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Effects of Treatment of Sorghum Stover Residue With Ammonium Hydroxide on Cell Wall Composition and *in vitro* Digestibility

Adnan B. Yousuf[1], A. Dismuke[1], M. Kering[1] & A. Atalay[1]

[1] Agriculture Research Station, Virginia State University, Virginia, USA

Correspondence: Adnan B. Yousuf, Agriculture Research Station, Virginia State University, P. O. Box 9061, Virginia 23806, USA. Tel: 1-804-524-6795. E-mail: ayousuf@vsu.edu

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Abstract

The study was undertaken to determine the effects of ammonium hydroxide (NH_4OH) treatment of sorghum stover residue on composition and in vitro dry matter disappearance (IVDMD) of two sorghum varieties, Dale (tall, sweet sorghum variety) and Brown mid rib (BMR) (Short, grain sorghum variety). The residue was treated with; water only (T00), 50 g NH_4OH kg^{-1} residue dry matter (DM) (T50), 100 g NH_4OH kg^{-1} DM (T100), and untreated control (neither water nor NH_4OH) (TUN) and allowed to react for one week before chemical analysis was performed. The fiber content (ADF and NDF) were not affected by the levels of alkali treatment but the crude protein (CP) and Soluble protein contents were both increased by alkali treatment. There was an increase ($P < 0.05$) in dry matter digestibility in vitro (IVDMD) by NH_4OH treatment from 529 g kg^{-1} to 651 g kg^{-1} in T50 and T100. The improvement in IVDMD may indicate that NH_4OH helps disrupt the lignin–carbohydrate complexes. The development of more economical and safe procedures which improve digestibility of the structural cell wall components would be very beneficial for improving the use of crop residue as feedstock for livestock and bioethanol production.

Keywords: sorghum stover, ammonium hydroxide, alkali treatment, IVDMD

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench), is a warm season grass that does well in a variety of soils and withstands hot and dry conditions. There are diverse species of sorghum that are grown for grain, biofuel, and forage. As a forage crop, most interest in sorghum has been concentrated on hybrid varieties, brown midrib genotypes, and sorghum-sudangrass (Houx III et al., 2013). This variety has distinct morphological characteristics including extensive root system, smaller leaf area with a waxy coating that limits water loss; it is the most widely adapted species among grasses for biomass and fuel production (Hons et al., 1986). The sweet sorghum varieties have been bred primarily for syrup production. Because of their high sugar content and large biomass yield, sweet sorghums have attracted the attention of researchers who have evaluated them as biofuel crops (Smith et al., 1987; Putnam et al., 1991; Rooney et al., 2007). If it is produced for biofuel, the acreage of sweet sorghum would likely increase. The sweet sorghum varieties tend to have higher yield, with lower lignin and higher digestibility than grain sorghums (Houx III et al., 2013). In the US, out of a total of 3.2 million hectares under sorghum in 2013, 2.6 million hectares were harvested for grain (USDA, 2014).

Unlike corn, sorghum continues to synthesize new vegetative material even after physiological maturity and its stover forage quality decreases at a lower rate (Bolsen et al., 1977). The left over residual material may have high lignin content and low forage quality, but its protein level is reported to remain above the minimum needed for gestating cow (Ward et al., 1979). In vitro dry matter disappearance (IVDMD) of sorghum stover was reported to be sufficient for cattle maintenance energy needs (Youngquist et al., 1990) and could offer maintenance energy to animals especially during period of forage deficits. Crop residues are poorly digested by ruminants because of their high cell-wall content. While forage sorghums can currently be grazed or hayed, residual stover from other sorghum types can be used as alternative hay source for animal.

The increasing global population will lead to increased demand for food and it is estimated that between 2003 and 2020, demand for livestock products will double (Delgado et al., 1999). To meet increased food demand with current agricultural land acreage, there is a need for new approaches on crop and livestock management strategies that include alternative forage sources. While genetic improvements may increase animal productivity, decreased availability of arable land may reduce acreage for forage production and lead to shortage of animal feed. Furthermore, increased erratic rainfall and drought incidences, production acreage of moisture stress tolerant crops like sorghum is likely to increase. Grain sorghum is produced for seed while sweet sorghums are produced to provide sugary juice for bioethanol plants. Both operations leave behind crop residual material that can be used for forage. Given current global climate-change prediction that paints a future dry and hot climate, these crops may be the choice of the future and therefore also a potential source of residual material for forage.

Meeting future animal forage demands will be challenging and alternative feed sources should be sought. One such alternative is crop residue; the non-edible plant parts that are left in the field after crops have been harvested and thrashed. Some researchers also include remains generated from crop-packing plants or that are discarded during crop processing (Ernest & Buffington, 1981). Despite their low digestibility, metabolizable energy, and mineral element contents and generally low forage quality (Nicholson, 1984; Doyle et al., 1986), crop residues have provided alternative forage in many parts of the world.

The forage quality of plant residual materials can be improved by use of chemicals. The chemical treatment of straws and other crop residues can increase the digestibility of the material for ruminants (Klopfenstein, 1978); it can be accomplished through the use of either alkaline compounds (Waiss & Guggolz, 1972; Rounds & Klopfenstein, 1974; Sundstol, 1984; Mason et al., 1990a; Goto et al., 1993) or oxidizing compounds (Ben-Ghedalia et al., 1980; Ben-Ghedalia & Miron, 1981; 1984; Bunting et al., 1984; Kerley et al., 1987; Amjed et al., 1992; Sultan et al., 1992).

The mode of action of alkaline compounds consists of a partial hydrolysis of the cell wall, which ruptures the ester bonds between hemicellulose and lignin without removing the latter (Jackson, 1977; Klopfenstein, 1978). Treatments with alkaline chemicals diesterifies lignin and xylan to release cellulose and hemicellulose and make them available for enzymatic breakdown (Sun & Cheng, 2002; Saha & Cotta, 2008; McIntosh & Vancov, 2010). Oxidizing agents or acids seem to cause a complete solubilization of hemicellulose and a reduction in lignin content of the treated material, possibly creating hollow spaces within the cellulose matrix that makes the cell walls more accessible to ruminal microbial action (Shefet & Ben-Ghedalia, 1982). By releasing cellulose and hemicellulose, such chemical treatment increases the forage quality of highly lignified plant material. In fact, ammonium pre-treatment of sorghum stover was reported to increase intake and degradation of sorghum stover in sheep (Ben Salem et al., 1994). This study was undertaken to determine the effects of alkali (ammonium hydroxide) treatment of residual material from grain- and sweet-sorghum production on composition and IVDMD.

2. Materials and Methods

2.1 Location of Study

The experiment was conducted at the Randolph Research Farm of Virginia State University (VSU) Small Ruminant Research Facility, located in the Tri-Cities area of Central Virginia (37.1° N; 77.3° W) at an elevation of 45 m above sea level.

2.2 Sorghum Stover

The study involved the use of stover residue recovered from two sorghum varieties; brown mid rib (BMR), short grain sorghum variety and Dale, a tall sweet sorghum variety. The residue from the two varieties was obtained from ongoing research projects at VSU Agriculture Research Station and included the leaves and stems obtained after the seed or panicle was harvested. The stem and leaves were cut into small pieces and dried to a constant weight in a conventional oven set at 60 °C. The dried Stover residue was ground through a 2 mm screen in a Willey Mill (Arthur A. Thomas Co., Philadelphia, PA).

Samples of ground stover (100 g DM) were placed in plastic bags and water was added to give a 50% DM. The treatments were (TUN) no ammonium hydroxide, (T00) water only, T50 and T100, 50 and 100 g $NH4OH$ per kg residue dry matter respectively. Each treatment was done in triplicates. The bags were immediately closed, the contents mixed thoroughly and allowed to react for seven days at room temperature (21 to 23 °C). After a week, all bags were removed and air dried for 48 h.

2.3 Sample Analyses

All samples were assayed for dry matter (DM), crude protein (CP), soluble protein (SolProt), acid detergent fiber (ADF), neutral detergent fiber (NDF), neutral detergent insoluble protein (NDIP), acid detergent insoluble/heat damaged protein (ADIPHD), starch, lignin, ash and IVDMD by ANALAB, Division of Agri-King, Inc.

2.4 Statistical Analyses

Data among treatments in composition and in vitro dry matter disappearance were analyzed using the General Linear Model procedure of SAS (2009). The model statement included treatment, variety and treatment × variety (Interaction) effects. When analysis revealed significant differences among treatment means, the Least Significant Differences ($P \leq 0.05$) were used to separate means among treatments and varieties.

3. Results and Discussion

Chemical composition of the stover residue from the BMR (Short) and Dale (Tall) varieties is given in Table 1. The CP (N X 6.25), NDF and ADF content ranged between 32-50, 389-616, and 255-372 g kg^{-1} DM, respectively, and were relatively higher in the BMR than in Dale. The Short variety contained higher ADF, NDF, Lignin and lower IVDM (501 vs 575 g kg^{-1} DM).

Table 1. Chemical composition of residue of two sorghum varieties

Item	Variety	
	BMR (Short) (g kg^{-1}DM)	Dale (Tall) (g kg^{-1}DM)
DM[1]	923.6	891.5
OM[2]	931.5	957.2
CP-[3]	50.3	32.4
Solprot[4]	222.2	224.6
ADIP_HD[5]	6.6	3.1
ADF[6]	372.3	255.1
NDF[7]	616.3	389.2
Lignin	53.0	45.2
Ash	68.5	42.8
Starch	33.4	106.2
IVDMD[8]	500.6	575.2

[1] DM, Dry matter; [2] OM, Organic matter; [3] CP, Crude protein; [4] Solprot, Soluble protein; [5] ADIP/HD, Acid detergent insoluble protein or Heat damaged protein; [6] ADF, Acid detergent fiber; [7] NDF, Neutral detergent fiber; [8] IVDMD, In vitro dry matter disappearance.

3.1 In vitro Dry Matter Disappearance and Crude Protein

There was no treatment × variety interaction ($P > 0.05$) in the IVDMD of the sorghum stover residue treated with NH_4OH. Ammonium hydroxide treatment but not its level increased ($P < 0.05$) the mean IVDMD by 25%, from 530 to 660 g kg^{-1} DM (Figures 1a and 1b). The IVDMD of the residue from the two varieties was affected differently by the levels of NH_4OH treatment. Ammoniation increased IVDMD of the residue in the tall variety 14 % over the short variety (550 to 630 g kg^{-1} DM). The tall variety appears to be more digestible compared to the short variety (635 vs 550 g kg^{-1} DM). The increase in IVDMD agrees with those of Hartley and Jones (1978) and Soleiman et al. (1979) although the increments obtained in this study were slightly higher.

Ammonium hydroxide treatment increased CP (N X 6.25) in sorghum residue in both varieties ($P < 0.05$) but did not affect CP similarly across varieties and treatments as indicated by treatment × variety interaction ($P <0.05$) (Table 2). Untreated sorghum residue CP in the short variety was 50 g kg^{-1} DM where as in the tall variety it was 32 g kg^{-1} DM (Table 2). When it was treated with 50 g kg^{-1} DM NH_4OH, the CP increased two folds in the short variety and three fold in the tall variety. Treatment with 100 g kg^{-1} DM NH_4OH further increased the CP three fold in the short variety from 50 to 133 and four fold in the tall variety from 32 to 138 g kg^{-1} DM. Similar effects of increased CP in poor quality forages as a result of ammoniation were reported in wheat straw (Soleiman et al, 1979), Barley straw (Graham & Aman, 1984), limpograss (Brown et al., 1987), and tall fescue straw (Kallenbach et al., 2006). The increased CP observed can be explained by both the nitrogen added and the ammonolysis reaction between the carbohydrates and the alkali as previously reported (Dryden & Kempton, 1983; Alibez et al., 1984; Mason et al., 1990b; Kondo et al., 1992).

There was no treatment × variety interaction ($P > 0.05$) in the soluble protein (Solprot) which is the fraction of protein rapidly degraded to ammonia in the rumen. There was, however, varietal as well as treatment difference ($P < 0.05$) in soluble protein (Figures 1c and 1d). The tall variety contained more soluble protein compared to the short (42.56 vs 33.57 g kg^{-1} DM). Treatment with NH_4OH at both levels resulted in similar soluble protein content compared to the untreated residue (T50 = 58.35; T100 = 63.01 g kg^{-1} DM) suggesting that the higher treatment may not have beneficial effects over that of the lower level.

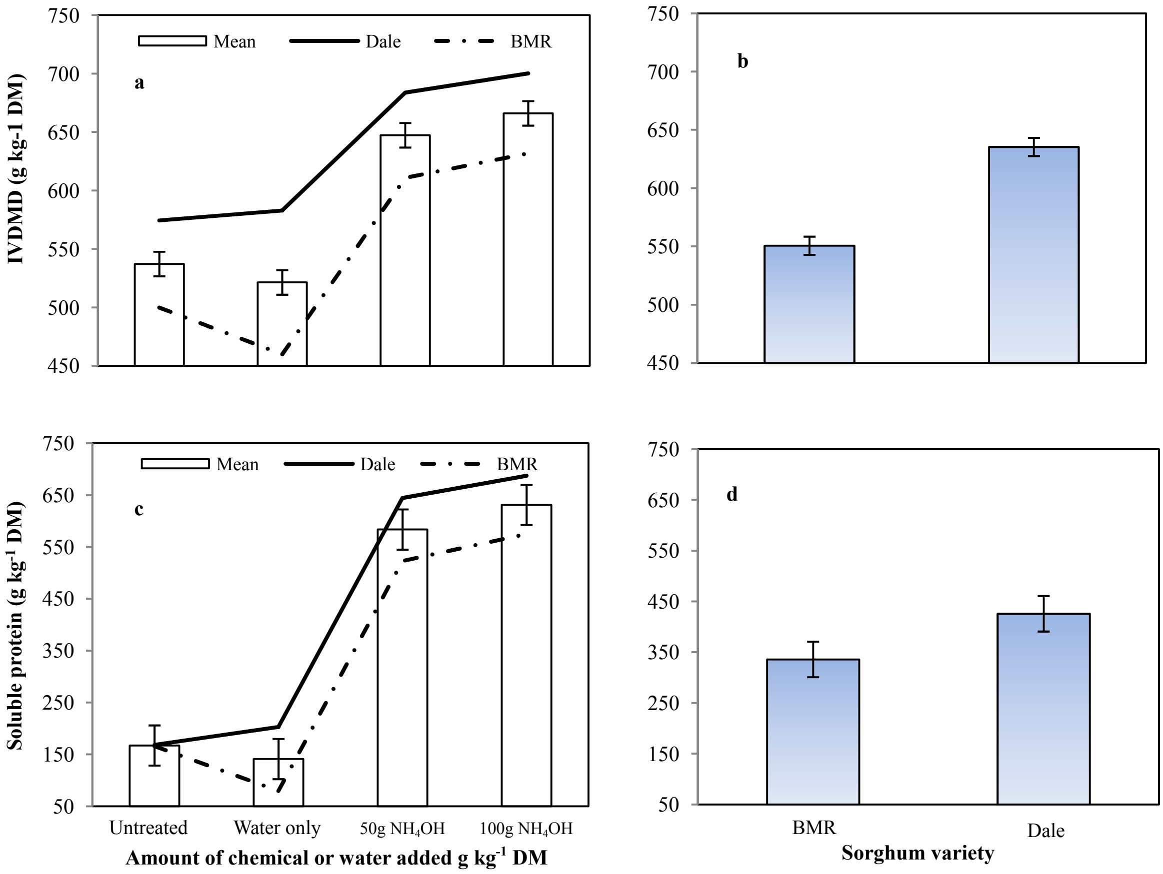

Figure 1. In vitro dry matter digestibility IVDMD and soluble protein as affected by treatments (a) and (c) and by sorghum variety (b) and (d), respectively

Acid detergent insoluble/heat damaged protein (ADIPHD) is estimated by the acid detergent insoluble nitrogen in the analysis. There was no treatment x variety interaction ($P > 0.05$) in heat damaged protein content. Ammonium hydroxide treatment did not change ($P > 0.05$) the heat damaged protein content of the residue (Figures 2c and 2d). The tall variety has lower ($P < 0.05$) HDP compared to the short variety (3.6 vs 7.8 g kg^{-1} DM).

3.2 Fiber Fractions

The concentrations of various fiber fractions (g/100 g DM) due to NH_40H treatment are shown in Table 3. There was no treatment x variety interaction ($P = 0.94$). The levels of NH_4OH treatment did not affect ($P = 0.59$) the NDF content of the sorghum stover residue (Table 3). There was difference in NDF content between the varieties. Averaged across varieties, the short variety has higher ($P < 0.05$) NDF content (597.6 g kg^{-1}) than the tall variety (383.2 g kg^{-1}) (Table 3). There was no interaction between treatment and variety ($P > 0.05$) in the ADF fraction of the stover residue. Treatment with NH_4OH did not affect the ADF content ($P > 0.05$) (Table 3). BMR had higher ADF ($P < 0.05$) than Dale at both NH_4OH treatment levels.

Table 2. Composition of ammonium hydroxide treated sorghum residues

Variety	Level of NH_4OH [1]	CP [2]	NDIP [3]	Starch
BMR (Short)	TUN (Water & NH4OH Untreated)	50.3e	28.1b	33.4f
	T00 (Water only treated)	49.3e	25.7bc	11.3g
	T05 (5 g NH_4OH kg^{-1} DM)	108.4c	35.9a	103.0d
	T10 (10 g NH_4OH kg^{-1} DM)	133.8b	35.7a	121.2c
Dale (Tall)	TUN (Water & NH_4OH Untreated)	32.4f	23.6c	106.2dc
	T00 (Water only treated)	32.2f	24.3c	88.2e
	T05 (5 g NH_4OH kg^{-1} DM)	103.9d	27.7b	144.9b
	T10 (10 g NH_4OH kg^{-1} DM)	138.2a	25.5bc	158.9a
		---------------- Probability	---------------	
Treatment		0.0001	0.0001	0.0001
Variety		0.0001	0.0001	0.0001
Treatment x Variety		0.0001	0.0044	0.0008

[1] NH_4OH Ammonium hydroxide; [2] CP Crude protein; [3] NDIP Neutral detergent insoluble protein; [4] Values in the same column followed by different letters differ significantly ($P < 0.05$).

Ammonium hydroxide treatment decreased lignin content in both varieties ($P<0.05$) but the reduction was not similar across varieties and treatments as indicated by treatment x variety interaction ($P< 0.05$) (Table 3). The lignin content of the NH_4OH treated residue in the short variety was reduced by 40% (from 53 to 32 g kg^{-1} DM) and by 33% in the tall variety (from 45 to 30 g kg^{-1} DM).

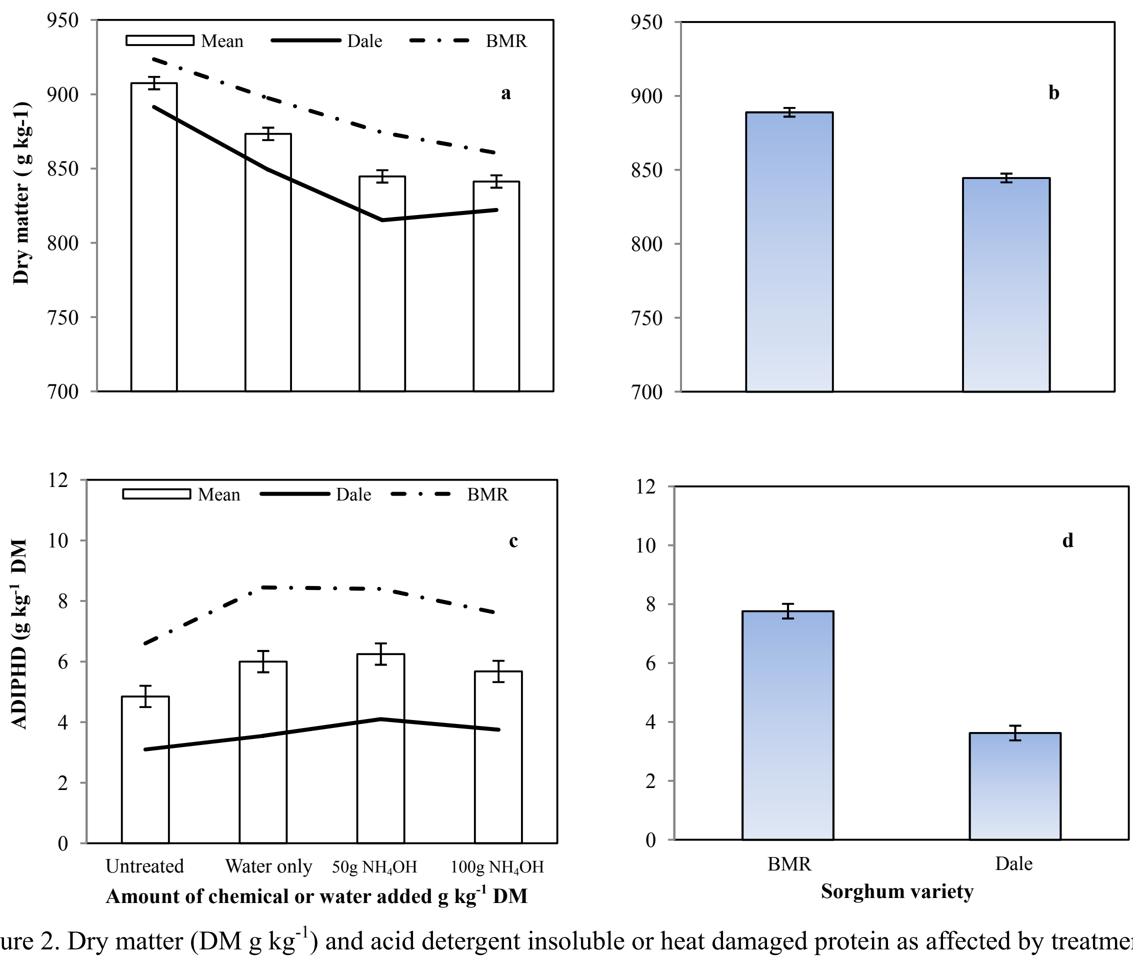

Figure 2. Dry matter (DM g kg^{-1}) and acid detergent insoluble or heat damaged protein as affected by treatments (a) and (b) and by sorghum variety (c) and (d), respectively

Table 3 composition of ammonium hydroxide treated sorghum residues

Variety	Level of NH_4OH [1]	ADF [2]	NDF [3]	Lignin	Ash
BMR	Water & NH_4OH Untreated	372.3a	616.3a	53.0a	49.6d
(Shorty)	T00 (Water only treated)	383.7a	590.7a	57.3a	68.5c
	T05 (5 g NH_4OH kg^{-1} DM)	370.5a	585.9a	40.2c	75.5b
	T10 (10 g NH_4OH kg^{-1} DM)	369.9a	597.6a	32.1de	89.1a
Dale	Water & NH_4OH Untreated	255.1b	399.2b	45.2b	42.8e
(Tall)	T00 (Water only treated)	265.8b	381.1b	40.9bc	70.0bc
	T05 (5 g NH_4OH kg^{-1} DM)	265.7b	373.7b	35.1d	75.2bc
	T10 (10 g NH_4OH kg^{-1} DM)	264.3b	389.0b	30.3e	90.8a
		-------------------- Probability ------------------			
Treatment		0.0852	0.5929	0.0001	0.0452
Variety		0.0122	0.0001	0.0001	0.0001
Treatment x Variety		0.1110	0.9401	0.0072	0.1081

[1] NH_4OH Ammonium hydroxide; [2] ADF Acid detergent fiber; [3] NDF Neutral detergent fiber; [4] Values in the same column followed by different letters differ significantly ($P < 0.05$).

4. Conclusions

Ammonium hydroxide treatment was an effective treatment to increase CP concentrations in sorghum stover residue and improve its usefulness as livestock feed. The IVDMD of this material was relatively low and differed between the residues of the two sorghum varieties. Treatment with NH_4OH increased IVDMD by reducing lignin content but increasing the fiber. There was also considerable diversity in the varieties in the fiber and lignin concentrations. For this reason researchers may be able to exploit the germplasm to develop cultivars with improved feed characteristics while maintaining traits for other uses (e.g., dry matter production, sugar content, lignocellulosic conversion, etc.). Subsequent studies should focus on evaluating other alkali and even acids for other feedstock (biofuel and livestock feed) traits. The development of more economical and safe procedures which improves digestibility of the structural cell wall components would be very beneficial for improving the nutritive value of low quality roughages. Use of crop residue as feedstock in bioethanol production can also be improved along the same lines for nutritional values.

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