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Evaluation of Composition and *in vitro* Dry Matter Disappearance of Alkali Treated Vegetable Soybean Residue

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Abstract

The study was undertaken to determine the effects of an alkali, ammonium hydroxide (NH_4OH) treatment of residue of two vegetable soybean varieties on composition and *in vitro* dry matter disappearance (IVDMD). Soybean residue was treated with no water and no NH_4OH (TUN), Water only (T00), and NH_4OH at 50 (T50), and 100 (T100) g kg^{-1}residue dry matter, and allowed to react for one week before chemical analysis was performed. The crude protein (CP) increased ($P < 0.05$) and *in vitro* dry matter disappearance improved ($P < 0.05$) with alkali treatment. The fiber content (ADF and NDF) was also reduced ($P < 0.05$) by alkali treatment but there was no difference ($P > 0.05$) in the effects of the alkali treatment levels. Ammonium hydroxide treatment increased ($P < 0.01$) *in vitro* dry matter disappearance (IVDMD) but no difference ($P > 0.01$) was observed between the alkali treatment levels. The development of more economical and safe procedures for handling the residue to improve digestibility of the structural cell wall components would be very beneficial for facilitating the use of soybean residue as potential source of feedstock for both feeding ruminant livestock and biofuel production.

Keywords: vegetable soybean stover, ammonium hydroxide, alkali treatment, IVDMD

1. Introduction

Soybean [*Glycine max* (L.) Merr.] is an important food legume native to China with a cultivation history of more than 5000 years. It was imported to North America in the early 1800's as a forage crop (Mease, 1804; Probst & Judd, 1973). Until the early 1940s, more than half of the surface area planted to soybean in the United States was used as forage, and a little less than half of the hundreds of cultivars available were forage types (Hartwig, 1973). Soybean is, however, currently mainly used in North America as a grain crop. At present, there are diverse species of soybean that are grown for grain, forage, oil and vegetable. Vegetable soybean is also known as Edamame, from a Japanese word meaning "immature soybean" (Mimura et al., 2007). Edamame is harvested and used at the immature R6 (fully expanded seed) stage (Fehr et al., 1971).

The growing global population and rising affluence is translating into increased demand for food and it is estimated that by the year 2020, demand for livestock products will double (Delgado et al., 1999). To meet this 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.

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 threshed and may sometimes also include remains generated from crop processing and packing plants (Ernest & Buffington, 1981). In the US, in 2013, 71.6 million metric tons of soybeans were harvested (USDA, NASS 2014). Assuming 50% of the biomass is left behind after harvest, 36 million metric tons of soybean residues will be available for use.

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., 1990; 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).

Alkaline compounds partially hydrolyze the cell wall and rupture the ester bonds between hemicellulose and lignin without removing the latter and by diesterifying lignin and xylan release cellulose and hemicellulose and make it available for enzymatic breakdown (McIntosh & Vancov, 2010; Saha & Cotta, 2008; Sun & Cheng, 2002; Klopfenstein, 1978; Jackson, 1977). Acids and oxidizing agents on the other hand 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). Chemical treatments increase the forage quality of highly lignified plant material by releasing cellulose and hemicellulose and ammonium pre-treatment is reported to increase intake and degradation of stover in sheep (Ben Salem et al., 1994). This study was undertaken to evaluate the effects of alkali (NH_4OH) treatment of vegetable soybean residue on composition and *in vitro* dry matter digestibility as measured by *in vitro* dry matter disappearance (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 Soybean Stover Residue

The study involved the use of stover residue recovered from two vegetable soybean varieties, Asmara and Randolph. The Asmara variety is a maturity group (MG) VI cultivar developed jointly by the Virginia State University and the USDA-ARS and was released in March 2003 as vegetable soybean with resistance to seed shattering (Mebrahatu et al., 2005a). The Randolph variety was also jointly developed by the Virginia State University and the USDA-ARS and was released at the same time with the Asmara as vegetable soybean with large seed size (Mebrahatu et al., 2005b). The residues were obtained from an ongoing research project at VSU Agriculture Research Station at the time and included the leaves and stems left after the pods were harvested. The stem and leaves were cut into small pieces and dried to a constant weight in a convection oven set at 60 °C. The dried soybean stover residue was then ground through a 2 mm screen in a Willey Mill (Arthur A. Thomas Co., Philadelphia, PA).

Representative samples of ground stover (100 g DM) were placed in plastic bags and water was added to all samples except the no water no alkali treatment to reconstitute a 50% DM. The treatments were no water no ammonium hydroxide (TUN), water only (T00), 50 (T50) and 100 (T100), g per kg residue dry matter NH_4OH respectively. The bags were immediately sealed, the contents mixed thoroughly and maintained at room temperature (21 to 23 °C) and allowed to react for seven days. After a week, all bags were removed and air dried for 48 h and samples stored at -21 °C until analyzed.

2.3 Sample Analyses

All samples were analyzed in triplicates for dry matter (DM), crude protein (CP), soluble protein (SolProt, protein which is rapidly degraded to ammonia in the rumen), acid detergent fiber (ADF), neutral detergent fiber (NDF), neutral detergent insoluble protein (NDIP a fraction of NDIP will both be degradable by rumen microbes and the intestine), acid detergent insoluble/heat damaged protein (ADIP/ADIPHD a protein fraction which is completely indigestible, Goering & Van Soest, 1970), starch, lignin, ash and IVDMD by ANALAB, a Division of Agri-King, Inc.

2.4 Statistical Analyses

Data collected for treatment composition and *in vitro* dry matter disappearances were analyzed using the General Linear Model procedure of SAS (2009). The model statement included treatment, variety and treatment × variety (interaction) effects. When analysis of data 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 soybean residue from Asmara and Randolph varieties is given in Table 1. The CP content in both varieties was high 92 g kg^{-1} DM as expected from a residue of leguminous plant. *In vitro* dry matter disappearance was 480 g kg^{-1} DM in the two varieties. Neutral detergent fiber, ADF and Solprot contents ranged between 560-629, 448-518, and 579-685 g kg^{-1} DM, respectively.

Table 1. Chemical composition of residue of two vegetable soybean varieties

Item	Variety	
	Asmara (g kg^{-1} DM)	Randolph (g kg^{-1} DM)
DM [1]	921.20	923.47
OM [2]	954.50	957.20
CP [3]	92.37	91.77
Solprot [4]	578.71	685.51
ADIP_HD [5]	10.34	11.24
NDIP [6]	17.30	12.90
ADF [7]	447.60	517.50
NDF [8]	560.30	629.30
Lig [9]	100.60	106.30
Ash	42.30	39.00
Starch	14.80	11.40
Oil	10.55	4.95
IVDMD [10]	480.70	479.80
Nitrate	70.60	960.00

[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]NDIP, Neutral detergent insoluble protein; [7]ADF Acid detergent fiber; [8]NDF Neutral detergent fiber; [9]Lig, Lignin; [10]IVDMD In vitro dry matter disappearance.

3.1 Protein Fraction

There was no treatment × variety interaction (P = 0.54) in the CP (N × 6.25) content of the soybean residue treated with NH_4OH, therefore main effect was considered. Treatment with NH_4OH but not the levels of treatment increased ($P < 0.05$) the mean CP of the residue from 92 to 170 g kg^{-1} DM (Figure 1a). Lack of treatment × variety interaction (Table 2) in mean CP of the residue from the two varieties (Randolph = 133 and Asmara = 135 g kg^{-1} DM) showed that CP content was not affected differently by the levels of NH_4OH treatment. Similarly increased CP levels were observed in a study with sorghum stover residue treated with 50 and 100 g kg^{-1} DM of ammonium hydroxide (Yousuf et al., 2014). Similar effects of increased CP in poor quality forages as a result of ammoniation were also reported in wheat straw (Soleiman et al., 1979), Barley straw (Graham and Aman, 1984), limpograss (Brown et al., 1987), and tall fescue straw (Kallenbach et al., 2006). The increased CP level observed could be explained by both the nitrogen added and the ammoniolysis reaction between the carbohydrates and the alkali as previously reported (Dryden & Kempton, 1983; Alibez et al., 1984; Mason et al., 1990; Kondo et al., 1992).

Table 2. Composition of ammonium hydroxide treated vegetable soybean residues

Variety	Level of NH_4OH[1]	CP[2]	Solprot[3]	ADIP[4]	NDIP[5]
Asmara	TUN (Water & NH_4OH Untreated)	92.37	578.71b	10.34f	17.30
	T00 (Water only treated)	107.14	308.71e	18.37a	51.63
	T50 (50 g NH_4OH kg^{-1} DM)	167.57	527.61bcd	14.47cd	50.80
	T100 (100 g NH_4OH kg^{-1} DM)	174.51	533.81bc	16.41b	51.30
Randolph	TUN (Water & NH_4OH Untreated)	91.77	685.51a	11.24f	12.90
	T00 (Water only treated)	94.34	438.81d	14.04d	52.80
	T50 (50 g NH_4OH kg^{-1} DM)	171.37	554.14bc	12.51e	52.27
	T100 (100 g NH_4OH kg^{-1} DM)	174.87	463.84cd	15.34bc	52.80
		-------------------------Probability---------------------			
Treatment		0.0001	0.0001	0.0001	0.0001
Variety		0.5890	0.0432	0.0001	0.4664
Treatment × Variety		0.5389	0.0228	0.0001	0.1655

[1]NH_4OH Ammonium hydroxide; [2]CP, Crude protein; [3]Solprot, Soluble protein; [4]ADIP, Acid detergent insoluble protein; [5]NDIP Neutral detergent insoluble protein; [6]Values in the same column followed by different letters differ significantly ($P < 0.05$).

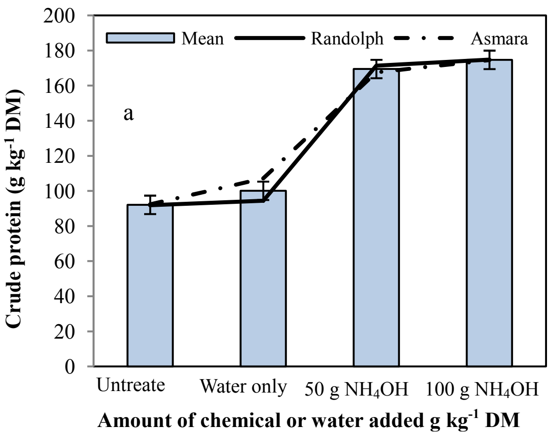
There was treatment × variety interaction ($P < 0.05$) in the solprot and ADIP contents (Table 2) indicating that treatment with NH_4OH did not affect Solprot and ADIP contents similarly in the two varieties. In the Asmara variety, increasing the level of NH_4OH from 50 to 100 showed an improvement in Solprot and ADIP. However, in the Randolph variety, there was a decrease in the Solprot and an increase in ADIP as the level of NH_4OH was increased from 50 to 100. Similar result was reported in a study with alkali treatment of sorghum stover (Yousuf et al., 2014).

3.2 Fiber Fractions

The various fiber fraction concentrations (g/100 g DM) due to NH_40H treatment are shown in Table 3. The treatment × variety interaction ($P < 0.05$) in NDF, ADF, and Lignin content shows that effects of alkali treatment were not the same for the treatment levels and the two varieties. The ADF content was the highest in the untreated residue and the lowest in the treatment with the 50 g kg^{-1} DM of alkali applied showing that alkali treatment but not level of alkali improved the fiber content of the residue. Similar effects of alkali treatment were also observed for the NDF where the untreated residue of the Randolph variety contained the highest level (Table 3). 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 × variety interaction ($P < 0.05$) (Table 3). The highest lignin content was exhibited by the treatment where no alkali was applied in both varieties (Table 3). Similar results of decreased fiber fractions were observed by Chaudhry (1994) and Amjed et al. (1992).

3.3 In vitro Dry Matter Digestibility

The *in vitro* digestibility of the soybean residue as measured by the *in vitro* dry matter disappearance is shown in Table 3. There was no variety and treatment × variety interaction ($P = 0.16$) in the IVDMD of the soybean stover treated with NH_4OH. There was no difference ($P > 0.05$) in the IVDMD between the two varieties. Ammonium hydroxide treatment but not level of NH_4OH ($P < 0.05$) increased the mean IVDMD by 38%, from 530 to 660 g kg^{-1} DM (Figures 2a and 2b). Ammoniation increased IVDMD of the residue in both varieties compared to the untreated samples.



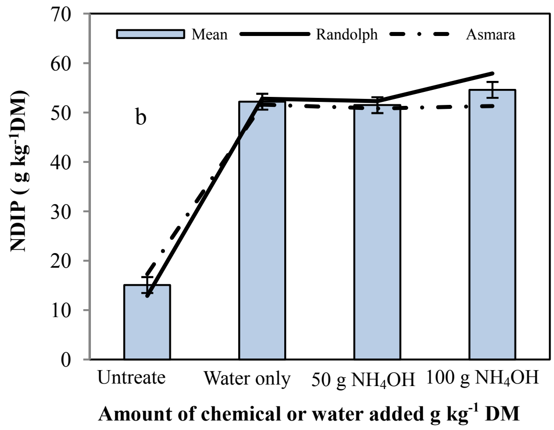


Figure 1. Crude Protein (a) and NDIP (b) content of vegetable soybean residue as affected by water or ammonium hydroxide treatments

Increased IVDMD values of ammoniated crop residues observed were in agreement with those of Amjed et al. (1992) and Brand et al. (1992). The improvement in the IVDMD observed may be a result of the increased CP and reduced fiber observed. Removal of core and noncore lignin fractions by alkali treatment is also associated with improved fiber digestion (Amjed et al., 1992).

Table 3. Composition of fiber and in vitro dry matter disappearance of ammonium hydroxide treated vegetable soybean residues

Variety	Level of NH_4OH[1]	ADF[2]	NDF[3]	LIG[4]	IVDMD[5]	Ash
Asmara	TUN (Water & NH_4OH Untreated)	507.60ab	624.83a	128.83a	411.20	42.30
	T00 (Water only treated)	447.60cd	560.30b	100.60d	480.70	60.00
	T50 (50 g NH_4OH kg^{-1} DM)	440.83cd	542.80b	106.17cd	714.60	86.47
	T100 (100 g NH_4OH kg^{-1} DM)	458.50cd	556.00b	103.87cd	726.23	99.67
Randolph	TUN (Water & NH_4OH Untreated)	534.70a	653.70a	119.33b	454.77	39.00
	T00 (Water only treated)	517.50a	629.30a	106.30cd	479.80	65.70
	T50 (50 g NH_4OH kg^{-1} DM)	432.20d	550.80b	109.10c	711.73	93.97
	T100 (100 g NH_4OH kg^{-1} DM)	472.40bc	555.47b	118.13b	731.87	114.57
		----------------------------Probability--------------------------				
Treatment		0.0001	0.0001	0.0001	0.0001	0.0958
Variety		0.0119	0.0026	0.0450	0.1609	0.0001
Treatment × Variety		0.0448	0.0189	0.0003	0.1575	0.3613

[1]NH_4OH, Ammonium hydroxide; [2]ADF, Acid detergent fiber; [3]NDF, Neutral detergent fiber; [4]LIG, Lignin; [5]IVDMD, In vitro dry matter disappearance; [6]Values in the same column followed by different letters differ significantly ($P < 0.05$).

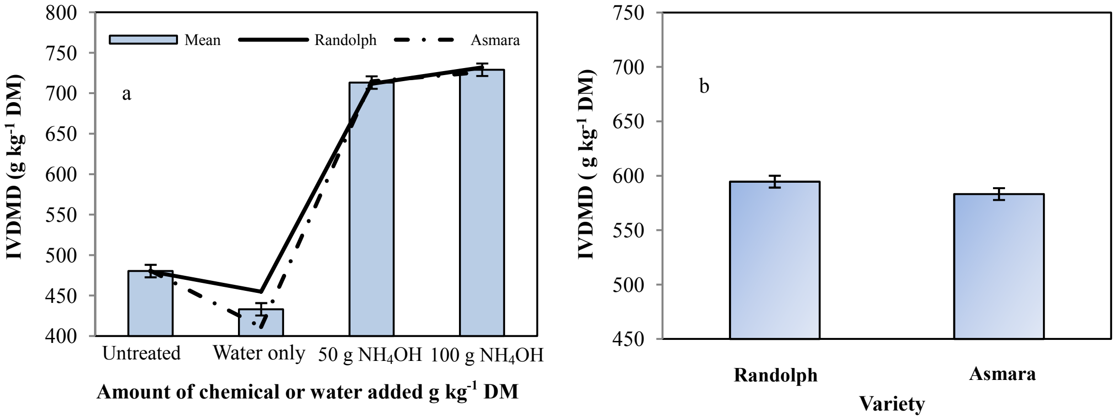


Figure 2. IVDMD (g kg^{-1} DM) of soybean stover (a) effects of NH_4OH level and (b) variety

3.4 Starch and Ash Content

There was no treatment × variety interaction (P > 0.05) in the starch content of the residue. There was treatment as well as variety difference (P < 0.05) in the starch content of the alkali treated soybean residue. Averaged across treatments, the Asmara variety contained higher (P < 0.05) starch than the Randolph. Averaged across varieties, the untreated (TUN) and the T50 treatments have significantly higher (P < 0.05) starch than the water only and T100. There was no treatment × variety interaction (P > 0.05) and also varietal effect (P > 0.05) on the ash content of alkali treated soybean residues. There were significant differences (P < 0.05) between the treatment levels in the ash (mineral content of the residue. The order of the ash content from the highest to the lowest was T100 > T50 > T00 > TUN.

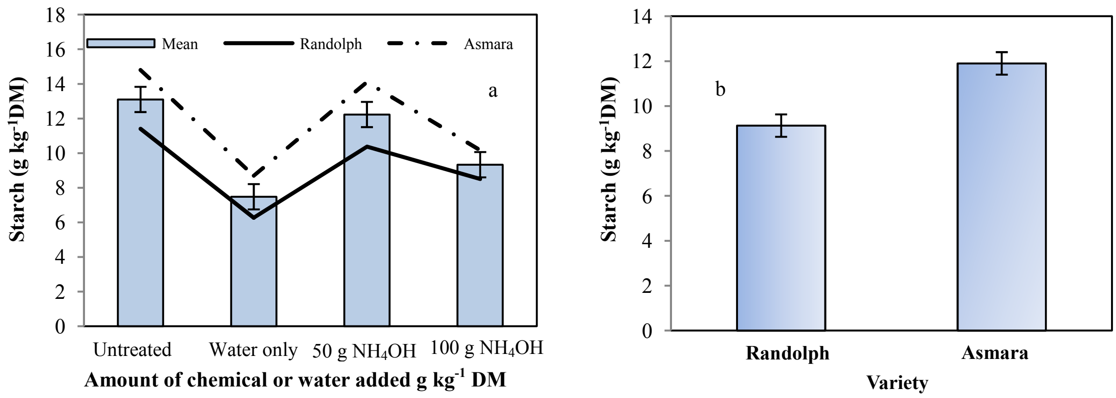


Figure 3. Starch content (g kg^{-1} DM) of soybean stover (a) effects of NH_4OH level and (b) variety

4. Conclusions

Ammonium hydroxide treatment markedly increased CP concentrations and improved IVDMD of the vegetable soybean residues, a potential underutilized feed resource. The two varieties did not respond similarly to the levels of NH_4OH as reflected in the interaction, however, the improvement in composition and digestibility by treatment with NH_4OH suggest that the residues can be used as potential ruminant livestock supplement feed during times of feed scarcity. The development of more economical and safe procedures which improve fiber, protein content and digestibility of the structural cell wall components would be very beneficial for improving the nutritive value of low quality roughages. Future studies need to focus on evaluating other potential crop residues and chemicals and the levels of inclusion for improving nutritional quality. Such findings can also be useful in bioethanol production since their use as feedstock can also be improved along the same lines for nutritional values.

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