LEAF PROTEIN CONCENTRATE QUALITY: THE EFFECT OF PASTURE SPECIES AND REDUCING AGENT

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SUMMARY

Leaf protein concentrates (LPC) were prepared from ryegrass (RG), white clover (WC), ryegrass/white clover (RG/WC) and lucerne (L). *In vivo* rat, true digestibilities of protein were lower for RG/WC and RG than WC and L. This was probably due to a higher proportion of the protein from the RG being the less digestible "chloroplastic" fraction (compared with the "cytoplasmic" fraction). Treatment of the juice with reducing agent ($Na_2S_2O_5$) significantly improved chemically available methionine and lysine, and protein quality of LPC.

INTRODUCTION

Compared with leaf protein concentrates (LPC) from ryegrass, those from white clover and lucerne showed superior digestibility and quality of protein when fed with methionine supplements to rats (Donnelly and McDonald, 1978). The nutritional availability of native methionine appeared low.

The possible contribution to these differences in digestibility between ryegrass and the legumes of variations in the proportion of the "cytoplasmic" and "chloroplastic" protein fractions, as observed by Byers (1971) for other species, has been examined and is reported here.

Aqueous sodium metabisulphite reduces oxidized methionine (Snow *et al.*, 1976), leading to improved protein quality (Bickoff *et al.*, 1975). It also inhibits protein-quinone condensation reactions (Pierpoint, 1970) which could reduce lysine availability (Davies *et al.*, 1978). These effects of the treatment of grass juice with metabisulphite were also investigated.

METHODS

LPC was prepared by methods previous outlined (Donnelly and McDonald, 1978) from pure stands of ryegrass (RG) (*Lolium perenne*), white clover (WC) (*Trifolium repens*) and lucerne (L) (*Medicago sativa*), and a stand of ryegrass/white clover (RG/WC), all after 5 weeks of regrowth in late summer-autumn 1978. Each load of herbage (400 to 800 kg) was divided into two equal

TABLE 1: CHEMICAL COMPOSITION OF JUICE AND LEAF PROTEIN CONCENTRATE

	Ryegrass		*White Clover*		*Lucerne*		*Ryegrass/ White Clover*		*SD of a Mean*	*Significance of:*	
	−MBS[1]	*+MBS*[1]	*−MBS*	*+MBS*	*−MBS*	*+MBS*	*−MBS*	*+MBS*		*Herbage Effect*	*Sulphite Effect*
Avialable lysine (g/16 g N)	4.77	4.91	5.73	5.79	5.43	5.53	5.11	5.28	0.16	***	*
Avail. methionine (g/16 g N)	1.72	1.94	1.52	1.60	1.82	1.94	1.83	1.88	0.09	***	*
Total methionine (g/16 g N)	2.8	2.8	2.6	3.0	3.1	2.9	3.3	3.0	—	—	—
Total cystine (g/16 g N)	0.5	0.6	0.4	0.5	0.4	0.5	0.4	0.4	—	—	—

[1] Without and with metabisulphite treatment.

portions before extraction and processing, and a solution of sodium metabisulphite (MBS) was sprayed on to one portion immediately prior to pulping to provide 1000 ppm of sulphur dioxide in the expressed juice. Results are presented for herbage botanical composition, percent "chloroplastic" protein of juice true protein, and available lysine, total and available methionine and total cystine in the LPC. Protein quality, expressed as Relative Nutritive Value (RNV), relative to lactalbumin, was measured using weanling rats and a slope-ratio growth procedure (Donnelly and McDonald, 1978). Where specified, methionine was added at 2 g/100 g protein. Four experiments were made. LPC (with and without metabisulphite) was compared from RG/WC and L (Experiment 1) and from RG/WC, RG and WC (Experiment 2). In Experiments 3 and 4, two aspects of the effects of MBS were examined. First, LPC prepared with MBS was compared with LPC supplemented with methionine, and secondly LPC prepared with MBS was supplemented with methionine to determine the effects of MBS on protein quality apart from its effects on methionine availability. Digestibility measurements were made in Experiments 1 and 2 only.

All chemical analyses, except total methionine and cystine, were carried out on the samples from each of the four replicates. Methionine, cystine and growth assays were carried out on pooled representative samples of the four replicates from each treatment.

RESULTS

The RG, WC and L herbages were 7, 92 and 92% pure, respectively. For RG the lower proportion was associated mainly with the presence of dead material (17%). The RG/WC herbage averaged 53% ryegrass, 20% white clover, and 27% other grasses, weeds and dead material.

There was a significant ($P < 0.001$) effect of herbage type on the "chloroplastic" proportion of the juice true protein. The results were RG 73%, WC 56%, L 49% and RG/WC 73% (SD $\pm$ 7%).

Available methionine also differed between herbages, beinig lowest for WC. Metabisulphite treatment significantly improved available lysine and available methionine by 2 and 7%, respectively, but did not alter total methionine or total cystine.

Methionine tended to be higher in RG/WC than in the other species.

Relative Nutritive Values were similar for LPC extracted without metabisulphite for all herbage types (Exp. 1 and 2, Table 2)

TABLE 2: RELATIVE NUTRITIVE VALUE (RNV) AND PROTEIN TRUE DIGESTIBILITY (PTD)

	Experiment 1		*Experiment 2*		*Experiment 3*	*Experiment 4*
	RNV[1]	*PTD%*	*RNV*	*PTD%*	*RNV*	*RNV*
Lactalbumin standard	1.00	94.0	1.00	97.3	1.00	1.00
RG			0.60	76.9		
RG + MBS[2]			0.71	76.0	0.79	
RG + met.[3]					0.82	
RG + MBS + met.					0.84	
WC			0.57	83.5		
WC + MBS			0.62	80.7	0.68	
WC + met.					0.84	
WC + MBS + met.					0.86	
RG/WC	0.62	77.3	0.57	77.4		
RG/WC + MBS	0.82	82.6	0.75	81.8		0.75
RG/WC + met.						0.81
RG/WC + MBS + met.						0.80
L	0.61	80.8				
L + MBS	0.75	83.1				0.66
L + met.						0.82
L + MBS + met.						0.86
Average SE of RNV ratios	0.03		0.02		0.02	0.03
Average SD of a mean		5.4		4.4		

[1] Calculated by expressing the coefficient, from the regression of body water gain on protein intake, for the test protein as a ratio of the coefficient for lactalbumin.
[2] Indicates treatment with metabisulphite.
[3] Indicates supplementation with methionine at feeding.

and were increased by metabisulphite treatment ($P < 0.05$). The smallest response to MBS was for WC.

Protein true digestibility ranged from 83.5 down to 76.9% and was higher for L than RG/WC (difference ± SE = +3.6 ± 1.5, $P < 0.05$), and higher for WC than RG (+6.5 ± 1.6, $P < 0.001$) and RG/WC (+6.1 ± 1.6, $P < 0.001$). MBS treatment improved the digestibility of protein for RG/WC only ($P < 0.05$).

Methionine supplementation at feeding lifted the RNV of LPC, made both without and with metabisulphite treatment, from 0.59 to 0.82 (on average) and from 0.72 to 0.84, respectively (Table 2).

DISCUSSION

The previous observation (Donnelly and McDonald, 1978) of higher protein digestibility in LPC from WC and L than RG was confirmed. This is most likely due to the different proportions of "chloroplastic" and "cytoplasmic" materials, which have differing digestibilities (Morris, 1977).

The "chloroplastic" protein is also lower in total and chemically available lysine than the "cytoplasmic" fraction (Byers, 1971; Allison, 1971). The herbage rankings for the "chloroplastic" proportion of true protein generally agree with the available lysine ranking for RG, WC and RG/WC. However, the digestibility of protein and available lysine results for lucerne are lower than those for WC despite a lower "chloroplastic" protein ratio for lucerne. This contrasts with previous results giving a higher digestibility and available lysine for lucerne than white clover (Donnelly and McDonald, 1978).

Metabisulphite treatment increased the chemically available methionine as confirmed by the improved RNV. Available methionine was, on average, 58% of total methionine and was improved to 63% with MBS treatment. However, since variations in amino acid availability are due mainly to changes in digestibility of the protein (Erbersdobler, 1976), even 63% is low compared with that which might be expected from an overall average protein true digestibiliity of 80%.

Metabisulphite treatment did not affect protein true digestibility for RG, WC or lucerne and had only a very small effect on lysine availability. Provided, then, that MBS treatment does actually inhibit protein quinone conjugation, this suggests that such reactions do not usually decrease these two measures of protein adequacy in forage fractionation and protein recovery as used here. It is not clear, however, if the improvement in methionine

availability associated with this treatment was due to the inhibition of quinone oxidation/condensation reactivity or to the prevention of methionine oxidation by other reaction systems.

There was no difference between herbage types in RNV when LPC was supplemented with methionine, regardless of whether treated with sulphite or not. This contrasts with earlier work which showed the legumes to have higher quality than ryegrass (Donnelly and McDonald, 1978).

In conclusion, these results show that there are variations between ryegrass and legumes in the composition of the true protein in expressed plant juice. These differences in proportions of "cytoplasmic" and "chloroplastic" fractions may be the source of variations in digestibiliity. Treatment of the juice with sodium metabisulphite improved the availability of methionine and gave a 28% improvement in Relative Nutritive Value. Other effects of this treatment were small.

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