IMPROVING THE NUTRITIVE VALUE OF STRAW

THE EFFECTS OF PHYSICAL AND CHEMICAL TREATMENTS ON THE NUTRITIVE VALUE OF OAT STRAW AS DETERMINED *IN VITRO* AND *IN VIVO*

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by

Teddy Athanasius Oluwole Chema Jones

Department of Animal Science,

Macdonald College of

McGill University,

Montreal.

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M.Sc. Nutrition

Teddy A.O.C. Jones

THE EFFECTS OF PHYSICAL AND CHEMICAL TREATMENTS ON THE NUTRITIVE VALUE OF OAT STRAW AS DETERMINED *IN VITRO* AND *IN VIVO*

ABSTRACT

In vitro experiments designed to establish a suitable level of alkali treatment to be applied to oat (*Avena sativa*) straw for an *in vivo* trial indicated the use of an 8% treatment level (60ml. 13.3% NaOH/100g. of straw).

Ground oat straw was treated chemically to make potential energy available and then pelleted to increase the voluntary intake. Ground untreated and pelleted untreated straw served as controls. The diets were fed in two 3-week periods to lambs, *ad libitum*. No supplements were given other than iodized salt licks.

Chemical treatment significantly ($P < .01$) increased energy digestibility but significantly ($P < .01$) depressed voluntary intake, while pelleting showed the opposite effect. Consequently, the nutritive value of the straw, as measured by digestible energy intake (NVI) was not increased by the physical and chemical treatment combination.

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I. INTRODUCTION.

Man's dependence on animals for food and clothing dates back to pre-historic times. However, it was not until the Greek and Roman civilizations that man began to record his observations on the feeding habits and characteristics of animals which he domesticated. Aristotle who lived about 400 B.C. recognized in his Historia Animalium that the alimentary tract of the ruminant contained a four compartment stomach whereas the stomach of other animals had only one compartment.

This difference in stomach structure is not merely one of anatomical significance but also of great physiological significance. The recognition of the role of the ruminant digestive system replaced the earlier held concept that the fibrous parts of feedingstuffs were totally indigestible to animals.

Ruminants, herbivores by nature, differ from other animals in having the capacity to consume large amounts of forage as the predominate portion of their daily ration, and also in being able to utilize holocellulose (cellulose and hemicellulose) the major component of forage. The ability of ruminants to utilize these complex carbohydrates is due to the presence in the reticulo-rumen (two of the four stomach compartments) of microorganisms which are capable of degrading holocellulose into materials which

can be used as nutrients by the host animal.

Cellulose, the most widely distributed single organic compound in the plant kingdom, forms the fundamental structure of all plant cell walls. Notwithstanding its abundant distribution in nature, only the herbivores, among the higher animals, can utilize cellulose as their primary source of energy. The recognition of this fact has encouraged the rearing of domesticated ruminants on forage crops in many parts of the world. While some ruminants such as the camel, yak and llama are hardy and can subsist on almost barren lands, others such as the cow, sheep and goat are ecologically suited to the more productive lands such as the Pampas, Prairies, Steppes and Velds.

Ruminants such as the sheep, cow, and goat have been domesticated by man so as to procure meat, milk, wool and skin. If only forage is fed to such ruminants with the aim of providing energy for maintenance, growth, or production, a large proportion of the plant cellulose must be in a form which is available to the cellulolytic rumen microorganisms. Low-quality forage (i.e. feedingstuffs which are high in cellulose but low in available energy) are often fed to ruminants. Low-quality forage includes feedingstuffs such as seed-coatings, cereal straws and mature herbage. These materials will not provide adequate energy for maintenance if fed unsupplemented since their

cellulose is highly lignified. Lignin is a plant constituent which encrusts cellulose and other nutrients as the plant matures and is not attacked by rumen microorganisms. Consequently, little energy can be derived from the cellulose of highly lignified forages.

In many developing countries found within the tropics ruminants are raised almost entirely on pasture. Within the tropics the climate is characterized by hot dry spells which last for about half of the year or more alternating with heavy rains. Pastures made lush by the heavy rains mature rapidly and become a highly lignified feed during the dry spells. Consequently, pasture utilization is greatly reduced. Breeding for herbage that will persist for longer periods may be a possible solution to increase pasture utilization and so provide a more nutritive feed during the dry spells. However, when it is considered that in tropical countries (e.g. Sierra Leone, The West Indies, India, Ceylon, the Phillipines) grain is a staple food primarily for humans it may be worth exploring the possibility of improving the nutritive value of low quality forages such as the straws of the cereals cultivated.

Literature on the use of alkali treatment as a means of improving the nutritive value of straw indicates that Eurcpean workers have been exploiting this possibility with success since the beginning of this century. A method of

alkali treatment of straw that achieved much popularity was that of Beckmann. Briefly, it involves the soaking of straw in 8 times its weight of 1.5% sodium hydroxide solution. However, because a large volume of water was required to wash the straw alkali-free and this caused loss of soluble nutrients, later workers turned their attention to alkali treatments which required little or no water for the removal of excess alkali. The term 'dry process' has been coined to refer to alkali treatments of straw involving the use of minimal or no water for washing the straw free of alkali. The 'dry process' has an added advantage in that it could be applied specifically in tropical areas where water shortage during the dry spells could be a serious limitation in the use of the Beckmann process.

The purpose of the research presented in this thesis was to investigate the effect of alkali treatment on the nutritive value of oat straw fed in two physical forms, viz-ground and pelleted. The first half of this research deals with the establishment of a suitable level of alkali treatment of oat straw which could be applied in sheep feeding trials. To obtain this level, <u>in vitro</u> cellulose digestion was used as a criterion for assessing the nutritive value resulting from various levels of alkali treatment. The second half of the research constituting the feeding trials describes the preparation of the oat straw, its feeding, and the <u>in vivo</u> results obtained as measured by digestion coefficients and voluntary intake.

II. REVIEW OF LITERATURE.

A. ASSESSING THE NUTRITIVE VALUE OF FORAGES FOR RUMINANTS.

1. General.

Domestic ruminants are reared for the products - meat, milk, wool and skin - which they provide. Therefore an assessment of the nutritive value of a forage must be reflected in the productivity of the animal. As the nutritive value[1] of a forage is influenced by factors such as its chemical composition and digestible nutrient content, any of these factors may be used as criteria for evaluation provided that the assessment made bears a close relationship with the productivity of the animal. The problem therefore which the ruminant nutritionist has been faced with in the evaluation of the nutritive value of a forage is one of devising a method that is relatively simple, precise and accurate and could be meaningfully related to the animal's performance.

2. Chemical Composition Data.

Chemical composition data are informative "but quantitatively none of it consistently correlates with significant animal performance criteria (Crampton 1957)." Chemical data on forage composition have been generally reported on analysis based on the Weende Proximate Principles devised by Heneberg

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[1] In this thesis, forage quality and forage nutritive value are used synonymously to refer to the contribution a forage makes in meeting the animals' nutritional requirements.

and Stohman at the Weende Experimental Station in Germany. This scheme of analysis partitions a feed into nitrogen-free- extract (NFE), ether extract (EE), crude protein, crude fibre, water, and ash fractions in an attempt to evaluate its feeding value. Criticisms relating to forage evaluation have been levelled mostly at the NFE and crude fibre fractions.

a) Crude Fibre and Nitrogen-Free-Extract.

According to the Weende scheme, crude fibre represents a portion of carbohydrates that is relatively undigestible such as cellulose and hemicellulose, whereas the nitrogen-free-extract (NFE) contains the more soluble and digestible carbohydrates such as the starches and sugars. Thus the crude fibre content was believed to inversely reflect the nutritive value of a forage. The above division of the carbohydrates has been demonstrated to be inadequate and unreliable (Norman, 1935; Crampton and Maynard, 1938; Ferguson, 1942; Ellis et al., 1946; Moxon and Bentley, 1953). Crampton and Maynard (1938) found out that in many cases crude fibre was highly if not more highly digestible than the NFE fraction. Norman (1935) demonstrated the variable composition of crude fibre and NFE in regard to lignin content with most of the lignin being found in the NFE fraction. Lignin, although not a carbohydrate, was grouped under the indigestible carbohydrates (crude fibre) in the

Weende scheme. Several workers are unanimous that the crude fibre analysis should be replaced by cellulose and lignin determinations which would be more meaningful to the ruminant nutritionist (Crampton and Maynard, 1938; Crampton and Whiting, 1942; Ellis et al.,1946; Gaillard, 1958; Matrone et al.,1946).

b) Lignin.

Lignin is not a well-defined chemical entity but its increased association with cellulose and the hemicelluloses in plant materials as the plant matures is well known. Whether its association with these carbohydrates is purely physical or chemical has not been fully elaborated. Some workers (Clarke,1938; Kamstra et al.,1958; Dehority and Johnson, 1961) are of the opinion that its relation is physical, that is, lignin forms an indigestible barrier to the action of rumen microbial enzymes and thereby prevents the utilization of cellulose and other nutrients. The depressing effect of lignification on the digestibility of plant materials has been illustrated by many workers (Norman, 1935; Crampton and Maynard, 1938; Drapala et al., 1947; Kamstra et al.,1955,1958; Dehority and Johnson, 1961).

Quicke and Bentley (1959) concluded from their study of lignin content in hays at different stages of maturity that lignin per se may not be the sole factor responsible for the differences in cellulose digestibility, but that the increased synthesis of non-lignin methoxyl-containing

components in mature forage plants and possible changes in either physical and chemical composition of the cellulose itself or in the association between lignin and cellulose in the cell wall may also be factors.

Forbes and Garrigus (1950), investigating the relationship between organic matter digestibility and the protein, crude fibre and lignin content of forages grazed by steers and wethers, found that the best correlation ($r = -0.95$ for steers, $r = -0.93$ for wethers at $P < .01$) between chemical composition and organic matter digestibility was obtained with lignin. There was also a significant inverse correlation between digestible organic matter intake and lignin content.

Data as to the digestibility of lignin are variable. The results of Crampton and Maynard (1938) and Gray (1947) indicated that lignin is practically non-digestible. But Sullivan (1955) noted that the digestibility coefficient of lignin could exceed 10% in some cases, and Nehring and Laube (1955) reported that in the case of straws it could rise to 20%. It has been observed by Balch et al., (1954) that the method of determination of lignin may lead to irregular digestibility results.

c) Crude Protein.

The amount of protein in a forage has been generally directly associated with the feeding quality of that forage.

However, Crampton and Jackson (1944) have indicated that the protein level of pasture forage is unlikely to limit its feeding value since the protein content is usually adequate to meet the needs of the ruminant.

3. Leaf to Stem Ratio.

It has been suggested (Woodman and Evans 1935) that the leaf to stem ratio may be used as a fair indication of the nutritive value of a forage. Crampton (1956) has stated that "leaves contain from two to two and a half times the concentration of protein as does the stem of the same plant regardless of the kind of plant." It is generally accepted that the stem contains more lignin than do leaves (Drapala et al.,1947; Mackenzie and Wylam 1957; Waite and Gorrod 1959; Hirst et al.,1959). Steppler (1948) observed that lignification was greatest at the top of the stem and least at the base. The difficulty in making measurements of leaf to stem ratio limits the use of this criterion in forage evaluation.

4. Digestibility Data.

It is logical to assume that if animal production should reflect the feeding value of a forage then the availability (digestibility) of the nutrients in a forage may be used as a criterion for evaluation.

Nutrient digestibility is generally expressed in one

of the following systems; Total Digestible Nutrients (TDN), Digestible Energy (DE), Digestible Organic Matter (DOM), and Digestible Dry Matter (DDM). A close interrelationship between these measurements has been shown by Heaney and Pigden (1963).

Crampton et al.,(1960) has pointed out that "the usefulness of quantitative digestion data, however, is limited because neither Total Digestible Nutrients nor the digestibility of calories (energy) consistently describes the effective feeding value of forages as measured by the performance of animals subsisting thereon.....measurements of the extent of digestibility do not include consideration of the total intake of the feed, a factor importantly concerned with the relative feeding values of forages."

5. Voluntary Intake.

Apparently, voluntary intake (feed eaten by animals when it is offered ad libitum) as a measure of the feeding value of a forage had long been suggested (Armsby, 1896). However, it was only within the last decade that forage workers made active investigations on the use of this criterion.

Based on the conclusions made by Crampton (1957) and subsequent research, Crampton et al., (1960) stated that "the effective nutritive value of a forage is determined jointly by the level of its maximum voluntary intake when

it constitutes the entire ration, and by the extent of its ultimate yield of digestible energy." From this statement these workers formulated the concept of the Nutritive Value Index (NVI) to be used as numerical index of the nutritive value of the forage. The NVI of a forage is calculated from the product of its relative intake (RI) and percent energy digestibility. The relative intake of a forage is expressed as the voluntary intake of a forage per unit of metabolic size ($W_{Kg}^{0.75}$) of the animal in relation to a standard forage and is represented accordingly by the equation:

$$RI = \frac{\text{observed intake} \times 100}{80(W_{Kg}^{0.75})}$$

It was assumed by Blaxter et al. (1961) in voluntary intake studies with sheep that intake varied with a fractional power of body weight close to 0.734; intake was governed by the rate of removal of digesta from the rumen; and the digestible energy consumed/day/$KgW^{0.734}$(E) can be related to intake (I) g/day/$KgW^{0.734}$ by the equation $E = 4.7$ (I - 31). In further studies with sheep, Blaxter et al., (1966) found that there was a positive correlation between maintenance requirement and voluntary intake.

Recently, Wilson et al. (1966) reported that intake was inversely and highly significantly related to herbage fibre content as measured by either crude fibre or modified acid detergent fibre.

Factors affecting the voluntary intake of forages have been extensively reviewed by Campling (1964), Van Soest (1965), and Conrad (1966).

6. Use of Data Obtained from *In Vitro* Rumen Fermentation Systems.

Recognition of the role of the microorganisms of the rumen as the actual source of cellulose utilization has led to the development of *in vitro* rumen fermentation systems. An *in vitro* system may be regarded as 'artificial reticulo-rumen' in which an attempt is made to simulate conditions such as pH, anaerobiosis, and temperature as found within the natural reticulo-rumen. In general, in forage evaluation studies such a system is composed of the following:

i) a substrate (forage) whose nutritive value is being evaluated;

ii) an inoculum which may be obtained from the expressed liquor from rumen ingesta or 'washed' or resuspended microflora from the rumen liquor;

iii) nutrient medium to meet the requirements of rumen microorganisms which include a source of readily available nitrogen, energy and certain fatty and amino acids, B-vitamins and inorganic elements.

The data obtained from *in vitro* fermentation systems are used to predict *in vivo* criteria (TDN, DMD, and DE) used in assessing the nutritive value of a forage. The advantage which the use of *in vitro* systems has over *in vivo* methods

is that it is time saving and obviates the use of large amounts of forage which would normally be fed to experimental animals. The development of *in vitro* systems has been reviewed by Donefer (1961) and Barnes (1966). Their role in evaluating forage nutritive value has also been reviewed (Barnes, 1965).

a) Energy Concentration.

i) Dry Matter Digestibility.

The main purpose of forages in ruminant diets is to provide energy (Reid, *et al.*,1959). A criterion of energy concentration commonly used, *in vivo*, to assess forage quality is that of dry matter digestibility. Asplund *et al*,(1958); Reid et al.,(1959); Clark and Mott (1960); Tilley *et al*. (1960); Bowden and Church (1962); Wilson and Pigden (1964); and Karn *et al*.(1967) have reported close correlations between *in vivo* and *in vitro* dry matter digestibility.

ii) Cellulose Digestibility.

Cellulose is a major constituent of plants which ruminants can utilize through symbiotic rumen microbial action. As most of the energy of a forage is derived from cellulose, attempts have therefore been made to predict *in vivo* criteria of energy concentration from *in vitro* cellulose digestion. Significant correlations have been demonstrated between *in vitro* cellulose digestion and *in vivo* dry matter digestibility (Reid *et al*., 1960; Baumgardt *et*

al.,1962; and Karn et al., 1967), digestible energy (Donefer et al.,1960; Reid et al., 1960; and Baumgardt et al.,1962), energy digestibility, and total digestible nutrients (Baumgardt et al.,1962). As a number of factors influence cellulose digestion, Barnes (1965) suggests that its use as a single criterion may be misleading. Packet et al.(1965) observed that the more readily digestible nutrients in a forage may be preferentially utilized by the in vitro microbial population. They found that a high ratio of the more readily digestible components in the in vitro system may in effect reduce cellulose digestion and thereby lead to a false classification of a forage having a high level of readily digestible substances such as hemicellulose, soluble proteins and carbohydrates.

iii) Volatile Fatty Acid Production.

The volatile fatty acids (VFA), i.e. acetic, propionic, and butyric acids, are produced as a product of cellulose digestion by the microorganisms of the rumen. Asplund et al. (1958) found a close correlation between total VFA production in vitro and in vivo dry matter digestibility. Gray et al. (1951) and Wilson and O'Shea (1964) have demonstrated that the amount of VFA production in vitro may be related to forage quality. In reviewing in vitro techniques for estimating forage quality, Barnes (1965) concluded that "the definition of optimum proportions of

VFA's required for efficient animal performance and subsequent study of VFA production *in vitro* may aid in evaluation of forage quality in the future."

iv) Anthrone Carbohydrate.

Pigden and Bell (1955) found that a good estimate of *in vivo* digestible organic matter (DOM) could be obtained from the fermentation of anthrone carbohydrate *in vitro*. Converting the per cent DOM to total digestible nutrients (TDN) they obtained estimates of TDN for 11 forages which were in close agreement with those derived conventionally with sheep.

b) Digestible Energy Intake Potential.

A relatively new *in vivo* criterion for assessing the nutritive value of a forage is the digestible energy intake potential. This criterion takes into consideration energy concentration in terms of per cent energy digestibility as well as the relative intake of a forage and is expressed as the Nutritive Value Index (NVI) of the forage (Crampton *et al.*, 1960). In an attempt to predict the NVI from *in vitro* data, *in vitro* cellulose digestion and dry matter disappearance (as measured by solubility methods) have been employed.

i. Cellulose Digestion.

Studying 9 forages of five different species and using *in vitro* technique, Donefer *et al.*(1960) related the differential lag phase of cellulose digestion existing between species to voluntary intake and the NVI. The 12-hour *in vitro* cellulose digestion (IVCD) was highly correlated with the relative intake ($r = 0.83$) and with the NVI ($r = 0.91$). He proposed that the NVI (Y) of a forage be predicted from the 12-hour IVCD (X) of that forage from the equation:-

$$Y = -7.8 + 1.314X$$

In further studies Donefer *et al.* (1962) showed that the 12-hour IVCD(X) was highly correlated with the NVI of 26 forages fed chopped ($r = 0.91$) and 16 forages fed ground ($r = 0.87$). They presented the following prediction equations for the NVI(Y) of chopped and ground forages, respectively:

$$Y = -3.5 + 1.23X \text{ and}$$
$$Y = 7.4 + 1.23X$$

The latter equation was also expressed as $Y = -3.5 + 1.23X + 10.9$ to illustrate the observed increase of 10.9 NVI units as a result of the grinding of the forage.

Rony (1964) reported that the digestible energy [intake] potential of chopped alfalfa and bromegrass as measured *in vivo* by the NVI was highly correlated ($r = 0.92$)

with their 12-hour *in vitro* cellulose digestibility.

ii) Solubility.

Using cupriethylene diamine as a solvent for cellulose, Dehority and Johnson (1963) obtained a highly significant correlation ($r = 0.84$) between the cellulose dissolved and the NVI of 8 grasses consisting of 4 species and 2 stages of maturity. Working with 14 forages (8 legumes and 6 grasses) of varying stages of maturity Donefer *et al.* (1963) found highly significant correlations of forage solubility in enzymic and combinations of enzymic-aqueous solutions with the NVI. Recently, Donefer *et al.* (1966) reported a highly significant correlation ($r = 0.95$) between *in vitro* dry matter disappearance by aqueous pepsin - HCl solution and the NVI of 35 grasses and 14 legumes grown in different climatic zones of the world. They presented the following regression equation for the prediction of a forage (hay) NVI (Y) from per cent dry matter disappearance (X):

$$Y = -0.75 + 1.60X.$$

B. DELIGNIFICATION OF FORAGES AND RELATED CONSIDERATIONS.

1. General.

The prime purpose of forage delignification is to make plant nutrients more available to the animal. Kellner and Kohler in Germany (Woodman and Evans, 1947) may be credited as being the first to take a step in this direction. In 1900, Kellner and Kohler reported the preparation of cellulose by removal of lignin from rye straw using a process similar to that in making paper from straw. The isolated cellulose which was known in Germany as Strohstoff proved to be highly digestible by ruminants.

These workers prepared Strohstoff by boiling 1000Kg. of rye straw in 2070 liters of a solution containing 55g. caustic soda, 20g. sodium carbonate and 22g. of a mixture of sodium sulphite and sodium thiosulphate, under 7 atmosphere pressure. At the end of 3½ hours the residue was filtered off, washed free of alkali, dried and ground into a meal (Woodman and Evans, 1947).

Analysis of Strohstoff showed that almost everything was removed from the original straw except cellulose. Therefore some less active treatment was seen to be needed. As Woodman and Evans (1947) put it, "it is not so much a question of actually dissolving out lignin from straw as of merely breaking down the intimate association of the cellulose with the incrusta, by which means the cellulose becomes more accessible to the digestive action of the rumen bacteria during which time the straw pulp remains in the rumen."

However, the work of Kellner and Kohler stimulated the investigations of a number of processes that has as their main object - making potential energy in cereal straw more available to the ruminant.

2. Chemical Methods.

Literature reviewed by the author indicates that although straw pulp[1] was, as a general rule, dried before feeding, because of the method of its preparation which may involve the use of either large or small quantities of alkaline solutions the term 'wet' and 'dry' treatments have been coined. For convenience, wet treatment is discussed in relation to temperature of the process used, hot or cold.

a) Hot 'Wet' Treatments.

The hot 'wet' treatment apparently formulated by Kellner to recover maximum cellulose at the expense of lignin loss unfortunately resulted in severe losses of soluble nutrients such as protein, Nitrogen free extract (NFE), minerals and vitamins (Woodman and Evans 1947, Arrazola 1950). Woodman and Evans (1947) boiled wheat straw with 5.9% NaOH solution for 7 hours under a pressure of 70lb./sq.in. The residue was washed free of alkali, pressed and dried. Analysis of the wheat pulp as compared with that of

[1]From hereon, the term straw pulp, treated straw or predigested straw are used synonymously to mean straw which has been subjected to some kind of alkali treatment.

rye pulp obtained by Kellner and Kohler (1900) and expressed on the basis of per cent of dry matter is as follows:

	Rye Pulp	Wheat Pulp
Nitrogen free extract (NFE)	19.96	16.22
Crude protein	0.62	0.36
Ether Extractives (EE)	0.20	0.49
Ash	2.44	3.11
Crude fibre	76.78	79.82
Cellulose (as defined by Norman & Jenkins 1933)		97.40

The overall cost of production of the process probably limited its widespread application following its introduction by Kellner and Kohler.

b) Cold 'Wet' Treatments.

This kind of treatment as introduced by Beckmann has achieved more popularity than the former. The Beckmann process, (Beckmann, 1921) as it is commonly referred to, consists of steeping chopped straw in 8 times its weight of 1.5% sodium hydroxide (NaOH) solution for at least 4 hours at atmospheric temperature and pressure. The treated straw is then washed free of alkali, drained, and is fed wet or dried before feeding. From 100Kg. of straw, 75 to 80Kg. of pulp are recovered. This represents a loss of 20 to 25% of the original material. The pulp consisted chiefly of NFE and crude fibre; and 66.86% and 86.24% were found to be digested, respectively.

The Beckmann process lends itself suitably and

economical farm scale operations and hence found widespread application, but with modifications, in many countries.

Some workers (Slade et al.,1939; Ferguson, 1943; Hvidsten and Homb, 1948; Stone et al.,1966) have used concentrations of NaOH solution ranging from 1.2 to 2% and reported an increase in the feeding value of the treated straw. Czadek (1941) replaced NaOH solution with 1.5% calcium hydroxide $Ca(OH)_2$ and observed a doubling effect of the starch equivalent of the treated straw over that of the untreated straw. Elpat'evskij (1962) also reported an increase in the nutritive value of treated straw when $Ca(OH)_2$ was used. Straw was treated with carbide sludge (Zaharjan, 1962), the residue after treatment of calcium carbide with water, and fed to stock for several years with no ill effect and was shown to increase the digestibility of rations, as well as adding minerals.

The digestion coefficient for crude fibre was only slightly reduced when the time of soaking was reduced from the usual 22-hour period (Ferguson, 1943) to a minimum of 3 hours (Watson, 1941; Ferguson, 1943; Williamson, 1941). The same effect on crude fibre was observed in varying the temperature from $40^{o}C$ to $0^{6}C$. (Ferguson, 1943).

Godden (1942) thought that chopping straw into lengths of 2-3 inches was an essential part in the pre-digestion process. However, no significant difference was found between straw treated whole and chopped straw in

composition (Hvidsten and Simonsen, 1953) or in digestibility (Watson, 1943; Ferguson, 1943; Hvidsten and Simonsen, 1953). The feeding of whole treated straw soon became the common practice in Norway (Homb and Nedkvitne 1957). No matter what modification was made in the Beckmann process, the principle of cold alkali treatment followed by washing prevailed.

An unmodified step in the Beckmann process was the washing process. Washing was necessary to make the pulp acceptable and as much as 500 gallons of slow flowing water was required for 200lbs. dry straw (i.e. approximately 20 liters of water/Kg. dry straw) as described by Watson (1941). Homb (1949) used 40 to 50 liters of water/Kg. dry straw for washing. Straw that was less well washed depressed appetite and caused scouring in dairy cows. The ammonia (NH_3) level in the rumen of these cows was low and the pH high. Washed straw should not contain more than 1.5g.NaOH/Kg. treated straw (Hvidsten, 1958). Homb (1949) suggested the feeding of A.I.V. silage along with pre-digested straw because of residual alkalinity.

In experiments involving dairy cows, the feeding of pre-digested straw had no adverse effect on the health of the cows (Homb, 1949). This was confirmed by Hvidsten, (1958) who used 3 pairs of twins. One of each pair was given pre-digested straw ad libitum and the other had the equivalent in energy value of hay and roots. Both groups were supplemented in their ration with minerals, corn silage

and concentrates to the same values. He found that milk yield, blood constituents (Ca, P, Mg, K, Na, sugar and hemoglobin) were not affected and there was little effect on acid-base equilibrium. Feeding straw pulp to horses doubled their urine output (Williamson 1941). Hvidsten (1947) reported that pre-digested straw can be given to horses in amounts up to 20Kg. to 25Kg. daily. Homb (1949) recommended the use of 15Kg. straw pulp/day for dairy cows, 7 to 8Kg./day for young cattle and 2 to 3Kg./day for sheep. Pre-digested straw was generally supplemented with mineral salts and crude protein to correct deficiencies (Watson, 1941; Ferguson, 1943; Nedkvitne, 1956; Stone et al.,1966). When straw was not supplemented,,Williamson (1941) observed a reduction of protein and fat digestion in horses whereas Homb (1949,1958) and Woodman and Evans (1947) reported negative values for protein digestibility in sheep.

Lampila (1964) being concerned about the cost of alkali, extravagant use of water, human labor involved, and the low protein content of the resulting pulp with regard to the procedure adopted in the Beckmann process, devised a method to obviate these obstacles which he believed were limitations to the wide-spread use of alkali-treated straw. He reported a treatment in which only 3 liters of alkali solution/Kg. straw would be required for the alkali treatment as opposed to approximately 8 liters of alkali solution/Kg. straw (since straw is steeped in 8 times its weight of alkali

solution) in the Beckmann process. He reduced both the solute and particularly the solvent component of his alkali solution so that his final alkali (NaOH solution) concentration was more than twice as concentrated than that used in the Beckmann process. Reduction in the amount of water required for the washing process was accomplished by packing the treated straw tightly in cylinders through which 4 liters of water/Kg. straw was allowed to percolate. By the Beckmann process about 40 to 50 liters of water/Kg. dry straw would be required for satisfactory washing (Homb 1949). The straw after washing contained 1g.NaOH/Kg. and this he considered satisfactory based on the observations of Hvidsten and Simonsen (1953). The digestibility of crude fibre of the straw pulp equated that of Beckmann's and that of the organic matter was somewhat better than Beckmann's. To improve the protein content of the pulp, urea was added as a supplement.

c) Cold 'Dry' Treatments.

The shortcomings of the Beckmann process became salient by the end of World War II and many workers who were interested in the alkali treatment of straw thereafter sought methods to ameliorate the Beckmann process. In Norway where the use of alkali-treated straw had become a common practice, Homb (1958) reported that water shortage was a frequent obstacle. The use of large volumes of water

to make the pulp alkali - free unfortunately caused a loss of dry matter ranging from 14% to 25% (Nesterowa, 1937; Williamson, 1941; Godden, 1942; Arrazola, 1950; Lucifero 1958).

Kormscikov (1945) performed laboratory tests which revealed that mere moistening and impregnation of wheat straw with 1% lime solution in the ratio of 250 parts to 300 parts of lime solution to 100 parts of straw for 24 hours was sufficient to make it more digestible than the untreated wheat straw. He fed the limed wheat straw to milk dairy cattle and wethers for over 62 days without previous washing and observed that the cows willingly consumed an average of 20Kg. daily and gave greater yield of milk than those fed untreated wheat straw. He also claimed that this method reduced the use of alkali solution [$Ca(OH)_2$] by 2 to 3 fold and saved labor compared with the procedure followed in the Beckmann process.

Magidov (1952) extended this principle of moistening and impregnation with weak alkali and reported satisfactory results with better utilization of nitrogen. His method consisted of sprinkling 1% NaOH solution (12Kg.NaOH in 1200 liters of water) for 5-10 minutes on 300Kg. straw placed in layers on a grating. The whole process lasted for 5 hours. Excess alkali was expressed and the product was fed as such to animals. Magidov reported that 'Self neutralization'occurred in the straw within 10 days.

A Russian worker, Zafren (1960,1962), was not only interested in reducing the amount of alkali solution but also in increasing the nitrogen content of the treated straw, simultaneously. Zafren (1960) explained that when straw is treated with alkali the alkali combines with acetyl groups from the straw forming acetates. If the usual NaOH, he further indicated, is replaced by ammonium hydroxide (NH_4OH) the ammonium acetate formed becomes a source of available nitrogen for rumen microorganisms. In a trial lasting 92 days he fed rations with untreated or ammonia-treated rye straw to young bulls and calculated, by comparing weights, that the feed value of the treated straw was 2½ times that of the untreated straw, expressed in oat feed units/100Kg. He claimed that the extra nitrogen provided by the treatment could replace 20 to 25% of the protein in the ration.

In 1962 Zafren reported the treatment of straw with only 120 liters of 25% ammonia solution/ton (3 parts ammonia/ 100 parts of straw). Such a treatment represents a drastic reduction in the amount of alkali solution used per Kg. straw. Treatment, Zafren noted, could be done in pits, trenches or stacks covered with plastic film. To expel unreacted ammonia (NH_3) the material was exposed to air for a few days before feeding the straw. Comparing the treated straw with untreated straw in feeding trials involving groups of young cattle, Zafren found that the treated

straw provided for one group almost half the total nitrogen intake and gave significantly greater gains than the untreated straw did even when supplements were omitted.

Wilson and Pigden (1964) also reported a method aimed at reducing the total volume of water used in the alkali treatment of straw. The salient feature about their method was the elimination of the washing process by which soluble nutrients were removed. As such they termed their treatment as a 'dry' process. In vitro digestibility of dry matter was used to evaluate the effect of the dry process which consisted of mixing finely ground wheat straw (Triticum sativum) or poplar wood (Populus alba)with 0 to 15g.NaOH in 30ml. water per 100g. straw. It is interesting to note that the treated material was stored for 13 to 21 days before in vitro studies were made. In vitro studies on both materials revealed that treatments up to 9% (i.e. 9g. NaOH/100g. material) increased dry matter digestibility, and beyond this level there was no further increase. "Residual alkali of the treated straw was estimated by titrating an aliquot of a water extract [of the straw] to pH 7.0. Alkali content of the straw decreased rapidly over the first 10 to 15 minutes, thereafter the level remained almost constant. After 21 days, wheat straw that was treated with NaOH at 6% level still had 30% of the NaOH unreacted (Wilson and Pigden, 1964)." They made preliminary feeding trials with straw treated at the 6% NaOH level mixed with either

corn silage or alfalfa hay, or neutralised with acetic acid, and found that sheep would readily consume such diets.

Wilson and O'Shea (1964) repeated the dry process as reported by Wilson and Pigden (1964) with the treated straw being stored for 26 days before *in vitro* determinations were made. Wilson and O'Shea examined the same 6 levels of treatment previously studied by Wilson and Pigden (1964) and used as criteria for evaluating the alkali-treated straw the *in vitro* determinations of crude fibre and dry matter digestibilities as well as the production of individual and total steam volatile fatty acids. They observed marked increases in the total steam volatile fatty acids and digestibility with alkali treatment up to the 9% level. They concluded that the 'dry' alkali treatment of wheat straw enabled it to be more fully utilized by rumen micro-organisms than the untreated straw.

3. Physical Methods.

The attempts made at forage delignification by physical methods have been fewer than those by chemical methods.

a) Steaming.

Honcamp (1932) and Kormanovskaya (1956) decomposed straw by steaming without the addition of chemicals. Honcamp (1932) reported that the digestibility coefficients of the

steamed straw were higher than those of the original straw and its starch equivalent value though higher was about that of poor quality hay, and contained no digestible protein.

b) Reduction of Particle Size.

In vitro studies carried out by Dehority and Johnson (1961) on forages at different stages of maturity showed that prolonged grinding (72 hours) in a ball-mill effected an increase in the total amount of cellulose digested. The increase became large with advancing maturity and lignification of forage. Rony (1964) also found in in vitro studies that ball-milled forages (whole plant or plant fractions) had higher cellulose digestion at all stages of growth than when ground. However, in in vivo studies Lloyd et al. (1960) indicated that grinding of a forage (early or late cut) caused a slight reduction in apparent digestibility of gross energy though it effected a marked increase in both intake and its Nutritive Value Index.

c) Radiation.

Inspired by the work of Garnett and Merewether (1960) who showed that lignin could be extracted from wood meal with 5×10^8 rads of gamma radiation, Pritchard et al.(1962) studied the effects of gamma radiation from Cobalt - 60 upon the feeding value of wheat straw. Assessments were

made by use of an *in vitro* rumen fermentation technique. They found that the greatest percentage increase in dry matter digestibility and volatile fatty acid (VFA) production occurred between 1×10^8 and 2.5×10^8 rads. Beyond this limit there was no increase in VFA production. Pritchard *et al.*(1962) suggested that above 2.5×10^8 rads "the carbohydrates are disintegrated to such a degree that they are no longer suitable substrates for rumen micro-organisms." They concluded that this method of treating straw was not feasible for commercial operations because of the high levels of radiation necessary to release the encrusted nutrients.

III. OBJECT OF RESEARCH

The current research trend in the improvement of the nutritive value of straw by alkali treatment involves the use of minimal amounts of water. This 'dry' alkali treatment has been shown to give comparable results with the Beckmann process in increasing the digestibility of straw.

It is well documented that the pelleting process increases the voluntary intake of a forage, particularly those of poor nutritive value. It can be suggested that a combination of pelleted treated straw could thus provide an increased voluntary intake and an increased availability of the nutrients consumed.

The major purpose of this research was to study the effect of a 'dry' alkali (NaOH) treatment of ground and pelleted oat (*Avena sativa*) straw on the following aspects:-

(a) Cellulose digestibility as determined by *in vitro* rumen fermentation and *in vivo* feeding trials;

(b) Voluntary intake and digestible energy intake as determined *in vivo*.

In vitro rumen fermentation trials were also conducted to determine the effect of 'dry' alkali treatment on cellulose digestibility of another low quality forage material viz. - sugar can bagasse.

IV. IN VITRO RUMEN FERMENTATION EXPERIMENTS.

A. GENERAL PROCEDURE USED IN FERMENTATION RUNS.

To assist in making a decision on a suitable choice of alkali treatment which would be applied to the straw for *in vivo* trials it was thought expedient to conduct screening tests by *in vitro* rumen fermentation experiments.

The procedure adopted for all fermentation runs was that reported by Donefer *et al.*(1960) with slight modifications.

1. In Vitro System and Substrates.

The *in vitro* system used consisted of 32 fermentation tubes (90ml., Pyrex 8260), each equipped with a rubber stopper fitted with a gas inlet tube through which CO_2 was passed throughout the fermentation period (24 hours) at the rate of approximately 160 bubbles per minute. Gas was exhausted by way of the clearance between the pouring lip of the tube and the rubber stopper. Fermentation tubes were maintained at a temperature of 40°C in a water bath. Total liquid volume in each tube was 50ml. with 200 to 700mg. of treated material and standards supplying a substrate level of approximately 200mg. cellulose.

Standard substrates were included in each run so that results from different fermentation runs could be compared. These standards consisted of alfalfa (Macdonald standard), bromegrass (Macdonald standard), Solka Floc SW40A,

and Avicel, the latter two being commercially prepared purified cellulose. All substrates to be tested were ground in a Raymond Laboratory Hammer Mill fitted with a screen having 0.024" (approximately 0.6mm.) diameter round holes (equivalent approximately to U.S.B.S. seive No.30), and stored in glass jars.

2. Preparation of Phosphate Buffer Extract (Inoculum).

A rumen fistulated steer, fed exclusively on a diet of high quality alfalfa hay, served as the source of rumen ingesta. The ingesta was pressed through several layers of cheese cloth, and 1.82Kg. of the resultant solid pulp were extracted with 1500ml.phosphate buffer (PB) at pH 7, according to the method described by Johnson et al.(1958). Preliminary to making the PB extract (PBE), the PB solution (1.059g. $Na_2 HPO_4$ + 0.436g. $KH_2 PO_4$ per liter) was preheated to 45°C. (to compensate for drop of temperature to approximately 40°C. during extracting procedure), and 25ml. saturated Na_2CO_3 solution was added to it and CO_2 bubbled through the mixture until the pH was 7, as measured by a pH meter (Beckmann Zeromatic). After moderate agitation, the pulp and PB mixture was re-pressed and the resultant PBE (inoculum) was transported to the laboratory in a pre-warmed thermos container and strained through 4 layers of cheese cloth.

3. Preparation of Basal Medium and Dispensation of Basal Medium and Inoculum.

All the components of the basal medium (Table 1) except iron and calcium, were premixed in a 2-liter Erlenmeyer flask in quantities necessary for the inoculation of 40 fermentation tubes, and conditioned (heated to 40°C., saturated with CO_2, adjusted to pH 7). Following this, 800ml. of the inoculum and 20ml. of iron and calcium mixture were added to the flask containing the basal medium and the total volume adjusted to 2 liters with distilled water. The flask was then placed on a magnetic stirer and attached to a Brewer Automatic Pipette which dispensed 50ml. of the mixed medium and inoculum to each fermentation tube (into which the substrate had been pre-weighed).

4. Initiation and Termination of Fermentation Run.

The addition of the inoculum and basal medium mixture to the substrates initiated the fermentation run. Two drops of mineral oil were added to each tube in order to prevent foaming, after which the tubes were connected to a CO_2 gas supply and placed in the water bath. At the end of 24 hours the tubes were removed from the bath, wiped and centrifuged immediately at 2200 r.p.m. for 8 minutes. The supernatant was discarded and the residue was immediately analyzed for cellulose or refrigerated for subsequent cellulose analysis.

TABLE 1.

COMPOSITION OF IN VITRO BASAL MEDIUM AND INOCULUM.

Solution	Volume used per tube	Amount used per tube
	ml.	mg.
Mineral mixture[a]	10.0	(see a)
Iron and Calcium ($FeCl_2 6H_2O$, 4.4mg./ml. $CaCl_2 2H_2O$, 5.29mg./ml.)	0.5	2.200
	...	2.645
Glucose (100mg./ml.)*	0.5	50
Urea (126mg./ml.)*	0.5	63
Biotin (10µg./ml.)	1.0	10µg.
PABA (100µg./ml.)	0.25	25µg.
n-Valeric acid (5mg./ml.)*	3.0	15
Casein hydrolysate-enzymatic[b] (20mg./ml.)*	2.5	50
Na_2CO_3 (200mg./ml.)	1.5	300
Phosphate buffer[c]* extract (Inoculum)	20.0	(see c)

[a] Na_2HPO_4, 5.65g.; $NaH_2PO_4 H_2O$, 6.27g.; KCl, 2.15g.; NaCl, 2.15g. $MgSO_4 7H_2O$, 0.582g.; and Na_2SO_4, 0.75g. per liter.

[b] Nutritional Biochemicals Corp.

[c] Na_2HPO_4, 1.059g.; KH_2PO_4, 0.436g. per liter.

*Prepared prior to each fermentation run.

5. Cellulose Analysis.

All cellulose analysis in this research was done by the following procedure which is the Crampton and Maynard (1938) method, slightly modified and reported by Donefer et al.(1961).

a) Acid Digestion.

The acid digestion mixture was prepared by mixing 650ml. of acetic acid, 150ml. of distilled water, and 80ml. of concentrated nitric acid. Using an automatic pipette (Machlett), 25ml. of the mixture was dispensed into each fermentation tube (tubes analyzed in series of 8). A glass stirring rod was inserted in each tube and the contents were well mixed, with the stirring rods left in the fermentation tubes during the entire digestion period.

Eight tubes, placed in a stainless steel wire basket were immersed in a boiling water bath for a 30-minute period. Contents of the tubes were mixed every 10 minutes. At the end of the digestion period the tubes were removed from the boiling water bath and allowed to cool for 5 minutes.

b) Filteration.

After the addition of 25ml. of 95% ethanol to each tube and mixing the tube contents were immediately transferred (quantitatively) to a filtering crucible (Selas - extremely coarse porosity), using a polyethylene wash bottle containing 95% ethanol to wash down the sides of

the tubes.

The precipitate in the crucible was then washed with approximately 10ml. each of acetone and ethyl ether, in succession.

c) Drying and Ashing.

The crucibles were next dried in a vacuum oven at 95°C for approximately 4 hours, after which they were cooled in a desiccator and weighed. They were then ashed overnight in a muffle furnace (600°C), cooled in a desiccator and reweighed.

d) Calculations of Cellulose Content.

The cellulose content of either the initial unfermented substrate or of the fermentation residue was calculated as the loss on ashing in the cellulose determination, as follows:

$$\text{Cellulose (g.)} = \text{Wt. (g.) dry crucible and contents} - \text{Wt. (g.) ashed crucible and contents}$$

$$\text{Cellulose (\%)} = \frac{\text{Wt. (g.) of cellulose}}{\text{Wt. (g.) of substrate}} \times 100$$

e) Calculation of Cellulose Digestibility.

$$\text{Cellulose digestibility (\%)} = \frac{\text{Wt. (g.) initial cellulose} - \text{Wt. (g.) cellulose residue}}{\text{Wt. (g.) initial cellulose}} \times 100$$

B. EXPERIMENT 1. THE EFFECT OF TYPE OF ALKALI, CONCENTRATION OF ALKALI SOLUTION, AND LENGTH OF TREATMENT ON IN VITRO CELLULOSE DIGESTIBILITY OF OAT STRAW.

1. Introduction.

A review of the literature on alkali treatment of oat straw indicates that different alkali compounds including lime, sodium hydroxide, and ammonia (Homb, 1949; Kormscikov, 1945; and El-Shazly, 1967), at various concentrations and dilutions (Wilson and Pigden, 1964; and Beckmann, 1921), and for varying lengths of time (Ferguson, 1943) have been used.

The main difference between the cold 'wet' and 'dry' alkali treatments lies in the ratio of water to alkali (solute) used for treatment. For example, the Beckmann process, which has been discussed under cold 'wet' treatments (section II,B), would require about 800ml. of water for a 12% (12g.NaOH/100g. straw) treatment whereas for this same level of treatment the 'dry' process devised by Wilson and Pigden (1964) requires only 30ml. of water.

The purpose of this experiment was to test the efficacy of 'wet' and 'dry' alkali treatments by measurements of in vitro cellulose digestion of oat straw. The variables being studied were the type of alkali used and the length of treatment. To give scope to the experiment certain 'wet' and 'dry' alkali treatments which have been proposed by

other workers were incorporated.

2. Experimental Procedure.

a) Sampling of Straw.

About 55 bales (approximately 1,250Kg.) of oat (Avena sativa) straw which had been reserved for subsequent feeding trials were sampled for in vitro studies as follows:- each bale was drilled with an electric drill fitted with a borer about 2.5cm. in diameter and long enough to penetrate into the centre of the bale. A minimum of three drillings was made on every bale, diagonally, breadthwise, and lengthwise so that about 5Kg. of chopped straw were collected. The straw was ground to pass through a No. 30 mesh screen (0.024" or approximately 0.6mm. in diameter), mixed by 'quartering' and then stored in a plastic bag.

b) Treatment.

The expression 'per cent level of treatment' or 'treatment level' which will be used refers to the weight of alkali solute in grams per 100 grams of untreated straw. Hence 8% treatment level refers to 8g. NaOH or NH_3/100g. of straw depending on the choice of alkali.

A 50g. sample of the ground straw was weighed into each of thirteen 1-liter beakers. Three of these beakers served as treatment controls with only water added instead of alkali solution. Thus the concentration of

alkali in the controls was zero. The NaOH solutions were on a weight by volume basis and the NH_3 solutions were on a volume by volume basis. All solutions were made from reagent grade chemicals.[1] In the case of the sodium hydroxide treatments, NaOH pellets (99.9% pure) were used for making the solutions; whereas for NH_3 treatments NH_4OH solution (NH_3 assay = 28%) was used. Different levels of alkali treatment were applied to the remaining beakers as outlined in Table 2. Treatments in which only 12 or 30ml. of alkali solution per 100g. straw were used represent the 'dry' treatments and those with 800ml. per 100g. straw of alkali solution are the 'wet' treatments. Thorough mixing of the straw with the alkali solution was done by hand using a spatula. The beakers were then covered with aluminium foil which was sealed to the side of the beaker with adhesive tape to prevent loss of treatment material either in the form of NH_3 or water. Finally the beakers were stored at room temperature. A 30-minute interval was provided between a set of two treatments to ensure adequate processing time so that the treatments could be terminated approximately 24 hours later.

A similar procedure was followed using another group of thirteen samples which were to undergo treatment for 5 days.

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[1]Reagent grade chemicals were obtained from Fisher Scientific Co. Ltd., Montreal.

TABLE 2.

ALKALI TREATMENTS OF OAT STRAW.

No.	Treatment Level (g.Alkali/50g. straw)	(%)	Solution Volume (ml./100g.straw)	Solution Concentration (%)
	AMMONIA TREATMENTS			
1	0.00	0.0	12	0.0
2	0.75	1.5	12	12.5
3	1.50	3.0	12	25.0
4	0.00	0.0	800	0.0
5	2.00	4.0	800	0.5
6	8.00	16.0	800	2.0
	SODIUM HYDROXIDE TREATMENTS			
7	0.00	0.0	30	0.0
8	2.00	4.0	30	13.3
9	4.00	8.0	30	26.6
10	8.00	16.0	30	53.3
11	2.00	4.0	800	0.5
12	4.00	8.0	800	1.0
13	8.00	16.0	800	2.0

It should be noted that Treatment 3 (Table 2) represents the NH_3 treatment ('dry') which Zafren (1961) used. The 'dry process' by Wilson and Pigden (1964) is midway between treatments 8 and 9 and treatment 13 simulates the Beckmann process.

c) Termination of Treatment.

At the end of 1 or 5 days, which ever period was applicable, the reaction was stopped by the removal of treatment solutions, as follows:

Samples treated with 30ml. of solution or less were transferred directly into clean aluminium pans and dried overnight in a forced air oven at approximately 40°C. Following overnight drying, the samples were exposed to the atmosphere for at least 4 hours to establish moisture equilibrium with the air before storage in jars.

For samples treated with 800ml. of alkali solution, the solution was withdrawn using an Oklahoma State filter screen[1] and the residue washed until the filtrate indicated a pH of 8 - 9 by paper pH indicator. This processing was an attempt to simulate the washing process in Beckmann's procedure. Washed treated samples with a pH of 8 - 9 were considered low in alkali content for subsequent *in vitro* studies. It was observed that unless such a procedure

[1]Oklahoma State filter screen - Filtering device (200 mesh stainless steel screen) Laboratory Construction Co., 8811 Prospect Ave., Kansas City, Mo., U.S.A.

was followed it would be impossible to terminate treatments at their proper time due to the washing process which was time consuming. The samples were then transferred to aluminium pans and dried and then stored in like manner as the others.

d) In Vitro Rumen Fermentation Runs.

Two fermentation runs were made two days apart resulting in a total of two replications of each treatment. Straw to which neither water nor alkali solution had been added was included in the runs as a control in order to compare results with the treated straws. As the 16% NaOH treated samples with 30ml. of solution were decidedly alkaline, a few drops of HCl were added to the respective fermentation tubes to bring the pH to 7 at the initiation of fermentation. At the end of the 24-hour fermentation period, the undigested (residual) cellulose was determined and the digestibility of cellulose was calculated for each treatment, based on original cellulose content (Table 3) of treated straw. Cellulose content was determined by the method already described in section IV,A.

3. Results and Discussion.

a) Physical Observations.

The reaction of the straw with NaOH solution was exothermic and the color of the straw changed progressively

to deep yellow with time. With the NH_3 treatments there was a color change, i.e. from straw yellow to deep yellow, but no heat production was observed.

The washing and filtering process was longer and more difficult with the NaOH treated samples whether treatment was for 1 or 5 days. The NaOH treated samples easily pulpified and this made filtering considerably difficult.

b) Cellulose Content.

The cellulose content of the treated samples as well as untreated are summarized in Table 3.

Regardless of length of treatment, in the case of samples treated with concentrated sodium hydroxide solution ('dry' treatments), there was a tendency for the cellulose content to decrease with increasing concentration of the alkali solution. The samples treated with concentrated alkali solutions were not washed, but dried directly since they contained only a small amount of water. Thus they contained after treatment residual alkali which increased the unit mass of the straw and hence the cellulose fraction was correspondingly decreased. However, this trend of decrease in cellulose content with increasing concentration of solution was not prominent with the NH_3 treatments, simply because any residual alkali would escape in the form of gas while the samples were being dried or even at room temperature.

TABLE 3

CELLULOSE CONTENT OF ALKALI-TREATED STRAW.

No.	Treatment Level	Solution Conc.	Cellulose Content (%)[1] Treatment periods (days)	
	(%)	(%)	(1)	(5)
		AMMONIA TREATMENTS		
1	0.0	0.0	39.5	39.3
2	1.5	12.5	39.4	40.1
3	3.0	25.0	39.4	40.4
4	0.0	0.0	40.8	42.3
5	4.0	0.5	42.6	45.8
6	16.0	2.0	46.9	51.4
		SODIUM HYDROXIDE TREATMENTS		
7	0.0	0.0	39.2	39.0
8	4.0	13.3	38.6	37.8
9	8.0	26.6	34.8	36.1
10	16.0	53.3	31.5	31.3
11	4.0	0.5	46.7	46.6
12	8.0	1.0	52.9	59.6
13	16.0	2.0	58.9	65.6

UNTREATED OAT STRAW.....39.3
(i.e. without water or alkali added).

[1]Each figure is the mean of two determinations.

The samples treated with dilute alkali solutions were washed to remove excess alkali. Washing would not only remove residual alkali but also water soluble substances and substances made soluble due to chemical action of the alkali. Washing reduced the unit mass of the straw and hence cellulose content was correspondingly increased.

The effect of the solvent action of dilute alkali and subsequent washing could be seen from the increase in the cellulose content by 2 to 6.7 units of the 5-day treated samples (5,6,12 and 13) over their cellulose content resulting from 1-day treatment (Table 3).

c) Cellulose Digestibility.

The averages of cellulose digestibility of the treated and untreated samples are presented in Table 4. Detailed cellulose digestibility data, and an analysis of variance of results are presented in Appendix Tables 1 and 2, respectively.

i) Cellulose Digestibility of Treatment Controls.

It appears that the addition of water alone to the straw (0% treatment level) decreased the digestibility of cellulose. This phenomenon was more pronounced in the 5-day treatments, although the difference was not statistically significant ($p < .01$). A possible explanation of this effect was the observed mold growth on the water surface of the

TABLE 4.

CELLULOSE DIGESTIBILITY OF ALKALI-TREATED OAT STRAW.

No.	Treatment Level	Solution Conc.	*In Vitro* Cellulose Digestibility (%)[1] Treatment periods (days)	
	(%)	(%)	(1)	(5)
		AMMONIA TREATMENTS		
1	0.0	0.0	24.0^{bcd}_{fg}	22.5^{abcd}_{de}
2	1.5	12.5	40.6^{ij}	26.3^{e}
3	3.0	25.0	51.7^{a}	30.2^{a}
4	0.0	0.0	19.1^{abc}	15.2^{cd}
5	4.0	0.5	19.9^{f}	24.7^{hi}
6	16.0	2.0	37.6	49.0
		SODIUM HYDROXIDE TREATMENTS		
7	0.0	0.0	24.0^{bcd}_{h}	18.8^{a}_{gh}
8	4.0	13.3	45.8^{jk}	43.0^{k}
9	8.0	26.6	56.5^{m}	59.7^{m}
10	16.0	53.3	75.6^{fgh}	79.6^{gh}
11	4.0	0.5	41.7^{l}	43.2^{m}
12	8.0	1.0	70.3^{m}	76.0^{n}
13	16.0	2.0	77.2	81.8
	UNTREATED OAT STRAW (i.e. without water or alkali added)		25.2	

[1]Each figure is the mean of two determinations.

Treatment means containing a common superscript are not significantly ($p < .01$) different.

control (No.4) treated with 800ml. of water/100g. straw for five days (Table 2). A musty odour was given out of this sample, the other controls, and also sample 11 of the 5-day treatments. It is likely that the molds exuded autotoxins which inhibited cellulose digestion, and that the alkali added to the straw in the other treatments had a fungicidal effect.

ii) Cellulose Digestibiltiy of NH_3 Treated Samples.

Statistical analysis indicates a significant ($p < .01$) difference between samples treated with alkali for 1 and 5 days, except in the case of sample 5. The trend indicated is that cellulose digestibility decreased at the end of five days in the samples treated with concentrated solutions ('dry' treatments) while the opposite effect appears to be the case for the samples treated with dilute solutions ('wet' treatments). The explanation offered here is that from the samples to which concentrated solutions were applied, the NH_3 passed out of solution at room temperature after five days with the result that it was no longer in contact with the straw but above it, being lighter than air. It is also possible that most of it escaped due to imperfect sealing of the foil to the side of the beakers after treatment was applied. It might be logical to assume that with the loss of the alkali (NH_3) there was mold growth in these 5-day treated samples and consequently

cellulose digestion was inhibited.

However, in the case of the samples treated with dilute solutions the alkali solution was at all times in contact with the straw. This had the effect of not only improving the degradation of the lignocellulose complex, but also suppressing the growth of molds.

Within treatment time periods there were marked increases in cellulose digestibility with increase in the concentration of the alkali. In the case of the highest alkali concentrations this resulted in 2-3 fold increases in digestibility when compared with their controls (Table 4).

iii) Cellulose Digestibility of NaOH Treated Samples.

Between time periods, with the exception of sample 8, the general trend in cellulose digestibility was towards a slight increase with time, regardless of the solution concentration. However, while the differences between cellulose digestibility for 1 and 5 - day treatments were not statistically ($p < .01$) significant with regards to the samples treated with concentrated solutions ('dry' treatments), the increased digestion with time was more pronounced in the case of the samples treated with dilute solutions ('wet' treatments), except for sample 11 in which mold growth was observed in the 5-day treated sample. This highly significant increase so demonstrated by the samples treated with dilute solutions for the long period of time reflects the importance

of the volume of the water in the reaction between the straw and the alkali. Zafren (1960) stated that when straw is treated with alkali, the alkali combines with acetyl groups from the straw and forms acetates. Since dissociation of the alkali (solute) must precede combination of the alkali with other groups, it follows that the greater the ratio of water to alkali (solute) is the greater the dissociation of the alkali which is an essential step in the degradation of ligno-cellulose. As the water to solute ratio was small in the samples treated with concentrated solutions ('dry' treatments), prolonging treatment for five days did not significantly ($p < .01$) affect the digestibility of cellulose whereas in the case of samples treated with dilute solutions ('wet' treatments) in which case the water to solute ratio was great, prolonging treatment for 5 days resulted in a better degradation of ligno-cellulose and hence digestibility of cellulose.

Within periods, there were marked increases in cellulose digestibility as the concentration of the alkali was increased in samples treated with either dilute or concentrated NaOH solution. These increases in all cases, i.e. for samples treated with either dilute or concentrated solutions, were highly significant (Table 4).

iv) 'Treatment x Time' Interaction.

The general pattern observed in cellulose digestibility

as length of alkali treatment was increased from 1 to 5 days was towards an increase. However, a decrease in cellulose digestibility was observed in two out of the four NH_3 treated samples and one out of the six NaOH treated samples, when treatment time was extended from 1 to 5 days. This departure from the general trend has been attributed to loss of alkali (NH_3) and the growth of molds and may account for the high significance ($p < .01$) of the 'treatment x time' interaction observed (Appendix Table 2).

v) Choice of Alkali.

The 'wet' and 'dry' treatments have been well represented by the use of concentrated and dilute solutions, and their efficacy on in vitro cellulose digestibility with reference to length of treatment and type of alkali has been discussed. NH_3 and NaOH had been originally chosen to find out which alkali would prove more satisfactory for future in vitro screening tests and also in the treatment of oat straw to be fed in in vivo trials with sheep.

On the whole, the samples treated with NaOH gave more consistent results than those treated with NH_3. The treatment applied to sample 3 (Tables 2 & 4) is representative of Zafren's NH_3 treatment of straw. 25% ammonia is almost reagent grade (28%) and this was very difficult to work with because of its pungent odour. Maybe, a lesser concentration at the same 3% treatment level would have

resulted in a higher digestibility comparable with that obtained for the 8 or 16% NaOH treatments (Table 4). However, because of the offensive smell of NH_3 and the absolute necessity of having treatment containers air-tight, NaOH was chosen as the alkali for future experiments.

C. EXPERIMENT 2. THE EFFECT OF NaOH 'DRY' TREATMENT ON IN VITRO CELLULOSE DIGESTIBILITY OF OAT STRAW.

1. Introduction.

Experiment 1. examined the effect of the use of different concentrations and type of alkali on *in vitro* cellulose digestibility of oat straw. NaOH was found to be more satisfactory than NH_3 for alkali treatments of straw and, in general, straw treated with dilute alkali solutions resulted in higher *in vitro* cellulose digestibility than straw treated with concentrated alkali solutions at the same level of treatment.

However, the use of dilute alkali solutions, 0.5 to 2.0%, (i.e. the 'wet' treatments) facilitated pulpification of the straw, which made the washing process long and filtering difficult. It also encouraged the growth of molds and caused loss of soluble matter as indicated by a corresponding increase in cellulose content.

The purpose of this experiment therefore was to conduct further investigations on *in vitro* cellulose digestion resulting from 'dry' treatment of oat straw using NaOH as alkali. Particular attention was directed to the level of treatment and concentration of solution in order to arrive at a suitable treatment level which may be applied to large batches of oat straw for animal feeding trials.

2. Experimental Procedure.

a) Sampling of Straw.

Ground oat straw to be treated as well as the control (untreated) was obtained from the contents of the plastic container as described in section IV,B, 2a.

b) Treatment.

The manner of treatment was identical to that described in section IV,B, except that all samples were stored for a reaction period of 24 hours after initiation of treatment. The 1-day period was chosen since it was found in Experiment 1. that the differences in *in vitro* cellulose digestibility resulting from 1 and 5-day 'dry' treatment of oat straw was not highly significant. Table 5 describes the treatments used in Experiment 2. As mentioned in the introduction of this experiment, investigations were chiefly directed to the effects of treatment level and solution concentration. To get a good picture of their effects it was thought expedient to choose treatment levels which were in geometric progression and to study each treatment level at three concentration levels in geometric progression, also.

In Experiment 1., the 4,8, and 16% treatment levels were used to represent NaOH 'dry' treatments (Table 2).

TABLE 5.

NaOH 'DRY' TREATMENTS OF OAT STRAW.

No.	Treatment Level (g.NaOH/50g.Straw)	(%)	Solution Volume (ml./100g.Straw)	Solution Concentration (%)
1	2.0	4.0	30	13.3
2	"	"	60	6.6
3	"	"	120	3.3
4	4.0	8.0	30	26.6
5	"	"	60	13.3
6	"	"	120	6.6
7*	"	"	240	3.3
8	8.0	16.0	30	53.3
9	"	"	60	26.6
10	"	"	120	13.3
11	16.0	32.0	60	53.3
12	"	"	120	26.6

*Treatment period was 6¼ hours and not 24 hours as all the others.

In this experiment a 32% treatment level was added so that in comparing *in vitro* cellulose digestibility against percent treatment level it can be seen at which level(s) of treatment maximum cellulose digestion was achieved. As it was observed in Experiment 1. that the ratio of water to alkali (solute) is an important factor in the degradation of lignocellulose complex, the solution volume (30ml./100g. straw) used for each treatment level in Experiment 1. was repeated in this experiment and then diluted so that several solution levels in geometric progression were obtained (Table 5).

c) Termination of Reaction.

As the treated samples were only slightly moist the reaction was stopped by spreading the samples in aluminium pans and drying overnight in a forced air oven at approximately 40°C. After drying, the samples were exposed to the air for at least 4 hours to establish moisture equilibrium with the air. They were then stored in tightly covered glass jars as were the others in the former experiment.

d) In Vitro Rumen Fermentation Runs.

In all, three fermentation runs were made each 7 days apart with a total of six determinations for each

sample treated and the control. The first run was considered as a preliminary trial in order to determine the amount of acid necessary to neutralize the tube contents. This step was necessary because of the requirements of the rumen microorganisms for a pH level of 7 or slightly lower. In this run, after the initiation of fermentation each tube was tested for its pH. The tubes containing samples treated at the 8, 16, and 32% levels were found to have a pH of 7.2 to 7.7. About 0.01 to 0.02ml. acetic acid (HOAc) was required to bring the pH down to between 7 and 6.85. HOAc was used because of the ability of rumen microbes to utilize acetate.

In the second and third runs the exact amount of HOAc required to neutralize excess alkali in each tube was added in between the pipetting of the first and second 25ml. of the basal medium and inoculum mixture. Besides these modifications the procedure used in the runs was that already described in section IV,A.

Cellulose analysis was done on all the treated samples as well as the control (untreated) before and after <u>in vitro</u> rumen fermentation by the method already described in section IV,A.

3. Results and Discussion.

a) Physical Observation.

The reaction of the alkali with the straw caused the color of the straw to change progressively from yellow to deep yellow with time and heat was given out.

b) The Effect of NaOH 'Dry' Treatment on Cellulose Content and Digestibility.

i) Cellulose Content.

A summary of cellulose content analysis of the treated samples and control are shown in Table 6.

Comparing the treated samples with the control it can be seen that the level of treatment increases the cellulose content decreases, particularly in the case of samples treated at the 16 and 32% treatment level. Since the washing process was replaced by neutralization, the mass of the straw was increased by the addition of NaOH with the cellulose content as a percent of the treated material correspondingly reduced.

ii) Cellulose Digestibility.

A summary of the digestibility data are presented in Table 6 and is the average of the second and third runs only. The data are presented in detail in Appendix Table 3. Appendix Table 4 contains the analysis of variance. The data from

TABLE 6

CELLULOSE CONTENT AND DIGESTIBILITY OF NaOH 'DRY' TREATED OAT STRAW.

No.	Treatment Level (%)	Solution Conc. (%)	Cellulose Content[1] (%)	Digestibility[1] (in vitro) (%)
1	4.0	13.3	38.3	41.4^{a}
2	"	6.6	38.2	45.2^{a}
3	"	3.3	38.9	47.9^{ab}
4	8.0	26.6	36.3	56.0^{bc}
5	"	13.3	37.5	62.0^{cd}
6	"	6.6	38.0	68.2^{de}
7*	"	3.3	37.0	71.1^{ef}
8	16.0	53.3	31.6	68.1^{de}
9	"	26.6	32.7	77.2^{fg}
10	"	13.3	32.5	81.4^{g}
11	32.0	53.3	26.2	77.8^{fg}
12	"	26.6	24.9	72.5^{ef}
13 Untreated straw..... (i.e. without water or alkali added.)			40.6	24.0

*Treatment period was $6\frac{1}{4}$ hours.

[1]Each figure is the mean of four determinations.

Treatment means containing a common superscript are not significantly ($p < .01$) different.

the first trial are not included as the conditions under which the run was made were not the same as in the second and third runs.

In the first run fermentation was considerably interrupted during the first few hours by frequent pH testing. After making a pH reading, the pH meter was washed with distilled water to remove any of the sample adhering to the stem of the pH meter. This washing caused an increase in the total volume of the basal medium and inoculum mixture. It was found that *in vitro* cellulose digestion was slightly increased in most of the samples on which pH readings were made.

Table 6 shows that there was a corresponding increase of *in vitro* cellulose digestibility with treatment level. However, at the 32% level there was a slight decline. This indicates that maximum *in vitro* cellulose digestion occurs at the 16% level and beyond this level there is no apparent increase.

With regard to the effect of alkali concentration on *in vitro* cellulose digestibility it was found that for any given level of treatment, except at the 32% level, the use of weaker concentrations of alkali resulted in relatively higher *in vitro* cellulose digestion and at the 8 and 16% treatment levels some of the differences were highly significant. It is not known why there was a

decrease in *in vitro* cellulose digestion at the 32% level when a weaker concentration of alkali was used (Treatment 12). However, the difference was not statistically highly significant (Table 6). Figure 1. shows the increase of *in vitro* cellulose digestibility with increasing level of treatment and the importance of the solvent, water, in alkali - straw reaction with regard to cellulose digestibility.

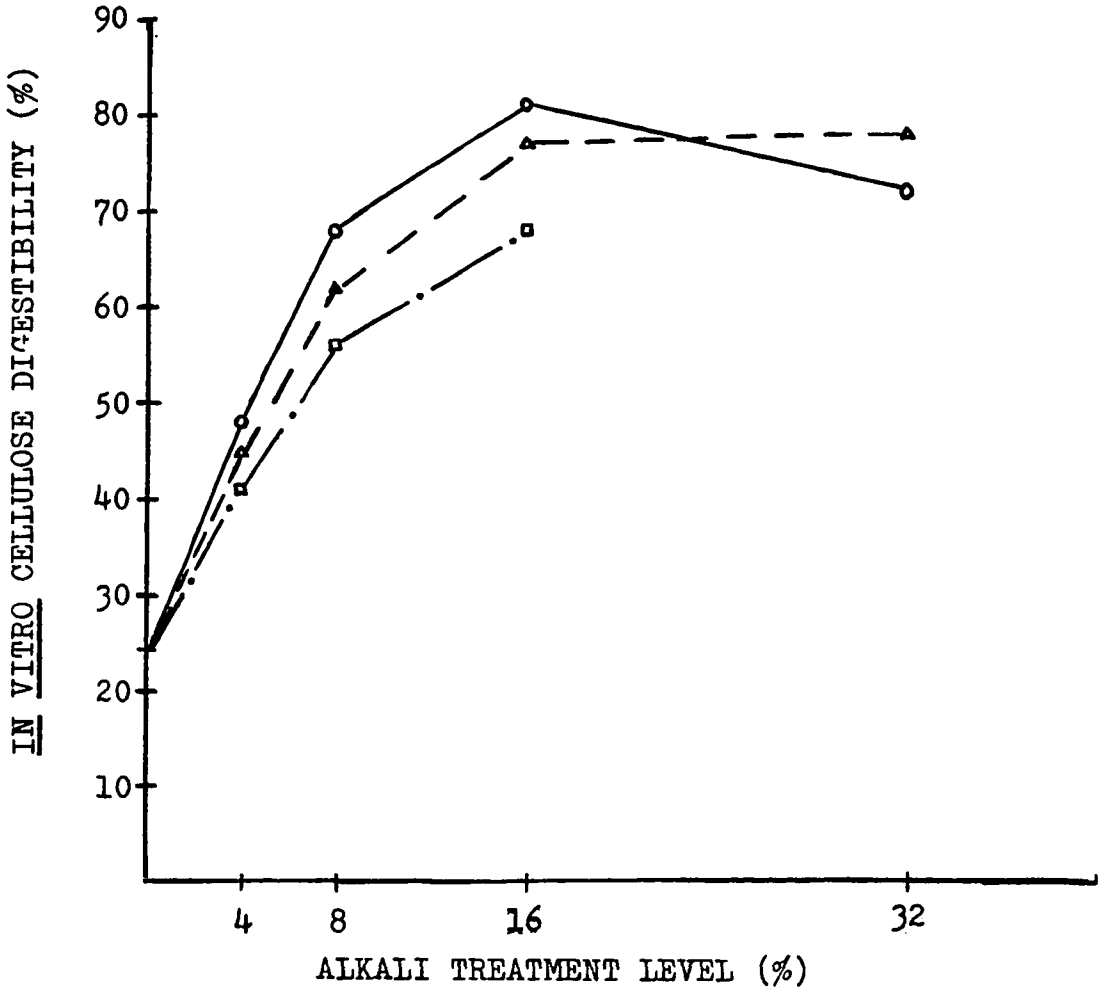
Treatment 7 (Table 5) whose reaction period was only 6¼ hours was introduced in this experiment to find out whether reducing the treatment time from 24 hours to about 6 hours would have an effect on *in vitro* cellulose digestion. Watson (1941) and Ferguson (1943) who used the 'wet' treatments have indicated that reducing the time to 3 hours was not critical on crude fibre digestibility. Wilson and Pigden (1967) observed in their NaOH 'dry' treatments of wheat straw that there was a rapid initial disappearance of alkali over the first 30 minutes and after which the loss was very slow.

The high digestibility of treatment 7 is an indication that the alkali - straw reaction occurred within a period of 6 hours or less.

c) Choice of Treatment for Feeding Trials with Sheep.

The 8% treatment level with 60ml. NaOH solution/ 100g. of straw was chosen for the following reasons: it resulted in 2.6 times the observed *in vitro* cellulose

Fig. 1. RELATIONSHIP BETWEEN SOLUTE TO SOLVENT RATIO AT A GIVEN LEVEL OF TREATMENT AND IN VITRO CELLULOSE DIGESTIBILITY OF OAT STRAW.



digestibility of the untreated straw (Table 6). Although the 16% treatment level with 60ml. NaOH solution/100g. of straw resulted in even greater *in vitro* cellulose digestion, it required twice the amount of alkali (solute) and neutralizing acid of the 8% level. Since water is to be kept at a minimum for a practical 'dry' treatment and also in order to reduce drying times the greater dilution of 120ml. NaOH solution/100g. of straw at the 8% level was not used as the doubling of added water only resulted in a slight increase of *in vitro* cellulose digestibility which was not significantly ($p < ,01$) different from that of the 60ml. NaOH/100g. straw at the 8% treatment level.

D. EXPERIMENT 3. THE EFFECT OF NaOH 'DRY' TREATMENT ON *IN VITRO* CELLULOSE DIGESTIBILITY OF SUGAR CANE BAGASSE.

1. Introduction.

Sugar cane is widely distributed in the tropics and the crushed juiceless residue, sugar cane bagasse, is a low quality forage which is sometimes used as feed or fuel. As a low-quality forage, sugar cane bagasse has a large potential for improvement. It has been stated previously that improving the nutritive value of low-quality forages by alkali treatment, 'dry' treatments may prove more advantageous in the tropics than 'wet' treatments because of the problem of seasonal water shortage. The aim of this experiment therefore was to investigate the efficacy in the use of NaOH 'dry' treatments on sugar cane bagasse in improving its nutritive value using measurements of *in vitro* cellulose digestibility. The *in vitro* cellulose digestibility of sugar bagasse and oat straw resulting from similar NaOH 'dry treatments was also compared.

2. Experimental Procedure.

a) Sampling of Bagasse.

One kilogram of chopped bagasse (obtained from a sample of about 3Kg. supplied by Innswood Sugar Estate, Spanish Town, Jamaica) was ground to pass through a coarse-mesh screen (approximately 3mm. in diameter) and then

through a fine-mesh screen (approximately 0.6mm. in diameter). After grinding, the bagasse was mixed by 'quartering' and then stored in a plastic bag.

b) Treatment.

Fifty grams of the sampled bagasse were weighed into each of eight 1-liter beakers and treatment was carried out as in section IV,B, with a 24-hour reaction time. Four levels of NaOH treatments were used each at two different solution concentrations (Table 7).

TABLE 7

NaOH 'DRY' TREATMENT OF BAGASSE.

No.	Treatment Level		Solution Volume	Solution Concentration
	(g.NaOH/50g.bagasse)	(%)	(ml./100g.bagasse)	(%)
1	2.0	4.0	60	6.6
2	"	"	120	3.3
3	4.0	8.0	60	13.3
4	"	"	120	6.6
5	8.0	16.0	60	26.6
6	"	"	120	13.3
7	16.0	32.0	60	53.3
8	"	"	120	26.6

c) Termination of Treatment.

At the end of 24 hours, the treated samples were immediately neutralized with acetic acid (HOAc). The equivalent of HOAc that would neutralize excess alkali in each 50g. treated sample was previously determined by titrating 5,10, and 20g. of the sample with 10% HOAc (v/v) to pH 7 as measured by a Beckmann Zeromatic pH meter. A linear relationship was obtained between the weights, 5, 10, 20g. of each treated sample and the equivalents of HOAc required for neutralization. The amount of HOAc required for the titration of each 50g. of treated sample was then calculated. The mixing of the HOAc with the treated straw was done with a mixer.[1] After mixing, the samples were dried in a forced-air oven at approximately 40°C. then exposed to the air for at least 4 hours and finally stored in tightly covered glass jars.

d) In Vitro Rumen Fermentation Runs.

Three runs were made at 7 days interval with three replications of each treatment and the control per run. The in vitro procedure used was that already outlined (section IV,A). Determinations of cellulose content were done on the neutralized bagasse as well as the control previous to and after fermentation runs. The method of cellulose analysis was that already described (section IV,A).

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[1]Kitchen Aid (Model K4 - B), product of the Hobart Mfg. Co., Troy, Ohio, U.S.A.

3. Results and Discussion.

a) Physical Observations.

Bagasse which was originally beige changed to yellow on alkali treatment. After neutralization and drying, the samples treated at the 16 and 32% level (Table 7) were very clumpy due to pulpification during mixing. It was necessary to break up the clumps by re-grinding before the material was used in fermentation runs.

b) Cellulose Content and Digestibility.

The cellulose content and *in vitro* cellulose digestibility of treated bagasse and control are summarized in Table 8. The digestibility results are illustrated in Figure 2. The data for *in vitro* cellulose digestibility and the analysis of variance are presented in Appendix Tables 5 and 6,,respectively.

From Table 8 and Figure 2 the following observations can be made:

(i) as the level of the treatment increases, the cellulose content decreases. This effect is due to the addition of the alkali which increases the unit mass of the bagasse. Consequently, the cellulose content as per cent of the treated bagasse is decreased;

(ii) as the level of treatment increases *in vitro* cellulose digestibility is correspondingly increased, except at the 32% level; maximum *in vitro* cellulose digestion

TABLE 8

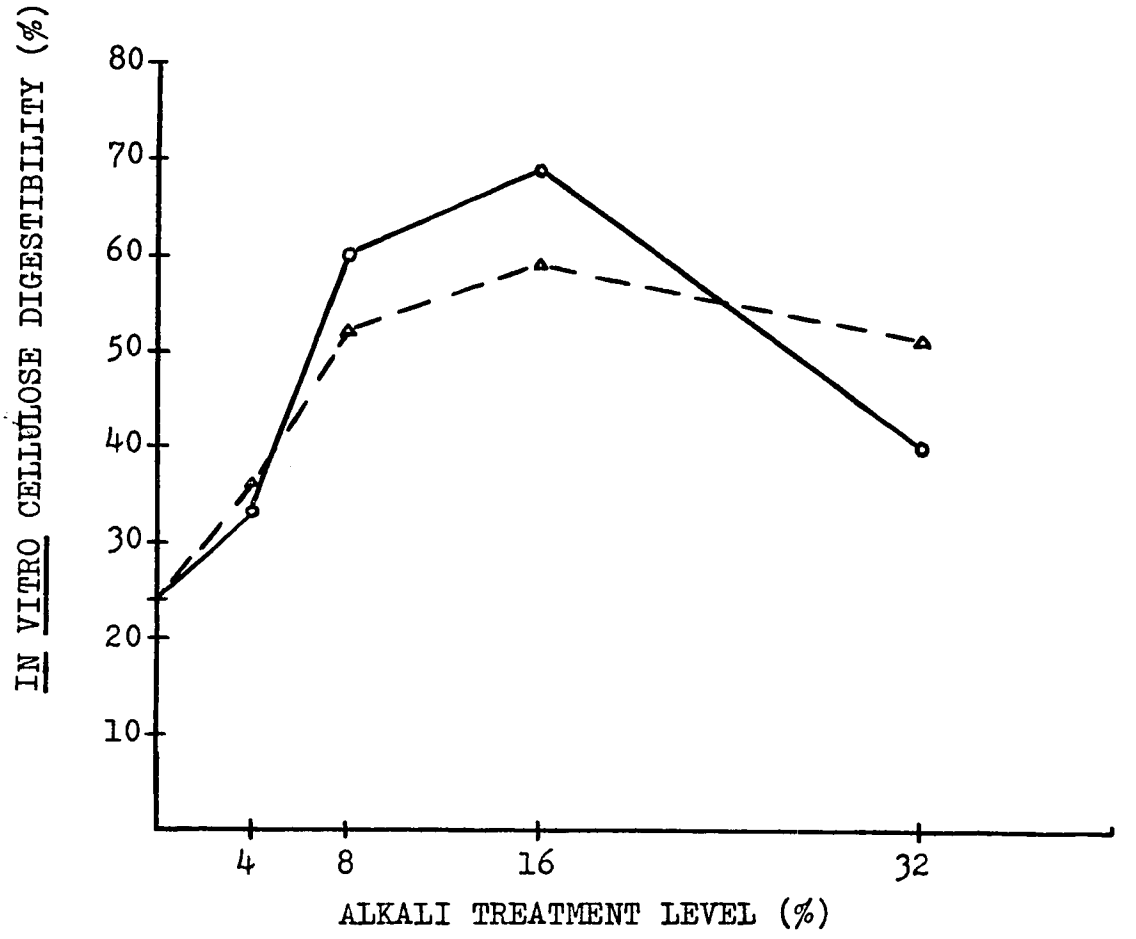
CELLULOSE CONTENT AND DIGESTIBILITY OF NaOH 'DRY' TREATED BAGASSE.

No.	Treatment Level	Solution Conc.	Cellulose Content[1]	Cellulose Digestibility[2] (In vitro)
	(%)	(%)	(%)	(%)
1	4.0	6.6	41.4	36.3^{a}
2	"	3.3	41.0	33.3^{a}
3	8.0	13.3	39.5	52.1^{c}
4	"	6.6	41.0	60.2^{d}
5	16.0	26.6	33.1	59.4^{d}
6	"	13.3	33.9	68.8^{e}
7	32.0	53.3	24.8	51.5^{c}
8	"	26.6	25.7	40.3^{b}
Untreated bagasse..... (i.e. without water or alkali added)			38.3	24.4

Treatment means with a common superscript are not significantly ($p < .01$) different.

[1]Each figure is an average of three determinations
[2] " " " " " " nine "

Fig. 2. RELATIONSHIP BETWEEN SOLUTE TO SOLVENT RATIO AT A GIVEN LEVEL OF TREATMENT AND IN VITRO CELLULOSE DIGESTIBILITY OF SUGAR CANE BAGASSE.



occurs at the 16% level;

(iii) except at the 4 and 32% levels, the weaker concentration of alkali solution at a given level of treatment gave higher *in vitro* cellulose digestibility.

The difference between *in vitro* cellulose digestibility resulting from the weaker and stronger alkali solutions at the 4% level was not highly significant, but that at the 32% level was. In Experiment 2. with oat straw, this same phenomenon of a decrease in *in vitro* cellulose digestibility resulting from a weaker concentration of alkali solution was observed at the 32% level also. It is not known why this reverse trend is observed at this treatment level.

c) Fermentation Runs.

The analysis of variance of *in vitro* cellulose digestibility for treated bagasse (Appendix Table 6) indicates a significant difference ($p < .01$) between fermentation runs. The difference so observed is attributed to variations in the *in vitro* procedures during the three runs. The variations possibly stemmed from the dilute consistency of the rumen liquor used in the second and third runs which may have resulted in inoculum of decreased microbial activity.

However, since the run differences are relatively small compared to observed differences due to treatment,

their importance can be minimized.

d) In Vitro Cellulose Digestibility of Alkali - Treated Bagasse and Oat Straw Compared.

Comparing in vitro cellulose digestibility of alkali-treated bagasse with that of oat straw (Table 9) the latter had a higher digestibility at any given level of treatment. A possible explanation of this lower response is that bagasse contains free residual sugars which arise from the extraction process of sugar from sugar cane. There is evidence that cellulose digestion may be reduced due to preferential attack of soluble carbohydrates which are rapidly fermentable by the in vitro microbial population (Hoflund et al.,1948; Packett et al.,1965). This preferential utilization may be the cause for the lowered in vitro cellulose digestibility of the bagasse as compared with oat straw.

Another possible explanation for the difference in response between straw and bagasse may be on the basis of the ligno-cellulose complex of the respective materials. The bagasse cell wall matrix could be more resistant to degradation as indicated by the lower cellulose digestibility results as compared to straw.

TABLE 9

CELLULOSE DIGESTIBILITY OF NaOH 'DRY'-TREATED BAGASSE AND OAT STRAW COMPARED.

No.	Treatment Level (%)	Solution Conc. (%)	Cellulose Digestibility (%) (In vitro) Oat Straw	Bagasse
1	4.0	6.6	45.2	36.3
2	"	3.3	47.9	33.3
3	8.0	13.3	62.0	52.1
4	"	6.6	68.3	60.2
5	16.0	26.6	77.2	59.4
6	"	13.3	81.3	68.9
7	32.0	53.3	77.8	51.5
8	"	26.6	72.5	40.3
Controls (i.e. Oat straw or bagasse without water or alkali added)			24.1	24.4

V. IN VIVO EXPERIMENT.

A. EXPERIMENTAL PROCEDURE.

1. General.

The experiment to be reported here relates to a feeding trial consisting of two periods using oat straw subjected to physical and chemical treatments.

In order to test the effect of treatment alone on *in vivo* cellulose digestion, the animals received only water, and iodized salt in addition to the straw diet.

Although the 8% alkali treatment level involving the use of 60ml. 13.3% NaOH solution/100g. of straw did not give the maximum treatment response as measured by *in vitro* cellulose digestibility, for reasons of a practical nature previously stated in section IV,C (Experiment 2.) it was used in this animal feeding trial to be reported.

As information on the feeding of treated straw was very limited, particularly in terms of the 'acceptability' of the diets by the animal, the trial to be reported was regarded as preliminary and thus designed to provide data for future more extensive trials.

2. Design of the Experiment.

The experiment was conducted as a 2 x 2 factorial design, viz, - physical forms of the straw (ground vs.

pelleted) and the chemical treatments of the straw (untreated vs. alkali-treated). A diagramatic presentation of the experiment is given in Table 10. Two sheep were assigned to each treatment with this design replicated to constitute two feeding periods.

TABLE 10

DESIGN OF IN VIVO TRIAL

	Alkali Treatment	
	Untreated (Control)	Treated
Physical Forms	(U)	(T)
Ground (G)	GU	GT
Pelleted (P)	PU	PT

GU = Ground, untreated oat straw
GT = Ground, treated oat straw
PU = Pelleted, untreated oat straw
PT = Pelleted, treated oat straw

3. Preparation of Diets.

a) Oat Straw.

In October 1966, all 55 bales of oat straw which had been sampled in June 1966 for the *in vitro* experiments, with the exception of 4 bales on which molds had grown, were ground in a Davis All Purpose Feed Granulator, Model GR-2[1] (hammer mill). Pelleting was done in a Templewood Junior Provender Press[2] (pellet mill) using a die size 7/16 inch (approximately 1.1cm.) in diameter. The length of the pellets obtained averaged about 2cm. Alkali treatment, neutralization and mixing were all done in a Davis Horizontal Batch Mixer,[1] Model S-20 (mixer) having a capacity of 75 cubic feet (approximately 2 cubic meters).

b) Grinding.

The baled straw was first ground to pass through a 3cm. diameter-mesh screen and then through a screen having a mesh of 1/8 inch (approximately 0.35cm.). The average length of the resulting ground straw was about 0.5cm. About 800Kg. of the ground straw was prepared for experimental use. Of this amount 100Kg. were set aside as

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[1]Manufactured by H.C. Davis and Sons Manufacturing Co. Inc. Box 395 Bonner Springs, Kansas, U.S.A.

[2]Manufactured by Templewood Hawksley Agricultural Division 2 Buckingham Ave., Slough Bucks, England and distributed in Canada by Northland Machinery Supply Co. Ltd., Fort William, Ontario.

ground untreated straw (GU), and another 100Kg. were pelleted to serve as pelleted untreated straw (PU). The remainder was reserved for treatment from which both ground treated (GT) and pelleted treated (PT) diets were obtained.

c) Laboratory Studies on the Neutralization of Alkali Treated Oat Straw.

These preliminary studies were conducted to determine the volume and concentration of HOAc which should be used to neutralize excess alkali in the treated straw without making the finished material too wet for drying.

To accomplish this end, two 50-gram samples of ground straw were treated with 13.3% NaOH solution and 10, 20, and 40g. portions of this alkali treated straw were titrated with 10% HOAc to pH 7 or below. By taking the pH down to 6.5 or 6.0, it was thought that this would ensure complete neutralization of unreacted alkali due to improper mixing. A slight excess of HOAc could be handled by the rumen microbes which function in a pH range of 5.0 to 7.5 (Barnett and Reid, 1961). Excess alkali on the other hand of more than 1.5g. NaOH/Kg. of treated straw has been shown to cause scours and depress appetite in dairy cows (Hvidsten, 1958).

The weights of 10, 20, and 40g. of the alkali treated straw plotted against the equivalents of HOAc required for their respective neutralization showed a

linear relationship. Making use of the linear relationship it was calculated that 16.7ml. of 25% HOAc would neutralize 50g. straw treated with 30ml. of 13.3% NaOH solution (8% treatment level). Hence 16.7 liters of 25% HOAc would neutralize 50Kg. straw treated with 30 liters of 13.3% NaOH solution. This quantity and concentration of HOAc was expected to bring the pH down to about 6.5.

d) Preparation of the 1st. Batch (50Kg.).

i) General.

Straw to be fed as ground or pelleted treated diet was prepared in two 50Kg. batches and one 100-Kilogram batch. It was thought that preparation in small batches rather than preparation of the total estimated amount required would facilitate correction or alteration of the method of preparation, if necessary.

ii) Treatment.

Thirty liters of 13.3% w/v NaOH solution which were required to treat 50Kg. of straw were prepared by dissolving 5.24 liters of 50% w/w NaOH solution in tap water and making up to volume. For convenience of handling and transportation the 13.3% NaOH solutions were prepared in two batches placed in polyethlene containers. However, instead of diluting 2.62 liters ($\frac{1}{2}$ x 5.24) of 50% w/w NaOH

solution to 15 liters, only 2.12 liters was diluted, by mistake. The resulting concentration of the alkali turned out to be 11.1% instead of 13.3%. This mistake was unfortunately realized after treatment. All 50Kg. of the straw were placed into the mixer after which agitation was started. As the straw was being mixed the alkali was dispensed from a tap fitted on the container. A considerable amount of alkali fell on the paddles and axle of the mixer and so dispersion of the alkali in the straw was reduced. Pre-mixing of the straw before the addition of the alkali caused a slight loss as dust. It was noted that the screw action of the mixer, only being partially loaded, caused the straw to be pushed to one end of the mixer (i.e. the end opposite to which the alkali was added). During mixing, some heat was evolved, but only for about 20 minutes after which there was no further rise in temperature. The temperature of the mixture rose slightly, but was not so high that the sides of the mixer could not be held with the hand. Mixing was continued for about 45 minutes and then stopped.

iii) Neutralization.

Seven liters of 50% HOAc instead of 16.7 liters of 25% HOAc was used for neutralization to make corrections for the lower concentration of alkali solution used by mistake and also to reduce the volume of acid as the straw

already appeared rather wet. The acid was dispensed from the tap of a polyethylene container with care so that most of it was deposited on the straw. Addition of the acid was made 24 hours following the initiation of alkali treatment of the straw. After one hour of mixing the straw was unloaded into large plastic bins and transported to the drying room.

iv) Drying and Pelleting.

The prepared straw[1] was spread 5 to 8cm. thick on polyethylene sheets of dimensions 3.6m. x 2.4m. and an electric fan was turned on to blow air over it. An exhaust fan at the other end of the room helped to speed up drying and the removal of HOAc fumes which became quite noticeable, once the fans were in motion. Uniform drying was facilitated by raking the material three times in 48 hours. After 72 hours, the material was dry enough for storage and/or pelleting.

Pelleting of the material was characterized by frequent breakdown of the machine. This problem was traced to the presence of caked materials inside the pellet mill which frequently blocked passages leading into the die. Due to losses during pelleting, 46Kg. of pellets were obtained from the original 50Kg. of straw and the pellets

[1]Prepared straw refers to straw which has been treated with alkali and neutralized with acid. For the sake of brevity the word material is used synonymously to refer to prepared straw.

were of a burnt color and appeared hard. The pH of the prepared pellets was 5.8 as measured by a pH meter instead of the expected 6.5. Excess acidity was attributed to the loss of alkali during the treatment. This batch was stored in burlap sacks for subsequent feeding as pelleted treated diet (PT) during the first period.

e) Preparation of the 2nd. Batch (50Kg.).

i) General.

In the preparation of this batch steps were taken to circumvent the difficulties encountered in the previous preparation especially in the mixing process.

ii) Treatment.

This time the correct volume of 50% w/w NaOH solution was used in making the alkali solution. 30 liters of 13.3% w/v NaOH solution were prepared by diluting each of 2.62 liters of 50% w/w NaOH solution to 15 liters with tap water in two polyethylene containers. However, the exothermic reaction of the alkali solution and the water caused the container to expand and extra water was added unwittingly in making up the required volumes. To overcome the problem previously encountered in dispensing the alkali solution and in mixing, the following procedure was adopted: about 10Kg. of straw were put into the mixer at the loading

end as during mixing the straw is gradually pushed towards the unloading end by the screw action of the paddles and axle; this straw was wetted by spraying the alkali from a small plastic bucket which had been perforated at the bottom with holes of about 3mm. in diameter; another two 10-Kilograms of straw were treated in like manner and the mixer was started. Very little loss of straw as dust resulted this time when mixing was commenced. When the straw had been pushed away from the loading end, the mixer was stopped and the remaining 20Kg. of straw were put in and treated with alkali solution in like manner as the previous 30Kg; the mixer was started again and after 30 minutes from the start of mixing several loads of the partially mixed straw were unloaded and put in again at the loading end while the mixer was in motion; one hour from the start of mixing, the mixer was stopped. This batch appeared to be more uniformly mixed than the former.

iii) Neutralization.

Twenty-four hours after alkali treatment was commenced the straw was neutralized with 8.4 liters of 50% HOAc. As most of the straw was then at the unloading end of the mixer about half of the acid was added at this end and mixed with the straw for 10 minutes. Then some of the straw was unloaded while mixing was still in motion and refed into the mixer at the loading end so that unneutralized

straw would be pushed down towards the unloading end. The mixer was then stopped while the remaining acid was added, after which mixing was continued for 20 minutes when more straw was again unloaded and refed into the mixer from the loading end. Mixing was allowed to go on for one and one-half hours, then the material was unloaded into plastic bins and transported for drying.

iv) Drying.

The material was dried in the same manner as was described in the previous preparation but was not pelleted as it was to be fed as the ground treated (GT) diet during the first period. Flakes of caked materials were observed in the prepared straw but no attempt was made to remove them. The pH of this batch was 6.1. Excess acidity was attributed to the slightly weak alkali solution used in treatment (due to error in dilution). This batch was also stored in burlap sacks until it was required for feeding. Forty-five Kilograms ground treated straw was obtained.

f) Preparation of the 3rd. Batch (100Kg.).

i) General.

Of the estimated 340Kg. of prepared straw to be required, 90Kg. had been prepared in the first two preparations. It was hoped that on completion of the third

batch enough prepared straw would be available for the first feeding period. As it turned out later, these three batches were more than sufficient for the entire 42 days of feeding, constituting two feeding periods.

ii) Treatment.

Sixty liters of 13.3% w/v NaOH solution which were required for this treatment were prepared by diluting with tap water each of 5.24 liters of 50% w/w NaOH solution to 30 liters in two polyethylene containers. The average concentration of the solutions was found to be 13.7% instead of 13.3%. No attempt was made to alter this concentration. The loading of the straw, dispensing of the alkali, and mixing were carried out in the same way as in the preparation of the second batch. The total time of mixing of the straw and alkali solution was intended to be one hour as in the second 50Kg. batch. Unfortunately, due to misinterpretation of instructions by an assisting technician, mixing was continued for four and three-quarters hours. At the end of this time an amount of steam had generated in the mixer, with a resultant rise in temperature. However, the material was not so hot that the sides of the mixer could not be touched with the hand. It was observed that a considerable amount of the material had fluffed and clung to the sides of the mixer.

iii) Neutralization.

Twenty-four hours after alkali treatment was initiated 16.7 liters of 50% HOAc were added to neutralize the alkali treated straw by the same method that was employed in the second batch preparation. Total mixing time for neutralizing was one and one-half hours after which the straw was unloaded into plastic bins and transported for drying.

iv) Drying and Pelleting.

Due to lack of space the material was spread 8 to 10cm. thick. It was observed that some of the material was in the form of balls. These balls were uniformly dispersed throughout the material and varied from about 0.5cm. to 3cm. in diameter. This balling effect may have resulted from the prolonged mixing and steaming during neutralization. Woodman and Evans (1947) who prepared fodder cellulose by the action of hot alkali and under pressure reported that "it contained however, a proportion of lumps consisting of materials which had balled together." Four days after drying the material was passed through slotted seive to separate the balls from the rest of the material. The balls were found to constitute about 4 to 5% of the prepared straw. On the third day of drying an attempt was made to pellet the relatively dry material, but this met with little succes due to too high a moisture content. On the fifth

day when the material was drier pelleting was uninterrupted. Fifty-one Kilogram were pelleted and stored to be fed as pelleted treated (PT) diet and the remaining 56Kg. were reserved as ground treated (GT) diet. Excluding the balled material, 107Kg. of prepared straw were obtained from 100Kg. of straw. This batch was used during the second feeding period. The pH of the ground treated straw was 6.0 and that for the pelleted treated straw was 6.1.

Significant aspects of the three batch-preparations are summarized in Table 11.

4. Feeding Trial.

a) Animals.

Sheep are traditionally used in this laboratory as a pilot animal for ruminant nutrition studies and thus sheep digestion stalls were available for this trial. Eight female lambs were used right through the two periods. The design of the stalls required female sheep to be used for ease of collection of urine and feces. Lambs about eight to ten months old were used because of their capacity for growth. Thus live weight gains, if any, could be detected during the trial.

b) Animal Preparation.

Within two weeks of the commencement of the first

Table 11. SOME PERTINENT DATA ON BATCHES OF STRAW PREPARED FOR FEEDING.

Batch No.	Weight of Straw	Treatment with NaOH Solution [1]		Neutralization with HOAc [2]		Mixing Time With NaOH Solution	Mixing Time With HOAc	Prepared Straw		
		Conc.	Vol.	Conc.	Vol.					
	(Kg.)	(%)	(Liters)	(%)	(Liters)	(Hrs.)	(Hrs.)	(pH)	Fed As	Nature
1	50	11.1	30	50	7.0	0.75	1.00	5.8	PT diet 1st. period	Dark brown and hard
2	50	12.5	30	50	8.4	1.00	1.50	6.1	GT diet 1st. period	Contained bits of caked materials
3	100	13.7	60	50	16.7	4.75	1.50	6.0 6.1	GT PT 2nd. period	Free from caked materials. Pellets were dark but not as hard as in Batch 1

[1] The intended treatment was with 30 and 60 liters of 13.3% NaOH, for 50 Kg. and 100Kg. of straw, respectively.

[2] The intended neutralization by acid was with 8.4 and 16.7 liters of 50% HOAc, for 50Kg. and 100Kg. of treated straw respectively.

feeding period the lambs were brought in from the barn, sheared, dewormed, and placed into digestion stalls so that they could get adjusted to close confinement. During these two weeks they received daily, 800g. of high quality alfalfa in the form of pellets, water and iodized salt lick. Randomization was also done to determine which set of two animals may received a particular diet during the first feeding period.

c) Feeding Practice.

In order to get a good estimate of voluntary intake and digestibility, the diets were fed for three weeks in each of two feeding periods. Lloyd et al.,(1956) has shown that variability in digestion coefficients was of minor importance following a 10-day preliminary period, and Lister (1957) found that the voluntary consumption of most of the forages he studied did not increase significantly after 10 days of feeding.

To commence the first feeding period, the lambs were gradually introduced to their diets. On the first day each lamb received 600g. of the alfalfa pellets and 200g. of her particular diet. On the following two days the ratio of alfalfa to diet was decreased and that of diet to alfalfa increased so that on the fourth day all lambs were receiving 800g. of their respective diets. The lambs were fed about 9.00 a.m., after weigh-backs of the

previous feed refused had been measured. On each day of feeding, 200g. in excess of feed consumed the previous day was given.

The second feeding period began immediately following the first period, but no alfalfa was given. The order of feeding was reversed so that the set of lambs which received ground untreated diet in the first period were given pelleted treated diet in the second period, and those previously on ground treated were switched to pelleted untreated (Table 10). However, for the first two days, the diet from the first period was fed along with the new one so as to cause a gradual change of diet. On the third day, each lamb received 800g. of her respective diet and thereafter 200g. in excess of what was consumed the previous day. During the last seven days of each feeding period samples of each diet fed were collected, ground to pass through a 1mm. diameter-mesh screen and stored in tightly covered glass jars to await analyses.

d) Salt and Water Consumption.

Salt licks (iodized salt) were made available to the animals at all times as was water. During the last seven days of each period water consumption was recorded for each lamb.

e) Liveweight Changes.

Each lamb was weighed at the beginning of each

period and at the end of the second and third week so that weight changes during the trial could be calculated.

5. Fecal and Urine Collection.

Total fecal collection was made during the last seven days of each period. Each day after collection, 10% by weight of the wet feces was sampled and dried in a forced-air oven at a temperature of approximately 40°C. The dried fecal aliquot from each lamb was collected for the seven-day period, ground to pass through a 1mm. diamerer mesh screen, and stored in tightly covered glass jars for subsequent analyses.

Urine output and pH were recorded daily during the last seven days.

6. Rumen Sampling.

At the end of each feeding period samples of rumen ingesta were removed from each lamb with the aid of a tube placed down the esophagus. The ingesta removed with the aid of a vacuum pump was squeezed through two layers of cheese cloth and the pH of the expressed fluid was measured with a pH meter.

7. Chemical Analyses and Gross Energy Determination.

Chemical analyses for dry matter, ash, and crude protein were done on both diet and fecal samples by the

A.O.A.C methods (A.O.A.C., 1960). Acid-detergent lignin was determined for the diets only, by the method of Van Soest, (1963). Cellulose content of both diet and fecal samples of each lamb was determined by the modified Crampton and Maynard method already described in section IV,A. Gross energy determinations were made on diets and feces using the Parr Oxygen Bomb Calorimeter[1] fitted with an automatic temperature recorder (described by Crampton, 1956).

8. Calculations.

a) Apparent Digestibility.

The apparent digestibility coefficients of dry matter, gross energy, crude protein, and cellulose were calculated from the following formula:

$$\text{Coefficient of Digestibility} = \frac{[(F_o \times A_o) - (F_1 \times A_1)]}{(F_o \times A_o)} \times 100$$

where, F_o = grams of feed consumed

F_1 = grams of feces excreted

A_o = Per cent 'nutrient' content of feed: dry matter, crude protein, or cellulose; or Kcal. gross energy/gram.

A_1 = Per cent 'nutrient' content of feces: dry matter, crude protein, or cellulose; of Kcal. gross energy/gram.

(All data were converted to dry matter basis)

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[1]Oxygen Bomb Calorimeter manufactured by Parr Instrument Co. Inc., Moline, Illinois.

b) Relative Intake.

The Relative Intake (RI) of a feed was calculated from the following equation (Crampton et al.,1960):

$$RI = \frac{\text{Observed intake x 100}}{80(W_{Kg.}^{.75})}$$

c) Nutritive Value Index.

The Nutritive Value Index of a feed was calculated by multiplying the per cent gross energy digestibility of the feed by its Relative Intake (Crampton et al.,1960).

NVI = RI x % gross energy digestibility.

B. RESULTS AND DISCUSSION.

1. General Observations.

During the two-week adjustment period when the animals were on high-quality alfalfa they showed no signs of stress and settled down easily to their new environment. However, when they were placed on their respective diets the animals displayed general anxiety, especially during the second period. This was manifested by the nibbling of the digestion stalls by most of the sheep, and those receiving the treated straw excreted soft masses of feces at irregular intervals.

2. Chemical Analyses of Diets.

Chemical analyses made on the feeds offered are presented in Table 12. In general, the dry matter, gross energy, crude protein, cellulose, and lignin contents of the chemically treated diets were lower than those of the chemically untreated diets regardless of physical form. This is due to the considerably higher ash content of the chemically treated diets, which resulted from the addition of NaOH and HOAc to the straw in the preparation of the treated diets.

There appears to be a period difference in the protein cellulose, lignin, and ash contents of both treated and untreated diets with the untreated diets showing an increase in their ash and protein contents and a slight

TABLE 12.

CHEMICAL ANALYSIS OF DIETS.
(Expressed on dry matter basis)

PERIOD I

Diets	Dry Matter (%)	Gross Energy (Kcal/g.)	Protein (%)	Cellulose (%)	Lignin (%)	Ash (%)
Ground Untreated	95.2	4.46	3.1	44.8	8.2	4.8
Ground Treated	92.6	4.06	3.6	39.9	7.1	14.0
Pelleted Untreated	94.2	4.38	3.9	43.0	8.2	5.4
Pelleted Treated	92.5	4.12	3.6	41.2	7.9	12.4

PERIOD II

Ground Untreated	95.3	4.42	4.3	42.0	7.9	6.2
Ground Treated	92.5	4.11	3.9	40.1	7.3	14.3
Pelleted Untreated	94.3	4.36	4.1	42.4	8.1	6.2
Pelleted Treated	92.9	4.08	3.8	40.2	7.3	14.4

decrease in their cellulose and lignin contents during the second period.

3. Apparent Digestibility.

a) Apparent Dry Matter Digestibility.

The dry matter digestibility data are presented in detail in Appendix Table 7, and a summary is given in Table 13. Statistical analysis is presented in Appendix Table 8.

TABLE 13.

SUMMARY OF APPARENT DRY MATTER DIGESTIBILITY DATA (%).

Chemical Treatment	Physical Form				
	Period I		Period II		
	Ground	Pelleted	Ground	Pelleted	Chem.Treat. Ave.
Untreated	37.7	30.3	35.1	33.2	34.1
Treated	61.8	45.0	46.9	36.9	47.6
Period Ave.	43.7		38.0		
Phy.Form Ave.	45.4(Ground)		36.3(Pelleted)		

A highly significant ($P < .01$) increase in dry matter digestibility was observed as a result of treatment. This difference is attributed to the effect of the treatment wherein lignin was made soluble in NaOH, thereby exposing the cell constituents to the digestive action of the microbes

of the rumen as well as the gastric juices of the abomasium. The whole philosophy behind the chemical treatment of straw with NaOH rests on this property of lignin, viz, its solubility in alkalis. Making use of this property many workers (Godden, 1942; Hvidsten and Homb, 1948; Lucifero, 1958; Laguta 1962; and Stone et al.,1966 have been able to show that the dry matter digestibility of straw is increased by alkali treatment.

With regard to physical form of the diets, pelleting was found to significantly ($P < .05$) depress dry matter digestibility. Although pelleting is known to decrease digestibility, the mechanism in the case of this experiment is not clear as both the ground and pelleted material were originally the same particle size. It is assumed that the pellets are quickly disintegrated through the action of prior chewing by the animal and water absorption in the rumen. However, it is also possible that the pelleted straw did not disintegrate completely in the rumen resulting in a smaller surface area for enzymatic degradation and thus decreased digestibility. The pelleting operation subjects the forage to high temperature and pressures and the possibility exists that these factors may have influenced the straw in some way as to reduce digestibility.

No significant differences were found between the first and second periods for dry matter digestibility. However, the digestion coefficients for dry matter

digestibility in the second period were lower than those of the first period especially in the case of the treated diets, regardless of the physical form (Appendix Table 7). This may be a reflection on the method of preparation of the treated diets offered in the second period (Table 11).

b) Apparent Gross Energy Digestibility.

The data on apparent gross energy digestibility and the analysis of variance are presented in Appendix Tables 7 and 8, respectively. A summary of the data is shown in Table 14.

TABLE 14.

SUMMARY OF APPARENT GROSS ENERGY DIGESTIBILITY DATA (%).

Chemical Treatment	Physical Form				
	Period I		Period II		
	Ground	Pelleted	Ground	Pelleted	Chem.Treat.Ave.
Untreated	35.4	27.1	32.0	29.7	31.0
Treated	57.3	38.5	40.7	30.0	41.6
Period Ave.	39.6		33.1		
Phy.Form Ave.	41.3(Ground)			31.3(Pelleted)	

The digestion coefficients of apparent dry matter digestibility were about 4 to 6 units higher than those of apparent gross energy digestibility (compare Tables 13 with 14). However, the pattern of the results are quite similar in that chemical treatment and physical form were

each shown to have a significant effect while no significant differences due to periods were obtained. The discussion presented in explanation of the differences observed for the dry matter digestibility data will suffice for the gross energy digestibility results.

c) Apparent Cellulose Digestibility.

The digestion coefficients and statistical analysis for apparent cellulose digestibility are given in Appendix Tables 7 and 10, respectively. A summary is shown in Table 15.

TABLE 15.

SUMMARY OF APPARENT CELLULOSE DIGESTIBILITY DATA (%).

Chemical Treatment	Physical Form: Period I Ground	Period I Pelleted	Period II Ground	Period II Pelleted	Chem.Treat.Ave.
Untreated	46.9	37.0	40.9	39.4	41.0
Treated	68.9	42.5	46.1	26.5	46.0
Period Ave.	48.8		38.2		
Phy.Form Ave.	50.7(Ground)		36.3(Pelleted)		

Although the in vitro trial (Experiment 2) indicated a large increase in cellulose digestibility as a result of treatment of straw with a 13% NaOH solution at the 8%

treatment level, these results were not confirmed in the *in vivo* trial.

It is clear that *in vitro* experiments only simulate to a certain extent what goes on in the intact animal. In *in vitro* rumen fermentations there is no passage of material; substrate particle size is greatly reduced; readily available sources of energy and protein are supplied to the micro-organisms via the nutrient medium, and any sort of physiological stress is removed.

The analysis of variance of the *in vivo* results indicates no significant difference between the chemically treated diets and untreated diets. The significant ($P < .05$) interactions involving chemical treatment suggest that the results have to be examined on a period and physical form basis with respect to chemical treatment.

On the basis of period, it was observed that chemical treatment resulted in a significant ($P < .05$) increase in cellulose digestibility of the ground treated straw during the first period. In fact the cellulose digestibility achieved in this case is similar to that observed in the *in vitro* trial (Table 6, Treatment 5). That the increase is not as large as observed in the *in vitro* trials is largely influenced by the high digestibility achieved for the untreated straw in the sheep trial as compared with the *in vitro* results (46.9% vs. 24.0%).

In the second period, however, chemical treatment did not significantly ($P < .05$) increase cellulose digestibility in the case of either ground or pelleted straw. This raises the question as to differences in the preparation of the straw used in the two periods.

An examination of batch preparation of the treated diets (Table 11) indicates that the method of preparation of the three batches differed in regard to the following:

(i) the concentration of NaOH solution used for treatment;

(ii) the volume of HOAc used for neutralization and

(iii) the individual time of mixing with alkali and acid. The outstanding difference between these preparations was the time of mixing (4.75 hours) with the alkali in the third batch preparation. Both heat and steam were produced during the long mixing of this batch and it was this batch that was fed during the second period. It has been noted previously that in the second period there was a decrease, though not statistically significant ($P < .05$), in both dry matter and gross energy digestibility of the treated diets. However, in the case of cellulose digestibility the decrease observed in the second period was statistically significant ($P < .05$).

From these observations the following can be inferred:

(i) that the third batch preparation which was fed during the second period was of an inferior nature

(in terms of digestion) to the first and second batch preparations which were fed during the first period;

(ii) that the inferior nature of the third batch stems from the prolonged mixing of the straw with the alkali;

(iii) that cellulose digestibility was more affected than either protein, dry matter, or energy digestibility.

It is suggested that the prolonged mixing during preparation of the treated diets fed in the second period may have in some way adversely affected the availability of cellulose.

Considering the results on the basis of physical form, it is suggested that depression in digestion due to pelleting counteracted any increase due to chemical treatment. A highly significant ($P < .01$) difference was found between the means of the ground diets (50.7%) and pelleted diets (36.3%). This depression in cellulose digestibility due to pelleting is similar to that observed for dry matter and gross energy digestibility.

d) Apparent Crude Protein Digestibility.

Data on apparent crude protein digestibility are presented in detail in Appendix Table 7 and the analysis of variance is to be found in Appendis Table 11. A summary of the data is shown in Table 16.

TABLE 16.

SUMMARY OF APPARENT CRUDE PROTEIN DIGESTIBILITY DATA (%).

Chemical Treatment	Physical Form				
	Period I		Period II		
	Ground	Pelleted	Ground	Pelleted	Chem.Treat.Ave.
Untreated	-25.2	- 4.0	17.3	11.1	- 0.8
Treated	- 9.7	-21.8	-37.3	-26.9	-23.9
Period Ave.	-15.2		-8.9		
Phy.Form Ave.	-13.7(Ground)		-10.4(Pelleted)		

The negative digestion coefficients obtained for the apparent crude protein digestibility are due to the small quantity of protein (3 - 4%) present in the diets (Table 12). None of the diets was supplemented with protein. The resulting negative digestion coefficients indicate that fecal nitrogen of metabolic (endogenous) origin exceed fecal nitrogen of dietary origin.

It would therefore be necessary to supplement alkali-treated straw with some source of protein especially a readily available source such as ammonia or urea, non-protein nitrogen compounds, which the rumen bacteria can easily utilize for protein synthesis. To increase the available nitrogen in alkali-treated straw and other roughages some workers (Zafren, 1960 and 1962; Chomyszyn et al.,1961; Laguta 1962; and El-Shazly, 1967) have turned their attention to the use of ammonia instead of

sodium hydroxide as the alkali for treatment.

Negative digestion coefficients for protein digestibility in the feeding of alkali (NaOH) treated straw to sheep have been reported by Woodman and Evans (1947) and Homb (1949). Williamson (1941) observed slightly reduced protein digestibility on feeding NaOH treated barley straw to horses and Honcamp (1932) who decomposed straw by steaming obtained no digestible protein. Honcamp (1932) stated that the small amount present in the fresh straw was lost during steaming.

The "chemical treatment x period" and "physical form x chemical treatment x period" interactions showed significance at the 5% level (Appendix Table 11). An examination of Table 16 indicates that the digestion coefficients of protein digestibility with reference to treated diets were lower in the second period than in the first period. This observation also supports the conclusion that the treated diets fed during the second period were of an inferior nature, in terms of digestion, to those fed in the first period.

4. Relative Intake.

The voluntary intake of the diets has been expressed as Relative Intake and data for individual lambs are presented in Appendix Table 7 and an analysis of variance of the data is shown in Appendix Table 12. A summary of the Relative Intake data is shown in Table 17.

TABLE 17

SUMMARY OF RELATIVE INTAKE DATA (%).

Chemical Treatment	Physical Form				
	Period I		Period II		
	Ground	Pelleted	Ground	Pelleted	Chem.Treat.Ave.
Untreated	45.0	55.9	43.5	59.5	51.0
Treated	38.0	51.1	27.0	47.6	40.9
Period Ave.	47.5		44.4		
Phy.Form Ave.	38.4(Ground)			53.5(Pelleted)	

The analysis of variance (Appendix Table 12) reveals that the chemical treatment of straw significantly ($P < .01$) depressed Relative Intake. Although interactions were not significant, the data (Table 17) indicates that the depression in voluntary intake due to chemical treatment was more pronounced in the case of ground as compared to the pelleted straw. The difference was also more pronounced in the second period as compared to the first.

It is not difficult to postulate that the taste of the diet (due to the addition of alkali and acid) resulted in decreased palatability of the chemical treated diets. The lower levels of alkali and acid used in the first period (Table 11) might account for the higher intake of the ground treated straw in that period.

Pelleting had a highly significant effect ($P < .01$) in increasing Relative Intake. Many workers (Heaney et al.,

1963; Minson, 1963; Campling, 1964; and Jordon and Hanke, 1965) have shown that pelleting increases the voluntary intake of a forage.

5. Nutritive Value Index.

The Nutritive Value Index (NVI) which is a numerical description of the "overall" nutritive value of a forage is computed from the product of the Relative Intake (RI) of the forage and its per cent gross energy digestibility (Crampton et al.,1960). Data for the Nutritive Value Index of each of the four diets are presented in detail in Appendix Table 13. A summary of the data is shown in Table 18.

TABLE 18.

SUMMARY OF NUTRITIVE VALUE INDEX DATA.

	Physical Form				
Chemical	Period I		Period II		
Treatment	Ground	Pelleted	Ground	Pelleted	Chem.Treat.Ave.
Untreated	15.9	15.3	14.0	17.4	15.6
Treated	21.9	19.7	10.9	14.1	16.6
Period Ave.	18.2		14.1		
Phy.Form Ave.	15.7(Ground)		16.6(Pelleted)		

The analysis of variance for the Nutritive Value Index reveals that there are no significant differences ($P < .05$)

between the means of ground and pelleted diets, treated and untreated diets, or the first and second period. The interactions were also found to be non-significant.

This implies that neither alkali treatment nor pelleting has caused an improvement of the original nutritive value of the ground untreated straw as measured by the NVI. Bearing in mind that the NVI of a forage is associated ($r = 0.88$ to 0.94) with body weight changes (Crampton, et al., 1960) it was therefore not surprising to find that there was no significant ($P < .05$) increase of the liveweight gain by the lambs when they received either treated or pelleted diets (Appendix Table 14).

In order to understand the lack of increase in the nutritive value of the straw when subjected to chemical treatment it is necessary to examine the two components from which the NVI is computed. Table 19 shows the means of the treated and untreated diets for both relative intake and percent gross energy digestibility as obtained from the summaries in Tables 17 and 14, respectively.

It is obvious from Table 19, that the failure of the treated straw to demonstrate a significant difference in the NVI resulted from a lower voluntary intake of the treated straw which counteracted the increased digestibility due to treatment. This observation emphasizes that in order to improve the overall nutritive value of a forage

TABLE 19

SUMMARY OF THE COMPONENTS USED TO CALCULATE NVI.

	Chemical Treatment	
	Untreated	Treated
Relative Intake (%)	51.0**	40.9
Gross Energy Digestibility (%)	31.0	41.6**
Nutritive Value Index	15.8	17.0

**Highly significant difference due to treatment ($P < .01$)

both its voluntary intake and energy digestibility must be increased.

In this *in vivo* experiment an attempt was made to increase the voluntary intake of the treated straw by pelleting. Although the Relative Intake of the pelleted treated straw was increased (Table 17) pelleting resulted in a depression of gross energy digestibility and this circumvented the use of this combination of treatments (chemical and physical) to increase the nutritive value of the straw.

As previously stated, the *in vivo* trial being of a preliminary nature was designed to obtain information on the acceptability of chemically treated oat straw by sheep. These studies thus established the importance of the following:

(i) minimizing treatment time (i.e. the individual

time of mixing the straw with alkali and acid) particularly in reference to overheating due to prolonged mixing and possibly pelleting.

(ii) the necessity for adding other nutrients such as protein to meet the complete energy requirements of the diets.

(iii) the advisability of adding ingredients (e.g. molasses) to mask the taste due to chemical treatment and thus increase the voluntary intake of the treated low-quality forage.

6. Liveweight Changes.

Data for liveweight changes are presented in Appendix Table 15. A summary of the data for the last week of each period is presented in Table 20.

TABLE 20.

SUMMARY OF LIVEWEIGHT GAIN (Kg.) FOR THE LAST WEEK OF EACH FEEDING PERIOD.

	Physical Form				
Chemical	Period I		Period II		
Treatment	Ground	Pelleted	Ground	Pelleted	Chem.Treat,Ave.
Untreated	0.68	0.45	0.75	0.34	0.55
Treated	1.70	0.56	0.68	0.56	0.87
Period Ave.	0.85		0.58		
Phy.Form Ave.	0.95(Ground)			0.48(Pelleted)	

Analysis of variance (Appendix Table 14) was done only for the last week of the two periods as it was felt that by that time the lambs were adapted to their diets and that there would be less fluctuations in liveweight gains. Also it was felt that by studying the liveweight changes during the last week only will ensure that the carry-over effect of the high quality alfalfa which was fed two weeks prior to commencement of the first period will be completely eliminated.

The statistical analysis shows no significant difference due to any of the factors studied. The high within treatment variability of the results (Appendix Table 15) account for the very high standard deviation and the resultant coefficient of variation of over 100%.

There was a tendency for slightly larger gain with the treated straw than with the untreated straw (0.87 vs. 0.55Kg./week) and also with the unpelleted straw than with the pelleted straw (0.95 vs. 0.48Kg./week). However, these gains were considerably less than would be expected, 1.3Kg./week, (N.R.C. 1964) if all the nutrient requirements (energy, protein, vitamins, and minerals) were being met.

Liveweight gain cannot be considered as an important criterion for evaluating the nutritive value of the treated diets in this feeding trial because of the following limitations:

(i) 'stress' of confinement in digestion stall is generally not conducive to normal weight gain;

(ii) the diets fed were not supplemented with deficient nutrients as the effect of chemical and physical treatment per se was being examined.

7. Water Intake, Urine Excretion, and Urine pH.

Data on the water intake, urine excretion, and urine pH for the last week of each period are presented in Appendix Table 16., and the average of the two periods are shown in Table 21.

TABLE 21.

AVERAGE DAILY WATER INTAKE, URINE EXCRETION, AND URINE pH.

Parameters	Treatment			
	Physical		Chemical	
	Ground	Pelleted	Untreated	Treated
Water Intake (liters)[1]	2.74	3.59	2.02	4.30
Urine Excretion (liters)[1]	1.53	2.11	0.68	2.96
Urine pH[1]	8.3	8.2	7.9	8.6
Rumen pH[2]	7.0	7.1	6.8	7.2

[1]Data were collected daily during the last 7 days of each period.

[2]Rumen pH determinations were made on the last day only of each feeding period.

a) Water Intake.

Water consumption was greater for lambs that

received alkali-treated diets than those on the control (untreated) diets. This higher intake resulted from the composition of the treated diets which had more than two times the ash content of the controls (Table 12). The increase in the ash content of the treated diets is due to the unreacted sodium hydroxide and to sodium acetate formed from the neutralization of NaOH with HOAc. It is well known that the consumption of high mineral content diets requires a larger than normal intake of water for normal kidney functions. Beacom (1959) has shown a close relationship between water intake and total diet ash content ($r = .83$), and total diet salt + ash content ($r = .94$), using sheep as experimental animals. Lloyd et al.(1962) also found that the average daily intake of water by sheep was highly correlated ($r = .98$) with the intake of ash from alfalfa and bromegrass.

With regards to the effect of physical form of the diet on water intake there was a slight but definite increase of water consumption due to pelleting. Lloyd et al.(1962) found a progressive increase in water intake of sheep fed alfalfa and bromegrass as the physical form was altered from chopped to ground and to pelleted form. This is not surprising since pelleting of a forage increases its dry matter intake (Heaney et al.1963; Minson, 1963; Campling, 1964;) and water intake is directly related to dry matter intake (Payne 1966). The increase in voluntary

intake of diets due to pelleting was found to be statistically highly significant ($P < .01$) as presented in Appendix Table 12.

b) Urine Excretion.

Treatment effect caused a more than four-fold increase in urine excretion over that of the controls. Studying the effect of Beckmann's treatment by sodium hydroxide on the digestibility and feeding value of barley straw for horses, Williamson (1941) found that urine output was doubled in horses receiving treated straw. Urine output due to treatment effect appears to parallel water intake. The large water intake which was necessary for the elimination of the high mineral matter consumed no doubt accounts for the high excretion of urine observed. The daily amount of urine excreted by sheep varies from 0.5 to 2.0 liters with an average of one liter (Ellenberger and Scheunert, 1925).

Pelleting caused a slight increase in the urine output of sheep. Again this is attributed to large intake of water by sheep that received pelleted diets.

c) Urine pH.

The slight increase of the pH (8.6) of the urine from lambs on treatment over that (7.9) from the controls suggests the presence of more alkaline cations in the urine

which may have arisen from the high sodium content of the treated diets. Physical form of the diets appears to have no effect on urine pH.

"In 40 sheep Healy, Bulard, and Spears found the urine to be acid only twice; in all other cases it was alkaline.... Foods of vegetable origin give rise to an alkaline urine because they contain excess of base-forming elements (sodium, potassium, calcium, magnesium) (Dukes, 1955)." This suggests that homeostasis of acid-base balance was not affected by either chemical treatment or physical form of the diet.

8. Rumen pH.

Alkali treatment caused a slight increase in rumen pH (7.2 vs. 6.8 for the control), which is probably a result of the inability of the animals to neutralize all the added alkali. That this increase is of minor importance is indicated by the fact that under normal circumstances the pH of the rumen of the completely healthy animal on a normal diet may vary from 5.0 to 7.5 (Barnett and Reid, 1961).

The pelleted diet as compared to the ground form appeared to have no effect on rumen pH (7.0 vs. 7.1), respectively.

VI. A STUDY OF CELLULOSE DIGESTIBILITY (*IN VITRO* AND *IN VIVO* OF THE DIETS OFFERED TO LAMBS.

1. Introduction.

The *in vitro* experiments (Experiments 1,2, and 3) previously described were done in the Summer and Fall of 1966, prior to the sheep trial in the Winter of the same year. The experiment to be reported here was carried out in the Spring of 1967, and based on samples of straw collected during the sheep feeding trial.

In vitro determinations of cellulose digestibility were made on samples of the four diets fed to see how the results compared with those of *in vivo* cellulose digestibility.

2. Experimental Procedure.

a) Sampling.

Feed intake for the calculation of *in vivo* cellulose digestibility and the other digestion coefficients studied was measured from the 12th. to 19th. day inclusive, assuming that it takes about two days for the feed to pass through the alimentary tract of the sheep. Accordingly, between the 12th. and 19th. day inclusive of each period, a daily sample representative of each diet fed, i.e. ground untreated (GU), ground treated (GT), pelleted untreated (PU), and pelleted treated (PT), (and weighing about 50g.) was collected and stored. The samples of each diet collected

were bulked for each period. The ground diets were re-ground using mesh screens 1.0 and 0.6mm. in diameter. The pelleted diets, however, required crushing in a hand-powered plate grinding mill prior to re-grinding to pass through the 1.0 and 0.6mm. diameter-mesh screens. Each re-ground sample was mixed by 'quartering' and stored in tightly covered glass jars.

b) Chemical Analysis.

Cellulose determinations were made before and after *in vitro* fermentation runs.

c) *In Vitro* Fermentation Runs.

Two fermentation runs were made four days apart with two replications of each diet per run. The procedure adopted was that already described for fermentation runs (section IV,A).

3. Results and Discussion.

The cellulose content and *in vivo* and *in vitro* cellulose digestibility of each diet are summarized in Table 22.

TABLE 22.

CELLULOSE CONTENT AND *IN VITRO* AND *IN VIVO* CELLULOSE DIGESTIBILITY.

PERIOD I

Treatment or Diet	Cellulose Content (%)	Cellulose Digestibility (%)	
		In Vivo	*In Vitro*
GU	42.6	46.9	20.2
GT	37.0	69.0	72.9
PU	40.5	37.0	25.5
PT	38.0	42.6	73.6

PERIOD II

GU	40.0	40.9	20.5
GT	37.1	46.1	77.8
PU	40.0	39.5	29.4
PT	37.4	26.6	82.1

There is a large variation between *in vitro* and *in vivo* results of cellulose digestibility for each diet and the *in vivo* results are not as consistent as the *in vitro* results with regard to diets and periods.

Of interest is the following observation. Regardless of whether the diet was treated or not, with a change in the physical form, from ground to pelleted, cellulose digestion was reduced *in vivo* but slightly increased *in vitro*. The substrates in both *in vitro* and *in vivo* experiments were

the same. The source of cellulolytic degrading microorganisms was not the same and neither was the environment in which cellulose digestion was done. If the microorganisms found in the rumen of the sheep and cattle are similar in type and in their ability to digest cellulose, this leaves 'environmental factors' as the only possible explanation for this contrast in cellulose digestibility observed.

The 'environmental factors' which may have possibly caused a decrease in *in vivo* cellulose digestibility due pelleting have already been suggested (section V, 3c) as being 'over-mixing' effect, heat and pressure effect of the pelleting process, and a small surface area due to incomplete disintegration of the pellets (pellets were re-ground for the *in vitro* study).

With regard to the slight increase observed in *in vitro* cellulose digestibility for the pelleted diets over the ground diets, to say that this is possibly the effect of the pelleting operation is to suggest that pelleting of a forage prior to *in vitro* rumen fermentation increases cellulose digestibility while pelleting prior to *in vivo* fermentation decreases cellulose digestibility. Further studies would have to be made to find out if this is the case, and if so, why?

Regardless of the physical form or period, *in vitro* results indicate that treatment effected approximately a three-fold increase in cellulose digestibility. However,

this was not observed for *in vivo* cellulose digestibility. Although reduced particle size and the enriched nutrient medium of the *in vitro* system might have played a role in this respect, the high cellulose digestibility of the *in vivo* control, ground untreated, (two times that of the *in vitro* control) cannot be overlooked. Perhaps a longer *in vitro* fermentation period (over 24 hours) should have been used. It may be that straw has a long lag phase for *in vitro* cellulose digestibility and consequently fermentation was not complete at the end of the 24-hour period used.

In vivo cellulose digestibility of the ground treated straw compared favorably with the *in vitro* result in the first period (69.0 vs. 72.9%). The reason for non-conformity in the second period of the *in vivo* result has been attributed to the difference in the method of preparation of the treated diets for the two periods (Table 11).

VII. SUMMARY AND CONCLUSION.

This research investigated the effect of subjecting oat (*Avena sativa*) straw to physical and chemical treatments in order to increase its nutritive value. The research work was divided into two parts, viz, a series of *in vitro* rumen fermentation experiments and an *in vivo* sheep feeding trial.

The *in vitro* rumen fermentation experiments were designed to establish a practical level of alkali treatment which could be used in the subsequent *in vivo* trial. *In vitro* cellulose digestibility was used as the criterion for evaluating the relative effectiveness of different alkali treatments. The effect of length of treatment period, and type and concentration of alkali were studied.

Whereas the *in vitro* cellulose digestibility of the untreated straw averaged 24.0%, treatment with alkali resulted in large increases in cellulose digestion. Maximum increases in cellulose digestion was obtained using NaOH at a 16% treatment level (16g.NaOH/100g. of straw). Comparing a 5-day versus a 1-day treatment period with respect to the effect of concentration and type of alkali, the longer interval only resulted in slight increases in cellulose digestion in the case of the NaOH and dilute NH_3 treatments and actually lowered digestion in the case of the concentrated NH_3 treatments. The latter depressions in cellulose digestion were attributed to mold growth on the

straw substrates during the prolonged treatment period. Because of the consistent results obtained with NaOH treatments and the ease to work with this alkali, NaOH was chosen as the more suitable alkali for the treatment of oat straw to be fed in the *in vivo* trial.

A further *in vitro* experiment with NaOH was designed to test treatment levels up to 32% and to establish a treatment level involving the use of minimal volume of alkali solution (i.e. a 'dry' treatment) for use in the *in vivo* trial. Volumes of treatment solutions were to be restricted in the *in vivo* trial, as the application of chemical treatment to large amounts of straw would be facilitated by minimizing the total amount of alkali solution needed. The *in vitro* results indicated that maximum cellulose digestion occurred at the 16% level of treatment (81.4 vs. 24.0% for the control) and that the greater the ratio of water to solute (alkali) the greater was the cellulose digestibility. For reasons of a practical nature, 8% treatment level (60ml. 13.3% NaOH solution/100g. of straw) was chosen for treatment of straw to be used in the *in vivo* trial.

To further test the efficacy of 'dry' treatments, *in vitro* cellulose digestion of bagasse, another low-quality forage, was also studied. As in the case of the oat straw, maximum *in vitro* cellulose digestion occurred at the 16% treatment level. Maximum *in vitro* cellulose digestibility

of the treated bagasse was two and a half times higher than the control (68.9 vs. 24.4% for the control). In vitro cellulose digestion was lower for treated bagasse than for treated oat straw at all levels of treatment.

The in vivo trial consisted of two 3-week feeding periods during which oat straw was fed in the following combinations to lambs approximately 10 months old: ground treated, ground untreated (control); pelleted treated, and pelleted untreated (control). Iodized salt was the only supplement added to these diets.

As information on the preparation of alkali treated straw for feeding was very limited, treated straw to be fed to lambs was prepared in three batches in order to facilitate correction or alteration of the method of preparation, if necessary. However, the third batch differed from the first two batches of treated oat straw prepared in that during treatment the time of mixing was accidentally extended from about 1.5 to 4.75 hours.

Chemical treatment effected a significant ($P < .01$) increase in dry matter and gross energy digestibility but no significant ($P < .05$) overall increase was shown for cellulose digestibility. Cellulose digestibility was significantly increased in the first period in the case of the ground treated straw (68.9 vs. 46.9% for the control). Crude protein digestion coefficients were predominantly negative due to the low protein content of all diets.

Pelleting of the straw significantly depressed dry matter, gross energy, and cellulose digestibility.

Chemical treatment significantly ($P < .01$) depressed voluntary intake while pelleting significantly ($P < .01$) increased it. Neither chemical treatment nor physical form had a significant ($P < .05$) effect on digestible energy intake (Nutritive Value Index) or liveweight gain.

In general, animal response to chemical treatment was lower in the second period than in the first. This decrease was attributed to the difference in preparation time of the batches of straw. The third batch which was fed in the second period was characterized by a longer mixing time.

A study of *in vitro* and *in vivo* cellulose digestibility of the diets fed to sheep indicated large variations between *in vitro* and *in vivo* results. Chemical treatment effected a three-fold increase in the *in vitro* results but less than a two-fold increase in the *in vivo* results. This is largely a reflection of lower digestibility for the untreated forage as observed *in vitro*. A longer *in vitro* rumen fermentation period might have resulted in closer *in vivo* - *in vitro* agreement between the results obtained for the controls. There was an apparent increase in *in vitro* cellulose digestibility due to pelleting, while the *in vivo* results showed a decrease, especially in the second period. In the first period, *in vitro* and *in vivo*

results compared favorably for the ground treated diet, but not in the second period.

In conclusion, the lack of an increase in the nutritive value of oat straw when subjected to a combination of chemical and physical treatments as measured by the Nutritive Value Index (NVI) resulted from the counteracting effects of the two treatments on the gross energy digestibility and voluntary intake, the product of these two criteria being used to calculate the NVI. Whereas there was an observed increase in energy digestibility due to chemical treatment, voluntary intake of the treated material was reduced.

It is suggested that voluntary intake of the chemically treated straw may be increased by the addition of an ingredient such as molasses which would mask the taste due to chemical treatment. To increase gross energy digestibility (i.e. make potential energy more available) it is suggested that the nitrogen content of the chemically treated straw be increased by the addition of urea from which nitrogen is readily available to the cellulolytic rumen microorganisms. Since pelleting was shown to decrease gross energy digestibility, it is suggested that this physical form of treatment may be unnecessary in subsequent trials.

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APPENDIX TABLE 1

IN VITRO CELLULOSE DIGESTIBILITY (%) DATA - EXPERIMENT 1.

Treatment No.	Trial 1	Trial 2	Subgroup Totals	Treatment Totals	Treatment Means
1a	24.7	23.3	48.0		24.0
1b	21.0	24.0	45.0	93.0	22.5
2a	42.2	39.0	81.2		40.6
2b	27.1	25.6	52.7	133.9	26.3
3a	51.5	52.0	103.5		51.7
3b	29.0	31.5	60.5	164.0	30.2
4a	18.9	19.3	38.2		19.1
4b	14.9	15.5	30.4	68.6	15.2
5a	20.4	19.4	39.8		19.9
5b	26.0	23.5	49.5	89.3	24.7
6a	38.9	36.3	75.2		37.6
6b	48.0	50.0	98.0	173.2	49.0
7a	24.0	24.0	48.0		24.0
7b	21.1	16.6	37.7	85.7	18.8
8a	47.5	44.1	91.6		45.8
8b	43.3	42.8	86.1	177.7	43.0
9a	54.8	58.3	113.1		56.5
9b	60.0	59.5	119.5	232.6	59.7
10a	77.6	73.6	151.2		75.6
10b	77.4	81.9	159.3	310.5	79.6
11a	40.2	43.2	83.4		41.7
11b	44.5	41.9	86.4	169.8	43.2
12a	67.8	72.9	140.7		70.3
12b	75.3	76.7	152.0	292.7	76.0
13a	76.5	78.0	154.5		77.2
13b	81.1	82.6	163.7	318.2	81.8

[a]Represents data for 1-day treatment.

[b] " " " 5-day "

APPENDIX TABLE 2.

ANALYSIS OF VARIANCE OF CELLULOSE DIGESTIBILITY - EXPERIMENT 1.

Sources	D/F	MS	F value obs.	F value 5%	F value 1%
Total	51				
Subgroups	25	949.7			
Treatments (Tr.)	12	1899.1	558.5	2.15	2.96
Time	1	14.5	4.3	4.23	7.72
Tr. x Time	12	78.1	23.0	2.15	2.96
Error	26	3.4			

SD = 1.8; $S_{\bar{x}}$ = 1.3; CV= 4.0%

L.S.R. (0.01) = 5.1 to 6.2 (for p = 2 to 26, n = 2)

APPENDIX TABLE 3

IN VITRO CELLULOSE DIGESTIBILITY (%) DATA - EXPERIMENT 2.

Treatment No.	Cellulose Digested (%)		Subgroup Totals	Treatment Totals	Treatment Means
1a	41.8	41.4	83.2		
1b	39.5	42.8	82.3	165.5	41.4
2a	45.9	44.5	90.4		
2b	44.2	46.4	90.6	181.0	45.2
3a	50.8	45.4	96.2		
3b	48.7	46.9	95.6	191.8	47.9
4a	57.4	55.2	112.6		
4b	53.3	58.2	111.5	224.1	56.0
5a	63.8	63.6	127.4		
5b	60.7	60.1	120.8	248.2	62.0
6a	69.6	68.5	138.1		
6b	67.3	67.6	134.9	273.0	68.2
7a	75.8	73.3	149.1		
7b	69.5	65.8	135.3	284.4	71.1
8a	69.0	69.4	138.4		
8b	68.1	65.9	134.0	272.4	68.1
9a	79.4	72.0	151.4		
9b	79.5	78.1	157.6	309.0	77.2
10a	84.3	78.9	163.2		
10b	81.6	80.7	162.3	325.5	81.4
11a	84.3	69.6	153.9		
11b	71.8	85.6	157.4	311.3	77.8
12a	79.1	63.7	142.8		
12b	71.0	76.1	147.1	289.9	72.5

[a]Represents data for the second fermentation run.
[b] " " " " third fermentation run.

APPENDIX TABLE 4

ANALYSIS OF VARIANCE OF IN VITRO CELLULOSE DIGESTIBILITY EXPERIMENT 2.

Sources	D/F	MS	F value obs.	F value 5%	F value 1%
Total	47				
Subgroups	23	353.9			
Treatments(Tr.)	11	732.4	40.7	2.21	3.10
Runs	1	6.3	<1		
Tr. x Runs	11	7.1	<1		
Error	24	18.0			

SD = 4.2; $S_{\bar{x}}$ = 2.1; CV = 6.5%.

L.S.R. (0.01) = 8.3 to 9.7 (for p = 2 to 12, n = 4).

APPENDIX TABLE 5.

IN VITRO CELLULOSE DIGESTIBILITY (%) DATA - EXPERIMENT 3.

Treatment No.	Subgroup Totals	Subgroup Means	Treatment Totals	Treatment Means
1a	113.3	37.8		
1b	104.0	34.7	326.5	36.3
1c	109.2	36.4		
2a	109.9	36.6		
2b	89.2	29.7	299.7	33.3
2c	100.6	33.5		
3a	165.2	55.1		
3b	149.1	49.7	469.2	52.1
3c	154.9	51.6		
4a	177.6	59.2		
4b	187.7	62.6	541.6	60.2
4c	176.3	58.8		
5a	189.2	63.1		
5b	175.8	58.6	534.8	59.4
5c	169.8	56.6		
6a	212.8	70.9		
6b	205.8	68.6	619.7	68.8
6c	201.1	67.0		
7a	175.0	58.3		
7b	152.4	50.8	463.5	51.5
7c	136.1	45.4		
8a	133.3	44.4		
8b	126.3	42.1	363.0	40.3
8c	103.4	34.5		

[a]Represents data for the first fermentation run
b " " " " second " "
c " " " " third " "

APPENDIX TABLE 6

ANALYSIS OF VARIANCE OF IN VITRO CELLULOSE DIGESTIBILITY EXPERIMENT 3.

Sources	D/F	MS	F value obs.	F value 5%	F value 1%
Total	71				
Subgroups	23	465.0			
Treatments (Tr.)	7	1433.3	152.48	2.22	3.05
Runs	2	170.2	18.10	3.20	5.10
Tr. x Runs	14	23.0	2.45	1.92	2.50
Error	48	9.4			

TREATMENTS:

SD = 3.1; $S_{\bar{x}}$ = 1.03; CV = 6.2%

L.S.R. (0.01) = 3.9 to 4.4 (for p = 2 to 8, n = 9)

RUNS:

$S_{\bar{x}}$ = 0.63

Multiple Range Test.

Range:	2	3
L.S.R. (0.01)	2.4	2.5

(n = 24)

3rd. Run	2nd. Run	1st. Run
48.0	49.6	53.2

APPENDIX TABLE 7.

APPARENT DIGESTIBILITY, RELATIVE INTAKE, AND NUTRITIVE VALUE INDEX DATA.

PERIOD I

Lamb No.	Treatment	Protein (%)	Cellulose (%)	Dry Matter (%)	Energy (%)	R I (%)	NVI
1	GU	-25.2	42.0	34.8	31.4	46.1	14.5
4	"	-25.3	51.8	40.6	39.4	44.0	17.3
2	GT	-17.4	62.4	56.8	53.1	34.3	18.2
6	"	- 2.1	75.5	66.9	61.5	41.7	25.6
7	PU	- 8.0	31.5	25.5	22.3	52.2	11.6
8	"	0.0	42.5	35.1	31.9	59.6	19.0
3	PT	- 8.9	45.6	47.7	41.7	53.2	22.2
5	"	-34.7	39.5	42.3	35.4	49.0	17.3

PERIOD II

3	GU	19.2	38.9	33.5	30.7	40.7	12.5
5	"	15.5	42.9	36.7	33.4	46.4	15.5
7	GT	-38.4	36.9	41.5	35.8	27.5	9.8
8	"	-36.3	55.4	52.3	45.6	26.5	12.1
2	PU	6.5	35.0	29.2	25.2	64.5	16.2
6	"	15.7	43.9	37.3	34.2	54.6	18.7
1	PT	- 8.6	27.6	41.4	35.1	44.8	15.7
4	"	-45.2	25.5	32.5	24.9	50.4	12.5

APPENDIX TABLE 8.

ANALYSIS OF VARIANCE OF APPARENT DRY MATTER DIGESTIBILITY

Sources	D/F	MS	F value obs.	F value 5%	F value 1%
Total	15				
Subgroups	7				
Physical	1	324.92	9.83	5.32	11.26
Chemical	1	738.49	22.35	5.32	11.26
Period	1	128.26	3.88	5.32	11.26
INTERACTIONS					
Phys. x Chem.	1	76.98	2.33	5.32	11.26
Phys. x Period	1	37.73	1.14	5.32	11.26
Chem. x Period	1	136.30	4.12	5.32	11.26
Phys. x Chem. x Period	1	1.47	<1		
Error	8	33.04			

Mean = 40.9; Standard Deviation = 5.7;
Coefficient of Variation = 13.9%

APPENDIX TABLE 9.

ANALYSIS OF VARIANCE OF APPARENT GROSS ENERGY DIGESTIBILITY

Sources	D/F	MS	F value obs.	5%	1%
Total	15				
Subgroups	7				
Phys. Treatment	1	402.00	11.59	5.32	11.26
Chem. Treatment	1	447.32	12.90	5.32	11.26
Period	1	167.70	4.84	5.32	11.26
INTERACTIONS					
Phys. x Chem.	1	88.36	2.55	5.32	11.26
Phys. x Period	1	49.00	1.41	5.32	11.26
Chem. x Period	1	148.84	4.29	5.32	11.26
Phys. x Chem. x Period	1	1.11	<1		
Error	8	34.67			

Mean = 36.3; Standard Deviation = 5.9;

Coefficient of Variation = 16.2%.

APPENDIX TABLE 10.

ANALYSIS OF VARIANCE OF APPARENT CELLULOSE DIGESTIBILITY

Sources	D/F	MS	F value obs.	F value 5%	F value 1%
Total	15				
Subgroups	7				
Phys. Treatment	1	822.26	15.16	5.32	11.26
Chem. Treatment	1	99.50	1.83	5.32	11.26
Period	1	448.38	8.27	5.32	11.26
INTERACTIONS					
Phys. x Chem.	1	300.15	5.53	5.32	11.26
Phys. x Period	1	58.14	1.07	5.32	11.26
Chem. x Period	1	310.64	5.73	5.32	11.26
Phys. x Chem. x Period	1	0.68	<1		
Error	8	54.23			

Mean = 43.5; Standard Deviation = 7.4;

Coefficient of Variation = 17.0%

APPENDIX TABLE 11.

ANALYSIS OF VARIANCE OF APPARENT CRUDE PROTEIN DIGESTIBILITY

Sources	D/F	MS	F value obs.	F value 5%	F value 1%
Total	15				
Subgroups	7				
Phys. Treatment	1	44.89	<1		
Chem. Treatment	1	2256.25	15.00	5.32	11.26
Period	1	156.25	1.04	5.32	11.26
INTERACTIONS					
Phys. x Chem.	1	68.89	<1		
Phys. x Period	1	6.25	<1		
Chem. x Period	1	1187.48	7.90	5.32	11.26
Phys. x Chem. x Period	1	1480.56	9.84	5.32	11.26
Error	8	150.38			

Mean = 12.1; Standard Deviation = 12.4;

Coefficient of Variation = 102.3%

APPENDIX TABLE 12.

ANALYSIS OF VARIANCE OF RELATIVE INTAKE

Sources	D/F	MS	F value obs.	F value 5%	F value 1%
Total	15				
Subgroups	7				
Phys. Treatment	1	916.58	49.81	5.32	11.26
Chem. Treatment	1	407.04	22.12	5.32	11.26
Period	1	38.14	2.07	5.32	11.26
INTERACTIONS					
Phys. x Chem.	1	11.72	<1		
Phys. x Period	1	40.00	2.17	5.32	11.26
Chem. x Period	1	69.29	3.76	5.32	11.26
Phys. x Chem. x Period	1	1.39	<1		
Error	8	18.40			

Mean = 46.0; Standard Deviation = 4.3;

Coefficient of Variation = 9.3%.

APPENDIX TABLE 13.

ANALYSIS OF VARIANCE OF NUTRITIVE VALUE INDEX

Sources	D/F	MS	F value obs.	F value 5%	F value 1%
Total	15				
Subgroups	7				
Phys. Treatment	1	3.71	<1		
Chem. Treatment.	1	4.11	<1		
Period	1	66.84	1.01	5.32	11.26
INTERACTIONS					
Phys. x Chem.	1	0.85	<1		
Phys. x Period	1	21.85	<1		
Chem. x Period	1	70.97	1.07	5.32	11.26
Phys. x Chem. x Period	1	0.39	<1		
Error	8	8.26			

Mean = 16.2; Standard Deviation = 2.9;

Coefficient of Variation = 17.9%

APPENDIX TABLE 14.

ANALYSIS OF VARIANCE OF LIVEWEIGHT CHANGES FOR THE LAST SEVEN DAYS OF EACH PERIOD.

Sources	D/F	MS	F value obs.	F value 5%	F value 1%
Total	15				
Subgroups	7				
Phys. Treatment	1	0.93	1.56	5.32	11.26
Chem. Treatment	1	0.38	<1		
Period	1	0.26	<1		
INTERACTIONS					
Phys. x Chem.	1	....	<1		
Phys. x Period	1	....	<1		
Chem. x Period	1	....	<1		
Phys. x Chem. x Period	1	....	<1		
Error	8	0.59			

Mean = 0.72; Standard Deviation = 0.77;

Coefficient of Variation = 106.0%

APPENDIX TABLE 15.

LIVEWEIGHT CHANGES DURING THE LAST SEVEN DAYS OF EACH PERIOD.

PERIOD I

Sheep No.	Diet Fed	Total Gain (Kg./Wk.)	Ave. Gain (Kg./Wk.)
1	Ground Untreated	0.45	0.68
4	" "	0.91	
2	Ground Treated	2.95	1.70
6	" "	0.45	
7	Pelleted Untreated	0.91	0.45
8	" "	0.00	
3	Pelleted Treated	1.13	0.56
5	" "	0.00	

PERIOD II

3	Ground Untreated	0.68	0.79
5	" "	0.91	
7	Ground Treated	0.23	0.68
8	" "	1.13	
2	Pelleted Untreated	0.45	0.34
6	" "	0.23	
1	Pelleted Treated	0.68	0.56
4	" "	0.45	

APPENDIX TABLE 16.

AVERAGE DAILY WATER CONSUMPTION, URINE EXCRETION, URINE pH[1] AND RUMEN pH[2]

PERIOD I

	Ground Untreated	Ground Treated	Pelleted Untreated	Pelleted Treated
Water Consumption (liters)	1.86	3.81	1.83	5.44
Urine Excretion (liters)	0.48	2.62	0.64	4.03
Urine pH	8.2	8.5	8.0	8.5
Rumen pH	6.7	7.0	6.9	7.1

PERIOD II

Water Consumption (liters)	2.37	2.92	2.03	5.05
Urine Excretion (liters)	1.12	1.90	0.50	3.29
Urine pH	8.0	8.7	7.5	8.7
Rumen pH	6.9	7.3	6.9	7.5

[1]Average daily water consumption, urine excretion and urine pH reported here are for the last seven days of each period.

[2]Rumen pH determination was done once only at the end of each period.