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Nutritional Evaluation of Sulfur Dioxide Treated Straw

D. Ben-Ghedalia, J. P. Van Soest, J. Miron

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Names of Investigators:

Principal Investigator: DANIEL BEN-GHEDALIA

Cooperating Investigators: PETER, J. VAN SOEST; JOSHUA MIRON

Name and Address of Affiliated Institutions:

Principal Institution: METABOLIC UNIT, THE VOLCANI CENTER, A.R.O.,

P.O. BOX 6, BET-DAGAN, 50250, ISRAEL

Cooperating Institution: CORNELL UNIVERSITY, DEPARTMENT OF ANIMAL
SCIENCE, ITHACA, NY, USA

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Signatures:

Principal Investigator

Institution's Authorizing Official

D. Ben-Ghedalia

Yuval Eshdat
Dr. YUVAL ESHDAT
Head, Div. for Research Projects and Budgets
Agricultural Research Organization

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ABSTRACT OF THE REPORT

This report extends our knowledge on the nutritional characteristics of SO₂ treated wheat straw including the following issues: 1) The interaction between SO₂-treated straw and concentrate. 2) The use of poultry litter as a complementary component in SO₂-treated straw rations. 3) Production trials with SO₂ treated straw. 4) The physico-chemical properties of treated straw.

In a series of studies with SO₂-treated straw, it has been shown that at the level of intake of 1000 g day⁻¹, up to 70% barley in the diet did not adversely affect the digestibility of straw organic matter and CW both in the rumen and in the entire tract. Increasing the proportion of straw in the rations was associated by an increase in the efficiency of microbial protein production and flow of N to the duodenum.

A study on the digestibility of ration containing 60% SO₂-treated straw + poultry litter (1:1) and 40% concentrate pointed to a synergistic interaction between the SO₂-treated straw and the poultry litter components. Based on limited data, macroelements absorption is not impaired in SO₂ treated straw diets. However, the availabilities of microelements and particularly that of copper is decreased in those diets.

Two lamb-growth studies have been conducted with the 1:1 mixture of either untreated or SO₂-treated straw + poultry litter (PL) replacing 60 or 70% of the concentrate of a 100% concentrate - (control) ration. With the 60% straw + PL rations body weight daily gains were 266, 356 and 363g for the untreated straw, SO₂-treated straw and 100% concentrate rations, respectively. The lambs fed the SO₂ treated straw ration were leaner, their meat was well accepted and received a per Kg higher price. The insensitivity of SO₂ treated straw to concentrate in the diet and the complementary nutritional characteristics with respect to poultry litter, explain the effectiveness of that mixture in replacing concentrate in intensive rations.

The findings of this research open the way for a massive use of recycled materials in intensive ruminant production. The American partner concentrated his studies on the physico-chemical properties of the treated straw in relation to their CW digestibility.

OBJECTIVES OF THE RESEARCH

The objectives of the research were:

I. To study the effect of the concentrate/straw ratio, on the digestibility of the SO_2 treated wheat straw (TWS) by sheep cannulated in the gastrointestinal. This point deserved rigorous examination, since if the digestibility of TWS is insensitive to the addition of concentrate, then its use in intensive ruminant diets could be enhanced. The scientific significance of this objective was in studying the effect of a starchy feed, on the degradation and digestion in vivo of the cell wall (CW) monosaccharides of the treated straw.

II. To assess the nutritional value of a diet consisting of TWS and poultry litter (PL), with sheep cannulated in the gastrointestinal. The possibility of the TWS to successfully match together with PL in ruminant diets was examined. Since energy availability of the TWS is markedly increased, PL can be considered as an complementary component with respect to nitrogen and minerals. Successful results in this direction will lead to increasing the use of straw and poultry manure in intensive diets. This point is of a particular interest for the Negev region in which the largest proportion of wheat is being produced and in which the sheep industry is striving for development.

III. To study the effect of feeding the TWS on the mineral availability in sheep. Including macro and micro elements and with particular reference to sodium and copper.

IV. To examine the capacity of diets containing TWS and PL to support intensive growth of lambs.

EVALUATION OF TREATED STRAW IN THE LABORATORY

Final Report to BARD

Submitted by

Peter J. Van Soest

Cornell University

Ithaca, NY 14853

INTRODUCTION

The most common treatments applied to cow quality straws and stovers has been sodium hydroxide (NaOH) and ammonia (NH₃). While other treatments such as SO₂, ozone etc. have been tried, discovery in the course of this work of indigestible soluble organic material from NaOH and NH₃ treatments led to the focus upon this particular problem as related to laboratory evaluation of treated forages. In general laboratory procedures have not performed as well in evaluating treated forages as for untreated ones.

Laboratory work in general has not followed the chemical changes that occur in treating straws so that most procedural evaluations are quite empirical at the present time. Chemical parameters e.g., fiber and lignin, provide different associations with digestibility as compared with untreated controls and other forages, but also a divergence between in vitro rumen and in vivo digestion exists that is not apparent in untreated forages (Berger et al., 1979, 1980). This difference has not been explained by any laboratory measurements, although it has been attributed to faster passage due to changed physical structure (Berger et al., 1980). Another possibility is that soluble cleaved lignin products remain digestible but are not measured in the Tilley Terry (1963) method (Van Soest et al., 1984). It is likely that many soluble lignin products are not digestible and could pass through the digestive tract (Neilson and Richards, 1978). A second problem is the possibility of heat-damage and the Maillard reaction whereby

ammonia or protein can become unavailable.

Our studies consisted of two parts. The first involving the collection of treated forages and their controls, followed by laboratory study. And a second one specifically dealing with the problem of heat damages in ammoniated forages. These are reported sequentially.

FIRST STUDY

Most of the treatments applied to low-quality forages with the intention of improving nutritional value have involved treatment with alkali (Jackson, 1977; Sundstol et al., 1978; Theander et al., 1984). Generally, the focus of effort has been to vary the conditions of treatment and then to measure the animal production response. Typically, there has not been a concurrent laboratory determination of the chemical changes that occur in treating straws and as a result most procedural evaluations used at the present are quite empirical.

The evaluation of chemically-treated straws offers special challenges. The first obstacle that must be overcome in evaluating the nutritional quality of alkali-treatment is to understand the causes of the variability in efficiency of treatment and its effect on animal response. It has not been determined if this variability is random, environmental, or intrinsic to the material being treated. Thus it is imperative that we must study the digestibility, chemical, and physical changes in forages that have been alkali-treated, relative to their controls, in order to account for and predict the nutritional properties of treated low-quality feeds.

The objective of the present study is to study these digestibility, chemical, and physical properties of alkali-treated forages, compared to their controls, in order to estimate the nutritional value of these feeds and the usefulness of chemical-treatment.

MATERIALS AND METHODS

Sample Description

The first study includes an examination of ammonia and sodium hydroxide-treated forages and their controls. Samples were obtained from researchers throughout the world, thus providing a broad range of species and environmental growing conditions. The forages and the type of chemical treatment they were exposed to are described in Table 1.

Chemical

The procedure followed is a modification of that previously employed by Lau and Van Soest (1981) and Van Soest et al. (1984). Approximately 1.0 g sample of feed was weighed into a 600 ml Berzelius beaker followed by 100 ml of neutral-detergent. The beaker contents were boiled for 60 min, then filtered under vacuum through a 30 ml coarse-porosity, frittered-glass crucible. The neutral-detergent solubles (NDS) were collected in a 500 ml vacuum Erlenmeyer and brought to a volume of 500 ml. The NDS were subsampled, flushed with 99.9% nitrogen, and refrigerated for subsequent spectrophotometric analysis. The NDF was recovered in the crucible, then dried at 100 C for only 1-3 h to minimize heat damage, and hot-weighed according to

Goering and Van Soest (1970). Next the NDF and the crucible were placed in a 100 ml beaker and 30 ml of 0.1000 N NaOH were added to the sample in the beaker. The beaker contents were boiled for 10 min to saponify the sample. At 10 min the beaker and contents were immediately placed in a vacuum dessicator and left to cool to room temperature. The dessicator was continuously flushed with 99.9% nitrogen to prevent CO₂ solubilization in the hot alkali. After cooling the beaker was removed from the dessicator, the crucible was placed on its side in a 600 ml Nalgene beaker. The contents were quantitatively rinsed from the 100 ml beaker into the Nalgene beaker with distilled, deionized water. The sample was continuously flushed with 99.9% nitrogen while being titrated to pH 7.0 (± 0.1) with 0.06 N HCl to determine the milliequivalents of base used in saponification. The milliequivalents of base/ g sample OM or

$$\text{Sap meq} = \frac{(\text{Vol. NaOH} \times \text{Molarity NaOH}) - (\text{Vol. HCl} \times \text{Molarity HCl})}{\text{Sample OM}}$$

The outside of the crucible was rinsed with distilled water before the crucible was transferred to a suction filtration apparatus where the sample was filtered through the same crucible. The saponification solubles (SS) were collected in a 500 ml vacuum Erlenmeyer, brought to a volume of 500 ml, subsampled, flushed with nitrogen, and refrigerated for subsequent spectrophotometric analysis. The crucible and contents were ashed at 550 C for a minimum of 6 h and then hot-weighed. The difference between this weight and the crucible with NDF weight could be used to calculate the NDF OM weight.

In vitro

Approximately 1.0 g sample was weighed into a 125 ml Erlenmeyer flask. The in vitro incubation, with rumen fluid, followed the method of Goering and Van Soest (1970). The 40 ml of buffer was added to the samples 12-24 h before the start of the incubation to allow time for sample hydration. The flask and

contents were then put in the refrigerator to limit the possibility of microbial growth. Rumen fluid was collected from a timothy hay fed, nonlactating, dairy cow 2-4 h after the first of her two daily feedings. After the 48 h fermentation, the sample residues were either: 1) removed from the bath and boiled in neutral-detergent to determine true organic matter digestibility or 2) left in the bath for an additional 24 h and 2 ml 6N HCl and .5 g pepsin were added per flask.

True undigested organic matter residues were measured on the first group of samples by boiling the flask contents for 60 min in neutral-detergent, filtering, drying overnight at 100 C, hot-weighing to determine the residue weight (RW), ashing at 550 C, and reweighing to determine the residue ash weight (RAW). The true organic matter residue was calculated by:

$$\text{NDOR} = \frac{(\text{RW} - \text{Ash}) - \text{RAW}}{\text{Sample OM}} .$$

The neutral-detergent residue solubles (NDRS) were collected in 500 ml vacuum Erlenmeyers and brought to 500 ml volumes, subsampled, flushed with nitrogen, and refrigerated for spectrophotometric analysis later.

The second group of samples referred to as the Tilley-Terry residues were filtered after pepsin treatment, dried overnight at 100 C, hot-weighed to determine the residue weight (TW), ashed at 550 C, and reweighed to determine the residue ash weight (TAW). The apparent organic matter residue was calculated by the following equation:

$$\text{TTOR} = \frac{(\text{TW} - \text{Ash}) - \text{TAW}}{\text{Sample OM}} .$$

The Tilley-Terry residue solubles (TTRS) were collected in 500 ml similarly to the NDRS.

NDS, SS, NDRS, and TTRS containers were removed from the refrigerator one

day before spectrophotometric analysis and left at 25-30 C to solubilize any precipitates, mainly the disodium lauryl sulphate from the neutral-detergent solution. Aliquots were taken, diluted as necessary, and absorption (optical density) was measured from 250 - 350 nm on a Scanning UV/VIS spectrophotometer. A 99.7 ppm Cu ⁺² in HNO₃ solution was used as a standard to adjust for any day-to-day variation. Optical densities (OD) were calculated for the peaks observed at 280 and 314 nm and expressed as units per milligram OM per milliliter volume by the following equation:

$$OD = \frac{\text{Absorbance} \times \text{dilution factor} \times \text{volume}}{\text{Sample OM}} \times 100$$

RESULTS

A total of 30 treated and 16 untreated samples were examined. Sixteen forages were treated with ammonia and the remaining fourteen were sodium hydroxide-treated. In general, ammoniation tended to decrease the NDF, but increased the ADF, causing a drop in hemicellulose content. There was tremendous variability in the availability of the additional nitrogen supplied to the forage from ammoniation, with 0 to 66% of that additional nitrogen being in the acid-detergent indigestible nitrogen (ADIN) pool which would render it unavailable to the animal and the microbes. The weaker alkali did not saponify as effectively so there often was an apparent increase in lignin percent. The sodium hydroxide-treated forages usually showed a greater loss of NDF with a similar increase in ADF. However, there was a concomitant greater neutral-detergent solubilization of both hemicellulose and lignin. Consequently, despite the greater loss in NDF, the percentage of Klason lignin still decreased. Ash content of treated samples were always different from the controls, were frequently observed to be 2-3 times greater than the controls, and sometimes differed by an order of magnitude. Thus it was imperative that

all values be corrected to an organic matter (OM) basis or interpretations would be compromised.

In vivo apparent OM digestibility measurements were regressed against in vitro apparent OM digestibilities (Table II). Sheep and cattle data were separated but discretion must be used in interpreting these results since the animals were not fed the same diets.

Correlation coefficients of in vivo and in vitro OM digestibilities with chemical properties are shown in Table III. In vitro correlations are higher than in vivo ones; however, the in vivo data is subject to both animal variation and experimental location while the in vitro results are not. Lignin content is inversely correlated with digestibility more strongly in control forages than in treated ones. Conversely, optical densities, which measure fractured lignin-carbohydrate bonds, are correlated positively with the treated samples. The saponification solubles optical densities of the treated forages estimated the intact lignin-carbohydrate bonds after alkali treatment that were still susceptible to cleavage by alkali and may be broken during anaerobic fermentation. These alkali-susceptible lignin-carbohydrate bonds are positively correlated with the increased digestibility of treated samples. The optical densities of the TTRS are biologically significant since they encompass the phenolic entities that were solubilized by microbial fermentation. Consequently, the relationship between OM digestibility and TTRS OD₂₈₀ and TTRS OD₃₁₄ are significant for the treated samples but not for the controls.

The Lucas test (Van Soest, 1967, 1982) is a classical technique to determine the true digestibility of a dietary entity and the endogenous excretion of that entity by adding it to the diet at different levels. The endogenous or metabolic component is calculated at a zero intake of the

entity. Cleavage of lignin-carbohydrate bonds decreased NDF values, thus increasing cell contents and causing a concomitant increase in OD. The Lucas test on the cell solubles is presented in Figure 1. The control forages split into two distinct populations both having a true digestibility of 96% but with metabolic components of -13 or -20. The metabolic component of -13 had been noted previously by several researchers (Aerts, 1978) but these equations were developed using better quality forages. A regression equation was developed which included 28 control forages where the amount of apparently digestible cell contents = $-16.0 + .86 \text{ cell contents}$ ($r^2=89.$, $n=28$). It should be noted that only the better quality forages such as Coastal bermuda grass hay, ryegrass hay, and a few straws fit previously published equations on good quality forages. If this population is omitted from the Lucas test then lower quality forages have a cell contents true digestibility of 96% and a metabolic component of -20. The treated forages were much more variable as indicated by the lower correlation coefficient ($r^2=31.$, $n=30$) and had true digestibility of the cell contents of only 37%. This suggests that the cleavage of lignin-carbohydrate bonds by alkali-treatment increases the cell contents but the amount of soluble lignin also increases to ultimately depress the digestibility of the cell contents.

When the Lucas test was applied to the OD₂₈₀ content of the forages, a similar response was observed (Figure 2). The total OD₂₈₀ in the sample was the sum of the neutral-detergent solubles (NDS) and the saponification solubles (SS). The amount of apparently digestible OD₂₈₀ was measured as the neutral-detergent residue solubles (NDRS) after 48 h in vitro fermentation. The control samples had a 96% true digestibility but yielded only a small metabolic component from the fermentation; whereas the treated samples had a greater amount of OD₂₈₀ but it was less digestible and thus contributed even less to the metabolic pool.

A similar situation was seen with the optical density at 314 nm (Figure 3). The OD_{314} of the controls was more digestible than was the optical density of the treated samples. The high correlation coefficient of the control samples and the 92% true digestibility coefficient indicate that the OD_{314} pool is nutritively uniform. However, the OD_{314} fraction of the treated forages had a lower true digestibility coefficient of 79%.

DISCUSSION

The effect of alkali-treatment is quite variable. There did not seem to be any consistent pattern of effectiveness of treatment that was intrinsic to species or geographical location of treatment. Application of aqueous alkali did seem to increase heat damage which would reduce the efficiency of treatment. This was especially true in the case of ammonia which is a weaker base and a potential participant in the Maillard reaction.

Lignin content was not significantly related to OM digestibility in treated samples, perhaps because the residual insoluble lignin is less frequently bound to the carbohydrate fraction. The appearance of soluble lignin and its lower correlation with OM digestibility than Lau and Van Soest (1981) noted could be attributable to the broader range of feed samples used, in conjunction with their more variable lignin contents. The number of lignin-carbohydrate bonds cleaved, the amount of residual insoluble lignin and its association with NDF, and the appearance of soluble optical density were very forage dependent. The net result was a very heterogeneous group of samples. The appearance of soluble lignin during Tilley-Terry in vitro fermentation, which is most analogous to the *in vivo* situation, had a high correlation with OM digestibility.

Lucas tests on the cell contents still pose unanswered questions. The primary concern is that the cell contents of low-quality forages generally

yield a microbial component of -20 units while previous work estimates the microbial component of better quality forages at -13. An interpretation of this difference could be that some soluble lignin is precipitating with the microbial fraction, possibly through a tanning reaction. It is important that one be able to distinguish if the true digestibility of the cell contents of low-quality forages differs from 96% or if the metabolic component differs from -13 in order to definitively interpret the effect of chemical treatments on nutritive value. Assuming for the time that the Lucas test of the control samples yields an equation that lies between these two situations (Figure 2.), then one observes a dramatic drop in digestibility of the cell solubles of the treated forages. Theoretically, this decrease in cell contents digestibility would be directly caused by the increased soluble lignin. Consequently, the nutritive value of treated feeds would be overvalued because of the contamination of the cell contents by soluble lignin.

A Lucas test on the soluble phenolics indicated that this was a nutritively uniform pool and that it had a true degradability coefficient close to 1.0. The Lucas test on the treated samples were less degradable, having a true digestibility coefficient of 79%. This is in agreement with the Lucas test on the cell contents which had a depressed digestibility. The treated samples also had a larger soluble phenolic pool which was associated with a lower extent of degradation. The additional phenolic absorbance is undoubtedly due to solubilized lignin.

Alkali-treatment of low-quality forages has variable effectiveness of treatment that becomes most apparent when one looks at the animal digestibilities. The OM digestibility depends on the degree of lignification of the forage, the number of lignin-carbohydrate bonds, and the susceptibility of these bonds to chemical and microbial degradation. Alkali-treatment

decreases the NDF and hemicellulose content of the forage. Consequently the proportion of cell contents increases but this pool is contaminated with soluble lignin and hemicellulose. It appears that the soluble lignin depresses the nutritive value of the cell contents, thus altering in vivo response from in vitro predictions; however there is a possibility that true digestibility of cell contents is less changed, rather it is the metabolic contribution that varies.

This survey of alkali and ammonia-treated forages indicates considerable variability in the results of treatment, which in turn implies a lack of control of quality. A reason for this deficiency in control of quality may be inadequate laboratory evaluation of forages, which would yield a more rapid and convenient assay of results than do animal feeding studies. While animal response is undoubtedly the final evaluation, quicker assays that will also evaluate chemical changes would allow for more rapid progress.

Relevant analyses that can be recommended include measurement of the extent of cleavage of lignin to carbohydrate bonds by the residual saponification (Lau and Van Soest, 1981) and the distribution of phenolic substances in the extracts obtained before and after saponification. The net amount of phenolic absorbances can be increased through Maillard (heat-damage) reactions that also render feed nitrogen unavailable. Since treatments with ammonia also have the objective of increasing crude protein, the unavailability of this nitrogen should be assayed via the nitrogen content of acid-detergent fibre.

Remaining problems requiring chemical study include the fate of the phenolic substances in rumen and post-rumen digestion. Fecal material should be assayed for these remnants and a better understanding of the influence of soluble lignin components upon animal digestion and metabolism is needed.

SECOND STUDY

The plant cell wall, or fractions of it, have long been the basis for predicting nutritive value of fibrous feeds, because the net cell wall generally contains the truly indigestible residues. In most normal forages the influence of lignification is restricted to the cell wall, and non cell wall components are available for digestion (Van Soest, 1982). This generalization may become restricted in the case of processed by-products and treated forages where heat and chemical treatments have been applied. Soluble lignin-like products can result from alkali and ammonia treatments of forages and straws through cleavage of lignin-carbohydrate bonds (Van Soest et al., 1984) and from the Maillard reaction (Theander, 1980).

Heating is often involved in the treatment or fermentation of feeds and can promote the Maillard reaction. This can greatly affect apparent lignin content and cell wall characteristics (Goering et al., 1972; Van Soest 1965). The principal reaction is the degradation of sugar components to phenolic products, which catalytically destroy amino acids and polymerise with the remnants of amino acids (Hodge, 1953; Theander, 1980). The most important carbohydrate sources are hemicelluloses and sucrose, and the principal products are a synthetic artifact lignin polymer of a distinctively high nitrogen content (Van Soest, 1965), as well as soluble components of lower molecular weight (Hodge, 1953; Theander 1980). Cell walls of heated forages and feeds are characteristically higher in lignin and lower in hemicellulose contents than unheated ones (Goering et al., 1973). The Maillard

polymer is lignin-like and is generally presumed to be indigestible. Analytically a high nitrogen content of crude lignin and acid-detergent fibre (ADIN) provides evidence of a history of heating, and can be related to lower digestibility of protein and dry matter in heated silages and hays (Goering *et al.*, 1972; Yu and Thomas, 1976). Maillard products are characteristic of many heat-dried feeds (Goering, 1976). It is such a significant quality problem that measurement of ADIN is recommended for heated and fermented feeds (AFRC, 1987).

A variety of products in addition to the artifact lignin polymer may occur in heated feeds (Theander, 1980). For example, the occurrence of bovine 'Bonkers' has been related to 4-methylimidazole, a Maillard product derived from the reaction of ammonia with sucrose (Perdock and Leng, 1989). Ammonia can apparently substitute for amino acids in the Maillard reaction. The occurrence of indigestible nitrogen associated with ADIN has been observed in high temperature ammonia-treated forages. (Mason *et al.*, 1989a,b,c).

Recently, however, the indigestibility of ADIN has been challenged (Britton *et al.*, 1986; Klopfenstein and Britton, 1987; Weiss *et al.*, 1989) on the basis that no significant decline in protein digestibility was observed when heated distillers grains were substituted for urea in isonitrogenous diets. We have obtained the data of Britton *et al.*, (1986) and of Klopfenstein and Britton (1987) and subjected it to mathematical analysis according to the model of Lucas (Van Soest, 1967; 1982). The details of this analysis is not a part of the BARD project and are published

elsewhere (Van Soest, 1989).

This paper presents the results of digestion trials conducted at the Institute of Grassland and Animal Production, Hurley, UK, on ammonia-treated forages (Mason *et al.*, 1989a,b,c). Samples were analyzed as described in the first study. In addition, other analysis from the digestion trials at Hurley were also utilized as described below. The statistical analysis consisted of correlating ADIN and other dietary nitrogen fractions with faecal nitrogen and deviations in digestibility from the regression of digestible amounts upon dietary contents (Van Soest, 1989). Estimates of true digestibility are obtained from the regression slopes. The principal problem in comparing digestibilities of nitrogen and soluble components is that their apparent digestibilities are confounded by metabolic and endogenous losses. These mathematical procedures are designed to remove the effects of the metabolic components to obtain estimates of true digestibility or indigestibility of the respective dietary components.

MATERIALS AND METHODS

Two digestion trials on ammonia-treated forages have been reported by Mason *et al.* (1989a,b,c). The first consisted of four grass species ryegrass, timothy, cocksfoot and tall fescue treated with ammonia in the oven method and at ambient temperature, making 4 controls and 8 treated products (Mason *et al.*, 1989a,b). The second investigated ryegrass at 3 maturities each treated in the oven method providing 3 controls and 3 treated (Mason *et al.*, 1989c). The combined studies provided 7 controls and 11 treated

forages. Digestion trials were with sheep. Full details of the forage compositions, treatments and digestion trials are provided in the three papers. Optical densities and saponification values were also measured. The data used here from Mason's studies include organic matter and nitrogen digestibilities; composition of the diet for total N, soluble non ammonia N (SNAN), neutral-detergent insoluble N (NDIN) and ADIN. Other faecal analysis included water-soluble (FSN) and insoluble nitrogen (FIN).

RESULTS

The relationship of dietary ADIN contents and faecal nitrogen are shown in Figure 4. Overall there is no significant relationship. However, the points can be statistically separated into two significantly differing groups. One including the controls and 4 treated forages, and the other the remaining 7 treated forages. The latter tend to be those with the highest SNAN and NDIN contents. The lower group shows a significant regression in the ADIN with a slope of 2.48, significantly greater than the unity required for faecal recovery. Clearly there is co-association with ADIN and the NDIN and SNAN in these samples. Regression of insoluble faecal N upon ADIN produced very similar results, the same seven treated samples deviating from those that will form a significant relationship with ADIN. This regression is $Y = 1.30X - 6$. Standard deviation of the regression coefficient is ± 0.30 , indicating that FIN may represent faecal recovery of ADIN. More definitive chemical evidence is needed on the nature of Maillard products in faeces and their recovery in ADIN.

A more important aspect is the existence of indigestible soluble components in the ammonia-treated forages, this is examined in Figure 5 by regression of apparently digestible neutral-detergent soluble organic matter upon the organic neutral-detergent soluble contents of the forages. Regressions of this type provide estimates of true digestibility from the slopes, and faecal endogenous losses from the negative intercepts (Van Soest 1982). Controls and treated samples have significantly different regression slopes. That for the controls is not significantly different from unity ($1.05 \pm .08$), and that for treated significantly less than unity ($0.73 \pm .04$).

Analogous regressions of digestible amount upon dietary contents were estimated for all of the nitrogen fractions. For NDIN and ADIN, it was assumed that FIN represented the indigestible quantity, and FSN for SNAN, respectively. The sum of NDIN and SNAN have also been tested as to the uniformity of the combination in respect to total faecal N. Estimation of the true digestibilities for these are given in Table IV for the controls and treated samples.

Estimates of true digestibility of controls are near unity for total N, SNAN, and NDIN + SNAN. That for NDIN is 0.81 and it can be expected that there is a small cell wall fraction containing indigestible nitrogen (Van Soest, 1982). In normal forages, ADIN did not give an estimate of true digestibility due to too large a variance in both controls and treated forages. Some of the reasons for this are apparent in Figure 4. It is evident that ADIN does not recover the insoluble indigestible N present in treated forage

and that NDIN although fairly digestible in normal forages may contain considerable unavailable N in treated ones. All of the N fractions in the treated forages except ADIN show true digestibilities significantly less than unity. The combination of NDIN plus SNAN largely account for the indigestibility of N in treated samples. Caution should be taken in interpreting the true digestibility of SNAN (.74) in treated samples, as it is likely that some low molecular weight products of the Maillard reaction could be absorbed and excreted in urine. Whilst urine was not examined for Maillard products in these experiments it should be mentioned that toxic 4-methyl imidazole was detected in all of the ammonia-treated forages and was generally higher in those that were oven-treated (Mason *et al.*, 1989a).

Since the treated samples show strong evidence of reduction in digestibility of soluble organic matter, as well as in nitrogen, a further relevant question is in regard to the composition of that organic matter of Maillard origin. The Maillard reaction involves the reaction of sugar and amino acid at unitary ratio and involving a net loss of 3 molecules of water from the aromatisation of the sugar and one mole of CO_2 from decarboxylation of the amino acid. Ammonia can also substitute for amino acid in the reaction (Hodge 1953, Theander 1980). The resistant polymer can expect to have a N content somewhat less than in normal proteins. Van Soest (1965) found a ratio of 8.9 of organic matter to N in the Maillard product in heated forages. Regression of the undigested soluble organic matter in faeces upon the elevation of faecal N gave a ratio of $8.83 \pm .11$ in reasonable agreement.

Previously, it had been reported that these samples contained soluble lignin measured spectrophotometrically at 280 nm in neutral-buffer extracts (Van Soest et al., 1984). Soluble lignin is likely to be indigestible and recoverable in faeces (Neilson and Richards, 1978). Samples that were treated and particularly those that were oven-treated, tend to contain more net phenolic matter than the untreated controls. The uv absorption at 280 nm is highly correlated with the SNAN ($r = +.92$) and with faecal soluble organic matter ($r = +.91$). Lignin-like phenolics have the structural requirements for participation in the Maillard reaction, and can substitute and compete with the phenolics derived from sugar degradation (Theander, 1980). Thus it is possible that the indigestible soluble organic matter contains soluble lignin components in addition to those phenolics derived from sugar, as well as nitrogen in its structure.

DISCUSSION

The potential interaction of soluble lignin in the Maillard reaction could be heterogeneous. One way is the ammoniolysis of lignin esters to produce lignin acid amides. Indigestible amide linkages have been noted in NIR spectra of ammonia-treated forages (F. H. Barton, pers. comm.). Another mechanism could be the condensation of phenol with sugar aldehydes. Still another, the reaction of 1-2 dihydroxyphenols in competition with sugar-derived products with ammonia or amino acid (Theander 1980). Sugars could disappear via either the Maillard reaction involving ammonia, or by aldehydic condensation with soluble phenolics released by

ammoniation. Verification of these processes will require further studies.

It would seem that the balance of component factors contributing to the soluble indigestible fraction may vary depending upon heat treatment. For the low temperature treatments, soluble lignin interactions with ammonia may dominate, whilst higher temperatures promote the conversion of sugar to phenol with subsequent interaction with ammonia. The correlation of sugar loss with elevation of SNAN is +.97 (Mason et al 1989a). In either case, nitrogen from ammonia or other sources is associated with the soluble fraction in a fairly consistent ratio.

For the phenolic-nitrogenous compounds to be both soluble and recoverable in faeces, molecular size cannot be too large (in order to be soluble) but must be in excess of 300-400 Daltons. Smaller components would be absorbed and probably excreted in urine. The occurrence of toxic 4-methyl imidazole is evidence for this.

The amounts of soluble indigestible matter that can be generated in treated forages is very considerable. The range in this study is up to 10 units of organic matter digestibility (as per cent). This matter will be generally lost in laboratory methods such as solubility with cellulase, rumen fluid, or in nylon bags, all based on insoluble gravimetric residues leading to over-estimation of the value of treated forages. Determinations of SNAN and the measurement of optical densities at 280 and 320 nm of the soluble matter for phenolic matter (Lau and Van Soest, 1981; Mason et al., 1989a) can be recommended to those who would make a more complete laboratory evaluation of treated forages.

CONCLUSIONS

In the first study, forty six samples of treated and untreated barley straw, coastal bermuda grass, oat straw, sugar cane bagasse, and wheat straw samples were evaluated by laboratory procedures involving saponification and the measurement of optical density. The 46 samples were collected from several research locations and had in vivo digestibility data available on them. Considerable variability in the effectiveness of treatment was observed. Saponification values measured the extent of cleavage of lignin-carbohydrate bonds by alkali-treatment. The use of Lucas tests on the cell solubles showed that the solubilization of lignin depressed the digestibility of the cell solubles, thus leading to an overvaluation of the nutritional quality of the treated material. In addition, it appears that some of the soluble lignin is associated with the microbial component, possibly through a tanning reaction, and negatively influences rumen and post-rumen digestion. Ammoniation of low-quality roughages also has the objective of increasing crude protein but 0 to 66% of the increase in nitrogen from the ammonia appeared in ADIN, and therefore heat-damaged, would be unavailable to the microbes and to the ruminant.

Results from the second study showed that heating forages and feeds will generate Maillard products of low digestibility, which are signalled by a rise in the nitrogen (N) content of acid-detergent fiber (ADIN). Data from digestion trials with sheep were also mathematically examined by the methods of Lucas. These data consisted of ammoniated forages with their untreated controls. The treated forages are shown to contain soluble indigestible

organic matter and nitrogen in the ratio of $8.83:1 \pm .11$, agreeing with the expected composition of Maillard polymers. With these forages the amount of faecal N loss considerably exceeds the ADIN estimate. The excess is due in part to the presence of indigestible soluble non-ammonia nitrogen (SNAN) that is associated with a soluble indigestible organic fraction. The existence of indigestible soluble N and organic matter constitutes the basis for severe criticism of gravimetric laboratory procedures, including nylon bags, used in evaluating treated forages and feeds, as these procedures will fail to recover the soluble indigestible components and thus over-value treated forages. The use of ADIN as a marker for heat damage and the Maillard reaction is validated, but in treated forages additional examination for phenolic absorbances at 280 and 320 nm, and soluble non-ammonia N (SNAN) is recommended.

The results from both studies are in agreement as to the occurrence of indigestible soluble residues arising from two sources; one from the cleaving of lignin esters to form soluble lignin. This is possible with both NaOH and NH_3 treatments. The second is the formation of soluble Maillard products. The latter formation is unique to samples having received NH_3 with some temperature treatment. However, many treated forages were observed also to have undergone spontaneous heating as evidenced by elevated ADIN. Theoretically, the use of SO_2 should also produce indigestible soluble fractions, although this was not investigated.

TABLE I

Description of samples, type of treatment, and its contributor

Contributor:	Sample	Treatment
Anon, 1982a (Setälä):	Oat straw	Untreated
	Oat straw	NH ₃
Anon, 1982b (Setälä):	Oat straw	Untreated
	Oat straw	NH ₃
Jewell et al., unpublished:	Barley straw	Untreated
	Barley straw	Aqueous NH ₃ stack
	Wheat straw cv. Bounty	Untreated
	Wheat straw cv. Bounty	Aqueous NH ₃ stack
	Wheat straw cv. Armada	Aqueous NH ₃ stack
	Wheat straw cv. Armada	Anhydrous NH ₃ oven
Masoud, 1983:	Coastal bermuda grass	Untreated round bale
	Coastal bermuda grass	Aqueous NH ₃ round bale
	Coastal bermuda grass	Untreated square bale
	Coastal bermuda grass	Aqueous NH ₃ square bale
Molina et al., 1983:	Sugar cane bagasse	Untreated
	Sugar cane bagasse	Aqueous NaOH
	Sugar cane bagasse	Aqueous NaOH
	Sugar cane bagasse	Aqueous NaOH
Smith et al., 1984:	Barley straw	Untreated
	Barley straw	Aqueous NaOH chopped silage
	Barley straw	Aqueous NaOH ground pellets
	Barley straw	Aqueous NH ₃ stack
Smith, unpublished:	Barley straw, spring	Untreated
	Barley straw, spring	Aqueous NH ₃
	Barley straw, winter	Untreated
	Barley straw, winter	Aqueous NH ₃
Steg et al., 1982:	Wheat straw	Untreated
	Wheat straw	Aqueous NaOH
Steg, unpublished:	Bagasse pith	Untreated pellets
	Bagasse pith	Aqueous NaOH pellets

TABLE 1 - continued

Description of samples, type of treatment, and its contributor

Contributor:	Sample	Treatment
Tuori, 1982:		
	Oat straw	Untreated
	Oat straw	Anhydrous NaOH
	Oat straw	Untreated
	Oat straw	Anhydrous NaOH
	Oat straw	Anhydrous NH ₃ stack
Manapat et al., unpublished:		
	Barley straw	Untreated
	Barley straw	Anhydrous NH ₃ stack
	Barley straw	Aqueous NH ₃ stack
	Barley straw	Anhydrous NH ₃ stack, vacuum
	Barley straw	Anhydrous NH ₃ oven
	Barley straw	Beckman NaOH
	Barley straw	Wet NaOH
	Barley straw	Dip NaOH
	Barley straw	Dip NaOH + urea
	Barley straw	Dry NaOH

TABLE II

Relationship between in vivo organic matter digestibility coefficients (OMD) of fibrous roughages fed to sheep or cattle and in vitro true organic matter digestibility coefficients (IVTOMD). Equations are given for alkali-treated and untreated forages (all), treated forages only (trt), and untreated forages only (cont).

Sheep and cattle data together:

all	OMD = $-7.7 + .72 \text{ IVTOMD}$,	$r^2 = .52$,	n = 46
cont	OMD = $-33.0 + 1.42 \text{ IVTOMD}$,	$r^2 = .61$,	n = 16
trt	OMD = $7.2 + .66 \text{ IVTOMD}$,	$r^2 = .75$,	n = 30

Sheep data only:

all	OMD = $-0.9 + .80 \text{ IVTOMD}$,	$r^2 = .79$,	n = 27
cont	OMD = $-16.8 + 1.08 \text{ IVTOMD}$,	$r^2 = .93$,	n = 8
trt	OMD = $11.4 + .65 \text{ IVTOMD}$,	$r^2 = .59$,	n = 19

Cattle data only:

all	OMD = $7.5 + .79 \text{ IVTOMD}$,	$r^2 = .90$,	n = 19
cont	OMD = $-55.1 + 1.84 \text{ IVTOMD}$,	$r^2 = .91$,	n = 8
trt	OMD = $22.3 + .58 \text{ IVTOMD}$,	$r^2 = .56$,	n = 11

TABLE III

Correlation between predictors of in vivo apparent organic matter (OM) digestion, in vitro apparent OM digestion, and in vitro true OM digestion. In addition, correlation coefficients are separated according to alkali-treated and untreated forages (all), alkali-treated forages only (trt), and untreated forages only (cont).

Predictor	in vivo apparent OM digestibility			in vitro apparent OM digestibility			in vitro true OM digestibility		
	all	trt	cont	all	trt	cont	all	trt	cont
number	46	30	16	46	30	16	46	30	16
Lignin %	11.2	17.9	25.8	27.7	39.9	49.7	14.3	32.1	65.0
NDS ¹ OD ₂₈₀	25.7	12.4	0.0	26.8	16.5	0.9	48.9	30.7	0.1
NDS OD ₃₁₄	6.6	0.7	12.4	5.3	0.7	21.2	23.7	10.8	7.2
Sap. meq. ²	38.8	12.8	14.0	35.9	12.6	18.5	56.4	19.0	32.8
SS ³ OD ₂₈₀	40.8	46.1	4.5	43.1	41.1	11.3	49.1	42.2	15.2
SS OD ₃₁₄	45.4	55.3	6.8	46.4	49.1	10.6	49.4	46.2	14.0
NDRS ⁴ OD ₂₈₀	9.4	4.5	0.2	14.0	0.9	4.6	44.1	12.9	2.2
NDRS OD ₃₁₄	4.3	7.8	0.6	6.0	0.4	9.0	28.6	3.5	0.1
TTRS ⁵ OD ₂₈₀	24.0	47.6	19.5	35.1	54.9	34.0	16.0	41.0	15.4
TTRS OD ₃₁₄	27.9	52.5	18.9	37.3	54.2	34.7	17.7	38.6	17.1

¹ Neutral-detergent optical density (units / mg OM).

² Saponification value of the neutral-detergent fibre (meq base / g OM).

³ Optical density of solubles after laboratory saponification (units / mg OM).

⁴ Optical density of neutral-detergent solubles of residue of 48 h in vitro fermentation (units / mg residue OM).

⁵ Optical density of hot H₂O solubles of residue of 48 h Tilley-Terry in vitro fermentation plus 24 h pepsin incubation (units / mg residue OM).

Table IV: Estimates of true digestibility of N fractions based on the regression of digestible components upon dietary contents.

Fraction	Controls (n = 7)			Treated (n = 11)		
	r	True dig.	SD	r	True dig.	SD
Total N	0.99	1.01	± 0.05	0.98	0.67	± 0.04
SNAN ^a	0.98	1.01	± 0.13	0.97	0.74	± 0.07
NDIN ^b	0.91	0.81	± 0.17	0.86	0.48	± 0.10
ADIN ^c	0.11	NS ^d	± 1.23	-0.23	NS ^d	± 2.01
NDIN + SNAN	0.98	1.01	± 0.04	0.97	0.52	± 0.04

^aSoluble non-ammonia N

^bNeutral-detergent insoluble N

^cAcid-detergent insoluble N

^dNo significant value

Fig. 1. Relationship between the percentage apparently digested neutral-detergent soluble organic matter and the percentage neutral-detergent soluble organic matter.

Fig. 2. Relationship between the apparent amount of digestible optical density (OD) per mg organic matter (OM) at 280 nm and the total units of optical density per mg OM at 280 nm.

Fig. 3. Relationship between the apparent amount of digestible optical density (OD) per mg organic matter (OM) at 314 nm and the total units of optical density per mg OM at 314 nm.

Figure 4. Relationship between dietary contents of ADIN and total faecal N for diets composed of forages treated with ammonia and their untreated controls. Calculated from the data of (Mason et al., 1989a,b,c). Values above the dashed line exceeded 3 standard deviations from the mean population and have been excluded from the regression.

Figure 5. Relationship between dietary contents of ADIN and total faecal N for diets composed of forages treated with ammonia and their untreated controls. Calculated from the data of (Mason et al., 1989a,b,c). Values above the dashed line exceeded 3 standard deviations from the mean population and have been excluded from the regression.

COOPERATION

Straw samples treated in Israel served as substrates and materials for the in-depth study of CW chemistry and physico-chemical properties in relation to straw digestibility. These studies were conducted in Cornell by the group of Prof. Van-Soest. exchange of data, discussions and continuous communication between the American and Israeli partners formed the basis of cooperation. The experience of the Israeli group was enriched with laboratory approaches to straw evaluation and that of the American group with practical aspects related to the feeding of SO₂-treated wheat straw.

ACHIEVEMENTS vs AIMS OF RESEARCH PROPOSAL

After admitting that: "Objectives have been met", BARD reviewer of project I-292-80 (previous stage of this research) appreciated that: "Ben-Ghedalia is overly optimistic about the application (of the SO₂ treatment, D.B.G.) to agriculture". Data presented in this report showing that lambs can grow intensively (360g/day) on a ration consisting of 60% SO₂-treated straw +poultry litter (1:1), and 40% concentrate, contradict strongly that pessimistic view. From the standpoint of production, the original title of this project "Conversion of straw into a concentrate-like feed" has been realized. We have shown that wheat straw when treated properly can be converted into a highly productive feed. We published our first (pioneering) paper on the SO₂ treatment of cereal straw in 1981; with the aid of BARD funding we gained a vast amount of knowledge regarding the effect of chemical treatments on the chemistry and biodegradability of straw CW both in vivo and in-vitro. Increasing interest of some of the relevant top scientific centers in the SO₂ treatment as reflected in recent literature is another evidence of the stimulatory impact of this project and its contribution to science. Ramification of this project into areas such as silage research and monogastric nutrition are additional fruits derived from our SO₂ studies. In terms of benefit to agriculture, the findings of this research open the way for a massive use of recycled materials in intensive ruminant production.