INFLUENCE OF VARIOUS FORMALDEHYDE TREATMENTS ON THE NUTRITIONAL VALUE OF CASEIN FOR WOOL GROWTH

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Abstract

Formaldehyde-treated casein was prepared by either soaking the casein in dilute aqueous formaldehyde followed by washing and drying, or by mixing the casein with small volumes of more concentrated formaldehyde solutions and allowing unreacted formaldehyde to remain in the product. The effectiveness of treatment was assessed by measurements of the amount of formaldehyde bound to casein and the resistance of the casein to degradation by rumen microorganisms *in vitro*. The utilization of the casein preparations by sheep was assessed by measurements of nitrogen digestibility, rate of wool growth, and wool fibre diameter.

The rate and extent of reaction of formaldehyde with casein depended on the duration of the reaction, the concentration of the formaldehyde solution, and the ratio of solution : casein. The extent of binding increased with formaldehyde concentration and was greater with a small volume of concentrated solution than with a larger volume of less concentrated solution containing the same amount of formaldehyde. Amounts of bound formaldehyde up to 3·8 g/100 g dry product were obtained.

Untreated casein was readily degraded to ammonia by rumen microorganisms *in vitro*. Dietary supplements of untreated casein were almost completely digested but produced only slight increases in wool growth rate. Formaldehyde-treated casein which contained 0·5–1·5% bound formaldehyde increased wool growth rate and fibre diameter substantially when included in the diet. The greatest wool growth response was obtained with casein preparations containing about 1% bound formaldehyde, regardless of the treatment procedure. Casein containing amounts of bound formaldehyde outside the range 0·5–1·5% was of little value for wool growth. The preparations that were most effective in stimulating wool growth corresponded to treatments that afforded good protection *in vitro* without producing an appreciable reduction in digestibility. Ineffective casein preparations were either incompletely protected in the rumen or had a lowered digestibility. Casein could be rendered virtually indigestible by excessive treatment with formaldehyde.

I. INTRODUCTION

Diets rich in proteins are inefficiently utilized by sheep because proteins can be extensively degraded to ammonia and volatile fatty acids by microorganisms in the rumen (McDonald 1968). In the absence of sufficient available energy in the diet, protein degradation will exceed protein synthesis by rumen microorganisms and thus reduce the quantity of amino acids available for absorption from the small intestine.

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When rumen fermentation is avoided by direct administration into the abomasum or duodenum, proteins are much more efficiently utilized. In particular, the rate of wool growth is markedly increased by abomasal administration of proteins (Colebrook and Reis 1969; Reis 1969).

Ferguson *et al.* (1967) showed that casein which had been treated with formaldehyde was resistant to microbial degradation in the rumen. Moreover, the addition of the treated casein to a roughage diet substantially increased wool growth rate. Subsequent work showed that the treated casein was of similar nutritional value to casein given via the abomasum (Reis and Tunks 1969, 1970). Casein treated with formaldehyde by methods similar to those of Ferguson *et al.* (1967) had an enhanced nutritional value, and increased wool growth rate in several breeds of sheep (Barry 1969, 1970, 1972; Hughes and Williams 1970; Langlands 1971*a*; Wright 1971). In other experiments with Romney and Corriedale sheep (Carrico *et al.* 1970; Henderson *et al.* 1970) the responses to formaldehyde treatment of casein have been variable.

The successful application of formaldehyde-treated proteins to practical ruminant feeding is dependent on a thorough examination of the technique and its effect on digestion and utilization of nutrients. Ferguson *et al.* (1967) soaked casein in dilute aqueous formaldehyde followed by washing and drying the product. In the present experiments this method (high-volume treatment) was compared with another procedure in which the casein was mixed with small volumes of more concentrated formaldehyde solutions, any unreacted formaldehyde being allowed to remain in the product (low-volume treatment). Particular attention was given to assessing the effects of treatment of casein with a wide range of amounts of formaldehyde by both procedures. The assessment of the amount of formaldehyde bound to the protein is a major problem. It is difficult to recover all of the bound formaldehyde by conventional methods such as distillation from hot concentrated phosphoric acid solution (Swain *et al.* 1948; Middlebrook 1949; Reddie and Nicholls 1971). [^{14}C]Formaldehyde was used in most of the present experiments to provide a simple and convenient way of assessing the total amount of formaldehyde carbon which remained in the products after subjecting them to a standard washing procedure. The amount of ^{14}C in these products was taken as an index of "bound formaldehyde".

The aims of these experiments were firstly, to define reaction conditions under which a reproducible, optimally protected casein suitable for nutritional experiments could be easily prepared; and secondly, to provide results which could be used as the basis for similar studies with other proteins and protein-rich supplements that might be used in practice. The nutritive value of the products was assessed by measuring their resistance to degradation by rumen microorganisms *in vitro*, their nitrogen digestibility *in vivo*, and their effects on the rate of wool growth.

II. Experimental

(*a*) *General*

Industrial-grade hydrochloric acid-precipitated casein (particles $<0{\cdot}6$ mm diam.) was used in all of the experiments. The casein was treated with formaldehyde solutions of varying concentrations prepared from analytical reagent (British Drug Houses; experiment 1) and from commercial-grade formalin (Monsanto Chemical Co.; experiments 2–4). In experiment 1, the reaction of

[^{14}C]formaldehyde with casein was measured. Based on the results of experiment 1, large batches of formaldehyde-treated casein were prepared for experiments 2–4, in which effects on wool growth and the digestibility of the nitrogen in casein were measured.

Medium Peppin Merino wethers (group 1, 32 sheep; group 2, 40 sheep) were used in experiments 2–4. Each sheep was maintained indoors in an individual pen and was given 500,000 i.u. of vitamins A and D_3 orally every 3 months. The sheep were fed once daily at about 8 a.m.; water was available *ad libitum*. The basal diet consisted of equal parts of lucerne and wheaten hays, and was offered either in a chopped form (experiment 4) or in a ground and pelleted form (experiments 2 and 3). Casein supplements were added to the basal diet during experimental periods either by mixing with the chopped hay or with the ground hay before pelleting. All sheep received 600 g/day of the basal diet during pre-experimental periods.

(*b*) *Outline of Experiments*

(i) *Experiment 1*

Casein was treated with [^{14}C]formaldehyde (*c.* 38 μCi/mole) solutions under three sets of conditions. Firstly, in experiment 1(*a*), casein samples (0·5 g dry matter) were mixed with 1·0 or 5·0 ml of either 0·4 or 4·0% w/v [^{14}C]formaldehyde solution and allowed to react for periods of 15 min to 17 days. Secondly, in experiment 1(*b*), casein was separated by sieving into coarse and fine fractions (>0·53 and <0·40 mm respectively) and samples (0·5 g dry matter) were mixed with 1·0 ml of solutions of varying [^{14}C]formaldehyde concentration (0·25–40% w/v) and allowed to react for 3 days. Finally, in experiment 1(*c*), casein samples (1 g dry matter) were mixed with 0·2 and 2·0 ml respectively of solutions of varying [^{14}C]formaldehyde concentration (5, 10, 20% and 0·5, 1·0, 2·0% w/v formaldehyde). One series of samples was allowed to react for 3 days and another for 12 days.

On each occasion casein was mixed with [^{14}C]formaldehyde solution in glass vials which were sealed and kept for the time intervals specified at a temperature of 20–25°C. After treatment, the casein from each vial was transferred to a filter paper (Whatman No. 1) and washed with water (20 × 5 ml) over a period of 1 hr. The treated casein was then dried at 70°C and its ^{14}C content measured.

(ii) *Experiment 2*

Large batches (20 kg) of casein were treated (high-volume treatment) in drums with solutions (2 litres per kilogram casein) of varying [^{14}C]formaldehyde concentration (0, 0·25, 0·5, 0·75, 1·0, 2·5, 5·0, and 20·0% w/v formaldehyde) such that the expected levels of bound formaldehyde in the product would be about 0, 0·25, 0·5, 0·75, 1·0, 1·5, 2·0, and 3·0% respectively. The specific radioactivity of the [^{14}C]formaldehyde was about 1·5 μCi/mole for the more dilute solutions (0·25–5·0% formaldehyde) and about 0·36 μCi/mole for the 20% (w/v) formaldehyde solution. After 3 days at ambient temperature (25–35°C) the treated casein was washed three times by filling the drum with tap water (about 180 litres) and siphoning off the free liquid; most of the remaining water was then removed in a basket centrifuge. The product, which contained about 50% water, was dried at about 50°C for 3 days.

Effects on wool growth were measured with 32 Merino wethers (group 1) which were randomly divided into 8 groups of 4 sheep. The experimental diets were prepared as pelleted mixtures of six parts ground basal roughage diet and one part casein (untreated or formaldehyde-treated); 700 g was offered daily to each sheep for 5 weeks. The digestibility of the nitrogen in casein, and the rate of degradation of casein *in vitro*, were measured after about 2 months and 18 months storage of the treated casein preparations. Digestibility was measured in eight sheep from group 1 after the wool growth experiment was completed, during faecal collection periods of 4 days.

(iii) *Experiment 3*

As the high-volume treatment method had the practical disadvantage of using relatively large volumes of formaldehyde solution, followed by washing and drying, the low-volume treatment was tested in experiment 3. Batches of casein (20 kg) were treated with [^{14}C]formaldehyde solutions (0·69 μCi/mole; 0·2 litre per kilogram casein) containing either 0, 10, or 20% (w/v) formaldehyde.

The casein and formaldehyde solutions were mixed in a dough mixer (Hobart Manufacturing Co. Ltd) and the treated mixtures were sealed in polyethylene bags for 12 days.

For the measurement of wool growth, three groups of four Merino wethers were randomly selected from the sheep of group 1 and allocated to the experimental diets, which were prepared as pelleted mixtures of six parts ground basal roughage diet and one part casein. Each sheep was offered 700 g daily for 5 weeks. The digestibility of the nitrogen in casein was measured in two of the above sheep after the wool growth experiment was completed, during faecal collection periods of 5 days.

(iv) *Experiment 4*

In experiment 4(*a*), batches of casein (30 kg) were treated by the low-volume method with solutions (0·1 litre per kilogram casein) of varying formaldehyde concentration (0, 2·5, 5·0, 7·5, 10·0, 12·5, 15·0, 20·0, 30·0, and 40·0% w/v formaldehyde). The formaldehyde solutions were sprayed on to the casein during mixing in a cone-shaped vessel fitted with a revolving planetary auger and lump breaker [Nauta-Mixer type D100, supplied by Mauri Bros & Thomson (Aust.) Pty Ltd]. After mixing was completed (about 10 min) the casein was stored at ambient temperature (10–20°C) in sealed polyethylene bags for 12 days.

Effects on wool growth were measured with 40 Merino wethers (group 2), which were randomly allocated to 10 groups of 4 sheep. During the experimental period of 5 weeks, each sheep received a daily ration of 600 g of the chopped basal roughage diet plus 100 g casein, which was either untreated or formaldehyde-treated. The digestibility of the nitrogen in casein was measured in all sheep during the wool growth experiment. Faeces were collected during a 9-day period near the end of the experiment.

In experiment 4(*b*), formaldehyde-treated casein mixtures were prepared as for experiment 4(*a*), except that, after being placed in sealed polyethylene bags, the preparations were heated in an oven at 70°C for 24 hr. Negligible weight loss occurred during heat treatment. The 40 sheep (group 2) were re-randomized into 10 groups of 4 sheep and effects on wool growth and the digestibility of the nitrogen in casein were measured, as in experiment 4(*a*).

(*c*) *Digestibility of Casein Supplements*

Faeces were collected into polyethylene bags attached to the sheep. Subsamples for analysis of dry matter, organic matter, and nitrogen were taken from a bulked sample representing several days' output. The nitrogen digestibility of the various casein preparations was assessed from the difference in faecal nitrogen output during the feeding of casein-supplemented diets and of the basal diet, prior to each experiment.

(*d*) *Wool Growth*

Wool growth rate was determined during periods of 2 weeks, both pre-experimentally and at the end of experiments 2, 3, and 4, by removing wool from tattooed areas (about 100 cm^2) with small animal clippers (Oster, size 40). The total amount of wool grown by each sheep was estimated from the previously measured ratio of fleece weight to tattooed area wool weight. The wool growth rates during the pretreatment periods were used to adjust the rates during the experimental treatments by covariance analysis.

The greasy wool samples were washed successively with a neutral detergent solution (0·3% Nonidet P40 detergent; from Shell Chemicals), warm water, and ethanol. The clean wool was conditioned at 20°C and 65% relative humidity and was then weighed. The water content of the clean wool was determined from the conditioned weight of samples of known dry matter content so that all the results could be expressed on a dry-weight basis. Average wool fibre diameter was determined by the method of Downes (1971).

(*e*) In vitro *Incubations of Casein with Rumen Contents*

Rumen contents were collected from a sheep about 24 hr after it had eaten its daily ration of 800 g chopped lucerne hay, and were strained through terylene cloth (porosity 150 μm) into a warmed vacuum flask. Samples of untreated and of formaldehyde-treated casein (100 mg dry matter), prepared for experiments 2–4, were incubated for 24 hr at 40°C with 20 ml of strained rumen con-

tents in tubes which were flushed with CO_2 and fitted with bunsen valves. Tubes of rumen contents (blanks) were also incubated without the addition of casein. After incubation, samples of the incubation mixture were treated with an equal volume of 0·2N H_2SO_4 and analysed for ammonia. The net conversion of casein-nitrogen to ammonia-nitrogen (substrate minus blank incubation) was used as a measure of casein degradation in rumen contents.

(*f*) *Analytical*

[^{14}C]Formaldehyde (Radiochemical Centre, Amersham, England) was diluted by appropriate addition of unlabelled formaldehyde (analytical reagent for experiment 1; commercial-grade formalin for experiments 2 and 3). The radiochemical purity of the [^{14}C]formaldehyde was shown to be at least 98% by comparing the total amount of ^{14}C in solution with the amount left in solution after quantitative precipitation of the formaldehyde as the dimedone derivative (Yoe and Reid 1941). The specific radioactivity of the dimedone derivative was measured by dissolving samples (20–50 mg) in a liquid scintillation solution, measuring the counting rates, and then measuring counting efficiency after the further addition of a known amount of ^{14}C as n-[1-^{14}C]hexadecane. The specific radioactivity of the formaldehyde was calculated from that of the dimedone derivative.

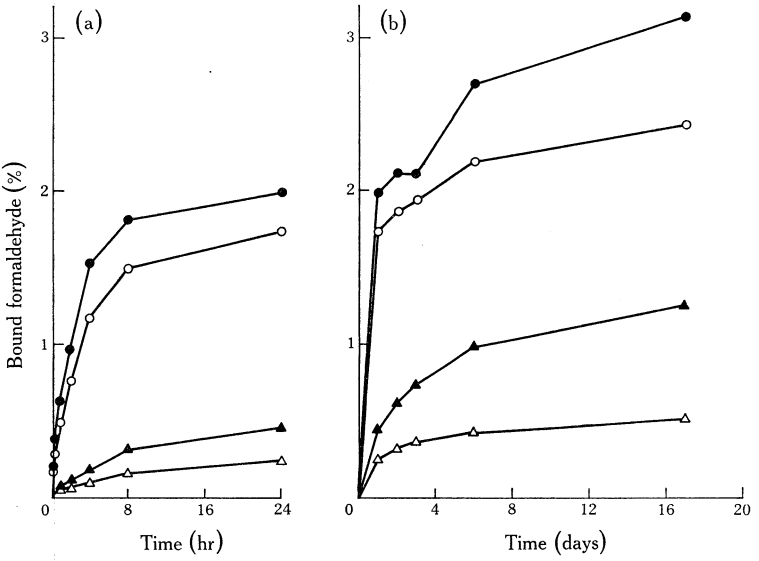

Fig. 1.—Effect of formaldehyde concentration on the rate of reaction of casein with formaldehyde [experiment 1(*a*)] over 24 hr (*a*) and over 17 days (*b*). The formaldehyde solutions contained 0·4 (△,▲) or 4·0% (○,●) (w/v) [^{14}C]formaldehyde. The volumes of [^{14}C] formaldehyde solution were 2 ml (△,○) or 10 ml (▲,●) per gram of casein.

Bound formaldehyde was estimated in washed casein samples that had been treated with [^{14}C]formaldehyde (experiments 1–3) by measuring the ^{14}C content (Downes *et al.* 1970) of samples that were combusted by a modification of the oxygen-flask method of Kalberer and Rutschmann (1961). In experiment 4, bound formaldehyde was assessed from the quantity of formaldehyde added minus the quantity removed from the treated casein by washing with water. The concentration of formaldehyde in aqueous solution was measured by the method of Bricker and Vail (1950).

Rumen contents were analysed for ammonia by steam distillation (McDonald 1952). Samples of casein, the experimental rations, and faeces were analysed for dry matter, organic matter, and nitrogen (Association of Official Agricultural Chemists 1960).

III. Results

(*a*) *Reaction of Formaldehyde with Casein (Experiment 1)*

Studies of the time course of the reaction of formaldehyde with casein over a 17-day period [experiment 1(*a*)] indicated that at least half of the formaldehyde binding occurred in the first 2 days, and Figure 1(*a*) shows the rapid reaction that

occurred in the first few hours, especially with the higher concentration of formaldehyde. A larger amount of formaldehyde was bound, at any given time, when the volume of solution added was 10 ml/g casein than when it was 2 ml/g casein, but the shapes of the curves were similar [Fig. 1(*b*)]. With 0·4% w/v formaldehyde solution, substantially less formaldehyde was bound than with 4% w/v formaldehyde, but for each volume of solution added the formaldehyde in the more dilute solution was used more efficiently (Table 1).

TABLE 1

REACTION OF [^{14}C]FORMALDEHYDE WITH CASEIN

Casein (0·5 g dry matter) was allowed to react with 1 or 5 ml of formaldehyde solution in sealed glass vials for 17 days; experiment 1(*a*)

Formaldehyde concentration (% w/v)	Volume of solution added (ml/g casein)	Formaldehyde added (g/100 g casein)	Formaldehyde bound	
			(g/100 g dry matter)	(g/100 g formaldehyde added)
0·4	2	0·8	0·53	67
0·4	10	4·0	1·26	32
4·0	2	8·0	2·43	31
4·0	10	40·0	3·15	8

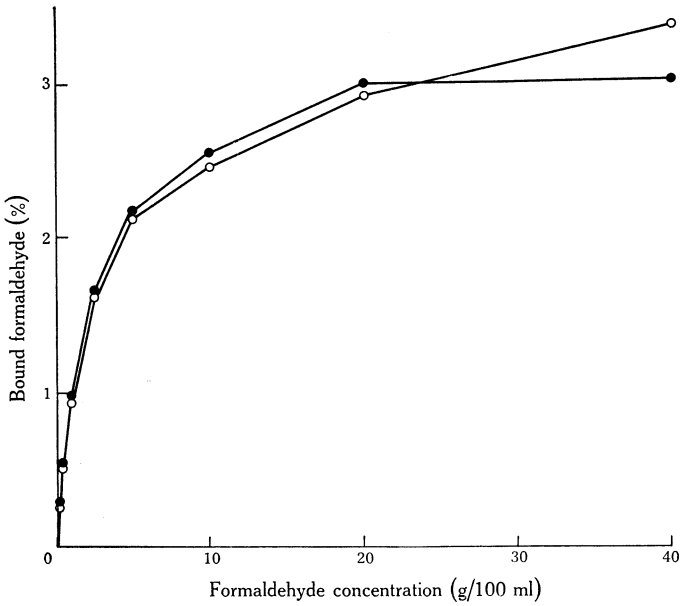

Fig. 2.—Effect of formaldehyde concentration and casein particle size on the reaction of casein with formaldehyde [experiment 1(*b*)]. Coarse (>0·53 mm diam., ●) and fine (<0·40 mm diam., ○) particles of casein were treated with [^{14}C]formaldehyde solution (2 ml per gram of casein) for 3 days.

In experiment 1(*b*) the amount of formaldehyde bound to casein after 3 days at ambient temperature increased with the concentration of formaldehyde used. With the most concentrated solution (40% w/v) the product contained about 3% bound formaldehyde (Fig. 2). There was no difference in the rates of reaction with fine and coarse particles of casein.

In experiment 1(*c*), a comparison was made between the effectiveness of formaldehyde binding achieved by adding the same range of amounts of formaldehyde (1, 2, 4 g/100 g casein), each at two concentrations. The amount of bound formaldehyde increased with formaldehyde concentration and increased slightly from 3 to 12 days (Table 2). The amount of formaldehyde bound was consistently greater with the low volumes of concentrated solution than with the high volumes of dilute solution. At least 55% of the added formaldehyde was bound to casein after 12 days reaction by the low-volume procedure (Table 2).

TABLE 2

REACTION OF [^{14}C]FORMALDEHYDE WITH CASEIN

Casein (1 g dry matter) was allowed to react with 0·2 or 2·0 ml of formaldehyde solution in sealed glass vials as indicated; experiment 1(*c*)

Formaldehyde concentration (% w/v)	Volume of solution added (ml/g casein)	Formaldehyde added (g/100 g casein)	Formaldehyde bound* (g/100 g dry matter)	
			3 days reaction	12 days reaction
0·5	2·0	1	0·47 (48)	0·59 (60)
1·0	2·0	2	0·90 (46)	1·09 (56)
2·0	2·0	4	1·49 (38)	1·77 (46)
5·0	0·2	1	0·63 (64)	0·71 (72)
10·0	0·2	2	1·21 (62)	1·29 (66)
20·0	0·2	4	1·88 (48)	2·12 (55)

* Values in parentheses are percentage of added formaldehyde bound.

(*b*) *Effect of Formaldehyde Treatment of Casein on Nitrogen Digestion and on Wool Growth*

The basal ration (600 g/day) used in experiments 2, 3, and 4 contained about 2% nitrogen (dry basis) and provided about 300 g/day of digestible organic matter.

(i) *Experiment 2* [*Fig. 3*(a)]

The batches of casein treated with increasing concentrations of formaldehyde solutions (0·25–20% w/v; 2 litres per kilogram casein) contained a range of amounts of bound formaldehyde up to 3·8%. These amounts represented 10–50% of the added formaldehyde.

Untreated casein was readily degraded by rumen microorganisms *in vitro* (86% converted to ammonia in 24 hr). With increasing proportions of bound formaldehyde up to 1% there was a rapid fall in the susceptibility of the treated casein to attack by rumen microorganisms. Products containing 1% or more bound formaldehyde were almost completely protected from degradation.

Digestibility of the casein-nitrogen *in vivo* was also reduced by treatment with formaldehyde. Untreated casein and products containing up to 1% bound formaldehyde were almost completely digested, whereas the digestibility of products containing larger amounts of bound formaldehyde was substantially reduced to as low as 37% for the product containing 3·8% bound formaldehyde.

The addition of 100 g/day of untreated casein to the basal diet produced only a small increase in wool growth (0·7 g/day above the basal value of 5·8 g/day). Diets containing casein (100 g/day) treated with formaldehyde solutions ranging in concentration from 0·75 to 5·0% w/v (0·5–2% bound formaldehyde) significantly increased wool growth ($P < 0·05$) by 3–4 g/day clean dry wool; fibre diameter was also significantly increased by 1–2 μm. Treatment of casein with smaller or larger amounts of formaldehyde gave a product of less value for wool growth.

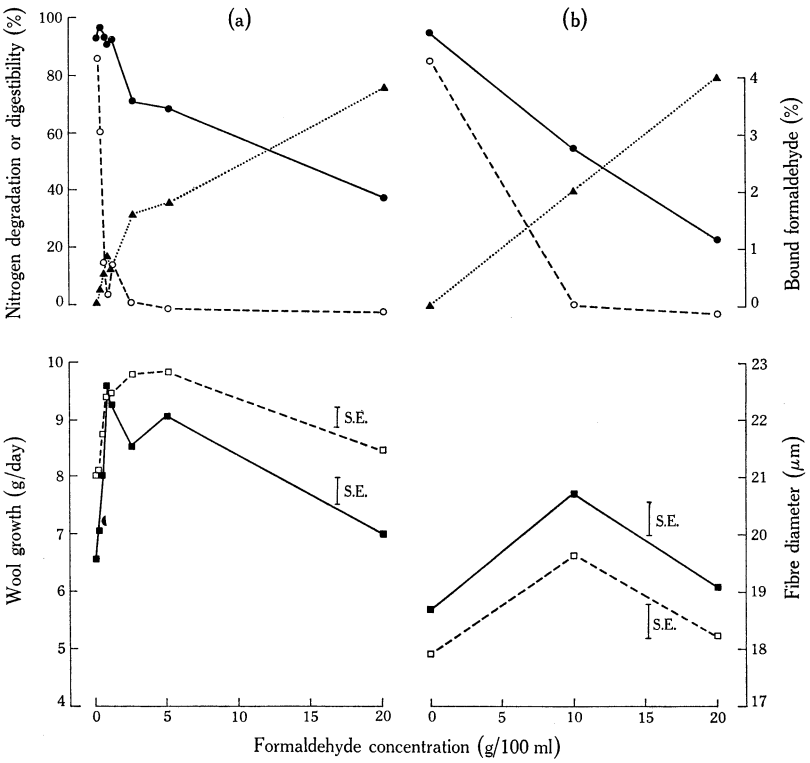

Fig. 3.—Effect of treating casein with 2·0 litres (*a*, experiment 2) or 0·2 litre (*b*, experiment 3) formaldehyde solution per kilogram casein on bound [^{14}C]formaldehyde content (g/100 g dry matter, ▲), on degradation of casein-nitrogen to ammonia-nitrogen by rumen microorganisms *in vitro* (○), and on casein-nitrogen digestibility (●). Effects on wool growth rate (g/day clean dry wool, ■) and on fibre diameter (□) were adjusted by covariance analysis for the pre-experimental wool growth rate and fibre diameter respectively. The mean pre-experimental wool growth rates for experiments 2 and 3 were 5·81 and 4·93 g/day respectively; the corresponding fibre diameters were 20·1 and 17·3 μm respectively. Each point is a mean for four sheep.

The results presented in Figure 3(*a*) were obtained about 2 months after the initial mixing of the formaldehyde with the casein. Measurement of degradation by rumen microorganisms *in vitro* and digestibility *in vivo* after 18 months storage gave essentially the same results. However, casein preparations which were partially degraded *in vitro* after 2 months storage were more readily degraded after 18 months storage.

(ii) *Experiment 3* [*Fig. 3*(b)]

The results of experiment 3 showed that the low-volume treatment (0·2 litre formaldehyde solution per kilogram casein) was effective for binding formaldehyde to casein and affording protection from degradation by rumen microorganisms *in vitro*. The amounts of bound formaldehyde were comparable to those found in experiment 2. However, digestibility was markedly reduced with both amounts of formaldehyde, and wool growth and fibre diameter were only increased significantly by the casein treated with the smaller amount of formaldehyde.

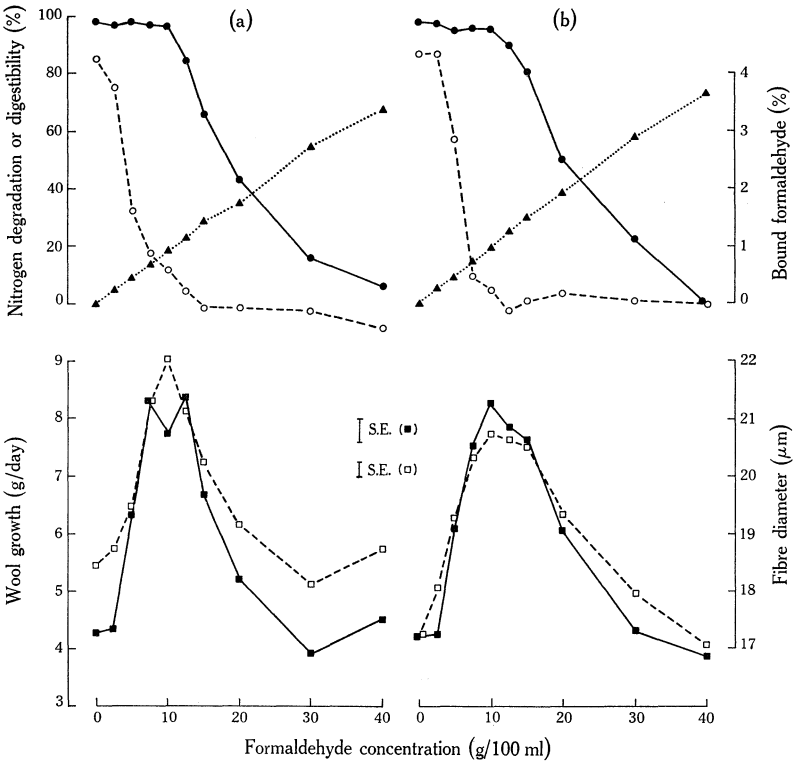

Fig. 4.—Effect of treating casein with 0·1 litre formaldehyde solution per kilogram casein on bound formaldehyde content (g/100 g dry matter, ▲), on degradation of casein-nitrogen to ammonia-nitrogen by rumen microorganisms *in vitro* (○), and on casein nitrogen digestibility (●). (*a*) Experiment 4(*a*), products of formaldehyde treatment unheated; (*b*) experiment 4(*b*), products heated for 24 hr at 70°C. Effects on wool growth rate (g/day clean dry wool, ■) and on fibre diameter (□) were adjusted by covariance analysis for the mean of the pre-experimental wool growth rates (3·44 g/day) and fibre diameters (17·7 μm) respectively for both experiments 4(*a*) and 4(*b*). Each point is a mean for four sheep.

(iii) *Experiments 4*(a) *and 4*(b) (*Fig. 4*)

Two further experiments were carried out using the low-volume treatment, but with a smaller proportion of solution (0·1 litre per kilogram casein). In experiment 4(*a*) [Fig. 4(*a*)] the amount of bound formaldehyde in the treated casein increased

with increasing concentration of formaldehyde in the reaction solution, in an approximately linear manner, to a maximum of 3·2%. Ruminal degradation of casein *in vitro* was rapidly reduced by treatment with increasing amounts of formaldehyde and was completely prevented with a 15% formaldehyde solution. Digestibility of casein *in vivo* was reduced at concentrations of formaldehyde above 10% and treatment with 40% formaldehyde rendered casein almost completely indigestible.

The addition of 100 g/day untreated casein to the basal diet again had only a small effect on wool growth. In contrast, the addition of 100 g/day casein, treated with concentrations of formaldehyde in the range 5–15%, resulted in substantial increases in wool growth; wool growth was more than doubled by casein treated with solutions containing 7·5–12·5% formaldehyde. The treatments that were most effective for stimulating wool growth corresponded to treatments that afforded good protection from ruminal degradation *in vitro* without an appreciable reduction in digestibility. Casein treated with smaller or larger amounts of formaldehyde was of little value for wool growth, and was either incompletely protected in the rumen or had a lowered digestibility *in vivo*. Wool fibre diameter responded to formaldehyde-treated casein in a similar manner to wool mass.

In experiment 4(*b*) [Fig. 4(*b*)] the casein was allowed to react with formaldehyde as in experiment 4(*a*), but with the additional treatment of heating at 70°C. Similar results to those in experiment 4(*a*) were obtained for all variables measured.

IV. Discussion

When casein is treated with formaldehyde the amount of bound formaldehyde can vary over a wide range, depending on the reaction conditions (Walker 1964). The present results show that, over the range of concentrations studied, casein containing about 1% bound formaldehyde (1 g per 100 g protein) was an optimally protected protein of improved nutritive value for wool production. The incorporation of 1% formaldehyde was achieved in various ways, using either dilute or concentrated formaldehyde solutions, and at temperatures of either 20 or 70°C. Irrespective of the method of preparation, casein containing 1% bound formaldehyde was substantially protected from ruminal degradation, its overall digestibility was not depressed appreciably, and the wool growth responses were the largest observed. With smaller amounts of bound formaldehyde there was more degradation of the protein by rumen microorganisms, and with larger amounts the overall digestibility was depressed and the wool growth responses were smaller.

It is apparent that similar products can be made by either the low- or high-volume procedure. In practice, the low-volume procedure has several advantages. Thus, the amount of bound formaldehyde can be easily controlled by adding the required amount of formaldehyde and allowing sufficient time for the reaction to occur; there is no need to remove unreacted formaldehyde; and after initial mixing of the reactants the material may be stored indefinitely in the containers in which the reaction is allowed to occur. Further, the results from experiment 4 indicate that a substantial increase in reaction temperature during the low-volume treatment does not influence the digestion of the casein or its utilization for wool growth.

There may be little advantage in completely protecting protein from microbial degradation in the rumen because digestibility in the intestines may be reduced. Our results showed that casein digestibility was reduced when the bound formaldehyde

content exceeded 1%. However, the decline in digestibility was not closely related to the amount of bound formaldehyde and appeared to be affected by the method of treatment. For example, in experiment 2 [see Fig. 3(*a*)] casein treated with a 20% formaldehyde solution had a digestibility of 37% and a bound formaldehyde content of 3·8%, whereas in experiment 4(*a*) treated casein with a similar digestibility (42%) had a bound formaldehyde content of only 1·7%. Despite these effects, the results indicate that the wool growth responses to formaldehyde treatment of casein can be largely explained by an increased supply of protein available for digestion in the intestines. Faichney and Weston (1971) treated casein with formaldehyde as described in our experiment 2 (2·0 litres of 1·5% w/v formaldehyde per kilogram of casein); it may be calculated from their results that about 80% of the treated casein was apparently digested in the intestines. Macrae *et al.* (1972) also studied the digestion of a formaldehyde-treated casein by sheep but provided no details of the treatment procedure. Their results showed that overall digestibility of the casein was only slightly reduced from 93 to 89% following formaldehyde treatment. They also showed that about 24% of the treated casein was digested in the large intestine and about 50% of the untreated casein appeared to escape degradation in the rumen. Other workers have reported substantial wool growth responses to supplements of untreated casein (Carrico *et al.* 1970; Barry 1972) which suggests that some of the supplements may escape degradation or alternatively stimulate microbial protein synthesis in the rumen under the conditions of their experiments.

Dr. D. M. Walker (personal communication) has studied the growth of rats fed some of the treated caseins from experiment 4(*a*). The casein containing about 1% bound formaldehyde was only slightly inferior to untreated casein in supporting growth of young rats. The slight depression in growth rate with the treated casein was due entirely to a lower food intake because rats pair-fed untreated casein grew at the same rate as those fed treated casein. Another treated casein, which had a low digestibility in sheep, was markedly inferior to optimally treated or untreated casein for growth of rats.

If formaldehyde-treated proteins are to be fed to ruminants over long periods it would be important to assess the amounts of formaldehyde released in the rumen and in other parts of the gastro-intestinal tract and to determine other possible effects of such a highly reactive compound. Mills *et al.* (1972) have shown that [^{14}C]formaldehyde bound to a sodium caseinate–oil mixture is rapidly metabolized by sheep tissues and excreted via expired air, urine, and faeces.

The result of the present experiments cannot be applied directly to the formaldehyde treatment of protein-rich feedstuffs normally fed to ruminants. Such materials consist of many compounds besides protein which could react with formaldehyde. The proteins may also vary in their susceptibility to ruminal degradation and may have biological values which are appreciably different from that of casein. Limited information indicates that the formaldehyde treatment of various proteins may produce different results from those obtained with casein. Cottonseed meal treated with varying amounts of formaldehyde by a low-volume procedure showed less decline in digestibility (Langlands 1971*b*) than that observed with casein in our experiment 4(*a*). Also, formaldehyde treatment has not enhanced the nutritional value of cottonseed meal for wool growth (Langlands 1971*b*; Saville *et al.* 1971). Rattray and Joyce (1970) observed that linseed meal and meat meal, treated with

formaldehyde, had quite different nitrogen digestibilities and rates of protein digestion *in vitro*. Treatment again did not improve the value of either protein as a supplement for wool growth. By contrast, formaldehyde treatment of artificially dried clover (Hemsley *et al.* 1970) significantly increased both protein digestion in the intestines and wool production in sheep. Because responses to formaldehyde treatment of feeds are likely to vary, it is important that treatment conditions are carefully controlled and recorded so that comparisons between experiments can be made.

V. Acknowledgments

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