

Effects of Liquid Ammonia and Urea Treatment on Chemical Compositions and *in vitro* Digestibility of Triticale Straw

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Abstract: A study was conducted to investigate changes in chemical compositions and *in vitro* digestibility of Triticale Straw (TS) treated with liquid ammonia and urea. TS chopped approximately 2 cm length. Liquid ammonia and urea were applied to 1000 g DM of chopped TS at rates of 0, 10, 20, 30 and 40 g kg⁻¹ DM. The chemicals were applied as aqueous solutions, which added 1500 g of water/1000 g of TS DM, thoroughly mixed, stored in plastic containers and kept for 45 days at ambient temperature (22-25°C). Chemical compositions and *in vitro* digestibility of test materials were determined. The amounts of Crude Protein (CP) and N-NH₃ increased ($p < 0.05$) when, liquid ammonia and urea were applied at higher levels (40 g kg⁻¹ DM) but decreased Neutral Detergent Fiber (NDF), however, effect of urea was higher than liquid ammonia. Acid Detergent Fiber (ADF) content decreased ($p < 0.05$) significantly only in Urea-treated TS (UTS). UTS had higher *in vitro* Digestibility of DM (IVDMD), Organic Matter (IVOMD) and OM in DM (IVDOMD) than Liquid ammonia-treated TS (LTS). As a point of digestibility, the rate of 10 in UTS treatments is equivalent to the rate of 30 in LTS treatments. Liquid ammonia and urea can improve characteristics chemical compositions and *in vitro* digestibility of TS but, urea was better than liquid ammonia.

Key words: Chemical compositions, digestibility, liquid ammonia, triticale straw, urea, Neutral detergent fiber

INTRODUCTION

Triticale (*Triticosecale*) is a man-made crop developed by crossing wheat (*Triticum turgidum* or *Triticum aestivum*) with rye (*Secale cereal*) (Martinek *et al.*, 2008). Triticale variety is stable, it will not revert back to produce rye or wheat plants. The objective behind making wheat/rye crosses is to capture the best traits of each species. Wheat yields and grain quality are better than rye. But rye has greater disease resistance and better tolerance of environmental stresses (Genying *et al.*, 2006). Interest in triticale has developed around two areas of potential use for grain. The first area of interest is for use as a feed grain because, it is proven to be a good source of protein, amino acids and B vitamins. It has shown promise as both a forage crop and as an alternative protein source in formulated rations for monogastrics, ruminants and poultry.

Straw is the most abundant of all agricultural residues in Iran and despite its very low digestibility, a significant amount of it is fed to ruminants. Ammoniating is one of the most studied chemical treatments to improve forage quality. Ammoniation improves forage digestibility due to

the hydrolytic action of ammonia on linkage between lignin and structural polysaccharide, which increases Organic Matter (OM) potentially available for utilization by ruminal microorganisms (Oji *et al.*, 2007). Ammoniation also increases the Crude Protein (CP) concentration of forage through fixation of applied N (Dean *et al.*, 2008). The average *in sacco* degradability of 5 varieties of triticale straw were 42.4 (29.8-51.9%) (Flachowsky *et al.*, 1991). The *in vitro* digestibility of triticale straw reported 46% DM basis (Narasimhalu *et al.*, 1989). When, cereal straws (containing triticale straw) is treated with urea (55 g kg⁻¹ air-dry straw, in sealed bags at 22°C for 60 days) the average of dry matter loss, were 137 g kg⁻¹ DM (range 87-192) (Dias-da-Silva and Guedes, 1990). Triticale grain contains more crude protein but considerably less crude fiber than barley grain and the compositions of the respective straws was similar (Charmely and Greenhalgh, 1987).

A lot of researchers have reported urea or ammonia treatment can improve the digestibility, crude protein content and other useful characteristics of cereal straws.

The objectives were to examine, the effects of liquid ammonia and urea treatment on the chemical compositions and *in vitro* digestibility of triticale straw.

MATERIALS AND METHODS

Triticale Straw (TS) used in the following experiment was obtained from non-irrigated, N fertilized, which had been grown under inland, cool temperate conditions in North-west of Iran (38°25'N, 48°30'E). TS collected from several fields then chopped (about 2 cm long) and mixed completely. Chemical composition of TS used in this study is shown in Table 1. Liquid ammonia (NH₃ 25%) and urea (NH₂-CO-NH₂ crystallized, N = 46%) were applied to 1120 g (1000 g DM) of chopped TS at rates of 0, 10-40 g kg⁻¹ DM. The chemicals were applied as aqueous solutions, which added 1500 g of water/1000 g of TS DM. The solutions were poured over the TS in a tub and mixed for approximately, 3 min. Then, the resulted material moved to the plastic containers and air was removed by compression. Treated TS was sealed and stored at ambient temperature (22-25°C) for 45 days. There were four replicates of each treatment.

At the end of the storage period, samples were exposed to free air, in shadow for 2 days for excess NH₃ to escape. They were then ground through 1 mm screen with a hammer mill. For determination of pH, 100 mL of deionized water added to 100 g of each fresh samples, mixed and shaken for 2 min then juice obtained by compression and measured with a pH meter (Wilson *et al.*, 2005). Samples were analyzed for concentrations of DM (60°C for 48 h or until, its weight become constant); Ash/Organic Matter (OM) (4 h at 550°C); Neutral Detergent Fiber (NDF) (without sodium sulfate or α-amylase; ash corrected), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) using the fibertec system (Foss tecator, 2010). Determination of N was conducted using the Kjeldahl method in an automated apparatus (Foss tecator, Kjeltec 2300 Analyzer unit. Crude Protein (CP) was calculated using N% × 6.25. Some minerals of TS measured with atomic absorption apparatus (902 Double Beam GBC) (Nielsen, 2006). Total Phenolic (TP) was determined using the Folin Ciocalteau method (Makkar, 2003). N-NH₃ was measured from fresh samples by the procedure described by Oji *et al.* (2007).

In vitro DM Digestibility (IVDMD), Organic Matter Digestibility (IVOMD) and Digestible OM in DM (DOMD) were determined according to the two-stage technique of Tilley and Terry (Oji *et al.*, 2007) with rumen liquor collected by stomach tube suction aided by vacuum pump from mature Iranian Taleshi cows. These cows with average weight of 350 kg were fed alfalfa hay and 2 kg each of common concentrate 2 weeks before commencement of the experiment and during collection period. Animals were not fed before rumen liquor was collected. The liquor was collected in flask immersed in

Table 1: Chemical compositions of triticale straw

Parameters	Amount
Dry matter (g kg ⁻¹)	893.2±35.7
Organic matter (g kg ⁻¹ DM)	895.2±39.6
Ash (g kg ⁻¹ DM)	104.8±4.2
Crude protein (g kg ⁻¹ DM)	21.1±0.6
Neutral detergent fiber (g kg ⁻¹ DM)	841.2±41.2
Acid detergent fiber (g kg ⁻¹ DM)	535.3±29.6
Acid detergent lignin (g kg ⁻¹ DM)	78±2.5
Total phenol (g kg ⁻¹ DM)	7.8±0.31
Calcium (g kg ⁻¹ DM)	4.14±0.16
Phosphorous (g kg ⁻¹ DM)	0.11±0.12
Magnesium (g kg ⁻¹ DM)	0.62±0.025
Potassium (g kg ⁻¹ DM)	4.53±0.19
Sodium (g kg ⁻¹ DM)	0.43±0.02
Iron (mg kg ⁻¹ DM)	69.95±3.79
Manganese (mg kg ⁻¹ DM)	10.25±0.61
Copper (mg kg ⁻¹ DM)	3.15±0.16
Zinc (mg kg ⁻¹ DM)	15.05±1.36

warm water maintained at 39°C, strained through three layers of cheese cloth and CO₂ bubbled slightly through it before dispensing into 100 mL tubes. Provided data was submitted to analysis of variance for completely randomized designs and LSD's test for multiple comparisons among means.

RESULTS AND DISCUSSION

Chemical compositions of untreated TS is shown in Table 1. The content of OM, CP, NDF, ADF and ADL of TS were in contrast with other results. This inconsistency may be due to TS varieties (genotype), different rate of leaf or stem, planting and location of agronomy. Chemical compositions of LTS and UTS are shown in Table 2. Liquid ammonia and urea increased (p<0.05) pH, from 4.77-5.74 and 9.39, respectively. The amounts of CP and N-NH₃ increased (p<0.05) when, liquid ammonia and urea were applied at higher levels (40 g kg⁻¹ DM) but, the effect of urea was higher than liquid ammonia. Liquid ammonia and urea increased CP content in TS at the range of 67.6 and 117.4% (for liquid ammonia) and 66.2 and 238.5% (for urea) for the rates of 10 and 40. Similarly, N-NH₃ increased in LTS and UTS, although LTS had low N-NH₃. The content of NDF decreased (p<0.05) when, liquid ammonia and urea were applied at higher levels. Also, ADF content decreased but it was significant only in UTS. There was a tendency for treatment to decrease ADL and TP but the reduction was no significant.

In vitro digestibility coefficients of treated triticale straw are shown in Table 3. Liquid ammonia and urea increased (p<0.05) IVDMD in TS in the range of 8.1 and 15.8% (for liquid ammonia) for the rates of 30 and 40 and 11.1 and 41.1% (for urea) for rates of 10 and 40. Similarly, IVOMD and IVDOMD increased in LTS and UTS. UTS had higher digestibility than LTS. The rate of 10 in UTS treatments is equivalent to the rate of 30 in LTS treatments.

Table 2: Chemical composition of treated triticale straw

Parameters	Liquid NH ₃					Urea					SE
	0	10	20	30	40	0	10	20	30	40	
pH	4.77f	5.12e	5.60d	5.72d	5.74d	4.77f	5.54d	6.50c	8.97b	9.39a	0.25
OM (g kg ⁻¹ DM)	894.9	889.4	889.8	891.2	891.7	895.1	890.1	889.5	891.1	890.5	0.43
Ash (g kg ⁻¹ DM)	105.2	110.5	110.2	108.7	108.3	104.9	109.9	110.5	108.9	109.5	0.44
CP (g kg ⁻¹ DM)	19.38g	32.48f	35.15ef	37.90de	42.13d	19.69g	32.72f	49.80c	56.33b	66.65a	2.33
NDF (g kg ⁻¹ DM)	840.8a	839.5a	835.3ab	836.3ab	826.5bc	842.4a	838.3ab	834.5ab	819.3c	821.0c	1.62
ADF (g kg ⁻¹ DM)	533.3ab	531.0ab	529.5ab	529.0ab	524.3ab	534.6ab	530.3ab	523.5ab	523.0ab	517.3a	1.64
ADL (g kg ⁻¹ DM)	78.0	76.5	75.8	76.0	74.0	78.1	76.0	74.5	75.3	73.0	0.94
TP (g kg ⁻¹ DM)	7.86	7.64	7.65	7.63	7.43	7.87	7.64	7.61	7.48	7.19	0.13
N-NH ₃ (mg kg ⁻¹ DM)	776i	1080h	1647g	1819f	2699d	779i	2137e	4067c	5591b	7455a	351.30

Means values in the same row with unlike letters are different (p<0.05). OM: Organic Matter, CP: Crude Protein, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, ADL: Acid Detergent Lignin, TP: Total Phenolic

Table 3: *In vitro* digestibility of treated triticale straw (g kg⁻¹ DM)

Parameters	Liquid NH ₃					Urea					SE
	0	10	20	30	40	0	10	20	30	40	
IVDMD	353.1f	353.4f	364.3ef	381.8de	408.8c	348.6f	387.2d	445.1b	489.5a	491.8a	8.64
IVOMD	342.4f	341.8f	353.7ef	373.0de	398.0c	337.0f	378.2cd	432.3b	478.8a	482.4a	8.61
IVDOMD	333.0f	328.3f	341.7ef	360.0de	387.2c	325.6f	365.5d	418.3b	464.6a	466.8a	8.42

Means values in the same row with unlike letters are different (p<0.05). IVDMD: *In vitro* Dry Matter Digestibility, IVOMD: *In vitro* Organic Matter Digestibility, IVDOMD: *In vitro* Organic Matter Digestibility in dry matter

Increasing for nitrogenous fractions and *in vitro* digestibility of TS are similar to previously reported research about TS (Dias-da-Silva and Guedes, 1990) and other cereal straws such as wheat, barley, oat and rice straws. Dryden and Leng (1986) recorded that the cell wall OM content of barley straw was the least at the highest levels of ammonia (45 g kg⁻¹ DM) used in their experiment. Mason *et al.* (1990) reported the lowest concentrations of cell wall constituents and the highest OM digestibility when, wheat straw was treated with 80 g ammonia kg⁻¹ DM and 400 g added water kg⁻¹ DM. Similar results were recorded by Zaman *et al.* (1994) who observed that the *in vitro* DM digestibility of barley straw was the highest after the addition of 80 g of urea kg⁻¹ of barley straw. Colucci *et al.* (1992) also, found that oat and barley straws of initially lower digestibility responded better to urea treatment. Similar results, in rice straw were reported by Vadiveloo (2003), who observed that the rice varieties with poorer straw quality responded better to urea treatment with results predicting that straw with an initial IVDMD of 45 or 55% would have an IVDMD of 59 or 62% after urea treatment. Oji *et al.* (2007) reported nitrogenous fractions and *in vitro* digestibility of maize residues increased (p<0.05) when, treated with aqueous ammonia and urea at 3% of DM. Also, in the case of N fractions there are reports elsewhere (Schneider and Flachowsky, 1990; Goto and Yokoe, 1996).

The urea, which is used was hydrolysed by urease enzyme in triticale straw to NH₃. It has been reported that urease activity demands optimal moisture content from over 375 g kg⁻¹ DM. Thus, at 1500 g kg⁻¹ DM moisture

level, urea hydrolysis could be extensive. The relatively high values for N-NH₃ in the treated materials, despite aeration to void free NH₃, could be explained by the physical changes in the cell wall of triticale straw that culminate in attachment of N as NH₃.

The significant increase in CP concentration of TS could be of practical benefits. The CP concentration of the untreated TS used in this experiment (21.1 g kg⁻¹ DM) would be inadequate for the requirements of maintenance and growing sheep (Ensminger, 2002) and so treating TS with urea would reduce the need to provide supplementary ruminal CP for these classes of livestock.

Additives make alkali condition in which, effects of urea are more than liquid ammonia. A trend for decreases in NDF, ADF and ADL fractions was observed in this experiment. Correspondingly, the *in vitro* digestibility increased with liquid ammonia-treated and urea-treated straw. These results are similar to earlier report about maize residues (Oji *et al.*, 2007). These declines (p<0.05) in concentrations of NDF are consistent with the generic action of ammonia on feedstuffs (Granzin and Dryden, 2003; Dean *et al.*, 2008). The reduction in fiber content after treatment with ammonia was due to reduction in hemicellulose, which was constituted in the cell wall together with cellulose and lignin. During ammonia treatment lignin dissociated from the ligno-cellulosic complex, but was still detected as lignin. Also, various workers have adduced reasons for the improvements observed after ammoniation of cereal residues. They include collapse of vascular bundle sheath cells in treated rice straw, rupture of inner cuticular surfaces and

separation from adjacent ground parenchyma when, wheat straw was ammoniated, alteration in the friability of the rigid layer covering the inner surface of cell walls (Goto *et al.*, 1993) and the ability of NH₃ to form an ammonia-cellulose complex and to decrease cellulose crystallinity. The latter workers argued further that any reduction in crystallinity of cellulose forming the microfibrils of the cell wall contributes to an increased fragility of the wall, which consequently increases susceptibility to attack by cellulolytic microbes. These facts may explain the observed increase in *in vitro* digestibility with treated residues. Also, it is known that NH₃ has the ability to dissolve parts of hemicellulose, cleave ester bonds of uronic acids with loss of acetyl groups, thus releases acetyl and phenolic acids, an effect that may possibly explain the decreases in NDF and ADF (Oji *et al.*, 2007).

Rumen fill is a major limitation to the productivity of ruminants eating straw. These reductions in NDF caused by ammonia treatment are encouraging because they imply that the degradability and intake of straw may be improved by chemical treatment. The total phenol content of triticale straw is very low (7.8 g kg⁻¹ DM), thus no significant decrease resulted in this experiment. Vitti *et al.* (2005) showed that urea treatment causes significant decrease of total phenol of collected browses. The decrease in TP with urea treatment is likely to be a result of the high pH caused by evolution of ammonia from urea (Makkar, 2003).

CONCLUSION

Triticale straw has very low quality as a feed for livestock. While, treatment of triticale straw with liquid NH₃ and urea caused changes in chemical compositions and *in vitro* digestibility, there were differences ($p < 0.05$) in effect between the liquid ammonia and urea. Liquid ammonia is definitely more technically cumbersome to handle and may expose the handler to health hazards while, urea does not have these problems. Moreover, it is cheap. Even though, ammonia sources are not as efficient as NaOH or KOH in improving the nutritive value of low quality roughages, the results obtained here and elsewhere, suggest the use of urea for upgrading the N of poor quality crop residues by rural farmers in developing countries and feeding same to ruminants.

ACKNOWLEDGEMENTS

We would like to express, the special thanks to the University of Mohaghegh Ardabili for financial support of the research through a scientific research grant offered.

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