

Effect of Urea Treatment of Roughages and *in vitro* Digestibility of Available Feed Resources in Maradi Area of Niger

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Abstract: The effect of urea treatment of roughages and the digestibility of feeds for ruminants was studied in southern Niger. Cereal straws, legume crop residues and concentrates were collected in the dry season. Cereal straws were millet stover, sorghum stover with its fractions (leaves and sheath, stems), *Diheteropogon hagerupii*, *Eragrostis tremula* and *Schizachyrium exile* and legume crop residues were groundnut haulms and cowpea husk. Cereal straws and cowpea husk were either untreated or treated with urea. *In vitro* digestibility characteristics were determined using rumen fluid from fistulated Jersey cows and metabolizable energy (ME) was determined through gas production after 24h of incubation. Cereal straws had different ($P<0.001$) apparent degradability (Apdeg), degradation rate (C), half-life to the maximum gas volume ($T_{1/2}$), degradation efficiency factor (DEF) and ME. Whereas, urea treated cereal straws had higher ($P<0.001$) (C) and increased ($P<0.01$) ME. Legume crop residues did not affect ($P>0.05$) *in vitro* parameters, however, treating cowpea husk decreased ($P<0.05$) gas production and ($T_{1/2}$) but increased ME ($p<0.01$). Concentrate degradation and kinetics parameters varied ($P<0.001$) strongly, while Apdeg and true degradability varied ($P<0.01$) leaving microbial yield (MY) unaffected. These findings will contribute to better utilization of the concerned feed resources by ruminants in Niger and related environment.

Keywords: Cereal Straws, Concentrates, Digestibility, Gas Production, Legume Crop Residues, Urea Treatment

1. Introduction

Nutritive value of feed is determined by its chemical composition and digestibility [1]. In Niger, high quality feeds are only available during the rainy season which lasts for four months per annum, whereas during the dry season feeds are scarce and have low nutritive value. Consequently, ruminant animals largely depend on cereal straws which are low in protein and available energy [2, 3]. Chemical treatment and/or supplementation with protein sources were used to improve the quality of these straws [2, 4]. However, research for development projects have accorded more attention on urea

treatment due to its advantage of improving intake, digestibility and nitrogen content and reducing the use of concentrate [2, 4].

The tendency these days is the use of agro-industrial by-products and crop residues for feeding ruminants in Niger [5] without due regard for nutritive values in West African countries. To evaluate nutritional attributes of these feed resources, *in vitro* degradability was used. *In vitro* digestibility has been widely used to assess the nutritional quality of feeds [6]. In the current study, cereal straws were treated with urea

in order to assess the effect of urea treatment on *in vitro* digestibility parameters. The objective of this study was to determine the effect of urea treatment on predominant roughages and to evaluate the quality of some feeds available in Southern Niger using *in vitro* digestibility techniques in order to contribute to better utilization of feed resources by ruminants in Niger.

2. Materials and Methods

2.1. Feeds

Feeds were brought from Maradi, South of Niger, with an annual rain fall of 350-600 mm; and a composite sample taken for urea treatment and laboratory analyses. Feeds were grouped into three classes: (1) cereal straws: millet stover (*Pennisetum typhoides*), sorghum (*Sorghum bicolor*) stover (whole sorghum stover, SS; sheath and leaves, SSL; stems, SSS), *Eragrostis tremula*, *Schizachyrium exile* and *Diheteropogon hagerupii*; (2) legume crop residues: groundnut (*Arachis hypogea*) haulms and cowpea (*Vigna unguiculata*) husk; and (3) concentrates: millet bran, wheat bran and cottonseed cake. All roughages (without SSL, SSS and groundnut haulms) were sprayed with 3% urea solution at 50% w/w of straw mixed and stored in plastic bags for 40 days. Concentrates used in this study are available to smallholder farmers in Southern Niger.

2.2. Gas Production

Two donors Jersey cows (~350 kg live weight) fitted with a rumen cannula were kept under natural grazing of kikuyu (*Pennisetum clandestinum*) pasture. These two cows had access to 2 kg of Lucerne per cow day in the afternoon, besides a mineral lick *ad libitum*. All roughages (treated with urea and untreated) and concentrates were used to determine gas production following the automated gas production technique [7]. Ingredients were ground (1 mm screen) and a total of 1.0 ± 0.0010 g DM of each sample was weighed into a 250 ml Duran bottle for *in vitro* incubation. Roughages and concentrates were incubated separately. During each run, there were three controls of Lucerne (alfalfa - *Medicago sativa*) and three blanks (only rumen fluid and buffer). Roughages were incubated in 4 runs in a completely randomised block design. Concentrates were incubated in 3 runs in a completely randomised design with 5 replicates per sample. A salivary buffer solution was prepared [8]. A 67 ml solution of the buffer was added to each sample and to each blank bottle. These bottles were kept in the incubator (39°C) for 1 hr to allow soaking of substrate prior incubation. Meanwhile, a mixture of rumen fluid was collected prior to morning feeding, squeezed and filtered through four layers of cheesecloth into a pre-warmed flask container (39°C) that had been flushed with carbon dioxide (CO₂) and transported to the laboratory. Inoculation was completed by adding 33 ml of the rumen fluid into each bottle under a stream of CO₂ and tightening bottle lids. Duran bottles were incubated and channels of pressure sensors were fitted. Pressure was logged at 20 min interval

during a 72-hours and 48-hours incubation period for roughages and concentrates, respectively, and was converted to volume using a predetermined calibration equation. The metabolizable energy value was calculated according to [9] as follows: ME (MJ/kg DM) = $2.20 + 0.136G_{24} + 0.057CP + 0.0029 CP^2$; Where CP = crude protein in g per 100 g DM, and G_{24} is the gas volume at 24 h after incubation (ml) of 200 mg. In this study gas volume after 24 hours was converted from 1000 mg to 200 mg of material incubated.

2.3. In vitro Digestibility

After 72-hours and 48-hours incubation period for roughages and concentrates, respectively as described above, the pH was measured and samples were centrifuged at 17,700xg for 20 minutes at 4°C. Then the supernatant was poured out, the residue transferred into a container (with known weight) and placed in the oven (70°C) for 5-6 days until dried constancy. Dry residues were corrected for blank incubation which contained only buffer and rumen fluid. The difference in mass between the dry residue and the mass of the incubated material represented apparent degradability (Apdeg). The residue was refluxed with neutral detergent solution (NDF) using ANKOM-A200/2220 (Macedon, NY, USA). www.ankom.com). The resulting weight of NDF (after correction) was subtracted from the mass of the incubated material which represented the true degradability (Trdeg) [10, 11]. The difference between Trdeg and Apdeg represented the microbial yield (MY) [12, 11].

The experimental design was a completely randomised block design (9 feeds x 4 runs) for roughages in Experiment 1 and (3 feeds x 5 pseudo replications x 5 runs) for concentrates in experiment 2. The model described by [13] was fitted to gas volumes to determine gas production kinetics following a two steps process:

$$Y = GP/(1+e(2+4C(Lt - t))) \quad [14]$$

Where Y is the total gas volume (mL) at time t, GP is the maximum gas production, C is the overall rate of gas production, and Lt is the bacteria colonization or Lag time (h). The cumulative gas production (GP) was used to calculate the maximum rate of GP at the point of inflection (μ) and the time taken to produce half of the gas volume ($T_{1/2}$) according to [15] as follows:

$$\mu \text{ (ml/h)} = GP \times C \text{ and}$$

$$T_{1/2} \text{ (h)} = Lt + 1/(2 \times C)$$

The partitioning factor (PF) [16, 17] and the degradation efficiency factor (DEF) [18] were calculated as follows:

$$PF = Trdeg/GP$$

$$DEF = Trdeg / T_{1/2} \times GP_{1/2} = 2PF / T_{1/2}$$

2.4. Statistical Analyses

Data were subjected to the General Liner Model of SAS to determine the effects of feeds, urea treatment and interaction

feed versus urea treatment on gas production parameters, the statistical significance being declared at $P < 0.05$. Means were compared by least squares means (LSMEANS). The models used were:

$$Y_{ijkl} = \mu + f_i + u_j + (fu)_{ij} + run_k + e_{ijkl} \text{ (Experiment1);}$$

Where, Y_{ijkl} is the independent variable (GP parameters, *in vitro* degradability), μ is the overall mean, f_i is the effect of feed roughages ($i=1-9$), u_j is the urea treatment effect ($j=1$), $(fu)_{ij}$ is the interaction between feeds and urea treatment, run_k is the effect of run ($k=1-4$) and e_{ijkl} the residual error.

$$Y_{ijkl} = \mu + f_i + r_j + run_k + e_{ijkl} \text{ (Experiment2);}$$

Where, Y_{ijk} is the independent variable (GP parameters), μ is the overall mean, f_i is the effect of feed concentrates (3 feeds x 5 pseudo replications) ($i=1-3$), r_j is the effect of replication ($j=1-5$), run_j is the effect of run ($j=1-5$) and e_{ijkl} the residual error.

3. Results

3.1. Dry Matter Degradability and Gas Production

The three classes of feeds had different ($P < 0.001$) apparent degradability (Apdeg), true degradability (Trdeg), microbial

yield (MY), rate of gas production (C), and maximum gas production (GP) but had no effect ($P > 0.05$) on pH (Table 1). These classes had different ($P < 0.001$) metabolisable energy (ME).

Within cereal straws, feed types affected Apdeg ($P < 0.001$), C ($P < 0.001$), Trdeg ($P < 0.01$), MY ($P < 0.001$) and GP ($P < 0.001$). Urea treatment increased Trdeg ($P < 0.01$), MY ($P < 0.05$) and C ($P < 0.001$). GP was highest in *Eragrostis* and lowest in millet stover and was affected by cereal type ($P < 0.01$). Cereal straws had different ($P < 0.001$) ME where fraction of sorghum stover leaves & sheath (SSL) and stems (SSS) had the highest ME and *Diheteropogon* and millet stover the lowest ($P < 0.001$). Treating cereal straws increased ($P < 0.01$) the ME content, with no indication of any interaction.

There was no effect ($p > 0.05$) of legume crop residues on all gas production parameters and ME. However, treating cowpea husk increased pH ($P < 0.05$) and ME ($P < 0.01$) but decreased ($P < 0.05$) the GP.

Concentrates affected ($P < 0.01$) Apdeg, Trdeg, highly affected ($P < 0.001$) pH, maximum gas production (GP), gas production rate (C) and ME. However, MY was similar ($P > 0.05$) among concentrates. The TrDeg in wheat bran was higher ($P < 0.01$) than in millet bran and cottonseed cake. The same trend ($P < 0.001$) was observed with GP.

Table 1. Degradability and gas production factors of feeds using *in vitro* digestibility.

Feeds	UT	Apdeg	TrDeg	MY	pH	GP	C	ME (MJ/kg DM)
Cereal straws class								
<i>Diheteropogon hagerupii</i>	-	377	623	246	6.83	128	0.021	3.61
	+	418	592	174	6.70	125	0.024	3.65
<i>Eragrostis tremula</i>	-	461	580	119	6.67	137	0.021	4.18
	+	480	626	146	6.71	128	0.023	4.23
<i>Schyzachiryum exile</i>	-	410	629	218	6.69	122	0.024	4.00
	+	366	682	317	6.77	129	0.027	4.12
Millet stover	-	382	550	169	6.78	82	0.026	3.55
	+	375	676	301	6.91	103	0.025	3.86
Sorghum stover (SS)	-	532	631	99	6.77	121	0.020	4.09
	+	462	684	222	6.71	120	0.022	4.21
SS leaves sheath	-	597	699	102	6.67	128	0.027	5.17
SS stems	-	508	579	71	6.70	140	0.019	4.53
Legume crop residues class								
Cowpea husks	-	505	703	198	6.68	156	0.045	6.62
	+	553	671	118	6.75	144	0.049	6.97
Groundnut haulms	-	537	761	224	6.72	152	0.042	6.73
Concentrates class								
Cottonseed cake	-	349	730	450	6.83	116	0.018	6.29
Millet bran	-	469	855	430	6.66	156	0.063	7.58
Wheat bran	-	438	873	505	6.64	180	0.073	8.49
Variation sources								
Between classes								
RMSE		87.58	79.87	115.14	0.11	21.044	0.014	0.69
Class effect		***	***	***	NS	***	***	***
Within cereal straws								
RMSE		40.28	53.03	72.42	0.10	11.61	0.0007	0.13
Feed effect		***	**	**	NS	**	***	***
Treatment effect		NS	**	*	NS	NS	***	**
Feed*treatment		NS	NS	*	NS	NS	**	NS
Within legume crop residues								
RMSE		79.17	44.23	117.98	0.02	4.65	0.002	0.12
Feed effect		NS	NS	NS	NS	NS	NS	NS
Treatment effect		NS	NS	NS	*	*	NS	**
Within concentrates								

Feeds	UT	Apdeg	TrDeg	MY	pH	GP	C	ME (MJ/kg DM)
RMSE		61.87	46.07	152.5	0.05	15.53	0.007	0.23
Feed effect		**	**	NS	***	***	***	***

UT: urea treatment; Apdeg: the apparent degradability (mg g^{-1} DM); Trdeg: true degradability (mg g^{-1} DM); MY: microbial yield (mg g^{-1} DM); GP: maximum gas production (ml g^{-1} DM); C: constant degradation rate of gas production (h^{-1}); ME: metabolizable energy; RMSE: Root mean square error; NS ($P > 0.05$); * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$).

3.2. Derived Factors of GP of Feeds

The three classes of feeds strongly ($P < 0.001$) affected halflife to the maximum gas volume ($T_{1/2}$), maximum rate of gas production at the point of inflection of the gas curve (μ) and degradation efficiency factor (DEF). They slightly ($P < 0.05$) affected the partitioning factor (PF) (Table 2).

Within cereal straws, feed type affected ($P < 0.001$) halflife to the maximum gas volume ($T_{1/2}$), maximum rate of gas production at the point of inflection of the gas curve (μ), the partitioning factor (PF) and degradation efficiency factor (DEF). Among cereal straws, *Diheteropogon* (34.9 h and 0.29) had the highest ($P < 0.001$) $T_{1/2}$ and lowest DEF, respectively, whereas SSL (22.4 h and 4.14 ml/h) had the lowest $T_{1/2}$ and highest ($P < 0.001$) (μ), respectively. Millet stover had the

lowest μ (2.22 ml/h) and highest PF (6.58), whereas SSL (0.46) had the highest DEF and SSS (0.46) had the lowest PF. However, urea treatment increased ($p < 0.05$) (μ) but did not affect any other factor. All these factors were not affected ($P > 0.05$) by legume crop residues. Treatment of cowpea husk with urea increased ($T_{1/2}$) but did not affect any other factor.

Concentrates highly ($P < 0.001$) affected halflife to the maximum gas volume ($T_{1/2}$), maximum rate of gas production at the point of inflection of the gas curve (μ) and degradation efficiency factor (DEF). They had different ($P < 0.01$) on partitioning factor (PF). Wheat bran had the highest ($P < 0.001$) (μ) and DEF, and the lowest ($P < 0.001$) ($T_{1/2}$). Inversely, cottonseed cake had the highest ($P < 0.001$) ($T_{1/2}$) and (PF), and the lowest ($P < 0.001$) (μ).

Table 2. Gas production and kinetic factors of feeds using in vitro digestibility.

Feeds	UT	$T_{1/2}$	μ	PF	DEF
Cereal straws class					
<i>Diheteropogon hagerupii</i>	-	34.9	2.77	4.97	0.29
	+	35.6	3.03	4.76	0.28
<i>Eragrostis tremula</i>	-	29.3	2.95	4.46	0.30
	+	30.0	2.95	4.88	0.33
<i>Schizachyrium exile</i>	-	28.8	2.97	4.94	0.33
	+	29.8	3.49	5.30	0.35
Millet stover	-	26.0	2.22	5.75	0.42
	+	28.2	2.59	6.58	0.47
Sorghum stover (SS)	-	29.6	2.47	5.26	0.36
	+	28.7	2.70	5.72	0.40
SS leaves sheath	-	22.4	4.14	5.44	0.46
SS stems	-	31.0	2.51	4.19	0.29
Legume crop residues class					
Cowpea husks	-	13.8	7.09	4.75	0.65
	+	15.1	7.15	4.64	0.62
Groundnut haulms	-	14.5	6.43	5.01	0.69
Concentrates class					
Cottonseed cake	-	31.4	2.05	6.3	0.40
Millet bran	-	12.4	9.83	5.5	0.88
Wheat bran	-	9.5	13.12	4.8	1.02
Variation sources					
Between classes					
Root MSE		5.92	2.74	0.65	0.17
Class effect		***	***	*	***
Within cereal straws					
RMSE		1.30	0.34	0.59	0.04
Feed effect		***	***	***	***
Treatment effect		NS	*	NS	NS
Feed*treatment		NS	NS	NS	NS
Within legumes crop residues					
RMSE		0.39	0.38	0.42	0.06
Feed effect		NS	NS	NS	NS
Treatment effect		*	NS	NS	NS
Within concentrates					
RMSE		2.73	1.13	1.17	0.21
Feed effect		***	***	**	***

UT: urea treatment; $T_{1/2}$: halflife to the maximum gas volume (h); μ : maximum rate of gas production at the point of inflection of the gas curve (ml/h), PF: partitioning factor; DEF: degradation efficiency factor; RMSE: Root mean square error; NS ($P > 0.05$); * ($P < 0.05$); ** ($P < 0.01$); *** ($P < 0.001$).

4. Discussion

Treating with urea generates gaseous ammonia and carbonic gas, which spread and treat forages and ensure good conservation and quality [4, 3]. This ensures the breakdown of chemical bonds between lignin, hemicellulose and cellulose, which are respectively indigestible, partially digestible or completely digestible.

Cereal straws, legume crop residues and concentrates had high effect on *in vitro* digestibility due to differences in physical and chemical composition in agreement with others [19, 20, 3]. The facts that urea treatment increased Trdeg, MY and (C) except with *Diheteropogon* and cowpea husk confirms an increase in quality and in rumen microbial activity and cell wall digestion of the feeds [20]. Gas production was highest in *Eragrostis* and lowest in millet stover and conversely millet stover had higher MY than *Eragrostis*. These results agree with [21] stipulating that GP and microbial yield are inversely related. Differences in ME among cereal straws can be attributed to differences in chemical composition of feeds.

The increase of ME of urea-treated cowpea husk could be due to increased nitrogen content [3] and probably ether extract. It was shown an improvement of nutritive value of moistened cowpea husk through increased microbial yield, nitrogen content and ether extract [18]. However, overflow of ammonia causes protein wastage when fermentable energy is insufficient to support microbial growth required to utilise the excess degraded protein [22], however reasons need to be sorted to explain why urea-treatment of cowpea husk decreased feed digestion and GP. Additionally, microbial fermentation in the rumen is highly dependent on the protein: energy (P/E) ratio [23].

Wheat bran had higher Trdeg and GP than millet bran in agreement a study [24] stipulating that nitrogen content is positively correlated to degradation parameters. The low Trdeg and GP of cottonseed cake compared to brans despite its high nitrogen content may be due to the presence of gossypol (phenolic organic compound) which could inhibit microbial activity in the rumen [25]. These findings were also supported by [26] who reported that free gossypol decreased crude protein content and *in vitro* digestibility. Furthermore, that condensed tannin constituted a limiting factor of *in vitro* gas production [27].

The large variation of kinetic factors, half time ($T_{1/2}$), maximum rate of GP (μ), PF and DEF within cereal straws is in agreement with [28]. Values of $T_{1/2}$ and DEF in maize stover and grass hay reported by [18] were lower and overlapped our findings on cereal straws. It may be conclude that our cereal straws are lower in nutritive values [3] than these grass hay and maize stover. That urea treatment increased (μ) for all straws may be explained by increased rate of degradation (C) due to increased rumen microbial activity and cell wall digestion [29]. It is possible that urea-treatment of cowpea husk may increase $T_{1/2}$ because ammonia interact with lignin producing phenolic compound that inhibit bacteria growth.

The kinetic factors were significantly affected by concentrates

due to differences in chemical composition among concentrates [30, 3]. Wheat bran with low NDF and high nitrogen content [2, 3] had higher (μ) and (DEF) and lower ($T_{1/2}$) than millet bran. The highest ($T_{1/2}$) and lowest (μ) and (DEF) observed in cottonseed cake may be due to its content of gossypol which decreased its degradability [25]. Additionally, feeds with high Trdeg, C, μ , DEF and low $T_{1/2}$ and Lt may predict good nutritive value and good microbial efficiency in agreement with [31, 18]. It should be stated that urea-treatment of cowpea husk emitted an objectionable odour which is yet unquantified. This suggests that either energy or nitrogen is unavailable for use or bacteria-inhibitory factor occur in urea-treated cowpea husk. Since, cowpea husk by itself constitutes a good source of protein and energy it should not be urea-treated.

5. Conclusion

Cereal straws are low in ME and *in vitro* digestion while legume crop residues and concentrates are high in ME and *in vitro* digestion parameters. Urea treatment did not conclusively increase the *in vitro* digestion and ME content of cereal straws as expected. To increase the feeding values of tropical cereal straws with low nutritive value, urea-treated straws supplemented with concentrates and legume crop residues are good strategies to curb the consequence of long dry season in this area.

Conflict of Interest

This manuscript is our own research with the original data. We acknowledge that all authors have contributed significantly; so there is no conflict of interest.

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