## Preliminary note on the utilization of alkaline hydrogen peroxide treated wheat straw by sheep

A.A. Brand, F. Franck and S.W.P. Cloete\* Elsenburg Agricultural Centre, Private Bag, Elsenburg 7607, Republic of South Africa

\* To whom correspondence should be addressed

Received 5 July 1988; accepted 3 October 1988

The effect of alkaline hydrogen peroxide (AHP) treatment and urea supplementation on the feeding value of wheat straw was investigated in a  $2 \times 2$  factorial experiment with sheep. Bales of wheat straw were dipped for a period of 24 h in an AHP solution consisting of 1% hydrogen peroxide and 0,55% sodium hydroxide, applied at a treatment solution : substrate ratio of 35:1. Dry matter and organic matter digestibilities of the wheat straw were increased by 20,8 and 18,1% ( $P \le 0.01$ ), respectively, by AHP treatment. The digestibilities of cell wall constituents, acid detergent fibre and hemicellulose of the wheat straw were increased by 26,0, 21,4 and 42,9%, respectively, as a result of AHP treatment. Peroxide treatment alone did not affect the voluntary intake of the straw significantly. Urea supplementation, however, stimulated voluntary intake of the AHP-treated wheat straw considerably ( $P \le 0.01$ ) by 57% to 81% above the other treatments.

Die invloed van alkaliese waterstofperoksied (AHP)-behandeling en ureumaanvulling op die voedingswaarde van koringstrooi is in 'n 2×2-faktoriaaleksperiment met skape ondersoek. Die behandeling is toegepas deur bale koringstrooi in 'n AHP-oplossing, bevattende 1% waterstofperoksied en 0,55% natriumhidroksied, vir 24 h te onderdompel teen 'n behandelingsoplossing : substraatverhouding van 35:1. Droë materiaal- en organiese materiaalverteerbaarheid van die koringstrooi is met 20,8 en 18,1% onderskeidelik verhoog (P≤0,01) deur AHP-behandeling. Die selwand-, suurbestande vesel- en hemisellulose-verteerbaarheid van die koringstrooi is onderskeidelik met 26,0, 21,4 en 42,9% verhoog (P≤0,01) deur AHP-behandeling. Peroksiedbehandeling alleen het nie die vrywillige inname van die strooi betekenisvol beïnvloed nie. Ureumaanvulling het egter die inname van die AHP-behandelde strooi aansienlik verhoog (P≤0,01) met 57% tot 81% bo die ander behandelings.

**Keywords:** Hydrogen peroxide treatment, in vivo digestibility, nitrogen balance, urea supplementation, voluntary intake, wheat straw.

Cereal straw includes approximately 80% carbohydrates mainly cell wall polysaccharides - which are potentially a source of energy for ruminants (Hartley & Jones, 1978). The structural polysaccharides, cellulose, hemicellulose and the pectinsaccharides are only partially degraded by rumen microbes, thus limiting the nutritional value of untreated straw (Theander & Aman, 1980; Thiago & Kellaway, 1982). The poor utilization of cereal straw may also be ascribed to the covalent bonds between polysaccharides and lignin (Hartley & Jones, 1978) and the crystalline structure of cellulose in plant cell walls (Kerley, Fahey, Berger, Merchen & Gould, 1986). Gould (1984) and Kerley, Fahey, Berger, Gould & Baker (1985) recently found that the treatment of wheat straw with alkaline hydrogen peroxide (AHP) resulted in partial delignification and decrystallization of the microfibrils, thereby increasing fibre digestibility to the extent that it may potentially be used for production in growing animals. These results were, however, obtained from small-scale laboratory experiments, using sophisticated apparatus and by evaluating the results using in vitro or in sacco techniques. Lewis, Kerley, Fahey, Berger & Gould (1987) also used AHP-treated wheat straw successfully in larger scale experiments, involving growing steers and lambs. The present experiment was conducted to evaluate the technique under South African conditions, and to extend it to practical farm conditions, whereby bales of wheat straw were dipped into an AHP solution. The effect of urea supplementation was investigated at the same

Firstly, 150 g of wheat straw was immersed in a 1% hydrogen peroxide ( $H_2O_2$ ) solution containing 0,55% sodium hydroxide (NaOH) at a pH of 11,5 and a treatment solution: substrate ratio of 50:1, as described by Gould (1984). The results of this laboratory trial were evaluated in terms of chemical composition and *in vitro* organic matter digestibility (IVOMD).

Based on these results, wheat straw was treated on a larger scale, involving the immersion of bales of wheat straw (ca. 10 kg) in an AHP solution in two cement pits for a 24-h period. The bales were placed in a shade net (nylon) container, as the baling twine disintegrated during

treatment. Treatment solution contained 0,98% H<sub>2</sub>O<sub>2</sub> and 0,55% sodium hydroxide at a pH of 11,5. After treatment, excess treatment solution was allowed to run off for a period of 12 h before the wheat straw was dried in the sun. Treatment solution was replenished to the required level before immersion of each bale. After treatment of approximately five bales/pit, the pH levels of both pits were found to decrease. Therefore, 5-10 ml sodium hydroxide (40% solution) was added to the replenishment solution, to maintain the pH at 11,5. Both pits were cleaned and filled with new treatment solution after treatment of 10 bales. In total, 40 bales of wheat straw were treated, at an overall treatment solution: substrate ratio of 35:1. After being hammermilled through a 18mm screen, the AHP-treated straw was mixed thoroughly before being included with untreated straw of the same batch in a  $2\times 2$  factorial experiment, in order to investigate the effects of AHP treatment and urea supplementation at a level of 1,5% (air-dry base), applied directly before feeding.

The experimental diets were evaluated with 20 adult SA Mutton Merino wethers in a voluntary intake, in vivo digestibility and nitrogen (N)-balance study, over a 35day period. During the collection period of 10 days, the experimental animals were fed at a constant level of 32 g/kg W<sup>0,75</sup>/day, to prevent differences in in vivo digestibility caused by different intake levels. The dry matter (DM), organic matter (OM), and crude protein (CP) content of representative feed and faeces samples were determined according to the methods of AOAC (1970). Cell wall constituents (CWC), acid detergent fibre (ADF), hemicellulose and lignin were determined according to the methods of Van Soest (1963) and Van Soest & Wine (1967). In vivo digestibilities and N balance were calculated according to standard procedures. The IVOMD of the samples obtained from the laboratory trial was determined as described by Engels & Van der Merwe (1967). Results of voluntary intake, digestibility and N balance were analysed according to standard procedures for a  $2 \times 2$  factorial design (Snedecor & Cochran, 1967). Individual contrasts, using t-test procedures, were used to test differences between treatment means for significance.

Results from the laboratory study suggested a DM loss of 26% in AHP-treated straw. The CP content of treated straw decreased by 47% (Table 1), suggesting a considerable washing out of N. The lignin content of the straw

**Table 1** The chemical composition of untreated and AHP-treated wheat straw in the laboratory study (DM basis)

Content (%)	Wheat straw				
	Untreated	AHP-treated			
СР	3,4	1,8			
CWC	82,9	84,3			
ADF	57,4	63,9			
Hemicellulose	25,5	20,4			
Lignin	8,7	4,9			
IVOMD	40,2	77,5			

was similarly decreased by 43,7% after AHP treatment. Gould (1984) and Lewis *et al.* (1987) correspondingly reported marked reductions in the lignin content of comparable products, resulting in a readily fermentable energy source. The IVOMD of the straw was accordingly substantially increased by 37,3 percentage units.

The treatment of straw bales, on the other hand, resulted in a less marked decrease in CP and lignin contents of wheat straw compared to the laboratory study (Table 2). This result suggests the possibility of a poorer treatment in the wheat straw treated with AHP on a large scale. This finding may possibly be related to the lower treatment level in the large-scale treatment. Reactions with soluble compounds may possibly have influenced the treatment solution, resulting in the observed drift in pH.

Treatment with AHP resulted in increases ( $P \le 0.01$ ) of 20,8 and 18,1%, respectively, in the DM and OM digestibilities of wheat straw (Table 3). Results reported by Lewis *et al.* (1987) also suggested improvements in the *in vivo* digestibility of AHP-treated wheat straw in comparison with untreated wheat straw. A further enhancement of 8,0% ( $P \le 0.05$ ) was observed in the DM digestibility of AHP-treated straw supplemented with urea. A significant

**Table 2** Effect of AHP treatment and urea supplementation on the chemical composition of wheat straw (DM basis)

Treatment:	Untro	eated	AHP-treated		
Supplementation:	Urea –	Urea+	Urea –	Urea+	
Content (%)					
CP	4,0	8,3	2,7	7,0	
CWC	81,8	81,6	78,5	78,7	
ADF	53,7	53,7	56,6	56,6	
Hemicellulose	28,1	27,9	21,9	22,1	
Lignin	7,7	7,8	7,0	6,9	

AHP treatment × urea supplementation interaction did not permit the independent evaluation of the main effects regarding CP digestibility. Supplementation with urea increased the CP digestibility of both the treated and untreated wheat straw, as was expected. The CP digestibility of unsupplemented AHP-treated wheat straw was, however, substantially lower than that of the untreated control, resulting in the significant interaction. The washing out of loosely bound N fractions by AHP treatment possibly contributed to this result. Treatment with AHP correspondingly resulted in increases ( $P \le 0.01$ ) of 26,0, 21,4 and 42,9%, respectively, in the CWC, ADF and hemicellulose digestibilities of wheat straw. Lewis et al. (1987) reported similar improvements in fibre digestibility after AHP treatment. Urea supplementation also resulted in smaller increases (P≤0,05) in CWC and ADF digest-

Voluntary intake of untreated wheat straw was independent of urea supplementation (Table 3). Treatment with AHP as such tended to improve voluntary intake by 107,4 g/day. Supplementation of the treated straw with urea, however, resulted in a marked ( $P \le 0.01$ ) improvement of voluntary intake compared to the other treatments, resulting in a highly significant ( $P \le 0.01$ ) AHP treatment × urea supplementation interaction. From these results, it appeared that a deficiency in N, aggravated by AHP treatment, may be associated with the lack of a response in voluntary intake of the straw treated with AHP solution. The extent and the rate of fibre digestion in AHP-treated straw were apparently improved by urea supplementation, resulting in a higher rate of passage and consequently in a higher voluntary intake. According to Jewell & Campling (1986), the voluntary intake of a diet will generally be improved by non-protein N supplementation at CP levels lower than 80 g/kg. This generalization obviously did not apply to the untreated diets included in the present study, probably as a result of an inadequate energy content.

**Table 3** Effect of AHP treatment and urea supplementation on the digestibility and voluntary intake of wheat straw

Treatment: Supplementation:	Untreated		AHP-treated		% Change due to	
	Urea –	Urea+	Urea –	Urea+	AHP treatment	Urea supple- mentation
Apparent digestibility						
DM	53,6ª	56,1 a	63,7 <sup>b</sup>	68,8°	20,8**	6,4*
OM	56,3ª	56,9ª	64,6 <sup>b</sup>	69,0 <sup>b</sup>	18,1**	4,1
CP	8,7 <sup>2</sup>	$61,4^{3}$	$-13,4^{1}$	$60,5^3$	_	_
CWC	57,8 <sup>a,1</sup>	$60,7^{a,1}$	$71,9^{b,2}$	77,4°,2	26**	6,5**
ADF	53,71	56,81	$64,8^{2}$	$69,3^{2}$	21,4**	6,4*
Hemicellulose	$63,8^{1}$	66,11	$90,6^{2}$	$95,0^{2}$	42,9**	4,3
Voluntary intake						
g/d	698,21	698,61	805,61	1261,4 <sup>2</sup>	_	_
g/kg W <sup>0,75</sup> /d	26,81	26,91	30,81	48,4 <sup>2</sup>	-	-

<sup>\*</sup> Significant (P≤0,05).

<sup>\*\*</sup> Significant (P≤0,01).

<sup>&</sup>lt;sup>a,b,c</sup> Denote significant differences ( $P \le 0.05$ ).

<sup>&</sup>lt;sup>1,2,3</sup> Denote significant differences ( $P \le 0.01$ ).

Treatment: Untreated AHP-treated % Change due to AHP Urea supple-Supplementation: Urea -Urea+ Urea-Urea+ treatment mentation N intake (g/d) 3,51  $7,5^{2}$  $2.8^{1}$  $8,1^{2}$ 147.7\*\* -1.0N excretion (g/d) Faeces 3,3 2,8 3,2 3,4 8.2 -4,62,8a,b Urine 1,3ª 5,0<sup>b</sup> 61,0\*\* 5,5° 31,7 N balance (g/d) -1,0-0.3-3,2-0.8-35,073,1

Table 4 Effect of AHP treatment and urea supplementation on the N balance of wheat straw

Urea supplementation markedly increased ( $P \le 0.01$ ) N intake (Table 4). Higher urinary N losses on these treatments, however, suggested poor utilization of the supplemented N, both in untreated and AHP-treated wheat straw. No significant differences in N balance were observed between the respective diets, but there was a tendency for urea supplementation to improve it.

The present study suggested that AHP treatment was less effective when applied on a larger scale. Limited penetration, the lower treatment solution: substrate ratio, and the exhaustion of the chemicals may be possible reasons for this result. The in vivo digestibility trial, nevertheless, suggested that AHP treatment is a promising technique, especially in the cropping areas, where an abundant supply of low-quality cereal straw is available. Inadequate voluntary intake may be a serious limitation when AHP-treated straw is fed without further supplementation. This problem may satisfactorily be solved by supplementation with urea, which resulted in a 57% improvement in this study. The continuous replenishment of chemicals in the dip treatment and the cost of the two chemicals involved, may prove to be major limitations of the treatment under practical conditions. The latter aspect is considered to be the most important limitation, since 4.0% sodium was analysed in treated samples. Lewis et al. (1987) correspondingly reported a sodium concentration of 5% in unwashed AHP-treated wheat straw. In future, researchers will have to discern between the individual effects of H<sub>2</sub>O<sub>2</sub> and NaOH, and should attempt to control the level of chemicals actually reacting with the substrate, from an economic point of view.

The authors thank the laboratory staff of the Animal Production Laboratory for assistance regarding chemical analyses of the samples.

## References

AOAC, 1970. Official methods of analysis (11th edn.).
Association of Official Analytical Chemists, Washington DC.

ENGELS, E.A.N. & VAN DER MERWE, F.J., 1967. Application of an *in vitro* technique to South African forages, with special reference to the effect of certain factors on the results. S. Afr. J. Agric. Res. 10, 983.

GOULD, J.M., 1984. Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification. *Biotechnol. Bioeng.* 26, 46.

HARTLEY, R.D. & JONES, E.C., 1978. Effect of aqueous ammonia and other alkalis on the *in vitro* digestibility of barley straw. *J. Sci. Food Agric*. 29, 92.

JEWELL, S.N. & CAMPLING, R.C., 1986. Aqueous ammonia treatment of wheat straw: Voluntary intake and digestibility in cattle. Anim. Feed Sci. Technol. 14, 81.

KERLEY, M.S., FAHEY, G.C., BERGER, L.L., GOULD, J.M. & BAKER, F.L., 1985. Alkaline hydrogen peroxide treatment unlocks energy in agricultural by-products. *Science* 230, 820.

KERLEY, M.S., FAHEY, G.C., BERGER, L.L., MERCHEN, N.R. & GOULD, J.M., 1986. Effects of alkaline hydrogen peroxide treatment of wheat straw on site and extent of digestion in sheep. *J. Anim. Sci.* 63, 868.

LEWIS, S.M., KERLEY, M.S., FAHEY, G.C., Jr., BERGER, L.L. & GOULD, J.M., 1987. Use of alkaline hydrogen peroxide treated straw as an energy source for the growing ruminant. *Nutr. Reports Int.* 35, 1093.

SNEDECOR, G.W. & COCHRAN, W.G., 1967. Statistical methods (6th edn.). The Iowa State University Press, Ames, Iowa, USA.

THEANDER, O. & AMAN, P., 1980. Chemical composition of some forages and various residues from feeding value determinations. J. Sci. Food Agric. 31, 31.

THIAGO, L.R.L. & KELLAWAY, R.C., 1982. Botanical composition and extent of lignification affecting digestibility of wheat and oat straw and paspalum hay. *Anim. Feed Sci. Technol.* 7, 71.

VAN SOEST, P.J., 1963. Use of detergents in the analysis of fibrous feeds. II. A rapid method of the determinations of fibre and lignin. J. Assoc. Off. Agric. Chem. 46, 825.

VAN SOEST, P.J. & WINE, R.H., 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. J. Assoc. Off. Agric. Chem. 50, 50.

<sup>\*\*</sup> Significant (P≤0,01).

a,b,c Denote significant differences (P≤0,05).

<sup>&</sup>lt;sup>1,2</sup> Denote significant differences ( $P \le 0.01$ ).