. Proc. Nebraska Feed and Nutr. Conf., p.31.

KRAUSE, V.E. 1973. Dehydrated alfalfa as a protein source in ruminant rations. PhD dissertation, Univ. Nebraska, Lincoln.

KRISHNAMOORTHY, P. 1982. Development of an in vitro technique to estimate rumen escape nitrogen in feedstuffs. PhD Thesis. Cornell Univ. Ithaca, N.Y.

KRISHNAMOORTHY, P., C.J. SNIFFEN and P.J. VAN SOEST. 1982. Nitrogen fractionation in ruminant feedstuffs for feed evaluation. Proc. Cornell Nutr. Conf., pp. 95—102.

LEWIS, D. and R.M. MITCHELL. 1974. Amino acid requirements of ruminants. In Protein Metabolism and Nutrition. Cole *et al* (eds.) pp. 417—424.

MAHADEVAN, S., J.D. ERFLE and F.D. SAUER. 1980. Degradation of soluble and insoluble proteins by bacteroides amylophilus protease and by rumen micro-organisms. J. Anim. Sci., 50:41.

MATSUBARA, H. and J. FEDER. 1971. Other bacterial, mold and yeast proteases. In: The Enzymes. 3rd Ed. Paul Boyer (ed.) pp. 721—793.

MCALLAN, A.B., R.J. MERRY and R.H. SMITH. 1975. Glycosyl ureides in ruminant feeding. Proc. Nutr. Soc., 34:90A.

ORSKOV, E.E. and C. FRASER. 1973. The effect of level of feeding and protein concentration on disappearance of protein in different segments of the gut in the sheep. Proc. Nutr. Soc., 32:68A.

PICHARD. G. 1977. Forage nutritive value: Continuous and batch in vitro rumen fermentations and nitrogen solubility. PhD Dissertation, Cornell Univ. Ithaca, N.Y.

PICHARD, G. and P.J. VAN SOEST. 1977. Protein solubility of ruminant feeds. Proc. Cornell Nutr. Conf. p.91.

RACHIS, J.J. and R.L. ANDERSON. 1964. Isolation of four trypsin inhibitors by DEAE-cellulose chromatography. Biochem. Biophys. Res. Comm., **15**; 230.

VAN SOEST, P.J. 1979. Personal communication. Cornell Univ. Ithaca, N.Y.

WALDO, D.R. and H.K. GOERING. 1979. Insolubility of proteins in ruminant feedstuffs by four methods. J. Anim. Sci., **49**; 1560.

WOHLT, J.E., C.J. SNIFFEN and W.H. HOOVER. 1973. Measurement of protein solubility in common feedstuffs. J. Diary Sci., **56**; 1052—1057.