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Münevver ARISOY

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# The Effect of Sodium Hydroxide Treatment on Chemical Composition and Digestibility of Straw

Münevver ARISOY

Hacettepe University, Faculty of Science, Department of Biology, Beytepe, Ankara - TURKEY

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**Abstract:** Digestibility *in vivo*, *in vitro* and *in situ* and effect on the chemical composition of barley straw following pilot-scale treatment with sodium hydroxide (NaOH) were investigated. A range of laboratory measurements were also verified. Laboratory methods included three estimates of digestibility *in vivo*, *in vitro* and *in situ*.

NaOH treatment reduced the hemicellulose content and this resulted in an increased content of cellulose and lignin in the remaining cell wall. The mean increased in organic matter digestibility. The study was designed also to compare the measurement of digestibility methods. It was determined that the result of the experiment *in vitro* digestibility method was more suitable for accurately predicting digestibility than that *in vivo* or *in situ*.

No significant differences were observed with regard to digestibility, between the NaOH treated straw samples either dried at 60°C or freeze dried samples.

**Key Words:** Sodium hydroxide treatment, barley straw, chemical composition, *in vivo*, *in vitro* and *in situ* digestibility.

## Sodyum Hidroksit Muamelesinin, Samanın Kimyasal Yapısına ve Sindirimine Olan Etkisi

**Özet:** Bu çalışmada sodyum hidroksit (NaOH) samanının kimyasal yapısına ve arpa samanının *in vivo*, *in vitro* ve *in situ* sindirimine olan etkisi araştırıldı. *In vivo*, *in vitro* ve *in situ* sindirim çalışmalarında uygulanan laboratuvar ölçümleri üç paralel halinde yapıldı. Samanın sodyum hidroksitle muamelesi organik madde sindirimindeki artış anlamına gelen hemiselüloz düzeyinde azalma, selüloz ve lignin düzeylerinde artma ile sonuçlandı. Bu çalışma aynı zamanda sindirim ölçüm metodlarını karşılaştırma açısından planlandı ve bu çalışmanın sonucunda *in vitro* sindirim metodunun *in vivo* ve *in situ* sindirim metodlarından daha uygun olduğu saptandı.

Ayrıca bu çalışma sonucunda sodyum hidroksitle muamele edilen samanların derin dondurucuda veya 60°C'de kurutulmasının sindirim açısından önemli bir fark yaratmadığı gözlemlendi.

**Anahtar Sözcükler:** Sodyum hidroksitle muamele, arpa samanı, kimyasal bileşim, *in vivo*, *in situ* sindirilebilirlik.

## Introduction

Straw and other fibrous crop residues have been used as feed for farm animals for centuries (1). Cellulose is the major component of straw, grass, stover, bagasse and many other plant as feed. In pure form e.g. cotton, cellulose are highly digestible in ruminants and other herbivores. In poor quality roughages, cellulose is associated with lignin and other compounds which make it more or less unavailable for the microbes of the intestinal tract. It has been known for almost 100 years that the digestibility of highly lignified materials may be improved by physical and chemical treatment (2). Microbiological treatment method for improvement of

poor quality forages and roughages has not been used in practice to date, but it may prove to be one of the most promising in future (2). Although microbiological treatment has not been used in commercial scale so far, physical treatment, such as chopping and grinding has been used in practice for a long time. But as energy costs like high temperature and high pressure have escalated the economic attractiveness of the physical treatment process has decreased and the present trend seems to be toward development of less expensive alternative treatment processes (2).

The first attempt to improve the digestibility of straw by chemical treatment was made towards the end of the

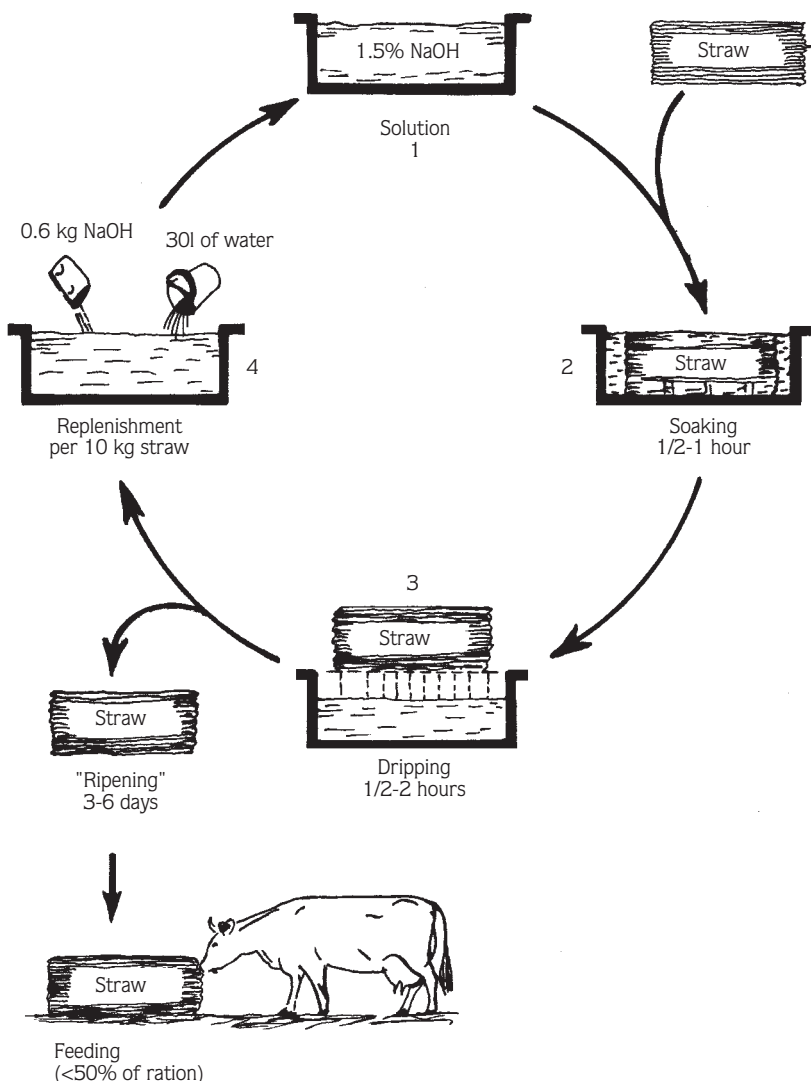


Figure 1. Methods for sodium hydroxide (Sunstøl, 1988 b).

nineteenth century by Lehman (2).

In the last years chemical treatments have been used to remove lignin. For chemical treatment beside Na-OH (sodium hydroxide) there are a great number of other chemicals, such as  $\text{Ca}(\text{OH})_2$ ,  $\text{KOH}$ ,  $\text{SO}_2$ ,  $\text{H}_2\text{SO}_4$ ,  $\text{Na}_2\text{CO}_3$ ,  $\text{O}_3$ ,  $\text{ClO}_2$  showing positive effect on nutritive value of the low quality forages and roughages, but for various reasons they are not used in practice appreciably. Treatment with sodium hydroxide has been shown to be an effective method for upgrading low quality straws, even though addition of NaOH exacerbates the nitrogen deficiency which already exists in straw (3). NaOH treatment of straw, undoubtedly gives the greatest improvement in the digestibility of straw in spite of all the difficulties arising from the corrosiveness of the chemical and the heavy final product obtained (4).

**Materials and Methods**

**Method For Treatment of Straw With Sodium Hydroxide**

Sunstøl dip treatment procedure was used for treatment of straw with sodium hydroxide (1,2,4). The principles of the method are given in Figure 1. After Na-OH treatment, the straw some of which was dried at 60°C for 24 hours and the other dried at deep-freeze were used as feed stuff.

**Method For In-vivo Digestibility**

In this study 9 sheep were used. During 21 days, 3 sheep were fed with untreated straw, the other 3 were fed with NaOH treated straw which was dried at 60°C and

Table 1. Chemical composition of treated and untreated straw.

	Untreated straw	NaOH Treated straw (Dried at 60°C)	NaOH Treated straw (Dried at deep freeze)	P
Drymatter (%)	92.6±0.80	26.6±0.12	25.8±0.15	0.000*
Ash (%)	5.18±0.01	13.9±0.58	13.8±0.10	0.000*
Crude Fat (%)	2.06±0.16	1.09±0.02	1.00±0.11	0.0005*
Crude Fiber (%)	45.7±0.21	44.30±0.48	43.8±0.47	0.0382*
Protein (%)	3.57±0.01	3.00±0.26	2.58±0.03	0.0112*
Total P (%)	0.037±0.01	0.033±0.00	0.028±0.00	0.7864
Total K (%)	1.70±0.03	1.27±0.01	1.26±0.04	0.000*
Total Ca (%)	0.37±0.01	0.28±0.01	0.26±0.04	0.0422*
Total Mg (%)	0.042±0.00	0.048±0.00	0.046±0.00	0.0481*
Total Na (%)	1.5±0.15	2.52±0.16	2.46±0.29	0.0239*
Cellulose (%)	40.2±0.42	42±0.58	43±1.32	0.0476*
Hemicellulose (%)	31.1.53	19±0.29	20±0.58	0.0002*
Lignin (%)	6±0.58	7±0.29	7.5±0.29	0.0483*

\*: Significant at the level  $P < 0.05$

the final 3 were fed with NaOH treated straw which was freeze dried. At the end of 10 days, it was started collecting feces of sheep. At the end of 21 days the digestibilities of dry matter (D.M.) %, organic matter (O.M.) %, crude fat %, crude fiber %, crude protein % and N-free extract % were calculated in feces of sheep some of which were fed with untreated straw and some others were fed with NaOH treated straw. These chemical analysis were made according to Official Methods of Analysis of the Association of Official Analytical Chemists (A.O.A.C.) (5).

#### Method for In vitro Digestibility

For in vitro digestibility, modified method of Tilley-Terry and Brezezinski was used (6, 7). According to the methods, the samples of straw (0.5 g) were placed in glass centrifuge tubes of 3.5 cm diameter and 10 cm length. Then 50 ml rumen fluid solution was added. After sweeping  $\text{CO}_2$  over the inoculated substrate mixture the tubes were tightly closed with rubber caps. Then the tubes were incubated at 38°C in dark for 48 hours, being shaken gently 4 times a day. At the end of the fermentation, the tubes were centrifuged immediately for 15 min at 1900 r.p.m. After discarding the supernatant 50 ml of fresh made pepsin solution was added to the residue in each tube and the tubes were incubated at 38°C for 48 hours. At the end of the incubation, tubes were centrifuged and the supernatants were discarded. The residue which consists mainly of lignin was washed with water. Percent of digestibility was calculated after drying the residue at 105°C.

#### Method for in situ

Modified method of Van Keuren- Heinemann and

Orskov was used for calculating the in-situ digestibility (8, 9). According to the method, the nylon bags made of nylon sail cloth (60 microns mesh size) of equal dry weight to the nearest 2 g. were used. For this method, cattle was used. The cannulae was opened large enough to be able to easily insert the nylon bags into the rumen. Before placing the bags into the rumen all bags which consist sample of straw were wetted by placing in water in order to allow mixing of water with samples of straw. For both treated and untreated straw samples, one bag was washed to provide an estimate of readily removed solubles and small particles. Bags were consequently removed from the rumen of the cattle at different incubation time. Following removal from the rumen surface of each bag was gently washed with water to remove fluid from the bag surface and allowed to drain off water which was finally dried to constant weight at 70°C. In situ digestibility was calculated by subtracting weight of empty nylon bag from the total weight of the bag and residual sample.

#### Method for Statistical Analysis

One way and two way analysis of variance was used (10).

#### Results

The chemical composition of the untreated and the treated straws is shown in Table 1.

The lower Dry Matter (DM) content recorded with NaOH treated straw was compared with untreated straw.

Ash is higher for the NaOH treated compared with the

	Untreated straw	NaOH Treated straw (Dried at 60°C)	NaOH Treated straw (Dried at deep freeze)	P
Dry Matter Digestibility %	54.35±1.30	71.25±0.43	69.7±0.18	0.0000*
Organic Matter Digestibility %	56.05±1.47	72.00±0.83	71.30±0.72	0.0001*
Crude Fad Digestibility %	47.5±4.33	20.1±0.75	19.3±0.84	0.0004*
Crude Fiber Digestibility (%)	65.05±1.07	85.55±4.02	87.50±0.31	0.0010*
Crude Protein Digestibility (%)	13.14±2.88	86.16±4.14	86.07±3.71	0.0000*
N-free extract Digestibility %	50.20±1.73	52.50±0.58	50.30±0.95	0.3646

Table 2. In vivo digestibility of straw.

Three sheep per treatment.  
\*: Significant at the level P<0.05

	Untreated straw	NaOH Treated straw (Dried at 60°C)	NaOH Treated straw (Dried et deep freeze)	P
Organic Matter Digestibility %	45.68±0.12	75.13±0.06	73.83±0.12	0.0000*

Table 3. In vitro Digestibility of Straw

\*: Significant at the level of P<0.05

untreated straw.

Table 2. shows the the digestibility of Dry Matter, Organic Matter, Crude Fiber, Crude Protein, in vivo significantly higher for NaOH treated straw compared with untreated straw.

There was not so much change in crude fiber content although crude fiber digestibility increased by an avarage of 21.47% for in vivo (Table 2).

The in vitro organic matterial digestibility is shown in Table 3.

According to Table 3 the organic matter digetibility measured in vitro method showed significant increases with NaOH treatment.

In situ digestibility of untreated and treated straw is shown in Table 4.

Weight removal (%) in situ increased significantly in the straw treated with NaOH compared with the untreated control (Table 4).

### Discussion

The lower Dry Matter (DM) content recorded with NaOH treated straw has already been reported by others

being related to NaOH treatment (3, 11).

The higher ash content for the NaOH treated was compared with the untreated straw. Smilary Moss et al. (1993) showed an increase in Ash for NaOH treated straw (11). Sodium behaved in a similar fashion as ash where the increase in Na indicated the increase in ash.

The reduction in crude fiber content after treatment with NaOH was due to a reduction in hemicellulose which was constituted in the cell wall together with cellulose and lignin. During NaOH treatment lignin dissociated from the ligno-cellulosic complex but was still detected as lignin.

According to the findings of wannapat et. al. (1985), no significant change was observed in crude fiber while crude fiber digestibility is increasing in vivo. The researcher explained the increase as being due to solubizlization of hemicellulose, increasing the extent rate of digestion of cellulose and the remaining hemicellulose (12).

According to table 2., digestibility of straw in vivo was significantly higher for NaOH treated straw compared with untreated straw. Smilary Moss et al. (1993) showed an increase of organic matter and dry matter digestibility in vivo for treated straw compared with untreated straw (11).

Table 4. In situ digestibility of straw.

Type of straw	Weight Removal (%)						
	Time (hr.)						
	4	8	16	24	48	72	96
Untreated	2.6±0.01	14.9±0.35	10.2±0.40	16.5±0.25	31.6±0.15	36.9±1.05	39.0±0.50
NaOH Treated (Dried at 60°C)	12.1±0.21	18.2±0.49	23.3±0.25	28.9±0.53	49.3±0.75	51.1±0.68	58.5±0.76
NaOH Treated (Deep freeze)	9.9±0.10	19.0±1.53	20.02±0.61	28.1±0.95	46.3±0.65	54.5±0.50	58.7±0.91

Significant at the level of  $p < 0.05$

The organic matter digestibility measured in vitro method showed significant increases with NaOH treatment. This has been also noted by Moss et al. (1993) (11). The organic matter digestibility contents in vivo for NaOH treated samples were an average of 71,65%, respectively whilst the organic matter digestibility in vitro were considerably higher, namely 74,5%. Evidence from work done by Berger et al. (1990) showed that lambs fed with diets at increasing levels of NaOH had increased rates of passage of a chromic oxide marker and decreased ruminal retention times compared with when they were fed with the control diet (13). The increased chromic oxide flow was assumed to indicate reduced retention time of potentially digestible fibre. The mean ruminal retention times were considerably shorter than the 48h used in the Tilley and Terry (1963) rumen fluid-pepsin in vitro digestibility technique which may partially explain the higher in vitro digestibility compared with that measured in vivo (7). Lower levels of Crude Fiber digestibility observation in vivo had been noted by other workers who gave the explanation that increased water intake owing to sodium levels diluted the bacterial population hindering substrate-enzyme contact and hence reducing fibre digestion (14).

There is also some evidence that soluble components such as ferulic and p-coumaric acid may be inhibitory to digestion (15). The observations made in vitro do not, however, establish whether inhibitory effects of soluble phenolics occur in vivo but should be considered a possibility. Another possibility to explain the slow rates of digestion observed in treated straw in vivo is that the high levels of solubilized lignin have the effect of removing much of the ionic structures from the plant cell wall, reducing the cation exchange capacity of the matrix which

affects the hydratability of the cell wall surface, and probably microbial attachment and induction of fermentation (16). All these factors may act together when NaOH applied to straw to give lower levels of digestibility in vivo compared with that in vitro.

In situ technique is a very useful tool when studying specific diet effects on the rumen environment, which may affect normal fermentation (16). These effects can be difficult to determine when using an in vitro method. Two of the problems involved with the in situ technique to measure Dry matter or fibre digestibility are build-up fermentation end-products in the bag and in-flow and out-flow of material through the pores. This work has shown that the treatment of straw with NaOH increased the digestibility of straw. Similarly Tuncer et al., (1986) showed an increase of digestibility of NaOH treated straw compared untreated straw (17). This upgrading had, however, considerable variability and appeared to be dependent on method of application and quantity of NaOH applied. Additionally the experiment was designed to compare the measurement of digestibility methods and it was found that in vitro digestibility method is the most suitable one for accurately predicting when compared with others.

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