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In vitro First Order Kinetic Disappearance of Dry Matter and Neutral Detergent Fiber of Chemically and Physically Treated Cottonseed Hulls

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Abstract: An *in vitro* experiment was conducted to determine the effect of chemical or physical treatments on disappearance kinetics of dry matter, Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) of Cottonseed Hulls (CH). For chemical treatment, CH were treated with NaOH as 20 g kg^{-1} DM (a 20% solution of NaOH was sprayed on CH and kept for 0.5 h (CH2S0.5) or 48 h (CH2S48)) or 40 g kg^{-1} DM (a 40% solution of NaOH was sprayed on CH and kept for 0.5 h (CH4S0.5) or 48 h (CH4S48) at room temperature). Physical processing was done using microwave irradiation (900 W) for 4, 6 and 8 min (CHm4, CHm6, CHm8, respectively). Fifty-five mL of medium was supplied into 100 mL bottle containing 0.45 g of each sample DM (4 replicates). Then, each bottle was inoculated under carbon dioxide with 5 mL of mixed rumen microbes 24, 48, 72 and 96 h. Then, contents of each bottle were filtered through a 22 μm filter, DM and Neutral Detergent Fiber (NDF) of the unfiltered medium was determined. Kinetic disappearance parameters were determined using first order exponential model of $D_{(t)} = D_{(i)}.e^{(-k.\ time)} + I$; $D_{(t)}$ is potentially digestible residues, $D_{(i)}$ is potentially digestible fraction, k is fractional rate constant of digestion (h^{-1}) and I is indigestible fraction. Chemical or physical treatment improved DM disappearance significantly ($p<0.05$). There was no significant difference in disappearance of NDF between microwave irradiated CH for 4, 6 and 8 min. Microwave irradiation for 8 min at 48 and 72 h incubation showed the highest ADF disappearance among the treatments. Physical or chemical treatment had no significant effect on first order DM disappearance of CH.

Key words: Cottonseed hulls, disappearance, microwave irradiation, neutral detergent fiber, associated lignin

INTRODUCTION

Limited supplies and/or high costs of conventional roughages and concentrates dictate that alternate sources of feed be utilized if certain handling and nutritional aspects of such feeds can be resolved (Brown *et al*., 1977). Cottonseed Hulls (CH) are a by-product of cotton processing, containing a large proportion of Neutral Detergent Fiber (NDF) and associated lignin and have been considered as a useful non-forage fibre source in ruminant rations (Hall and Akinyode, 2000). Improvement of low quality forages with NaOH has received a great deal of attention (Canale *et al*., 1988). The close physical and chemical association between cellulose, plant cell wall matrix sugars (primarily hemicellulose) and lignin, as well as the crystalline arrangement of native cellulose polymers, prevent rapid and extensive degradation of plant structural carbohydrates by cellulolytic micro-organisms in the ruminant gastrointestinal tract (Northcote, 1972; Bailey, 1973; Van Soest, 1973; Cowling, 1975). Haddad *et al*. (1998) showed that inclusion of wheat straw treated with 3% NaOH plus 3% $Ca(OH)_2$ at up to 40% of the dietary DM had no effect on ruminal pH, osmolality, in situ digestion kinetics of the NDF of wheat straw, or ruminal VFA and NH_3 concentrations. Lesoing *et al*. (1981) concluded that *in vitro* Dry Matter Disappearance (IVDMD) of wheat straw was increased 29% by chemical treatment with 1% NaOH plus 3% $Ca(OH)_2$ and by as much as 86% by a 4% NaOH plus 1% $Ca(OH)_2$ treatment. Kerley *et al*. (1986) demonstrated that digestibilities of DM, NDF and cellulose were higher ($p<0.05$) when sheep were fed alkaline hydrogen peroxide treated wheat straw diets than when fed the non-treated wheat straw diets.

Microwave energy is non-ionizing and causes a rise in the temperature within a penetrated medium as a result of rapid changes of the electromagnetic field. Heat is generated throughout the material, leading to faster heating rates and shorter processing times compared with conventional heating, where heat is usually transferred

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from the surface to the interior. Sadeghi and Shawrang (2007) showed a decrease in water soluble fraction and degradation rate but an increase in the potentially degradable fraction of crude protein of cottonseed meal by microwave irradiation. They also showed that irradiation for 2-4 min increased ($p<0.05$) intestinal digestibility of ruminally undegraded crude protein but extending to 6 min lowered ($p<0.05$) the beneficial effect that 4 min had on intestinal crude protein digestibility. Microwave irradiation of barley grain for 3 min increased ruminal starch degradability and over 5 min, decreased rate and extent of CP and starch degradation. The decrease in CP degradation increased as microwave irradiation time increased.

The aim of this experiment was to determine the effect of chemical and physical treatments on disappearance kinetics of dry matter, neutral detergent fiber and acid detergent fiber of cottonseed hulls.

MATERIALS AND METHODS

Cottonseed hull samples and treatment procedures: Cottonseed hull samples were collected from industries located in north east, Iran. Samples were treated chemically using NaOH. Cottonseed hulls were treated as 20 g kg^{-1} DM (a 20% solution of NaOH was sprayed on CH and kept for 0.5 h (CH2S0.5) or 48 h (CH2S48)) or 40 g kg^{-1} DM (a 40% solution of NaOH was sprayed on CH and kept for 0.5 h (CH4S0.5) or 48 h (CH4S48) at room temperature). Physical processing was done using microwave irradiation (900 W) for 4, 6 and 8 min (CHm4, CHm6, CHm8, respectively).

***In vitro* procedure:** The fermentation medium was prepared according to that described by Arroquy *et al.* (2005), included 400 mL cell-free ruminal fluid, cellobiose (0.05 g), K_2HPO_4 (0.45 g), KH_2PO_4 (0.45 g), NaCl (0.90 g), $(NH_4)_2SO_4$ (0.90 g), $MgSO_4 \cdot 7H_2O$ (0.09 g), $CaCl_2$ (0.09 g), resazurin (0.01 g), $NaHCO_3$ (4 g) and cysteine-HCl (0.5 g) per liter of medium. Rumen fluid was obtained from three sheep (49.5±2.5 kg body weight) fitted by rumen fistulae, before the morning feeding (The animals were fed 1 kg day^{-1} of DM alfalfa hay and 0.3 kg day^{-1} DM concentrate (165 g CP kg^{-1} DM)) and immediately strained through four layers of cheesecloth and centrifuged at 3000 rpm for 5 min. Then, the supernatant was centrifuged at 15000 rpm for 15 min. Forty-five milliliter of medium were distributed into a 100 mL bottle containing each experimental sample and autoclaved at 120°C for 20 min. Then, each bottle was inoculated with 5 mL of cloth-cheese strained rumen fluid and finely bubbled with CO_2, sealed and incubated. Previous to the inoculation, the rumen fluid was incubated for 1 h in an incubation chamber at 39°C (to allow large feed particles to rise to the top) and in time introducing inoculum taking care not to include the large particles that rose to the top nor that which sedimented in the bottom and was introduced anaerobically into the fermentation bottles. Chemically and physically treated cottonseed hulls were incubated for 24, 48, 72 and 96 h at 39°C (3 bottles per each sample and each time, n = 96). After each incubation, bottle contents were filtered through a 22 μm filter paper. Unfiltered samples were analyzed for DM (using air forced oven at 80°C, 48 h), ADF and NDF as described by Van Soest (1973).

Statistical analysis: Data of DM, NDF and ADF disappearance of the samples were analyzed using a complete block design model as:

$$Y_{ijk} = \mu_i + B_j + T_k + e_{ijk}$$

Where:

Y_{ijk} = The dependent variable
B_j = Effect of time
T_k = Effect of treatment (chemical or physical procedure)
e_{ijk} = Experimental error

A non-linear first order model was used to estimate the disappearance kinetic parameters of DM. The model was:

$$D_{(t)} = D_{(i)} \cdot e^{(-k \cdot time)} + I$$

Where:

$D_{(t)}$ = Residual at any time
$D_{(i)}$ = Potentially digestible fraction
k = Fractional rate constant of digestion (h^{-1})
I = Indigestible fraction

RESULTS AND DISCUSSION

Dry matter disappearance: Extent of dry matter disappearance of chemically and physically treated CH is shown in Fig. 1 and 2, respectively. Chemical or physical treatment improved DM disappearance significantly ($p<0.05$). There was no significant difference between DM disappearances of chemical or physical treatment at 24 h incubation.

It is shown in Fig. 1 that CH2S48 at 48 h incubation demonstrated a slight decrease in disappearance among chemical treatments. Microwave irradiation for 8 min showed the highest DM disappearance at 96 h incubation. There was no significant difference among chemical as for physical treatments, separately. Ghebriel *et al.* (1981) ensiled sorghum silage with

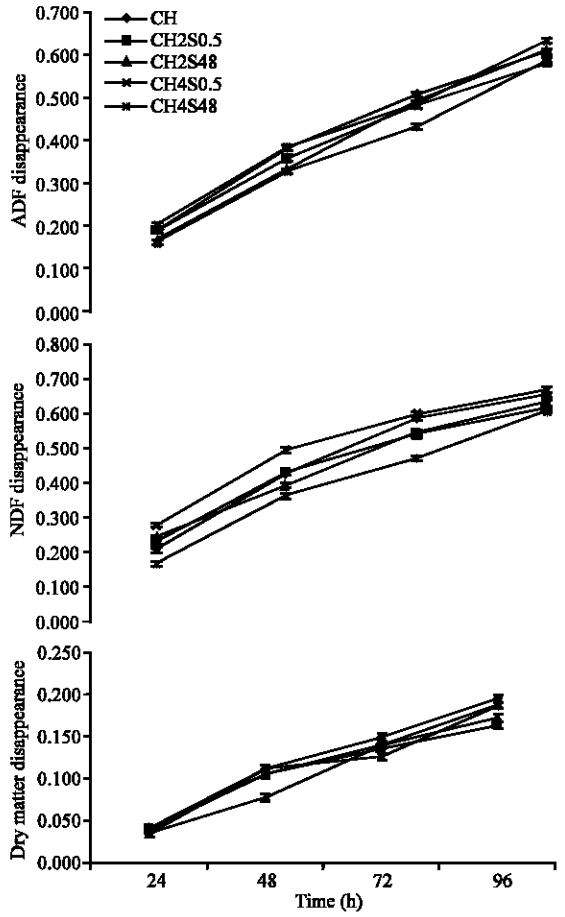

Fig. 1: Dry matter, NDF and ADF disappeaqrance values of sodium hydroxide treated Cottonseed Hulls (CH: untreated cottonseed hulls; CH2S0.5: Sodium hydroxide treated cottonseed hulls as 20 g kg^{-1} DM and kept for 0.5 h; CH2S48: Sodium hydroxide treated cottonseed hulls as 20 g kg^{-1} DM and kept for 48 h; CH4S0.5: Sodium hydroxide treated cottonseed hulls as 40 g k^{-1} DM and kept for 0.5 h; CH4S48: sodium hydroxide treated cottonseed hulls as 40 g kg^{-1} DM and kept for 48 h); p-value: Time effect: Dry matter ($p<0.05$), NDF ($p<0.05$) and ADF ($p<0.05$); Treatment effect: Dry matter ($p<0.05$), NDF ($p<0.05$) and ADF ($p<0.05$)

NaOH as 40 g kg^{-1} and reported that *in vitro* dry matter disappearance of sorghum silage increased (37%) by addition of NaOH. Canale *et al.* (1988) performed an *in vivo* study with NaOH (40 g kg^{-1} of forage DM) treated alfalfa and orchardgrass and concluded that sodium hydroxide treatment increased the proportion of potentially digestible DM and NDF compared with that of untreated forage.

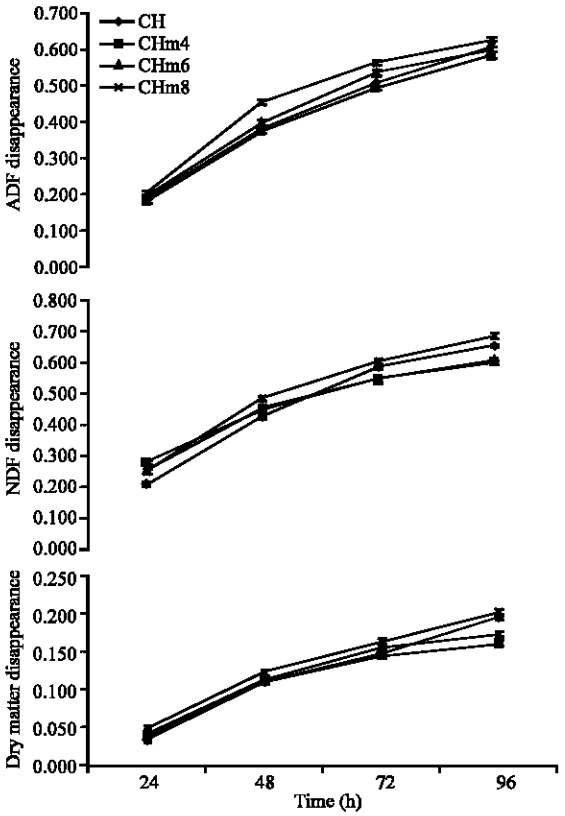

Fig. 2: Dry matter, NDF and ADF disappearance values of microwave irradiated cottonseed hulls (CH: Untreated Cottonseed Hulls; CH2S0.5: Sodium hydroxide treated cottonseed hulls as 20 g kg^{-1} DM and kept for 0.5 h; CH2S48: Sodium hydroxide treated cottonseed hulls as 20 g kg^{-1} DM and kept for 48 h; CH4S0.5: Sodium hydroxide treated cottonseed hulls as 40 g k^{-1} DM and kept for 0.5 h; CH4S48: SODIUM hydroxide treated cottonseed hulls as 40 g kg^{-1} DM and kept for 48 h); p-value: Time effect: Dry matter ($p<0.05$), NDF ($p<0.05$) and ADF ($p<0.05$); Treatment effect: Dry matter ($p<0.05$), NDF ($p<0.05$) and ADF ($p<0.05$)

Neutral detergent fiber disappearance: Data of neutral detergent fiber disappearance of chemically and physically treated CH is shown in Fig. 1 and 2, respectively. Chemical or physical treatment caused an increase in NDF disappearance of CH in each time of incubation. Chemical Treatment of CH for 48 h compared with 30 min significantly improved the disappearance of NDF ($p<0.05$). There was no significant difference in disappearance of NDF between microwave irradiated CH for 4, 6 and 8 min. Haddad *et al.* (1994) compared $Ca(OH)_2$, NaOH, NH_4OH and urea at different levels and combinations and reported that the largest *in vitro* and

Table 1: Non-linear first order parameters of *in vitro* DM disappearance of non treated or chemically and physically treated cottonseed hulls

	Treatments**									
Items*	CH	CH2S0.5	CH2S48	CH4S0.5	CH4S48	CHm4	CHm6	CHm8	SEM	p-value
Di	0.310	0.310	0.260	0.340	0.350	0.300	0.260	0.290	0.088	>0.05
Kd	0.007	0.016	0.011	0.006	0.009	0.008	0.011	0.010	0.005	>0.05
I	0.140	0.140	0.190	0.110	0.110	0.150	0.190	0.160	0.091	>0.05
R2	0.970	0.930	0.980	0.960	0.960	0.980	0.970	0.970		

**CH: Untreated Cottonseed Hulls; CH2S0.5: Sodium Hydroxide treated cottonseed hulls as 20 g kg^{-1} DM and kept for 0.5 h; CH2S48: Sodium Hydroxide treated cottonseed hulls as 20 g kg^{-1} DM and kept for 48 h; CH4S0.5: Sodium Hydroxide treated cottonseed hulls as 40 g k^{-1} DM and kept for 0.5 h; CH4S48: Sodium Hydroxide treated cottonseed hulls as 40 g kg^{-1} DM and kept for 48 h); *D_i = Potentially digestible fraction; K_d = Fractional rate constant of digestion; I = Indigestible fraction; R^2 = Coefficient of determination

in vivo NDF digestibilities were observed with 5% NaOH combined with 2.5% $Ca(OH)_2$. The authors also concluded that $Ca(OH)_2$ apparently enhanced fiber digestion at low pH. Berger *et al*. (1979) showed that the increasing levels of NaOH treatment caused marked decreases in the diet NDF but only slight decreases in ADF concentration.

Acid detergent fiber disappearance: The extent of acid detergent fiber disappearance of chemically and physically CH is shown in Fig. 1 and 2, respectively. Disappearance of ADF among physically and chemically treated CH was not statistically different at 24 and 96 h incubation. Microwave irradiation for 8 min at 48 and 72 h incubation showed the highest ADF disappearance among the treatments ($p<0.05$). Haddad *et al*. (1994) resulted that the ADF content of wheat straw was not greatly affected by chemical treatment. These results agree with Jackson (1977), who summarized >100 research papers dealing with alkali treatment of low quality forages and concluded that NDF content was decreased by alkali treatment, whereas the ADF content remained the same. Waller (1976) found that 18.0% of the corn cob hemicellulose was solubilized by 4.0% NaOH treatment with no reduction in ADF or Acid Detergent Lignin (ADL).

Non-linear first order dry matter disappearance: Non-linear first order parameters of *in vitro* DM disappearance of the samples are presented in Table 1. Results of the present study indicated that non-linear first order parameters of *in vitro* DM disappearance of CH were not influenced by the chemical or physical processing. It was previously indicated that DM digestion of forage might be affected by NaOH treatment (Canale *et al*., 1985). Lesoing *et al*. (1981) showed that *in vitro* dry matter disappearance of NaOH-treated wheat straw was significantly greater than untreated. Haddad *et al*. (1998) showed that inclusion of up to 20% wheat straw treated with 3% NaOH plus 3% $Ca(OH)_2$ in diets of lactating cows resulted in ruminal function and performance that were similar to those of cows fed diets that contained alfalfa haylage only. There are indications that the response to NaOH treatment depends on the type of roughage. Summers and Sherrod (1975) treated sorghum stover, forage sorghum hay, CH and peanut hulls with NaOH and found that digestibility of peanut hulls, CH and forage sorghum hay was not improved.

Sadeghi and Shawrang (2008) demonstrated that microwave irradiation increased ($p<0.05$) the washout fraction and decreased ($p<0.05$) the potentially degradable fraction and degradation rate of starch. They also showed that microwave irradiation for 2 and 4 min increased *in vitro* CP digestibility of ruminaly undegraded canola meal at 8 and 12 h pre-incubation (linear effect, $p<0.001$) and 24 h pre-incubation (quadratic effect, $p<0.001$). Microwave irradiation for 6 min decreased *in vitro* digestibility of CP compared to 4 min.

CONCLUSION

Chemical or physical treatment can improve DM disappearance. Microwave irradiation for 8 min showed the highest DM disappearance at 96 h incubation. Chemical or physical treatment caused an increase in NDF disappearance of CH in each time of incubation. Acid detergent fiber disappearance of CH among treatments was not significantly different. Results of the present study indicated that non-linear first order parameters of *in vitro* DM disappearance of CH were not influenced by the chemical or physical treatment.

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REFERENCES

Arroquy, J.I., R.C. Cochran, T.G. Nagaraja, E.C. Titgemeyer and D.E. Johnson, 2005. Effect of types of non-fiber carbohydrate on *in vitro* forage fiber disappearance of low-quality grass hay. Anim. Feed Sci. Technol., 120: 93-106.

Bailey, R.W., 1973. Structural Carbohydrates. In: Chemistry and Biochemistry of Herbage, Butler, G.W. and R.W. Bailey (Eds.). Academic Press, New York, pp: 157-211.

Berger, L.T., T. Klopfenstein and R. Britton, 1979. Effects of sodium hydroxide on efficiency of rumen digestion. J. Anim. Sci., 49: 1317-1323.

Brown, W.H., F.M. Whiting, B.S. Daboll, R.J. Turner and J.D. Schuh, 1977. Pelleted and nonpelleted cottonseed hulls for lactating dairy cows. J. Dairy Sci., 60: 919-923.

Canale, C.J., S.M. Abrams, L.D. Muller, W.L. Kjelgaard, P.M. Anderson and H.W. Harpster, 1988. Alkali treated forage for early lactation dairy cows: Effect on lactation performance and nutrient digestibility. J. Dairy Sci., 71: 2166-2174.

Cowling, E.G., 1975. Physical and chemical constraints in the hydrolysis of cellulose and lignocellulosic materials. Biotechnol. Bioeng. Symp., 5: 163-181.

Ghebriel, A.W., D.W. Kellogg and D.D. Miller, 1981. Effect of sodium hydroxide treatment of sorghum silage on lactation of cows and on *in vitro* digestion. J. Dairy Sci., 64: 792-797.

Haddad, S.G., R.J. Grant and S.D. Kachman, 1998. Effect of wheat straw treated with alkali on ruminal function and lactational performance of dairy cows. J. Dairy Sci., 81: 1956-1965.

Haddad, S.G., R.J. Grant and T.J. Klopfenstein, 1994. Digestibility of alkali-treated wheat straw measured *in vitro* or *in vivo* using Holstein heifers. J. Anim. Sci., 72: 3258-3265.

Hall, M.B. and A.M. Akinyode, 2000. Cottonseed hulls: Working with a novel fiber source. Proceedings of the 11th Annual Florida Ruminant Nutrition Symposium, Jan. 13-14, Gainesville, Florida, pp: 179-186.

Jackson, M.G., 1977. Review article: The alkali treatment of straw. Anim. Feed Sci. Technol., 2: 105-130.

Kerley, M.S., G.C. Jr. Fahey, L.L. Berger, N.R. Merchen and J.M. Gould, 1986. Effects of alkaline hydrogen peroxide treatment of wheat straw on site and extent of digestion in sheep. J. Anim. Sci., 63: 868-878.

Lesoing, G., T. Klopfenstein, I. Rush and J. Ward, 1981. Chemical treatment of wheat straw. J. Anim. Sci., 51: 263-269.

Northcote, D.H., 1972. Chemistry of the plant cell wall. Annu. Rev. Plant Physiol., 23: 113-132.

Sadeghi, A.A. and P. Shawrang, 2007. Effects of microwave irradiation on ruminal protein degradation and intestinal digestibility of cottonseed meal. Anim. Feed Sci. Technol., 106: 176-181.

Sadeghi, A.A. and P. Shawrang, 2008. Effects of microwave irradiation on ruminal dry matter, protein and starch degradation characteristics of barley grain. Anim. Feed Sci. Technol., 141: 184-194.

Summers, C.B. and L.B. Sherrod, 1975. Sodium hydroxide treatment of different roughages. J. Anim. Sci., 41: 420-420.

Van Soest, P.J., 1973. The uniformity and nutritive availability of cellulose. Fed. Proc., 32: 1804-1808.

Waller, J.C., 1976. Evaluation of sodium, calcium and ammonium hydroxides for treating crop residues. M.Sc. Thesis, University of Nebraska, Lincoln.