The effectiveness of formaldehyde treatment in protecting dietary protein from rumen microbial degradation

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The ruminant derives its amino acid supply jointly from the dietary protein which escapes rumen degradation and from microbial protein synthesized in the rumen (Ferguson, 1975). The former process varies greatly between feeds, depending on protein solubility, whilst the most important factor regulating microbial protein synthesis is the quantity of plant organic matter fermented in the rumen, provided that rumen ammonia concentration and mineral elements are not limiting. In some situations the animal's requirement for amino acids cannot be fully met from normal sources (see Ferguson, 1975), and this has led to the development of techniques to protect a much greater proportion of dietary protein from rumen degradation. The present paper evaluates treatment of protein with formaldehyde (HCHO) for this purpose.

Chemistry of the reactions of HCHO with proteins

Van Dooren (1972) has surveyed the world's literature on reactions between HCHO and proteins. In most instances the initial step appears to be the rapid formation of a methylol compound (1):

$$R\text{–}XH + HCHO \rightleftharpoons R\text{–}X\text{–}CH_2OH. \quad (1)$$

XH can be terminal amino groups ($-NH_2$), the ε-amino group of lysine, the primary amide groups of asparagine and glutamine, the guanidyl group of arginine, the hydroxy groups of threonine and serine, the sulphydryl group of cysteine, the phenol group of tyrosine, the phenyl group from phenylalanine, the indole group of tryptophan and the imadazole group of histidine. Condensation reactions (2,3) then take place slowly over time, with the formation of stable methylene cross-linkages between protein chains:

$$R\text{–}X\text{–}CH_2OH + R'\text{–}\overset{\alpha}{N}H_2 \rightarrow R\text{–}X\text{–}CH_2\text{–}\overset{\alpha}{N}H\text{–}R' + H_2O; \quad (2)$$

$$R\text{–}XH + R'\text{–}\overset{\alpha}{N}H\text{–}CH_2OH \rightarrow R\text{–}X\text{–}CH_2\text{–}\overset{\alpha}{N}H\text{–}R' + H_2O. \quad (3)$$

These reactions of HCHO with the amino acid groupings take place under differing conditions of pH and temperature. At neutral pH and room temperature Ferguson (1975) considered the principal reactions to be those involving terminal amino groups, the primary amide groups of asparagine and glutamine and the ε-amino and guanidyl groups of lysine and arginine, respectively.

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Effect of HCHO treatment of feedstuffs on amino acid supply

Ferguson, Hemsley & Reis (1967) first demonstrated that these chemical reactions could be utilized to protect casein from rumen degradation, the methylene linkages being hydrolysed under acid-pepsin conditions in the abomasum and the animal's amino acid supply therefore being increased. The effects of treatment of a range of feedstuffs with HCHO on the quantities of amino acids leaving the abomasum and absorbed from the intestines are shown in Tables 1 and 2. The differences in amino acids leaving the abomasum (Table 1) and amino acids absorbed from the intestines (Table 2) between untreated and HCHO-treated diets have been expressed as a percentage of the amino acids supplied in the diet by the component that was HCHO-treated. These expressions are subsequently referred to as the net increases due to HCHO treatment, and give an over-all measure of the effectiveness of HCHO treatment in increasing amino acid supply in six specific situations; the net increases cannot be extrapolated to different diets. It should also be clearly realized that in addition to representing increases in the quantity of dietary protein escaping rumen degradation, the responses to HCHO treatment include any changes in microbial protein synthesis or reductions in enzymic digestion in the intestines (Table 2 only) caused by treatment with HCHO.

The quantities of total amino acids and total essential amino acids (g/d) leaving the abomasum (Table 1) were markedly increased by HCHO treatment of casein and of groundnut meal added at the higher level, the increases being greater for the more soluble casein. HCHO treatment of groundnut meal added at the low level produced only very small increases in these parameters. Net increases for individual essential amino acids were generally of the same order as for total essential amino acids, with the exceptions of arginine which was consistently higher, threonine which was consistently lower, and cysteine-cystine which were negative in two instances.

HCHO treatment also increased the quantities of total amino acids and total essential amino acids (g/d) absorbed from the intestines (Table 2). The smallest increases occurred on the diet of silage (Beever, Thomson, Cammell & Harrison, 1976), and were associated with reduced microbial protein synthesis due to over-protection of dietary protein by the high rate of HCHO applied. Net increases in amino acid absorption due to HCHO treatment were below the mean for total essential amino acids on all three diets for lysine, for threonine on one diet and for methionine in the one instance where this was measured. On all three diets the net increases for arginine and phenylalanine were above the mean for total essential amino acids.

Chemical damage due to reaction with HCHO is probably responsible for the smaller net increases in lysine absorption (Table 2). The lower net increases in flow from the abomasum or in absorption from the intestines for threonine and for total sulphur-containing amino acids (SAA) generally occurred where either a large part of the nitrogen consumed was HCHO-treated or the ration total N content was low. It seems that either HCHO treatment in these situations reduces microbial

Table 1. *Effect of formaldehyde (HCHO) treatment of casein and groundnut meal supplements on the quantities of amino acids leaving the abomasum of sheep (calculated from Faichney (1974))*

Component of diet treated . . .	Casein (12 g HCHO/kg CP)		Groundnut meal (9 g HCHO/kg CP)			
Nitrogen intake (g/d)	43·1		36·1		20·9	
HCHO-treated N (mg/g N intake)	400		500		330	
Natural protection of supplementary protein (%)*	9		37		37	
Amino acids leaving abomasum (g/d)	Untreated diet	Response to HCHO treatment†	Untreated diet	Response to HCHO treatment†	Untreated diet	Response to HCHO treatment†
Total amino acids	135·6	90·7 (77)‡	154·3	42·6 (40)‡	131·4	6·6 (16)‡
Essential amino acids§	69·9	40·2 (74)	74·1	17·0 (38)	61·1	4·7 (27)
Threonine	6·9	2·6 (51)	7·3	0·6 (21)	6·1	0·1 (5)
Lysine	10·1	5·8 (65)	8·2	2·3 (58)	6·7	0·6 (35)
Leucine	13·5	8·5 (81)	8·7	1·6 (22)	7·0	0·4 (13)
Isoleucine	8·2	4·8 (81)	3·5	0·8 (22)	3·1	0·2 (13)
Valine	9·4	5·8 (78)	9·3	1·5 (32)	7·7	0·7 (35)
Histidine	3·0	2·7 (84)	11·2	−0·2 (−6)	8·8	0·6 (59)
Arginine	7·0	3·4 (82)	9·8	6·8 (54)	7·8	2·1 (42)
Phenylalanine	7·9	4·7 (82)	12·9	2·5 (45)	11·4	−0·2 (−10)
Methionine	3·9	1·9 (62)	3·2	0·9 (70)	2·6	0·4 (69)
Cysteine–cystine	1·6	−0·6 (−15)	1·9	0·9 (67)	2·3	−0·9 (−159)
Cysteine equivalents	4·7	0·9 (31)	4·5	1·7 (68)	4·4	−0·6 (−61)

CP, crude protein.

*From Hume (1974); refers to component of the diet which was subsequently HCHO-treated.

†Calculated as difference in amino acids leaving the abomasum between sheep fed on HCHO-treated and those on untreated diets.

‡Net increase due to HCHO treatment, calculated as: response to HCHO treatment (g/d)/100 g amino acid(s) supplied in the diet by the component that was HCHO-treated.

§Excluding cysteine–cystine.

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Table 2. *The effect of formaldehyde (HCHO) treatment of ensiled grass and casein on the net absorption of amino acids from the small intestine of sheep, and of HCHO treatment of soya-bean meal on the net absorption of amino acids from the small and large intestine of yearling cattle*

Authors	Beever, Thomson, Cammell & Harrison (1976)		MacRae, Ulyatt, Pearce & Hendtlass (1972)		Verité, Poncet, Chabi & Pion (1976)	
Component of diet treated . . .	Ensilaged grass (60 g HCHO/kg CP)		Casein (30 g HCHO/kg CP)		Soya-bean meal (6 g HCHO/kg CP)	
Nitrogen intake (g/d)	32·5		20·6 (29·4)		232	
HCHO-treated N (mg/g N intake)	1000		300		550	
Natural protection of supplementary protein (%)	15		9•		61•	
Amino acids absorbed from intestine (g/d)	Untreated diet	Response to HCHO treatment†	Untreated diet ‡	Response to HCHO treatment†	Untreated diet	Response to HCHO treatment†
Total amino acids	100·0	13·1 (9)§	75·8	36·7 (69)§	751·4	146·1 (19)§
Essential amino acids‖	49·3	6·8 (10)	38·9	16·5 (69)	345·3	67·4 (19)
Threonine	5·1	1·0 (12)	4·5	1·5 (68)	42·8	2·9 (9)
Lysine	8·6	−1·5 (−39)	5·6	1·6 (44)	56·7	5·8 (12)
Leucine	8·2	2·5 (17)	6·8	3·7 (79)	64·0	7·5 (12)
Isoleucine	7·6	−0·6 (−6)	4·3	1·7 (68)	35·2	5·6 (15)
Valine	7·0	1·1 (9)	5·1	2·1 (66)	40·4	9·4 (23)
Histidine	2·0	0·4 (15)	1·7	0·7 (44)	15·6	4·9 (25)
Arginine	4·9	2·2 (26)	4·4	1·9 (100)	37·2	17·5 (31)
Phenylalanine	5·9	1·7 (17)	4·5	2·3 (85)	41·1	13·6 (35)
Methionine	nd	nd	2·0	1·0 (71)	12·3	0·1 (1)
Cysteine–cystine	nd	nd	nd	nd	12·4	2·3 (18)
Cysteine equivalents	nd	nd	nd	nd	22·3	2·4 (11)

CP, crude protein; nd, not determined.

•From Hume (1974); refers to component of the diet which was subsequently HCHO-treated.

†Calculated as difference in net absorption of amino acids from the intestine between animals fed on HCHO-treated and those on untreated diets.

‡In results of MacRae *et al.* (1972), untreated diet refers to dried grass only, and HCHO-treated diet to dried grass+HCHO-treated casein.

§Net increase due to HCHO treatment, calculated as: response to HCHO treatment (g/d)/100 g amino acid(s) supplied in the diet by the component that was HCHO-treated.

‖Excluding cysteine–cystine.

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synthesis of these amino acids by reducing the amount of N fermented in the rumen below a critical level, or else HCHO reacts with these amino acids to a greater extent than is generally realized. The consistently high net increases for arginine suggest that reactions between this amino acid and HCHO must be completely reversible and that microbial synthesis or arginine is not inhibited by HCHO treatment of the diet.

In production trials the best possibilities for responses to HCHO treatment must exist where there is a potential for high rates of protein synthesis in the animal tissues (i.e., lactation and wool growth) but where amino acid supply from the digestive tract is low. Low supply of amino acids will occur in situations where dietary protein contributes little to the amino acids arriving at the duodenum (i.e., diets of low protein concentration and those where most of the N is either non-protein-N (NPN) or very soluble protein), and situations where microbial protein production is restricted due to low levels of energy intake. Two such situations will now be discussed.

Wool growth

SAA are first limiting for wool growth and many experiments in Australia and New Zealand have shown that with sheep restricted to maintenance or even sub-maintenance levels of energy intake, large increases in wool growth can be obtained from postruminal or intraperitoneal supplementation with these amino acids (Reis & Schinckel, 1964; Reis, 1967; Barry, 1973). About 1 g methionine/d is sufficient to give the maximum response (Ferguson, 1975). The wool growth response to HCHO treatment of casein supplements given under such conditions (Reis & Tunks, 1969) can be explained from the increases in SAA supply produced by this treatment (see Tables 1 and 2). HCHO treatment of forages and concentrate meals has given variable responses in wool growth (Ferguson, 1970, 1975; Barry, 1973, 1976). Barry (1976) summarized some of these data for sheep fed at the maintenance level of energy intake and concluded that the wool growth response to HCHO treatment increased with the concentration of protein-bound SAA in the diet, and that large wool growth responses to HCHO treatment of normal forages were unlikely unless the concentration of SAA in the forage proteins could be increased through genetic selection. From wool S retention studies Barry & Andrews (1973) thought it unlikely that HCHO treatment of a ryegrass–clover hay had increased the quantities of SAA available to the sheep, although the treatment produced considerable protein protection in vitro; the data in Tables 1 and 2 support this conclusion and show that in some instances the increases in SAA supply from HCHO treatment can be disproportionately low, and in some instances negative.

Silage diets

Wilkinson, Wilson & Barry (1976) concluded that N utilization and intake were low on diets of direct-cut, untreated silage, due to extensive protein degradation and carbohydrate fermentation in the silo. It was thought that rumen microbial protein

synthesis was probably low, because over half the N consumed was NPN and all the readily available energy had already been fermented to organic acids in the silo. Beever *et al.* (1976) found that only 15% of the protein arriving at the duodenum was of dietary origin in sheep fed on a diet of untreated silage.

Treatment of herbage with solutions containing HCHO has been used to increase intake and animal production (including N utilization) from silages fed to sheep (Barry, Fennessy & Duncan, 1973; Wilkins, Wilson & Cook, 1974), growing cattle (Waldo, 1975) and dairy cows (Valentine & Radcliffe, 1975). Wilkinson *et al.* (1976) showed that further responses in animal production, though not necessarily in intake, could be obtained from postruminal supplementation with amino acids in animals given HCHO-treated silages *ad lib.*; this showed that whilst HCHO treatment improved animal performance, the amounts of some limiting essential amino acids absorbed were still below the levels necessary for optimum production.

Where the protein-N in the material ensiled is very low, another approach is to supplement the animals with a HCHO-treated protein concentrate. In this respect Verité & Journet (1976) obtained responses of approximately 1 kg milk/d from HCHO treatment of rapeseed (Brassica sp.) and soya-bean meals fed at 1–1·5 kg/d to high-yielding dairy cows given a basal ration predominantly of maize silage.

Effect of HCHO treatment of silage upon food conversion efficiency (FCE)

The increase in cattle growth rate from HCHO treatment of herbage before ensiling is slightly greater than can be explained from the increase in intake (Wilkinson *et al.* 1976). This increase in FCE (g body-weight gain/g food intake) ranged from 6% where material treated at 28 g HCHO/kg crude protein (CP) (N×6·25) was fed to yearling cattle (Kossila & Lampila, 1974) to 12% for material treated at 100 g HCHO/kg CP fed to calves 3–6 months old (Taylor & Wilkins, 1976); HCHO treatment of silage fed to dairy cows has produced similar improvements in efficiency of milk production (Valentine & Radcliffe, 1975).

Beever *et al.* (1976) found that HCHO treatment of herbage before ensiling increased amino acid absorption from the small intestine by 13%, increased the proportion of the energy digested in the rumen which could be accounted for as volatile fatty acids from 56 to 74%, and increased the amount of absorbed energy that was available for metabolism from 10·6 to 11·9 MJ/kg dry matter (DM). In comparative slaughter experiments, HCHO treatment of herbage before ensiling increased carcase protein retention, but had no effect on the efficiency with which metabolizable energy was used above maintenance (C. R. Lonsdale, personal communication). It therefore seems that the improvement in FCE could be jointly due to increases in the supply of available energy/unit DM consumed and to increases in carcase protein retention.

Desired levels of HCHO application

HCHO application rates in situations where satisfactory responses have been obtained are summarized in Table 3. The rates show considerable variation in g

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Table 3. *Formaldehyde (HCHO) application rates to feeding-stuffs in relation to the quantity of crude protein (CP) (nitrogen×6·25) treated and to the quantity of true protein which would normally be degraded in the rumen of ruminants*

Reference	Type of material treated	Proportion of dietary true protein degraded in rumen on the untreated diets (g/kg)	HCHO application rate		
			g/kg CP	g/kg true protein	g/kg degradable true protein
	(Situations where optimum levels of application have been determined)				
Wilkinson, Wilson & Barry (1976)	Low-DM grass silage (F)	850*	30–50	39–66	46–78
Ferguson (1975)	Chaffed lucerne hay (I)	550†	30–40	43–58	78–105
Ferguson (1975)	Dried, chaffed lucerne (I)	270‡	20	29	107
D. J. Thomson & D. E. Beever (unpublished results)	Dried grass (I) (ground and pelleted)	270	10	13	49
	(Situations where increased amino acid absorption, or responses in production, or both have been observed)				
Hemsley, Hogan & Weston (1970)	Dried, chaffed legume forage (I)	550†	40	58	105
Barry (1976)	Lucerne hay (F)	550†	80–130	116–188	211–342
Waldo (1975)	Soya-bean meal (I)	390§	12	14	36
Verité, Poncet, Chabi & Pion (1976)	Soya-bean meal (I)	390§	6	7	18

DM, dry matter; I, HCHO applied under controlled conditions indoors; F, HCHO applied under field conditions.

*Results of Beever, Thomson, Cammell & Harrison (1976)
†From Ferguson (1975).
‡Assumed to be the same as for dried grass.
§Results of Hume (1974).

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HCHO applied/kg CP treated; however, HCHO applied as a proportion of true protein which is not naturally protected from rumen degradation is considered to be a more meaningful expression. Where HCHO has been applied under controlled conditions indoors, higher rates of application appear necessary for the chaffed legume materials than for the dried, ground feedstuffs, possibly due to ease of penetration of the HCHO. Losses of HCHO during field application in silage making are in the region of 20% (R. F. Wilson, personal communication) and, using $H^{14}CHO$, Kreula (1973) found that 12 and 6% of the HCHO which was actually placed on the grass was lost from the silo as carbon dioxide and effluent, respectively. Taking total losses therefore as 34% of that originally applied to low-DM grass silages, the optimum amount of HCHO available for binding with protein would appear to be 30–52 g/kg degradable true protein. This is similar to the value found for dried grass and is lower than the range for legume materials treated in the long form (Table 3); this is in line with the deduction of Wilkinson *et al.* (1976) that the maximum amount of HCHO that can be added at ensiling before silage intake declines is less for ryegrass (Lolium sp.) (80 g/kg CP) than for lucerne (*Medicago sativa* L.) (150 g/kg CP). Comparing the results for lucerne hay treated under indoor and field conditions it would seem that losses of HCHO in excess of 50% probably occurred during the field application.

Laboratory estimates of protein protection

The amount of HCHO released upon distillation with dilute mineral acid has been used as a measure of protein protection in concentrate meals (Ferguson, 1970). However, when the technique is applied to silage, the proportion of the HCHO actually applied which can be recovered progressively declines with time and after 100 d is only 20% (R. F. Wilson, personal communication), which contrasts with an 82% recovery for the radioactivity applied as $H^{14}CHO$ (Kreula, 1973). Reactions 2 and 3 (p. 221) probably take longer to proceed to completion in silage than in concentrate meals, due to the moisture content diluting the reactants. Progressive protein binding lowers HCHO recoveries by acid distillation due to the formation of cyclic structures between HCHO and tyrosine, tryptophan and histidine in acid conditions (P. Van Dooren, personal communication); this could be responsible for the apparently declining HCHO content in HCHO-treated silage and may not be an accurate guide to enzymic hydrolysis of amino acid–HCHO bonds in the intestines. The amount of N released in acid pepsin after incubation with rumen fluid (Barry, 1973, 1976) is preferred by the author as a laboratory measure of protein protection and attempts are currently being made at the Grassland Research Institute, Hurley, to modify this procedure in vitro by also measuring the amount of N synthesized as microbial protein (T. N. Barry, D. E. Beever & R. J. Wilkins, unpublished results).

Conclusions

HCHO treatment of a range of feedstuffs increased the amounts of total essential amino acids that were available to the animal in all instances, but for

lysine, threonine and SAA in many instances the increases were very low and in some decreases were seen. The low increases for SAA are considered to be especially serious, in view of the low concentrations of these amino acids in forage proteins, and it is probable that these factors account for the poor wool growth responses to HCHO treatment of forages. HCHO treatment of herbage before ensiling has been shown to increase FCE, as well as voluntary intake; however, the further increases in production obtained from postruminal supplementation with amino acids show that the amount of some essential amino acids absorbed in animals fed on HCHO-treated silage is still below the requirement for maximum production. Optimum HCHO application rates for several feedstuffs are presented, and the best method of expressing these is considered to be as g HCHO/kg true protein treated which is not naturally protected from rumen degradation.

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