AGRICULTURAL AND FOOD SCIENCE

Agricultural and Food Science (2022) 31: 260–281

https://doi.org/10.23986/afsci.115262

Chemical treatment of straw for ruminant feeding with NaOH or urea – investigative steps via practical application under current European Union conditions

Siriwan D. Martens[1], Vicki Wildner[1,2], Johanna Schulze[1,2], Wolfram Richardt[3], Jörg M. Greef[4], Annette Zeyner[2] and Olaf Steinhöfel[1,2]

[1]Department of Animal Husbandry, Saxon State Office for Environment, Agriculture and Geology (LfULG), Am Park 3, 04886 Köllitsch, Germany

[2]Institute of Agricultural and Nutritional Sciences, Martin Luther University Halle-Wittenberg, Theodor-Lieser-Str. 11, 06120 Halle (Saale), Germany

[3]Landwirtschaftliche Kommunikations- und Servicegesellschaft mbH (LKS), August-Bebel-Str. 6, 09577 Niederwiesa, Germany

[4]Institute for Crop and Soil Science, Julius Kühn-Institut (JKI), Bundesallee 58, 38116 Braunschweig, Germany

e-mail: siriwan.martens@smekul.sachsen.de

Weather extremes in parts of Europe have led to a renewed search for alternative feeds for ruminants. Cereal straw presents one source of fibre, which is hard to digest due to its lignin-carbohydrate complexes. Chemical and biological treatments have been investigated to improve digestibility. Here, the applicability of alkaline treatments for farming conditions under EU legislation and their efficacy were checked. Thus, we tested caustic soda (60, 120 g kg^{-1}straw) and urea (15, 30, 45, 60 g kg^{-1}straw without and with urease addition) applications both at laboratory scale and using a mixer-wagon. The nutritive value was evaluated analyzing chemical parameters including fibre components and estimating *in vitro* digestibility. The *in vitro* digestibility indicated by gas production, enzymatically soluble substrate and neutral detergent fibre digestibility (30h) was highest for the NaOH treatments, which did not differ by dose. Remoistening the straw to 600 g DM kg^{-1} was a precondition for the effectiveness of both treatments. Urease addition enhanced the intended ammonification when urea was applied at ≥ 30 g kg^{-1}. An ambient temperature for urea treatment ≥ 25 °C was necessary and had to be maintained for at least 14 d post treatment. The determination of crude ash in NaOH treated feeds by the standard procedure and time overestimated the mineral fraction and had to be modified. This systematic approach provides guidance for feasible straw treatments for EU farmers. However, trials for feed acceptance and *in vivo* digestibility are needed to demonstrate the real effect in animals.

Key words: wheat straw, urease, fibre components, *in vitro* digestibility, gas production

Introduction

Lack of roughage for ruminants due to drought in parts of Europe in 2018 and 2019 has reactivated the search for fibre sources other than “traditional” forages such as grass.

Cereal straw is widely available throughout the year in Europe. The continent had a share of almost 35 % of the world wheat straw production with 213 million tonnes in 2019 (calculated with a grain to straw ratio of 1:0.8; FAO 2021). However, the main obstacle for extensive straw use in animal feeding is its low digestibility due to the high lignin content (Jung 1989, Adesogan et al. 2019).

The plant cell wall is a very complex construction. Cellulose, hemicellulose and lignin are interlinked (Lee et al. 2014). By special treatments these bonds can be loosened so that cellulose and hemicellulose are more accessible to the rumen microflora (Jung and Deetz 1993). The objective of physical, chemical and biological treatments, alone or in combination, is either to increase feed intake, digestibility or both, especially for ruminants (Flachowsky 1987). In contrast to the more recent developments aimed at bioethanol production from straw (Yoswathana et al. 2010), this does not include the conversion of cellulose to sugar prior to the rumen.

In the 1980s intensive research efforts were directed towards chemical treatment of straw in Germany (Flachowsky 1987, Ochrimenko et al. 1987, Schneider et al. 1987). Research and implementation worldwide have been reported (e.g. Owen et al. 2012, Adesogan et al. 2019). Even though some of those approaches were promising, under the current environmental regulations of the EU, the way NaOH or NH_3 were applied in the past is not now feasible. For example, NaOH was predominantly washed out of the straw resulting in the production of contaminated residual water as a by-product. Ammonia was often applied in a gaseous form contributing to gas

Received 4 March 2022 / Accepted 7 December 2022
The Scientific Agricultural Society of Finland

emissions. In the European Union Register of Feed Additives, NaOH is currently listed in the Annex I (released on 21 January 2021) as an acidity regulator for cats, dogs and ornamental fish (Code 1j524) (EC 2021). However, in the sense of the specific use here, it can be defined as a processing aid (EC 2003). On the other hand, feed grade urea is registered for ruminants (EC 2012). The first objective of these trials was to test alternative methods of applying NaOH and urea with regard to their applicability on farm. A secondary objective was to identify simple and inexpensive laboratory indicators for *in vitro* digestibility to allow for a screening of a range of treatments. It was hypothesized that dosage, the humidity of the straw, and in the case of urea also storage temperature, duration and pressure plus the use of an external urease would contribute to the increase of *in vitro* digestibility of cereal straw when treated with either NaOH or urea .

Material and methods

Winter wheat straw was harvested in July 2018 in Köllitsch, Northern Saxony, Germany, with excellent sensory quality because of preceding dry weather conditions and stored as square bales in a dry environment.

Trial 1 – NaOH addition

During the first half of 2019, caustic soda (NaOH microbeads, technical grade, WHC GmbH, Hilgertshausen, Germany) was dissolved in tap water to obtain the respective concentrations and masses to be added to the dry wheat straw, following the Sodagrain process of Orskov et al. 1979. Personal protection measures recommended by the manufacturer such as protective gloves and eyewear as well as sufficient fresh air circulation were followed. In June 2019, the following treatments were applied, partly derived from Flachowsky et al. 1984 and Block et al. 1985, in triplicates:

1. Control (air dry straw, ~880 g DM kg^{-1})
2. 60 g NaOH kg^{-1} air-dry straw + H_2O to remoisten the straw to 600 g kg^{-1} dry matter (DM)
3. 60 g NaOH kg^{-1} air-dry straw + H_2O to remoisten the straw to 450 g kg^{-1} DM
4. 120 g NaOH kg^{-1} air-dry straw + H_2O to remoisten the straw to 600 g kg^{-1} DM
5. 120 g NaOH kg^{-1} air-dry straw + H_2O to remoisten the straw to 450 g kg^{-1} DM

The dissolved NaOH was added to 1.0 kg of chopped straw (2 – 5 cm) in a concrete mixer (120 l, 550 W, 230 V; CM120L, HORNBACH Baumarkt AG, Bornheim, Germany). The straw was mixed for 5 min. The pH and DM content at 105 °C (for > 12 h) were determined. The straw was filled into plastic bags (120 l, 69 cm × 107 cm, polyethylene), which were kept open. Each treatment was stored for either 2 or 7 d at ambient temperature and then frozen to –20 °C for further analysis. Weight was determined before and after storage.

Trial 2 – Urea addition with loose or compact storage

In March 2020, in a second approach, urea (feed grade, 90 %)(SALVANA Tiernahrung GmbH, Klein Offenseth-Sparrieshoop) without or with urease (MAXAMMON®, Harbro Ltd, Turriff, Aberdeenshire, UK) was applied. The urease product contained a mixture of extruded soybeans, pea meal and a by-product of *Aspergillus niger*. For each treatment, a self-propelled diet feeder (SILOKING, 12 m^3, 2006, SILOKING Mayer Maschinenbau GmbH, Tittmoning, Germany) was filled with 100 kg air dry wheat straw (~880 g DM kg^{-1}). The dry ingredients were added first, followed by water. The following treatments were applied:

1. 1.5 kg urea 100 kg^{-1} air-dry straw, the straw remoistened to 800 g DM kg^{-1}
2. 1.5 kg urea + 0.5 kg Maxammon per 100 kg air-dry straw, the straw remoistened to 800 g DM kg^{-1} (following the recommendations of the Maxammon manufacturer)
3. 1.5 kg urea 100 kg^{-1} air-dry straw, the straw remoistened to 600 g DM kg^{-1}
4. 1.5 kg urea + 0.5 kg Maxammon per 100 kg air dry straw, the straw remoistened to 600 g DM kg^{-1}

The straw was subsequently mixed thoroughly for 5 min. The mixture was divided in two parts: one part was loosely (L) filled into plastic bags (120 l, 69 cm × 107 cm, polyethylene), closed with a rubber ring (around 6 and 8 kg treated straw per bag for higher and lower DM), and the other part was compacted (C) by feet in drums of 120 l and closed with an airtight lid (around 14 and 19 kg treated straw drum^{-1} for higher and lower DM). A data logger for temperature (TG-4080; Gemini Data Loggers Ltd, Chichester, UK) was enclosed in each of the triplicates. The straw was stored at about 25 °C for 14 d.

Trial 3 – Increasing urea addition with two storage temperatures

This trial resembled Trial 2. However, we remoistened the straw to 500 g kg^{-1} DM in June 2020. The treatments were applied in laboratory scale by dissolving the ingredients in the liquid first and then applied to the air-dried straw, which was mixed by hand, wearing chemical protection gloves:

1. 33 g urea (90%) kg^{-1} air-dried straw, dissolved in water
2. As 1), +5 g Maxammon kg^{-1} air-dried straw
3. 50 g urea (90%) kg^{-1} air-dried straw, dissolved in water
4. As 3), +5 g Maxammon kg^{-1} air-dried straw
5. 67 g urea (90%) kg^{-1} air-dried straw, dissolved in water
6. As 5), +5 g Maxammon kg^{-1} air-dried straw

About 1 kg of treated straw was packed into side seal bags (50cm × 30 cm, 90 µm, polyethylene) in triplicates and closed using rubber rings.

The straw was stored at about 25 °C and 40 °C, respectively for 14 d and 28 d respectively, resulting in 24 treatments in total. Sufficient ventilation was provided, especially at opening of the treatments.

An overview of the chemical treatments is given in Table 1.

Table 1. Chemical treatments in the different trials

		Trial 1	Trial 2	Trial 3
Reagent	Substance	NaOH	Urea (feed grade)	Urea (feed grade)
	Application rate	60; 120 g kg^{-1} straw	15 g kg^{-1} straw ± urease	30; 45; 60 g kg^{-1} straw ± urease
Straw	Target DM (g kg^{-1})	600; 450	600; 800	500
Storage	Ambient °C	22 °C	25 °C	25; 40 °C
Sampling	days after treatment	2; 7	14	14; 28

Chemical analysis

Samples of untreated and treated straw were analyzed for DM (at 105 °C for 12 h), crude ash, neutral detergent fibre assayed with a heat stable amylase and expressed exclusive of residual ash (aNDFom), acid detergent fibre expressed exclusive of residual ash (ADFom), acid detergent lignin (ADL), crude fat (EE), crude protein (CP), enzymatically soluble organic substance (ELOS), gas production according to the Hohenheim Gas Test (HFT). Analytical methods of the Association of German Agricultural Analytic Research Institutes (VDLUFA 1976) were applied (for the principle of the methods and further references see Appendix 1). The parameter aNDFom digestibility was determined after 30 h ($NDFD_{30h}$). The latter is using the DAISY II-Incubator® (ANKOM Technology, Macedon NY, USA) applying the *In Vitro* True Digestibility Procedure suggested by ANKOM implemented by the Landwirtschaftliche Kommunikations GmbH Lichtenwalde (LKS) (LKS FMUAA 223, Rev. 02/02/2018) (ANKOM 2006, Goeser and Combs 2009). Standard time for NDFD determination by LKS is 30 h. As extraordinary high crude ash values were determined in NaOH treated samples at the beginning applying the standard procedure for 3 h, subsequently different time periods for ashing were tested for 15 selected samples treated with NaOH. Wheat straw treated with 120 g NaOH kg^{-1}DM with varying amounts of water and storage duration were subjected to different ashing times in

a muffle furnace and compared: 3 h, 13 h, 17.5 h and 24 h. Additionally a combination of ashing +NH_4NO_3 (solution of 20 % w/v as described in VDLUFA III, 8.1 [paragraph 7.1]) was applied after 6 h and 9 h in the muffle furnace.

As one aim was to identify simple analytical parameters, which have a high correlation to *in vitro* digestibility of straw, the following were calculated:

digestible aNDFom (g kg^{-1}DM) = $NDFD_{30h}$ (in %)/100 × aNDFom (g kg^{-1} DM),

indigestible aNDFom = aNDFom (g kg^{-1}DM) – digestible aNDFom (g kg^{-1} DM),

Non-fibre carbohydrates (NFC) = (1000 − [aNDFom+CP+EE+ash]),

when urea was applied NFC = 1000 − ash − EE − aNDFom − (CP − CP_u + U) where CP_u is the CP from urea and U is the urea content (Hall 2000, Detmann and Valadares Filho 2010), N share in urea at 950 g kg^{-1} DM = 0.45, i.e. each kg of urea = 2.81 kg CP_u),

cellulose = (ADFom − ADL), hemicellulose = (aNDFom − ADFom), the ratio ADL/ADFom as indicator of degree of lignification (Zeyner 1995). For comparison with the literature, the indicators total digestible nutrients (TDN), relative forage quality (RFQ) and estimated DM intake (DMI) have been calculated and are presented in the appendix (Tables A1–A8).

In the urea treated samples NH_3-N of total N (VDLUFA III, 4.8.1) was determined.

Non-starch polysaccharides (NSP) were analyzed by the Julius Kühn-Institut in Braunschweig (AOAC 2000).

The BfUL (State owned company for Environment and Agriculture, Nossen) determined minerals and trace elements according to DIN EN 15510 (DIN 2017) using ICP-OES (inductively coupled plasma optical emission spectrometry).

Statistical analysis

The effects of the NaOH treatments on chemical composition and digestibility in Trial 1 were analyzed using the model:

$$Y_{ijk} = \mu + NaOHconc_i + DM_j + NaOHconc \times DM_{ij} + \varepsilon_{ij}$$

where μ = general mean, i = 1, 2 (60 g, 120 g NaOH kg^{-1}straw), j = 1, 2 (450, 600 g DM kg^{-1} straw), ε_{ij} = residual error

The effects of the urea treatments on chemical composition and digestibility in Trials 2 and 3 were analyzed using the models:

Trial 2:

$$Y_{ijk} = \mu + DM_i + Max_j + Comp_k + DM * Max_{ij} + DM * Comp_{ik} + Max \times Comp_{jk} + DM \times Max \times Comp_{ijk} + \varepsilon_{ijk}$$

where μ = general mean, i = 1, 2 (600, 800 g DM kg^{-1} straw), j = 1, 2 (0, 5 g Maxammon kg^{-1} straw), k = 1, 2 (compact, loose), ε_{ijk} = residual error

Trial 3:

$$Y_{ijk} = \mu + Urea_i + Max_j + Temp_k + Per_l + Urea \times Max_{ij} + \varepsilon_{ijkl}$$

where μ = general mean, i = 1, 2, 3 (30, 45, 60 g urea kg^{-1} straw), j = 1, 2 (0, 5 g Maxammon kg^{-1}), k = 1, 2 (25, 40 °C), l = 1, 2 (14, 28 d), ε_{ijkl} = residual error

Variance analysis using the univariate and multivariate procedures was performed for the treatments after the respective storage time, while the posthoc Tukey test included the untreated straw. The Pearson correlation was calculated. The software IBM® SPSS® Statistics (Version 19, SPSS, Inc., IBM Company©) was used.

Results

Crude ash determination when NaOH is used

Figure 1 shows the crude ash concentrations at different times of ashing for straw samples, which were all treated with 120 g NaOH kg^{-1}. The shorter the ashing period in the muffle furnace, the higher the standard deviations within replicates (n = 3) of a treatment. With increasing time both the variation within a treatment and the variation between treatments diminished apart from a decreasing absolute value for crude ash.

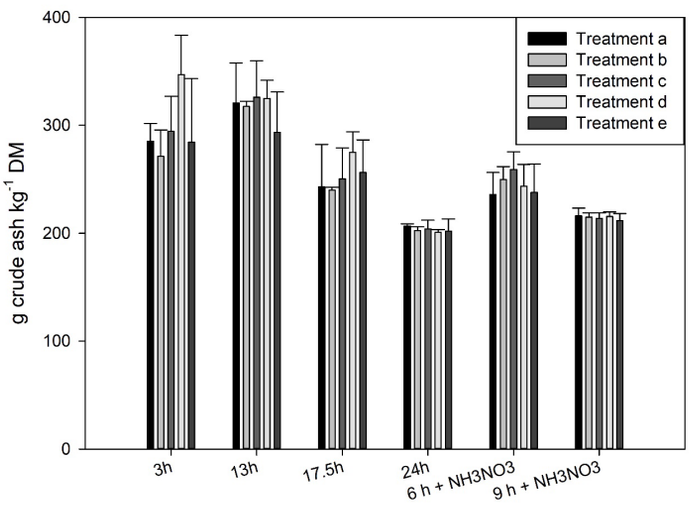

Fig. 1. Crude ash concentrations in different NaOH treated samples following increasing ashing times at 550 °C (a–e, all 120 g NaOH kg^{-1}; a: 450 g DM kg^{-1}, 2 d storage; b: 600 g DM kg^{-1}, 2 d; c: 600 g DM kg^{-1}, 7d; d: like a [other batch]; e: 450 g DM kg^{-1}, 7 d). Error bars show the standard deviation.

Trial 1 – NaOH addition

Results for chemical composition are presented in Table 2. Values of the different storage duration (2 d and 7 d) were averaged because of their similarity. The pH varied between 11 and 12 depending on the NaOH application rate. In order not to confuse the replacement of organic matter by sodium with the possible effect of NaOH, the fibre components including the cell wall carbohydrates and NFC are given in g kg^{-1}organic matter (OM). The crude ash almost doubled and tripled when adding 60 or 120 g NaOH kg^{-1}straw respectively. While aNDFom decreased with increasing NaOH concentration, ADFom increased compared to the control. ADL was lower with the highest application rate at 600 g DM kg^{-1}. Thus the cellulose: ADL ratio increased likewise (further calculated parameters see Appendix, Table A1). All three parameters for *in vitro* digestibility ($NDFD_{30h}$, HFT, ELOS) were higher compared to the control. There was no difference in these parameters between the NaOH treatments. The different proportions of the fibre fractions (cellulose/ADL, ADL/ADFom) of the high NaOH treatment were significantly higher or lower respectively than the control. The main cell wall carbohydrate monomers which were analyzed, namely glucose and xylose, did not change with treatments. Only the minor sugar monomers arabinose and mannose increased with NaOH addition. The xylose:arabinose ratio lowered (7.3 vs. 6.2 vs. 5.9 with 0, 60 and 120 g NaOH kg^{-1} DM). OM losses were highest with high moisture (450 g kg^{-1}) and the lower NaOH application rate (60 g kg^{-1}), and amounted only one quarter with the high application rate and 600 g kg^{-1} DM (Table 2).

When correlating the different indicators of digestibility with each other (HFT, ELOS, $NDFD_{30h}$) the correlation coefficient R was < 0.2 with $p > 0.1$ whith regard to the NaOH treatments alone (Appendix, Fig. A1).

Main and significant differences in the mineral composition were present depending on the NaOH addition, i.e. 31 g vs. 62 g Na kg^{-1} DM for the 60 and 120 g treatment respectively while untreated straw contained about 0.2 g Na kg^{-1} DM.

Table 2. Chemical composition and *in vitro* digestibility of NaOH treated wheat straw (2 and 7 d storage duration resumed) (Trial 1)

g NaOH kg^{-1}	0 (untreated)	60	60	120	120	SEM	Treatment effects (Significance level)		
Target DM [g kg^{-1}]	900	450	600	450	600				
n	11	12	7	11	7		NaOH	DM	NaOH x DM
pH	6.20^{c}	10.7^{b}	10.8^{b}	12.1^{a}	12.3^{a}	0.12	***	n.s.	n.s.
DM [g kg^{-1}]	906^{a}	432^{e}	593^{c}	462^{d}	614^{b}	2.5	***	***	n.s.
Crude ash [g kg^{-1} DM]	79^{c}	140^{b}	151^{b}	215^{a}	230^{a}	6.0	***	n.s.	n.s.
Crude protein [g kg^{-1} OM]	38.8	45.8	47.3	44.6	42.8	1.29	n.s.	n.s.	n.s.
Ether extract [g kg^{-1} OM]	9.28^{c}	16.3^{b}	13.1^{bc}	24.8^{a}	25.3^{a}	0.82	***	n.s.	n.s.
aNDFom [g kg^{-1} OM]	832^{a}	765^{b}	740^{b}	681^{c}	694^{c}	6.3	***	n.s.	n.s.
ADFom [g kg^{-1} OM]	468^{b}	521^{a}	514^{a}	531^{a}	529^{a}	4.6	n.s.	n.s.	n.s.
ADL [g kg^{-1} OM]	62.7^{a}	64.5^{a}	54.9^{a}	45.1^{ab}	30.2^{b}	2.77	***	*	n.s.
NFC [g kg^{-1} OM]	120^{b}	165^{ab}	179^{a}	148^{ab}	160^{ab}	6.6	n.s.	n.s.	n.s.
Ratios									
dig/indig aNDFom	0.46^{b}	1.22^{a}	1.06^{a}	1.55^{a}	1.42^{a}	0.080	*	n.s.	n.s.
Cellulose/ADL	6.55^{c}	7.23^{c}	8.66^{bc}	14.1^{ab}	19.9^{a}	0.90	***	o	n.s.
ADL/ADFom	0.134^{a}	0.124^{a}	0.106^{ab}	0.084^{bc}	0.056^{c}	0.0048	*	*	n.s.
$NDFD_{30h}$ [g kg^{-1} NDF]	310^{b}	533^{a}	512^{a}	591^{a}	572^{a}	13.5	*	n.s.	n.s.
HFT [ml 200 mg^{-1} DM]	32.1^{b}	43.1^{a}	41.6^{a}	42.8^{a}	41.4^{a}	0.24	n.s.	**	n.s.
ELOS [g kg^{-1} DM]	346^{b}	516^{a}	496^{a}	494^{a}	511^{a}	5.9	n.s.	n.s.	n.s.
Polysaccharides [g kg^{-1} OM]	(n = 3)							n.s.	n.s.
Arabinose	29.3^{b}	37.2^{a}	37.1^{a}	38.5^{a}	38.7^{a}	0.51	n.s.	n.s.	n.s.
Xylose	215	233	225	225	229	1.9	n.s.	n.s.	n.s.
Mannose	1.70^{c}	2.55^{b}	2.61^{b}	3.33^{a}	3.27^{a}	0.087	***	n.s.	n.s.
Galactose	7.03	7.11	7.25	7.68	7.82	0.122	*	n.s.	n.s.
Glucose	248	268	269	258	254	2.8	*	n.s.	n.s.
Losses	(n = 6)								n.s.
DM losses [g kg^{-1}]		137^{a}	94.4^{ab}	51.5^{b}	31.0^{b}	11.67	***	o	n.s.
OM losses [g kg^{-1}]		118^{a}	80.4^{ab}	40.7^{bc}	24.4^{c}	10.03	***	o	n.s.

DM = dry matter; aNDFom = neutral detergent fibre analyzed with heat-stable amylase and expressed without residual ash; ADFom = acid detergent fibre expressed without residual ash; ADL = acid detergent lignin; NFC = non-fibre carbohydrates; $NDFD_{30h}$ = aNDFom *in vitro* digestibility in 30 h; dig/indig = digestible/indigestible; HFT = Hohenheim Feed value Test; ELOS = enzymatically soluble organic substance; SEM = standard error of the mean. Variance analysis excluding the untreated control (n.s. = not significant, o $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Treatments with different letters are significantly different (Tukey test, $p < 0.05$).

Trial 2 – Urea addition with loose or compact storage

When monitoring the temperature of the urea treated straw (15 g kg^{-1}) stored in drums and bags at 25 °C ambient, a small peak was recognized during the second day, especially in the loose treatments with 600 g kg^{-1} DM. The treatment without Maxammon peaked after 25 h with 5.8 K above ambient, while the treatment with Maxammon peaked only after 36 h with 7.9 K above ambient. The treatments with 800 g kg^{-1} DM adapted to the ambient temperature (25 °C), and the compact lower dry matter treatments remained below 29 °C. The chemical composition of the urea treated straw is presented in Table 3. After 14 d the pH was highest in the high DM treatments while it was more differentiated in the lower dry matter treatments with approximately 8.4 without and 8.9 with Maxammon treatment. There was a significant influence of the urease treatment after 14 d on almost all parameters, with DM playing the second most important role while compaction was subordinated (detailed results in the Appendix, Table A2). Ether extract content decreased with urease application. The aNDFom and ADFom values of the treatments with urease was similar to the original straw while they were lower with the urea only additive treatment. ADL content did not change significantly compared to the original straw. Gas production and ELOS increased with urea application without urease. In general, those nutritional quality parameters were highest with 600 g kg^{-1} DM without Maxammon. Also, the DM losses were highest with this treatment (43 g kg^{-1} on average). NH_3-N was negatively correlated to pH after 14 d of storage in the range of pH 8.5–9.4 ($R = -0.68$, $p < 0.001$).

Table 3. Chemical composition and *in vitro* digestibility of urea treated wheat straw (15 g urea kg^{-1}) at different DM levels, after 14 d of storage (compact and loose resumed) (Trial 2)

Target DM [g kg^{-1}]	880#	600	600	800	800	SEM	Treatment effects (Significance level)						
Maxammon [g kg^{-1}]	0	0	5	0	5		Max.	DM	Comp.	Max. × DM	Max. × Comp.	DM × Comp.	Max. × DM × Comp.
storage [d]	0	14	14	14	14								
n	3	6	6	6	6								
pH	6.74^{d}	8.46^{c}	8.90^{b}	9.33^{a}	9.32^{a}	0.012	***	***	n.s.	***	n.s.	**	n.s.
DM [g kg^{-1}]	877^{a}	623^{d}	607^{e}	796^{c}	815^{b}	0.4	o	***	*	***	***	**	o
Crude ash [g kg^{-1} DM]	73.9^{a}	63.8^{cd}	70.0^{b}	61.6^{d}	64.7^{c}	0.24	***	***	n.s.	**	n.s.	n.s.	n.s.
Crude protein [g kg^{-1} DM]	34.7^{c}	79.0^{a}	62.3^{b}	75.7^{a}	67.3^{b}	0.87	***	n.s.	n.s.	*	n.s.	n.s.	n.s.
Ether extract [g kg^{-1} DM]	17.4^{ab}	20.4^{a}	12.5^{c}	16.3^{b}	12.0^{c}	0.31	***	**	n.s.	*	n.s.	*	*
aNDFom [g kg^{-1} DM]	793^{a}	746^{c}	791^{a}	775^{b}	802^{a}	1.1	***	***	o	**	n.s.	o	n.s.
ADFom [g kg^{-1} DM]	475^{a}	440^{c}	486^{a}	455^{b}	475^{a}	1.7	***	n.s.	n.s.	**	n.s.	n.s.	n.s.
ADL [g kg^{-1} DM]	60.7^{abc}	57.5^{bc}	61.0^{ab}	53.1^{c}	68.3^{a}	0.87	***	n.s.	n.s.	**	n.s.	n.s.	n.s.
NFC [g kg^{-1} DM]	81.3^{c}	116^{a}	91.1^{bc}	98.9^{b}	80.6^{c}	1.19	***	***	n.s.	n.s.	n.s.	n.s.	n.s.
NH_3-N [g kg^{-1} N]	17.1^{e}	29.8^{b}	37.4^{a}	19.5^{c}	17.6^{d}	1.60	***	***	n.s.	***	n.s.	*	n.s.
In vitro digestibility													
$NDFD_{30h}$ [g kg^{-1} NDF]	328	360	328	361	318	1.9	***	n.s.	**	n.s.	***	**	**
HFT [ml 200 mg^{-1} DM]	33.8^{c}	39.6^{a}	33.6^{c}	35.5^{b}	32.8^{c}	0.122	***	***	n.s.	***	**	n.s.	n.s.
ELOS [g kg^{-1} DM]	354^{c}	459^{a}	340^{c}	404^{b}	358^{c}	1.7	***	***	n.s.	***	o	n.s.	n.s.
DM losses [g kg^{-1}]		43.3^{a}	8.53^{b}	15.1^{b}	2.18^{b}	0.192	***	***	n.s.	*	**	n.s.	n.s.

= no urea; DM = dry matter; aNDFom = neutral detergent fibre analysed with heat-stable amylase and expressed without residual ash; ADFom = acid detergent fibre expressed without residual ash; ADL = acid detergent lignin; NFC = non-fibre carbohydrates; $NDFD_{30h}$ = aNDFom *in vitro* digestibility in 30 h; HFT = Hohenheim Feed value Test; ELOS = enzymatically soluble organic substance; SEM = standard error of the mean. Variance analysis excluding the untreated control (n.s. = not significant, o $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Treatments with different letters are significantly different (Tukey test, $p < 0.05$).

Trial 3 – Increasing urea addition with two storage temperatures

The components pH and crude protein were ranked according to urea concentration, especially the latter increasing with rising urea addition, while aNDFom content decreased at the highest urea dosage in contrast to NFC content (Table 4). The urease application had the strongest impact on gas production (Table 4; Appendix, Table A6). The Appendix (Tables A3–A5) gives more details according to temperature and storage time.

Table 4. Chemical composition and *in-vitro* digestibility of urea treated wheat straw (30, 45, 60 g kg^{-1}) after ≥ 14 d of storage (14 & 28 d storage period and 25 & 40 °C storage temperature resumed) (Trial 3)

Urea [g kg^{-1}]	0 (untreated)	30	30	45	45	60	60		Treatment effects (Significance level)				
Maxammon [g kg^{-1}]	0	0	5	0	5	0	5						
n	15	12	12	12	12	12	12	SEM	Urea	Max	Urea x Max	Temp	Per
pH	6.45^{c}	8.51^{b}	8.57^{ab}	8.61^{ab}	8.74^{a}	8.61^{ab}	8.77^{a}	0.020	***	***	n.s.	***	**
DM [g kg^{-1}]	895^{a}	529^{b}	508^{b}	515^{b}	503^{b}	521^{b}	505^{b}	3.8	n.s.	o	n.s.	o	n.s.
Crude ash [g kg^{-1} DM]	77.4^{b}	79.7^{ab}	81.9^{a}	80.9^{ab}	78.5^{ab}	79.4^{ab}	79.9^{ab}	0.44	n.s.	n.s.	o	**	n.s.
Crude protein [g kg^{-1} DM]	35.6^{d}	79.7^{c}	78.5^{c}	106^{b}	89.2^{bc}	143^{a}	113^{b}	3.6	***	***	***	***	o
Ether extract [g kg^{-1} DM]	10.6^{b}	13.2^{ab}	13.6^{a}	13.3^{ab}	12.7^{ab}	13.2^{ab}	13.2^{ab}	0.11	n.s.	n.s.	n.s.	n.s.	n.s.
aNDFom [g kg^{-1} DM]	779^{a}	774^{ab}	772^{abc}	758^{bcd}	777^{a}	753^{d}	755^{cd}	1.9	***	*	*	o	n.s.
ADFom [g kg^{-1} DM]	451	472	470	476	486	456	477	2.0	**	**	*	n.s.	**
ADL [g kg^{-1} DM]	61.6	60.3	59.5	59.8	63.0	60.6	55.9	1.02	n.s.	n.s.	n.s.	*	n.s.
NFC [g kg^{-1} DM]	97.4^{c}	108^{bc}	108^{bc}	124^{ab}	124^{ab}	120^{bc}	149^{a}	3.0	***	**	**	***	**
NH_3-N [g kg^{-1} N]	27.2^{c}	470^{ab}	497^{ab}	465^{ab}	558^{a}	379^{b}	517^{a}	13.8	**	***	**	***	*
In vitro digestibility													
$NDFD_{30h}$ [g kg^{-1} NDF]	303^{ab}	299^{b}	303^{ab}	316^{ab}	334^{ab}	308^{ab}	343^{a}	4.5	*	*	n.s.	*	***
HFT [ml 200 mg^{-1} DM]	32.9^{cd}	33.6^{bc}	35.1^{ab}	33.9^{bc}	36.5^{a}	31.3^{d}	36.8^{a}	0.30	*	***	***	n.s.	***
ELOS [g kg^{-1} DM]	347^{b}	353^{b}	375^{ab}	372^{ab}	374^{ab}	377^{ab}	396^{a}	3.5	*	*	n.s.	*	n.s.
DM losses [g kg^{-1}]		17.1	-35.8	-6.6	-13.7	20.3	-15.1	6.88	n.s.	*	n.s.	n.s.	o

Per =storage period; Temp = storage temperature; Max = Maxammon; DM = dry matter; aNDFom = neutral detergent fibre analysed with heat-stable amylase and expressed without residual ash; ADFom = acid detergent fibre expressed without residual ash; ADL = acid detergent lignin; NFC = non-fibre carbohydrates; $NDFD_{30h}$ = aNDFom *in vitro* digestibility in 30 h; HFT = Hohenheim Feed value Test; ELOS = enzymatically soluble organic substance; SEM = standard error of the mean. Variance analysis excluding the untreated control (n.s. = not significant, o $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Treatments with different letters are significantly different (Tukey test, $p < 0.05$).

In all three urea levels (30, 45, 60 g kg^{-1}) the pH increased immediately after urease was added in contrast to the treatments without Maxammon (Appendix, Tables A3–A5, 0 d of storage). The same was true for the NH_3-N concentration, which rose when urease was applied; it further increased during storage. In most of the cases, final NH_3-N concentration in the straw was lower when stored at 40 °C while CP was higher (significant effect of temperature, Table 4; Appendix, Tables A3–A6). The fibre fractions ADFom and ADL did not differ significantly among treatments. A significant increase in ELOS compared to the original straw and to 30 g urea only was achieved with the highest urea dosage plus urease. $NDFD_{30h}$ did not increase significantly compared to the original straw (Table 4). In general, longer storage times increased $NDFD_{30h}$ (significant effect of period, Table 4; Appendix,

Table A6). This corresponded also to the ratio of digestible/indigestible aNDFom. The result was more defined with gas production (HFT), which was lowest immediately after applying urea and significantly higher than the original straw in the treatments with 45 and 60 g urea plus urease (Table 4; Appendix, Tables A4–A6). The ratio of cellulose/ADL was highest with 60 g urea plus urease and stored for 28 d at 25 °C. At the same time, it corresponded to the lowest ADL/ADF ratio (Appendix, Table A5).

The correlation of digestibility indicators with each other (HFT, ELOS, $NDFD_{30h}$, TDN, DMI, RFQ) was at the highest ≥ 0.5 with RFQ on HFT and digestible NDF on HFT (Appendix, Fig. A2) (with coefficients of determination R = 0.59 at maximum whether in- or excluding the control treatment [0 g urea]), whereupon all correlations were significant.

Table A7 (Appendix) gives an overview of the potential digestibility values with the different treatment groups. Graphically it can be viewed in the supplemental figures A1 and A2. The $NDFD_{30h}$ and the ratio of digestible/indigestible NDF are higher with NaOH, and similar between urea and control. The NaOH treatment showed a much higher variability in the ratio of cellulose/ADL than urea and control treatment, while the mean was slightly higher. Gas production (HFT) and ELOS were highest with NaOH, while urea treatment only gave a slightly higher mean than the control.

Discussion

Protocol for crude ash determination

Caustic soda reacts with carbon dioxide from the air: $2\ NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$. Pure sodium carbonate is very water soluble, but has a high melting point of 851 °C. That is probably the cause of the difficulties in determining the crude ash by the standard method of 3 h at 550 °C.

As a result of our comparison of different ashing times without or with ammonium nitrate a safe protocol for NaOH treated straw apparently is either ashing for 24 h at 550 °C or for 9 h plus an application of a solution of NH_4NO_3.

Chemical composition of NaOH treated straw (Trial 1 – NaOH addition)

It can be assumed that NaOH is washed out in the process of NDF and ADF analysis and that the values are consequently actually "exclusive of residual ash" as declared. The increase in ADFom while aNDFom decreased means a reduction of hemicellulose and a rise in cellulose. The reduction of hemicellulose was also observed with urea (Harada et al. 1999, Vadiveloo and Fadel 2009) and after white-rot fungal treatment (Nayan et al. 2018). It was probably partly solubilized (Canale et al. 1990). The main component of hemicellulose in monocotyledons are xylans. These are long chains of xylose with side branches of arabinose and uronic acid, among others (Sauermost and Freudig 1999). Cellulose consists of a linear chain of several glucose units (Klemm et al. 1998a, Klemm et al. 1998b). However, it is not quite clear why ADF increased and how NaOH could have caused it. Although arabinose has been found in ADF (overview see Südekum 1994), compared to the magnitude of the difference between ADF and NDF here, the rise in arabinose (aldopentose) and mannose (aldohexose) concentrations measured in our samples are negligible. On the other hand, the xylose/arabinose ratio was improved with rising NaOH dosage. It may improve microbial digestion in the rumen (Agbagla-Dohnani et al. 2012). According to Zeyner (1995), the ratio of ADL/ADFom (and its common logarithm) is an indicator of the lignification of a forage and thus of its digestibility. Similarly, this may also apply to cellulose/ADL (Nayan et al. 2018). According to this, the high NaOH treatment should result in the highest NDF digestibility and gas production. However, NDFD30h did not increase linearly in this experiment, and this supports with the mediocre correlations between the concentrations of chemical components and organic matter digestibility found by Galvao et al. 2008. Nevertheless, the ratio of digestible/indigestible NDF doubled and tripled compared to the original straw.

The *in vitro* digestibility of aNDFom, the gas production and the enzymatically soluble organic matter increased by adding NaOH independent of the dose applied, and a poor correlation (R < 0.1) was found between those parameters when the NaOH treated samples were considered exclusively. This shows that an alignment with *in vivo* values is essential in order to classify the values obtained in the laboratory.

Trial 2 – Urea addition with loose or compact storage

The contents of crude protein, NH_3-N and the pH changed as expected. At the low level of application (15 g urea kg^{-1}), the ratio between fibre components did not change significantly. However, ether extract content declined

with urea + urease. That might be explained by the fact that the cuticle waxy layer, which is a component of crude fat (Sun and Tomkinson 2003) was broken down as observed in a trial with rice straw (Shen et al. 1999). In our case this might have been enhanced by the urease. This may also contribute to a higher digestibility (Shen et al. 1999). However, the effect could not be observed in Trial 3.

In vitro parameters for digestibility (HFT, ELOS) apart from NDFD30h were highest at 600 g kg^{-1} DM without Maxammon before and after storage (Appendix, Table A2). The immediate change was surprising as a storage time of at least 14 d is recommended for urea application (Ochrimenko et al. 1987). Only NDFD30h improved with time in this treatment. The high DM and application of Maxammon was unfavorable for the potential digestibility at the low urea dosage of 15 g kg^{-1}.

Trial 3 – Increasing urea addition with two storage temperatures

In contrast to the trial with only 15 g urea kg^{-1}, this trial showed a positive effect of urease with increasing urea dosage (≥ 45 g urea kg^{-1} DM) on gas production. Urease has an accelerating effect to increase organic matter digestibility (Jayasuriya and Pearce 1983). Urease is an enzyme naturally occurring in many plants, bacteria, fungi and algae. According to earlier findings, soybean, which was also an ingredient of the product used in the Trials 2 and 3, seems to be an adequate source of urease for effective application in cereal straw (Jayasuriya and Pearce 1983, Khan et al. 1999).

When comparing the absolute values of *in vitro* digestibility across the two trials (Trial 2 and 3), no clear urea dose-effect relationship was observed. As mediocre correlations between different indicators for potential digestibility were found, *in vivo* trials have to confirm the feeding potential. The figures of DM-losses are inconsistent. Probably the DM determination at 105 °C with highly volatile components (NH_3 at pH > 5.0) is the cause of the error (Weissbach and Kuhla 1995). The principle of straw treatment with urea is based on the reaction $H_2N\text{-}CO\text{-}NH_2 + H_2O \rightarrow 2NH_3 + CO_2$, which is catalyzed by urease. Overall, approximately 50% of the total nitrogen was present in the form of ammonia after storage. The treatments stored at 40 °C maintained a higher CP content than the ones at 25 °C, while the ammonia-N portion in the DM was similar. Ammonia can be utilized by the ruminal microflora as non-protein nitrogen. However, the volatility of NH_3 is enhanced with increasing pH.

General considerations and comparison of the treatments with urea and NaOH

The true NDFD of the different treatments may have varied more widely than in the results presented with only 30 h incubation. Krämer et al. (2012) determined the indigestible NDF in forages after 288 h *in situ* in cows (i.e. 10 times longer incubation time). The real retention time in the rumen depends on the whole ration including its structure. For a first screening the 30 h incubation might have been sufficient.

NaOH seems to give a clear advantage over urea when comparing the *in vitro* digestibility parameters $NDFD_{30h}$, HFT and ELOS to the untreated control. In a palatability trial with sheep, there was no difference in the DM intake of either NaOH or urea treated barley straw (60 g or 40 g kg^{-1}) as the only feed component after three weeks of adaptation (data not shown). In a feeding trial with sheep with the same straw as component of a total mixed ration the NaOH treatment increased the *in vivo* fibre digestibility in contrast to the urea treatment (Bachmann et al. 2022).

The high content of sodium has to be considered in the formulation of the ration for cattle when feeding NaOH treated straw. The daily requirement of sodium of a dairy cow giving 30–40 kg milk per day can be met by an intake of 31–38 g Na per cow per day (700 kg live weight) (Kirchgessner et al. 2014). Therefore, that 1 kg of soda straw (60 g NaOH kg^{-1} DM) is already sufficient to meet the daily sodium requirement of a cow. Thus a sufficient quantity of drinking water has to be provided to facilitate the endogenous regulation and excretion of surplus cations to prevent nephritis (Suttle 2010). Furthermore, the alkaline pH should be neutralized by combining the straw with acidic feeds such as silages. As a general recommendation, alkali-treated feeds should not be used for transition cows because they may increase the incidence of milk fever (Suttle 2010).

Conclusions

A moisture content of 400 g kg^{-1} clearly contributed to the effectivity of both NaOH and urea treatments. While there was a dose-response relationship with urea, doubling the NaOH concentration at an already elevated level did not further increase *in vitro* digestibility. The differences in pressure produced manually were marginal.

Storage temperature played a subordinate role while an extended storage period increased gas production and $NDFD_{30h}$ with the urea application. Urease addition was only advantageous in terms of increased gas production and $NDFD_{30h}$ when applied at urea dosages ≥ 30 g kg^{-1}.

Treatment of wheat straw with 60 g kg^{-1} NaOH or ≥ 45 g kg^{-1} urea at 600 or 500 g DM kg^{-1} respectively increased the *in vitro* digestibility. Urea treated straw had to be stored for at least two weeks (preferably 4) at ≥ 25 °C ambient. Studies on voluntary feed intake and *in vivo* digestibility are needed to confirm the expected benefit in ruminant animals. Chemical straw treatment might be an option to overcome situations of forage scarcity; then the cost-benefit relationship also in terms of the environmental impact may be justified.

Acknowledgements

The experiments were part of the project “Environmentally sound dairy cattle nutrition”, financed by the Saxon State Office for Environment, Agriculture and Geology. Thanks go to Dirk Hillegeist of the JKI Braunschweig for the analysis of non-starch polysaccharides and to our technical assistant Frank Püschel (LfULG). Furthermore, we thank Dr. Ulf Müller (LfULG) for his advice in the statistics and Dr. David Davies for editing the language.

References

Adesogan, A.T., Arriola, K.G., Jiang, Y., Oyebade, A., Paula, E.M., Pech-Cervantes, A.A. Romero, J.J., Ferraretto, L.F. & Vyas, D. 2019. Symposium review: Technologies for improving fiber utilization. Journal of Dairy Science 102: 5726−5755. https://doi.org/10.3168/jds.2018-15334

Agbagla-Dohnani, A., Cornu, A. & Broudiscou, L.P. 2012. Rumen digestion of rice straw structural polysaccharides: effect of ammonia treatment and lucerne extract supplementation in vitro. Animal 6: 1642−1647. https://doi.org/10.1017/S175173111200050X

Anderson, T. & Hoffman, P. 2006. Nutrient composition of straw used in dairy cattle diets. Focus on Forage 8. https://fyi.extension.wisc.edu/forage/files/2014/01/StrawFOF.pdf

ANKOM 2006. Analytical Methods - Daisy Incubators. ANKOM Technology. https://www.ankom.com/analytical-methods-support/daisy-incubators

AOAC 2000. AOAC Official Method 994.13: Total Dietary Fiber. In: Horwitz, W. (ed.). Official Methods of Analysis. AOAC International. Gaithersburg, MD, USA. p. 84–88.

Bachmann, M., Martens, S.D., Le Brech, Y., Kervern, G., Bayreuther, R., Steinhöfel, O. & Zeyner, A. 2022. Physicochemical characterisation of barley straw treated with sodium hydroxide or urea and its digestibility and *in vitro* fermentability in ruminants. Scientific Reports. https://doi.org/10.1038/s41598-022-24738-w

Block, H.J., Weissbach, F. & Prym, R. 1985. Untersuchungen zum Feuchtaufschluss von Stroh mit Natronlauge. 1. Mitteilung: Veränderungen der Verdaulichkeit und der Energiekonzentration. Archives of Animal Nutrition 35: 61−80. (in German). https://doi.org/10.1080/17450398509426968

Canale, C.J., Abrams, S.M., Varga, G.A. & Muller, L.D. 1990. Alkali-Treated Orchardgrass and Alfalfa: Composition and *In Situ* Digestion of Dry Matter and Cell Wall Components. Journal of Dairy Science 73: 2404−2412. https://doi.org/10.3168/jds.S0022-0302(90)78924-2

Detmann, E. & Valadares, Filho, S.C. 2010. On the estimation of non-fibrous carbohydrates in feeds and diets. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia 62: 980−984. https://doi.org/10.1590/S0102-09352010000400030

DIN 2017. Animal feeding stuffs- Methods of sampling and analysis- Determination of calcium, sodium, phosphorus, magnesium, potassium, iron, zinc, copper, manganese, cobalt, molybdenum and lead by ICP-OES; German version EN 15510:2017.

EC 2003. Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. Official Journal L 268:29, 2003. http://data.europa.eu/eli/reg/2003/1831/oj

EC 2012. Commission Implementing Regulation (EU) No 839/2012 of September 2012 concerning the authorisation of urea as feed additive for ruminants. Official Journal of the European Union L252:11, 2012. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:252:0011:0013:EN:PDF

EC 2021. European Union Register of Feed Additives. https://ec.europa.eu/food/safety/animal-feed/feed-additives/eu-register_en

FAO 2021. Crops primary 2019. In: FAOSTAT. http://www.fao.org/faostat/en/#data/QCL

Flachowsky, G. 1987. Physikalische, chemische und biologische Methoden der Strohaufbereitung und Möglichkeiten der praktischen Nutzung. Wissenschaftliche Zeitschrift der Karl-Marx-Universität Leipzig 36: 232−247. (in German).

Flachowsky, G., Wolf, I., Löhnert, H.J., Möller, E., Lampe, W. & Bach, H. 1984. Investigations on the digestibility of untreated and alkali-treated sunflower husks. Beiträge zur tropischen Landwirtschaft und Veterinärmedizin 22: 293−298.

Galvao, L., Guedes, C.M., Rodrigues, M.A.M., Silva, S.R., Valentim, R., Moreira, O., Ribeiro, J.R. & Sequeira, C.A. 2008. Prediction of apparent digestibility of hays from natural pastures of the Northeast region of Portugal. Livestock Research for Rural Development. http://www.lrrd.org/lrrd20/8/galv20124.htm

Goeser, J.P. & Combs, D.K. 2009. An alternative method to assess 24-h ruminal in vitro neutral detergent fiber digestibility. Journal of Dairy Science 92: 3833−3841. https://doi.org/10.3168/jds.2008-1136

Hall, M.B. 2000. Neutral Detergent Soluble Carbohydrates Nutritional Relevance and Analysis: A Laboratory Manual. University of Florida.

Harada, C., Nakamura, Y. & Minato, H. 1999. Effect of Sodium Hydroxide Treatment of Rice Straw on Cell Wall Composition and Digestibility of Dry Matter. Nihon Chikusan Gakkaiho 70: 61−66. https://doi.org/10.2508/chikusan.70.61

Jayasuriya, M.C.N. & Pearce, G.R. 1983. The effect of urease enzyme on treatment time and the nutritive value of straw treated with ammonia as urea. Animal Feed Science and Technology 8: 271−281. https://doi.org/10.1016/0377-8401(83)90048-2

Jung, H.G. 1989. Forage Lignins and Their Effects on Fiber Digestibility. Agronomy Journal 81: 33−38. https://doi.org/10.2134/agronj1989.00021962008100010006x

Jung, H.G. & Deetz, D.A. 1993. Cell Wall Lignification and Degradability. In: Jung, H.G., Buxton, D.R., Hatfield, R.D. & Ralph, J. (eds.). Forage Cell Wall Structure and Digestibility. ASA, CSSA, and SSSA Books. p. 315−346. https://doi.org/10.2134/1993.foragecellwall.c13

Khan, M.J., Scaife, J.R. & Hovell, F.D. 1999. The effect of different sources of urease enzyme on the nutritive value of wheat straw treated with urea as a source of ammonia. Asian-Australasian Journal of Animal Sciences 12: 1063−1069. https://doi.org/10.1016/0377-8401(83)90048-2

Kirchgessner, M., Stangl, G., Schwarz, F. J., Roth, F. X., Südekum, K.-H. & Eder, K. 2014. Tierernährung. 14 ed. DLG-Verlag, Frankfurt am Main. 635 p. (in German).

Klemm, D., Philpp, B., Heinze, T., Heinze, U. & Wagenknecht, W.1998a. Comprehensive cellulose chemistry. Volume 1: Fundamentals and analytical methods. Wiley-VCH Verlag GmbH, Weinheim. 263 p. https://doi.org/10.1002/3527601929

Klemm, D., Philpp, B., Heinze, T., Heinze, U. & Wagenknecht, W. 1998b. Comprehensive cellulose chemistry. Volume 2: Functionalization of cellulose. Wiley-VCH Verlag GmbH, Weinheim. 406 p. https://doi.org/10.1002/3527601937

Krämer, M., Weisbjerg, M.R., Lund, P., Jensen, C.S. & Pedersen, M.G. 2012. Estimation of indigestible NDF in forages and concentrates from cell wall composition. Animal Feed Science and Technology 177: 40–51. https://doi.org/10.1016/j.anifeedsci.2012.07.027

Lee, H.V., Hamid, S.B.A. & Zain, S.K. 2014. Conversion of Lignocellulosic Biomass to Nanocellulose: Structure and Chemical Process. The Scientific World Journal: https://doi.org/10.1155/2014/631013

Nayan, N., Sonnenberg, A.S.M., Hendriks, W.H. & Cone, J.W. 2018. Screening of white-rot fungi for bioprocessing of wheat straw into ruminant feed. Journal of Applied Microbiology 125: 468−479. https://doi.org/10.1111/jam.13894

Ochrimenko, W.I., Flachowsky, G., Richter, G., Löhnert, H.-J. & Hennig, A. 1987. Feuchtstrohkonservierung mit Harnstoff und Einsatz von Feuchtstroh in der Fütterung. Wissenschaftliche Zeitschrift der Karl-Marx-Universität Leipzig 36: 260−266. (in German). https://doi.org/10.1080/17450398609425324

Orskov, E.R., Stewart, C.S. & Greenhalgh, J.F.D. 1979. The effect of sodium hydroxide and urea on some storage properties of moist grain. The Journal of Agricultural Science 92: 185−188. https://doi.org/10.1017/S0021859600060639

Owen, E., Smith, T. & Makkar, H. 2012. Successes and failures with animal nutrition practices and technologies in developing countries: A synthesis of an FAO e-conference. Animal Feed Science and Technology 174: 211−226. https://doi.org/10.1016/j.anifeedsci.2012.03.010

Sauermost, R. & Freudig, D. 1999. Lexikon der Biologie. Spektrum Akademischer Verlag, Heidelberg. (in German). https://www.spektrum.de/lexikon/biologie/

Schneider, M., Richter, G. & Flachowsky, G. 1987. Zum Futterwert von NH3-begastem Weizenstroh. Wissenschaftliche Zeitschrift der Karl-Marx-Universität Leipzig 36: 248−253. (in German).

Shen, H.S., Sundstol, F., Eng, E.R. & Eik, L.O. 1999. Studies on untreated and urea-treated rice straw from three cultivation seasons: 3. Histological investigations by light and scanning electron microscopy. Animal Feed Science and Technology 80: 151−159. https://doi.org/10.1016/S0377-8401(99)00045-0

Südekum, K.H. 1994. Monosaccharide composition of cell-wall carbohydrates. Digestion and absorption. Livestock Production Science 39: 71−79. https://doi.org/10.1016/0301-6226(94)90155-4

Sun, R.C. & Tomkinson, J. 2003. Comparative study of organic solvent and water-soluble lipophilic extractives from wheat straw I: yield and chemical composition. Journal of Wood Science 49: 0047−0052. https://doi.org/10.1007/s100860300008

Suttle, N. 2010. Mineral nutrition of livestock. 4th ed. CABI, Wallingford. 579p. http://www.ucv.ve/fileadmin/user_upload/facultad_agronomia/Producion_Animal/Minerals_in_Animal_Nutrition.pdf

Vadiveloo, J. & Fadel, J.G. 2009. The response of rice straw varieties to urea treatment. Animal Feed Science and Technology 151: 291−298. https://doi.org/10.4141/cjas96-037

VDLUFA 1976. VDLUFA Book of Methods Volume III The chemical analysis of feedstuff. 3rd ed. VDLUFA-Verlag, Darmstadt.

Weissbach, F. & Kuhla, S. 1995. Substance losses in determining the dry matter content of silage and green fodder: arising errors and possibilities of correction. Übersichten zur Tierernährung 23: 189−214.

Yoswathana, N., Phuriphipat, P. Treyawutthiwat, P. & Eshtiaghi, M.N. 2010. Bioethanol production from rice straw. Energy Research Journal 1: 26−31. https://doi.org/10.3844/erjsp.2010.26.31

Zeyner, A. 1995. Ermittlung des Gehaltes an verdaulicher Energie im Pferdefutter über die Verdaulichkeitsschätzung. Übersichten zur Tierernährung 23: 55−104. (in German).

Appendix 1: Analytical methods VDLUFA III and calculation forage quality indicators

Principles

8.1	Crude ash	at 550 °C for at least 3 h
6.5.1	aNDFom	adapted from Mertens 2002
6.5.2	ADFom	adapted from Van Soest et al. 1991
6.5.3	ADL	adapted from Goering and Van Soest 1970
5.1.1	EE	extraction with petroleum ether, see also Mattsson 1978
4.1.2	CP	combustion according to Dumas
6.6.1	ELOS	using pepsin, HCl and cellulose, see also De Boever et al. 1986
25.1	HFT	see Menke et al. 1979, Menke and Steingass 1987, Steingass and Menke 1986
4.8.1	NH3-N	by microdiffusion, fresh sample extracted with water, compare Conway 1962, Conway and Byrne 1933

Calculations

total digestible nutrients TDNgrass = (NFC1 × 0.98) + (CP × 0.87) + (FA × 0.97 × 2.25) + (NDF × 0.93 × (22.7 + 0.664 × NDFD30h) / 100) – 10 (in % of DM, FA fatty acids = ether extract – 1; equation for grass (Moore and Undersander 2002); NDFD48h replaced with NDFD30h),

estimated dry matter intake (DMIgrass) = –2.318 + 0.442 × CP – 0.01 × CP2 – 0.0638 × TDN + 0.000922 × TDN2 + 0.18 × ADFom – 0.00196 × ADF2 – 0.00529 × CP × ADFom (for grass, Moore and Kunkle 1999),

relative forage quality RFQ = (DMIgrass, % of BW) × TDNgrass, % of DM) / 1.23 (Undersander and Moore 2004).

1NFC Non-fiber carbohydrates = (1000 – [aNDFom+CP+EE+ash]),

when urea was applied NFC = 1000 – ash – EE – aNDFom – (CP – CPu + U) where CPu is the CP from urea and U is the urea content (Hall 2000, Detmann and Valadares Filho 2010), N share in urea at 950 g kg^{-1} DM = 0.45, i.e. each kg of urea = 2.81 kg CPu)

References

Conway, E.J. 1962. Microdiffusion Analysis and Volumetric Error. 5th edition, C. Lockwood & Son. 467 p.

Conway, E.J. & Byrne, A. 1933. An absorption apparatus for the micro-determination of certain volatile substances: The micro-determination of ammonia. Biochemical Journal 27: 419–429.

De Boever, J.L., Cottyn, B.G., Buysse, F.X., Wainman, F.W. & Vanacker, J.M. 1986. The use of an enzymatic technique to predict digestibility, metabolizable and net energy of compound feedstuffs for ruminants. Animal Feed Science and Technology 14: 203–214. https://doi.org/10.1016/0377-8401(86)90093-3

Detmann, E. & Valadares Filho, S.C. 2010. On the estimation of non-fibrous carbohydrates in feeds and diets. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia 62: 980–984. http://dx.doi.org/10.1590/S0102-09352010000400030

Goering, H.K. & Van Soest, P.J. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). US Agricultural Research Service, Washington D.C.

Hall, M.B. 2000. Neutral Detergent Soluble Carbohydrates Nutritional Relevance and Analysis: A Laboratory Manual. University of Florida. 77 p.

Mattsson, P. 1978. Crude fat determination in feedingstuffs: some studies of extraction and hydrolysis methods. Meddelande - Statens Lantbrukskemiska Laboratorium (Sweden) 49.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D. & Schneider, W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro. Journal of Agricultural Science 93: 217–222. https://doi.org/10.1017/S0021859600086305

Menke, K.H. & Steingass, H. 1987. Estimation of the energy feeding value from gas formation estimated in vitro with rumen fluid and from chemical analysis. 2. Regression equations. Ubersichten zur Tierernahrung 15: 59–93.

Mertens, D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. Journal of AOAC International 85: 1217–1240. https://doi.org/10.1093/jaoac/85.6.1217

Moore, J.E. & Kunkle, W.E. 1999. Evaluation of equations for estimating voluntary intake of forages and forage-based diets. Journal of Animal Science (Suppl. 1) 204.

Appendix

Moore, J.E. & Undersander, D.J. 2002. Relative forage quality: An alternative to relative feed value and quality index. Proceedings 13th Annual Florida Ruminant Nutrition Symposium. p. 16–32. https://animal.ifas.ufl.edu/apps/dairymedia/rns/2002/moore.pdf

Steingass, H. & Menke, K.H. 1986. Estimation of the energy feeding value from gas formation estimated in vitro with rumen fluid and from chemical analysis. 1. Studies of the method. Übersichten zur Tierernährung 14: 251–270.

Van Soest, P.J., Robertson, J.B. & Lewis, B.A. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science 74: 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Undersander, D. & Moore, J.E. 2004. Relative Forage Quality (RFQ) - Indexing legumes and grasses for forage quality. In: Proceedings, National Alfalfa Symposium, 13-15 December, 2004, San Diego, CA, UC Cooperative Extension. University of California. Davis. https://alfalfa.ucdavis.edu/+symposium/proceedings/2004/04-193.pdf

Table A1. Chemical composition and *in vitro* digestibility of NaOH treated wheat straw (2 and 7 d storage duration resumed) (Trial 1)

g NaOH kg^{-1}	0 (untreated)	60	60	120	120				
Target DM [g kg^{-1}]	900	450	600	450	600	SEM	Significance level		
n	11	12	7	11	7		NaOH	DM	NaOH x DM
Calculated forage quality									
TDN [g kg^{-1} DM]	345^{c}	437^{a}	418^{ab}	373^{bc}	371^{bc}	7.5	**	n.s.	n.s.
RFQ	37.2^{c}	51.5^{a}	48.8^{ab}	41.5^{bc}	39.2^{c}	1.27	***	n.s.	n.s.
DMI [g kg^{-1} BW]	13.2	14.4	14.4	13.4	13.0	0.18	**	n.s.	n.s.

aNDFom = neutral detergent fiber analysed with heat-stable amylase and expressed without residual ash; ADFom = acid detergent fiber expressed without residual ash; ADL = acid detergent lignin; dig/indig = digestible/indigestible; TDN = total digestible nutrients; RFQ = relative forage quality; DMI = dry matter intake; BW = body weight; SEM = standard error of the mean. Variance analysis excluding the untreated control (n.s. = not significant, o $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Treatments with different letters are significantly different (Tukey test, $p < 0.05$).

Table A2. Chemical composition and *in vitro* digestibility of urea treated wheat straw (15 g urea kg^{-1}) at different DM levels, compact and loose, after 0 and 14 d of storage (Trial 2)

Target DM [g kg^{-1}]	880 (no urea)	600	600	600	600	600	600	800	800	800	800	800	800	
Maxammon [g kg^{-1} DM]	0	0	5	0	0	5	5	0	5	0	0	5	5	
compaction	L	L	L	L	C	L	C	L	L	L	C	L	C	SEM
storage [d]	0	0	0	14	14	14	14	0	0	14	14	14	14	14
n	3	3	3	3	3	3	3	3	3	3	3	3	3	
pH	6.74^{e}	7.24^{d}	8.37^{c}	8.45^{c}	8.47^{c}	8.85^{b}	8.94^{b}	8.37^{c}	7.21^{d}	9.37^{a}	9.29^{a}	9.38^{a}	9.26^{a}	0.076
DM [g kg^{-1}]	877^{a}	646^{e}	602^{h}	617^{g}	630^{f}	608^{h}	607^{h}	805^{c}	814^{b}	794^{d}	797^{cd}	818^{b}	813^{b}	19.9
Crude ash [g kg^{-1} DM]	73.9^{a}	61.9^{e}	70.1^{abc}	63.8^{de}	63.7^{e}	70.6^{ab}	69.3^{abcd}	63.5^{cd}	66.2^{bcde}	61^{e}	62.3^{e}	64.6^{de}	64.7^{cde}	0.68
Crude protein [g kg^{-1} DM]	34.7^{f}	91.8^{a}	45.8^{f}	79.7^{ab}	78.3^{abc}	61.6^{e}	62.9^{de}	76.1^{bcd}	75.2^{bcde}	78.2^{abc}	73.2b^{cde}	65.9^{cde}	68.6^{bcde}	1.59
Ether extract [g kg^{-1} DM]	17.4^{abc}	18.5^{ab}	18.3^{ab}	21.1^{a}	19.6^{a}	12.5^{cde}	12.6^{cde}	17.1^{abcd}	18.3	14.0^{bcde}	18.6^{ab}	12.3^{de}	11.7^{e}	0.79
aNDFom [g kg^{-1} DM]	793^{abc}	753de	813^{a}	747de	746^{e}	790^{abc}	791^{abc}	779^{bc}	796^{ab}	780^{bc}	771^{cd}	806^{a}	798^{ab}	4.5
ADFom [g kg^{-1} DM]	475^{abcd}	425^{f}	490^{a}	442^{ef}	439^{ef}	486^{a}	487^{a}	454^{cde}	477a^{bc}	451de	460^{bcde}	472^{abcd}	478^{ab}	4.0
ADL [g kg^{-1} DM]	60.7^{abc}	44.6^{d}	64.4^{abc}	58.1^{abcd}	57.0^{abcd}	62.2^{abc}	59.8^{abc}	60.3^{abc}	67.5^{ab}	54.9^{bcd}	51.2^{cd}	65.7^{abc}	70.9^{a}	1.42
NFC [g kg^{-1} DM]	81.3^{defg}	99.7^{abcd}	63.8^{g}	114^{ab}	118^{a}	91.9^{cdef}	90.3^{cdef}	91.9^{cdef}	72.5f^{g}	95^{bcde}	103^{abc}	77.6^{efg}	83.6^{cdefg}	2.90
NH_3-N [g kg^{-1} N]	17.1^{h}	107^{g}	451^{a}	295^{c}	302^{c}	369^{b}	379^{b}	137^{fg}	155^{ef}	202^{d}	188^{d}	175de	177de	16.83
Ratios														
dig/indig aNDFom	0.488^{bc}	0.498^{bc}	0.577^{ab}	0.599^{a}	0.527^{abc}	0.461^{cd}	0.516^{abc}	0.481^{bc}	0.515^{abc}	0.605^{a}	0.526^{abc}	0.381^{d}	0.561^{ab}	0.0149
Cellulose/ADL	6.83^{abc}	8.73^{a}	6.65^{bc}	6.61^{bc}	6.72^{bc}	6.85^{abc}	7.18^{abc}	6.52^{bc}	6.08^{bc}	7.21^{abc}	7.98^{ab}	6.25^{bc}	5.76^{c}	0.157
ADL/ADFom	0.128^{abc}	0.105^{c}	0.131^{abc}	0.132^{abc}	0.130^{abc}	0.128^{abc}	0.123^{abc}	0.133^{abc}	0.142^{a}	.122^{abc}	0.111^{bc}	0.139^{ab}	0.148^{a}	0.0027
Calculated forage quality														
TDN [g kg^{-1} DM]	354^{fg}	410^{bc}	376de	435^{a}	421^{ab}	370^{ef}	382de	392^{cd}	389^{cde}	416^{ab}	410^{bc}	346^{g}	391^{cde}	5.9
RFQ	35.9^{h}	62.5^{a}	40.6^{fg}	63.7^{a}	61^{a}	43.9^{ef}	45.8^{de}	53.9^{bc}	49.8^{cd}	58.6^{ab}	55.5^{b}	43.2^{ef}	49.1^{cd}	1.61
DMI [g kg^{-1} BW]	12.5^{f}	18.8^{a}	13.3^{f}	18.0^{ab}	17.8^{abc}	14.6^{e}	14.7^{e}	16.9^{bcd}	15.8^{de}	17.3^{bc}	16.6^{cd}	15.4^{e}	15.4^{e}	0.28
In vitro digestibility														
NDFD30h [g kg^{-1} NDF]	328^{cd}	332^{bcd}	366^{abc}	374^{ab}	345^{abcd}	316de	340^{abcd}	325^{cd}	338^{abcd}	377^{a}	344^{abcd}	276^{e}	359^{abc}	6.65
HFT [ml 200 mg^{-1} DM]	33.8^{bcd}	38.0^{a}	35.0^{bc}	39.2^{a}	39.9^{a}	34.0^{bcd}	33.2^{cd}	34.0^{bcd}	32.8^{d}	35.0^{bc}	35.9^{b}	33.8^{d}	32.4^{d}	0.56
ELOS [g kg^{-1} DM]	354^{cd}	463^{a}	344^{d}	450^{a}	468^{a}	341^{d}	338^{d}	389^{bc}	361^{cd}	403^{b}	406^{b}	358^{cd}	358^{cd}	8.7
DM losses [g kg^{-1}]				55.9^{a}	30.8^{ab}	2.81c	14.2^{bc}			19.7^{bc}	10.5^{bc}	0.825^{a}	3.53^{c}	3.95

DM = dry matter; aNDFom = neutral detergent fiber analysed with heat-stable amylase and expressed without residual ash; ADFom = acid detergent fiber expressed without residual ash; ADL = acid detergent lignin; NFC = non-fiber carbohydrates; dig/indig = digestible/indigestible; TDN = total digestible nutrients; RFQ = relative forage quality; DMI = dry matter intake; LW = live weight; NDFD30h = aNDFom *in vitro* digestibility in 30 h; HFT = Hohenheim Feed value Test; ELOS = enzymatically soluble organic substance. Treatments with different letters are significantly different (Tukey test, $p < 0.05$).

Appendix

Table A3. Chemical composition and *in vitro* digestibility of urea treated wheat straw (30 g urea kg^{-1}) at different temperatures and for varying storage duration (Trial 3)

Urea [g kg^{-1} DM]	0	30	30	30	30	30	30	30	30	30	30	
Maxammon [g kg^{-1} DM]	0	0	0	0	0	0	5	5	5	5	5	
Nominal temperature [°C]			25	25	40	40		25	25	40	40	
Storage [d]	0	0	14	28	14	28	0	14	28	14	28	SEM
n	15	3	3	3	3	3	3	3	3	3	3	(30g urea)
pH	6.45^{b}	6.05^{b}	8.60^{a}	8.48^{a}	8.53^{a}	8.42^{a}	8.13^{a}	8.68^{a}	8.63^{a}	8.46^{a}	8.51^{a}	0.141
DM [g kg^{-1}]	895^{a}	521^{b}	518^{b}	526^{b}	530^{b}	545^{b}	513^{b}	506^{b}	497^{b}	508^{b}	522^{b}	4.9
Crude ash [g kg^{-1} DM]	77.4	76.2	78.7	81.1	78.7	80.2	79.4	85.7	83.8	80	78.1	0.719
Crude protein [g kg^{-1} DM]	35.6^{e}	133^{a}	63.7^{d}	72.0^{cd}	92.6^{b}	90.6^{b}	125^{a}	68.7^{cd}	78.3^{bcd}	81.7^{bcd}	85.5^{bc}	4.15
Ether extract [g kg^{-1} DM]	10.6	12.9	13.4	12.8	13.6	13.2	12.8	13.3	14.4	13.4	13.2	0.18
aNDFom [g kg^{-1} DM]	779	765	781	776	770	769	761	776	763	775	775	2.2
ADFom [g kg^{-1} DM]	459	451	467	464	486	471	441	456	473	473	478	2.9
ADL [g kg^{-1} DM]	61.6	56.9	66.7	55.2	56.3	63.2	67	53.3	56.6	67.7	60.5	1.52
NFC [g kg^{-1} DM]	97.4^{ab}	66.9^{b}	118^{a}	113^{a}	99.4^{ab}	101^{ab}	76.4^{ab}	111^{a}	115^{a}	104^{ab}	102^{ab}	3.4
NH_3-N [g kg^{-1} N]	27.2^{f}	15.8^{f}	563^{a}	524^{ab}	374^{d}	421^{cd}	146^{e}	555^{a}	528^{ab}	440^{bcd}	466^{bc}	32.7
Ratios												
dig/indig aNDFom	0.440	0.378	0.415	0.451	0.393	0.448	0.438	0.443	0.471	0.360	0.477	0.0105
Cellulose/ADL	6.55	7.05	6.03	7.41	7.71	6.53	5.66	7.66	7.5	6.01	7.07	0.199
ADL/ADFom	0.134	0.126	0.143	0.119	0.116	0.134	0.152	0.117	0.119	0.143	0.126	0.0033
Calculated forage quality												
TDN [g kg^{-1} DM]	338^{b}	379^{ab}	385^{a}	392^{a}	382^{a}	394^{a}	393^{a}	389^{a}	402^{a}	369^{ab}	400^{a}	2.6
RFQ	35.3^{b}	50.8^{a}	48.7^{a}	51.8^{a}	48.6^{a}	53.1^{a}	56.4^{a}	51.1^{a}	53.1^{a}	48.5^{a}	52.4^{a}	0.67
DMI [g kg^{-1} BW]	12.8^{b}	16.5^{a}	15.6^{ab}	16.3^{a}	15.6^{ab}	16.5^{a}	17.6^{a}	16.2^{a}	16.2^{a}	16.1^{a}	16.1^{a}	1.46
In vitro digestibility												
NDFD30h [g kg^{-1} NDF]	303	274	293	310	281	310	305	314	320	262	322	5.3
HFT [ml 200 mg^{-1} DM]	32.9^{b}	27.6^{c}	33.7^{ab}	34.4^{ab}	32.3^{b}	34^{ab}	28.0^{c}	33.9^{ab}	34.3^{ab}	36.0^{a}	36.2^{a}	0.56
ELOS [g kg^{-1} DM]	347	353	353	351	360	347	376	361	359	392	380	4.4
DM losses [g kg^{-1}]			58.2	9.6	2.77	-2.71		-4.87	1.51	-21.4	-74.6	9.237

DM = dry matter; aNDFom = neutral detergent fiber analysed with heat-stable amylase and expressed without residual ash; ADFom = acid detergent fiber expressed without residual ash; ADL = acid detergent lignin; NFC = non-fiber carbohydrates; dig/indig = digestible/indigestible; TDN = total digestible nutrients; RFQ = relative forage quality; DMI = dry matter intake; LW = live weight; NDFD30h = aNDFom *in vitro* digestibility in 30 h; HFT = Hohenheim Feed value Test; ELOS = enzymatically soluble organic substance. Treatments with different letters are significantly different (Tukey test, $p < 0.05$).

Appendix

Table A4. Chemical composition and in-vitro digestibility of urea treated wheat straw (45 g urea kg^{-1}) at different temperatures and for varying storage duration (Trial 3)

Urea [g kg^{-1} DM]	0	45	45	45	45	45	45	45	45	45	45	
Maxammon [g kg^{-1} DM]	0	0	0	0	0	0	5	5	5	5	5	
Nominal temperature [°C]			25	25	40	40		25	25	40	40	
Storage [d]	0	0	14	28	14	28	0	14	28	14	28	SEM
n	15	3	3	3	3	3	3	3	3	3	3	(45 g urea)
pH	6.45^{b}	6.06^{b}	8.79^{a}	8.60^{a}	8.49^{a}	8.55^{a}	8.51^{a}	8.85^{a}	8.73^{a}	8.76^{a}	8.60^{a}	0.153
DM [g kg^{-1}]	895^{a}	508^{b}	510^{b}	511^{b}	526^{b}	515^{b}	514^{b}	489^{b}	513^{b}	505^{b}	508^{b}	5.6
Crude ash [g kg^{-1} DM]	77.4	72.7	82	82.2	79.2	80.1	74.9	79.1	76.9	81.2	76.7	0.84
Crude protein [g kg^{-1} DM]	35.6^{f}	184^{a}	87.9^{de}	80.1^{de}	132^{b}	1222^{bc}	167^{a}	75.9^{e}	83.2^{de}	101^{cd}	96.8^{de}	6.7
Ether extract [g kg^{-1} DM]	10.6	12.3	13.3	13.3	13.5	13	13.5	12.6	12.2	13.2	12.6	0.17
aNDFom [g kg^{-1} DM]	779	759	765	751	755	760	770	786	780	774	768	2.7
ADFom [g kg^{-1} DM]	459	442	465	484	472	484	476	481	492	481	490	3.6
ADL [g kg^{-1} DM]	61.6	62	58.5	55	61.9	63.7	64.8	52.1	70	70	60.1	1.59
NFC [g kg^{-1} DM]	97.4^{b}	54.2^{c}	133^{ab}	155^{a}	101^{b}	107^{b}	56.5^{c}	128^{ab}	129^{ab}	112^{b}	128^{ab}	5.94
NH_3-N [g kg^{-1} N]	27.2^{d}	11.7^{d}	565^{a}	584^{a}	314^{cd}	398^{bc}	182^{d}	608^{a}	588^{a}	505^{ab}	530^{ab}	36.50
Ratios												
dig/indig aNDFom	0.440^{ab}	0.461^{ab}	0.488^{ab}	0.479^{ab}	0.412^{b}	0.477^{ab}	0.381^{b}	0.476^{ab}	0.520^{ab}	0.422^{ab}	0.599^{a}	0.0130
Cellulose/ADL	6.55	6.18	6.94	8.09	6.68	6.68	6.35	8.63	6.10	5.91	7.41	0.260
ADL/ADFom	0.134	0.14	0.126	0.115	0.131	0.132	0.136	0.108	0.142	0.145	0.123	0.0035
Calculated forage quality												
TDN [g kg^{-1} DM]	338^{c}	426^{ab}	430^{ab}	437^{ab}	417^{ab}	429^{ab}	402^{b}	417^{ab}	433^{ab}	409^{b}	455^{a}	3.3
RFQ	35.3^{c}	42.0^{bc}	60.1^{a}	57.6^{a}	52.4^{ab}	53.9^{ab}	36.6^{c}	54.5^{ab}	55.6^{a}	53.7^{ab}	59.8^{a}	1.49
DMI [g kg^{-1} BW]	12.8^{bcd}	12.1^{cd}	17.2^{a}	16.2^{a}	15.5^{abc}	15.4^{abc}	11.2^{d}	16.0^{ab}	15.8^{ab}	16.1^{ab}	16.2^{a}	0.37
In vitro digestibility												
NDFD30h [g kg^{-1} NDF]	303^{ab}	316^{ab}	327^{ab}	324^{ab}	290^{b}	323^{ab}	276^{b}	322^{ab}	342^{ab}	296^{ab}	374^{a}	6.1
HFT [ml 200 mg^{-1} DM]	32.9^{c}	26.9^{d}	33.5^{c}	35.3^{bc}	32.0^{c}	34.7^{bc}	27.4^{d}	35.0^{bc}	34.5^{bc}	37.1^{ab}	39.3^{a}	0.72
ELOS [g kg^{-1} DM]	347	385	364	378	372	372	345	345	357	380	405	5.9
DM losses [g kg^{-1}]			5.55	-10.2	5.82	-27.6		-27.6	-3.33	-2.20	-21.7	8.754

DM = dry matter; aNDFom = neutral detergent fiber analysed with heat-stable amylase and expressed without residual ash; ADFom = acid detergent fiber expressed without residual ash; ADL = acid detergent lignin; NFC = non-fiber carbohydrates; dig/indig = digestible/indigestible; TDN = total digestible nutrients; RFQ = relative forage quality; DMI = dry matter intake; LW = live weight; NDFD30h = aNDFom *in vitro* digestibility in 30 h; HFT = Hohenheim Feed value Test; ELOS = enzymatically soluble organic substance. Treatments with different letters are significantly different (Tukey test, $\mathbf{p} < 0.05$).

Appendix

Table A5. Chemical composition and in-vitro digestibility of urea treated wheat straw (60 g urea kg^{-1}) at different temperatures and for varying storage duration (Trial 3)

Urea [g kg^{-1} DM]	0	60	60	60	60	60	60	60	60	60	60	
Maxammon [g kg^{-1} DM]	0	0	0	0	0	0	5	5	5	5	5	
Nominal temperature [°C]			25	25	40	40		25	25	40	40	
Storage [d]	0	0	14	28	14	28	0	14	28	14	28	SEM
n	15	3	3	3	3	3	3	3	3	3	3	(60 g urea)
pH	6.45^{c}	6.20^{c}	8.75^{a}	8.87^{a}	8.40^{a}	8.43^{a}	7.57^{b}	8.91^{a}	8.76^{a}	8.73^{a}	8.67^{a}	0.151
DM [g kg^{-1}]	895^{a}	533^{b}	498^{b}	517^{b}	520^{b}	549^{b}	505^{b}	495^{b}	500^{b}	502^{b}	524^{b}	6.1
Crude ash [g kg^{-1} DM]	77.4^{ab}	72.4^{b}	78.5^{ab}	81.6^{ab}	79.8^{ab}	77.9^{ab}	77.2^{ab}	84.9^{a}	79.9^{ab}	77.9^{ab}	76.7^{ab}	0.74
Crude protein [g kg^{-1} DM]	35.6^{e}	197^{ab}	128^{cd}	95.1^{d}	187^{ab}	164^{bc}	208^{a}	104^{d}	95.6^{d}	125^{cd}	126^{cd}	7.99
Ether extract [g kg^{-1} DM]	10.6	13.8	13.5	13.7	11.9	13.6	15.9	13.6	13.4	12.3	13.3	0.29
aNDFom [g kg^{-1} DM]	779	752	751	763	744	754	745	759	758	763	739	2.4
ADFom [g kg^{-1} DM]	459	445	457	471	437	459	442	463	482	469	494	3.7
ADL [g kg^{-1} DM]	61.6	57.0	58.1	62.3	58.2	63.6	49	52.5	47.6	62.4	61.0	1.50
NFC [g kg^{-1} DM]	97.4^{cde}	73.1^{e}	138^{abc}	155^{a}	86.7^{de}	99.8^{bcde}	62.8^{e}	148^{ab}	162^{a}	130^{abcd}	153^{a}	7.01
NH_3-N [g kg^{-1} N]	27.2^{d}	11.9^{d}	483^{a}	569^{a}	185^{d}	278^{bc}	153^{cd}	574^{a}	579^{a}	424^{ab}	490^{a}	37.12
Ratios												
dig/indig aNDFom	0.440	0.459	0.460	0.525	0.389	0.429	0.418	0.534	0.546	0.461	0.567	0.0166
Cellulose/ ADL	6.55^{ab}	6.90^{ab}	6.89^{ab}	6.60^{ab}	6.53^{ab}	6.25^{b}	8.10^{ab}	7.84^{ab}	10.4^{a}	6.61^{ab}	7.12^{ab}	0.345
ADL/ADFom	0.134	0.128	0.127	0.132	0.133	0.139	0.111	0.113	0.099	0.133	0.124	0.0033
Calculated forage quality												
TDN [g kg^{-1} DM]	338^{b}	456^{a}	458^{a}	466^{a}	437^{a}	445^{a}	447^{a}	466^{a}	478^{a}	449^{a}	488^{a}	4.17
RFQ	35.3^{cd}	35.8^{cd}	64.3^{a}	66.8^{a}	41.7^{bcd}	50.4^{abc}	27.6^{d}	68.7^{a}	66.5^{a}	60.9^{ab}	62.0^{ab}	2.99
DMI [g kg^{-1} BW]	12.8^{abcd}	9.69^{cd}	17.2^{ab}	17.6^{a}	11.7^{bcd}	13.8^{abc}	7.67^{d}	18.1^{a}	17.2^{ab}	16.7^{ab}	15.6^{ab}	0.747
In vitro digestibility												
NDFD30h [g kg^{-1} NDF]	303	314	313	343	279	297	292	347	362	312	361	7.9
HFT [ml 200 mg^{-1} DM]	32.9^{bcd}	25.1^{f}	29.9^{de}	34.1^{abc}	29.2^{de}	31.8^{cd}	26.0^{ef}	36.0^{ab}	37.6^{a}	36.2^{ab}	37.7^{a}	0.85
ELOS [g kg^{-1} DM]	347	363	381	359	376	394	350	385	375	405	408	5.5
DM losses [g kg^{-1}]			72.3	3.73	46.3	-41.3		$^{-1}$6.3	$^{-1}$8.6	-5.4	-20.1	10.71

DM = dry matter; aNDFom = neutral detergent fiber analysed with heat-stable amylase and expressed without residual ash; ADFom = acid detergent fiber expressed without residual ash; ADL = acid detergent lignin; NFC = non-fiber carbohydrates; dig/indig = digestible/indigestible; TDN = total digestible nutrients; RFQ = relative forage quality; DMI = dry matter intake; LW = live weight; NDFD30h = aNDFom *in vitro* digestibility in 30 h; HFT = Hohenheim Feed value Test; ELOS = enzymatically soluble organic substance. Treatments with different letters are significantly different (Tukey test, $p < 0.05$).

Appendix

Table A6. Significance of the effect of the factors urea concentration (30, 45, 60 g kg^{-1}), maxammon application (0, 5 g kg^{-1}), storage period (14, 28 d) and temperature (25, 40 °C) on the nutritional composition of wheat straw (Trial 3)

	Significance level									
	Urea	Per	Temp	Max	Urea×Max	Urea×Temp	Urea×Per	Per×Max	Per×Temp	Max×Temp
pH	***	**	***	***	n.s.	*	n.s.	n.s.	n.s.	o
DM [g kg^{-1}]	n.s.	n.s.	o	o	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Crude ash [g kg^{-1} DM]	n.s.	n.s.	**	n.s.	o	n.s.	n.s.	*	n.s.	n.s.
Crude protein [g kg^{-1} DM]	***	o	***	***	***	***	**	*	n.s.	***
Ether extract [g kg^{-1} DM]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
aNDFom [g kg^{-1} DM]	***	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.
ADFom [g kg^{-1} DM]	**	0.001	n.s.	***	**	o	*	n.s.	n.s.	n.s.
ADL [g kg^{-1} DM]	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	o
NFC [g kg^{-1} DM]	***	**	***	**	**	*	o	n.s.	n.s.	***
NH_3-N [g kg^{-1} N]	**	*	***	***	**	*	n.s.	n.s.	o	***
Ratios										
dig/indig aNDFom	**	***	o	**	n.s.	n.s.	n.s.	n.s.	*	n.s.
Cellulose/ADL	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
ADL/ADFom	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Calculated forage quality										
TDN [g kg^{-1} DM]	***	***	n.s.	n.s.	n.s.	n.s.	n.s.	o	n.s.	n.s.
RFQ	***	n.s.	**	o	o	**	n.s.	n.s.	n.s.	o
DMI [g kg^{-1} BW]	n.s.	n.s.	**	o	n.s.	**	n.s.	n.s.	n.s.	*
In vitro digestibility										
NDFD30h [g kg^{-1} NDF]	*	**	*	*	n.s.	n.s.	n.s.	n.s.	o	n.s.
HFT [ml/200 mg DM]	*	***	n.s.	***	***	o	n.s.	*	n.s.	***
ELOS [g kg^{-1} DM]	*	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	o
DM losses [g kg^{-1}]	n.s.	o	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Per = storage period; Temp = storage temperature; Max = Maxammon; DM = dry matter; aNDFom = neutral detergent fiber analysed with heat-stable amylase and expressed without residual ash; ADFom = acid detergent fiber expressed without residual ash; ADL = acid detergent lignin; NFC = non-fiber carbohydrates; dig/indig = digestible/indigestible; TDN = total digestible nutrients; RFQ = relative forage quality; DMI = dry matter intake; LW = live weight; NDFD30h = aNDFom *in vitro* digestibility in 30 h; HFT = Hohenheim Feed value Test; ELOS = enzymatically soluble organic substance. n.s. = not significant, o $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table A7. Ranges of potential digestibility indicators plus pH overall and in the different treatment groups (Trial 1, 2, 3)

Treatment	All					Control					Urea					NaOH				
	n	Min	Max	Mean	SEM	n	Min	Max	Mean	SEM	n	Min	Max	Mean	SEM	n	Min	Max	Mean	SEM
dig/indig aNDFom	189	0.268	2.584	0.652	0.030	17	0.286	0.674	0.452	0.028	133	0.268	0.655	0.479	0.007	39	0.658	2.584	1.325	0.080
Cellulose/ ADL	196	3.99	29.5	7.84	0.273	19	5.08	8.68	6.47	0.179	133	4.75	16.3	7.01	0.119	44	3.99	29.5	10.9	1.04
ADL/ADF	195	0.033	0.200	0.123	0.002	18	0.103	0.164	0.133	0.003	133	0.058	0.177	0.128	0.002	44	0.033	0.200	0.106	0.006
TDN (g kg^{-1} DM)	189	288	520	406	3.11	17	293	388	343	5.77	133	341	500	415	3.00	39	288	520	402	8.14
RFQ	189	17.4	75.3	49.7	0.770	17	28.5	44.2	35.7	1.22	133	17.4	75.3	52.7	0.851	39	29.3	68.9	45.7	1.38
DMI (g kg^{-1} LW)	189	4.55	19.2	15.0	0.172	17	10.7	15.9	12.8	0.349	133	4.55	19.2	15.6	0.212	39	12.2	16.4	13.9	0.172
$NDFD_{30h}$ (g kg^{-1} NDF)	190	311	721	370	7.81	17	222	403	315	12.2	133	211	396	322	3.26	40	397	721	554	12.5
HFT (ml 200 mg^{-1} DM)	185	22.3	45.3	35.2	0.359	15	29.6	35.1	32.5	0.419	132	24.1	41.2	33.6	0.316	38	22,3	45,3	41.9	0.578
ELOS (g kg^{-1} DM)	184	286	581	400	4.72	15	323	382	348	5.19	131	311	473	378	3.16	38	286	581	499	8.12
pH	165	6.01	12.5	8.93	0.113	3	6.16	6.74	6.53	0.186	132	6.01	9.41	8.41	0.067	30	9.50	12.5	11.5	0.177

dig/indig aNDFom = digestible/indigestible neutral detergent fiber analyzed with heat-stable amylase and expressed without residual ash; ADL = acid detergent lignin; ADFom: acid detergent fiber expressed without residual ash; TDN = total digestible nutrients; RFQ = relative forage quality; DMI = dry matter intake; LW = live weight; NDFD30h = aNDFom *in vitro* digestibility in 30 h; HFT = Hohenheim Feed value Test; ELOS = enzymatically soluble organic substance; SEM = standard error of the mean.

Appendix

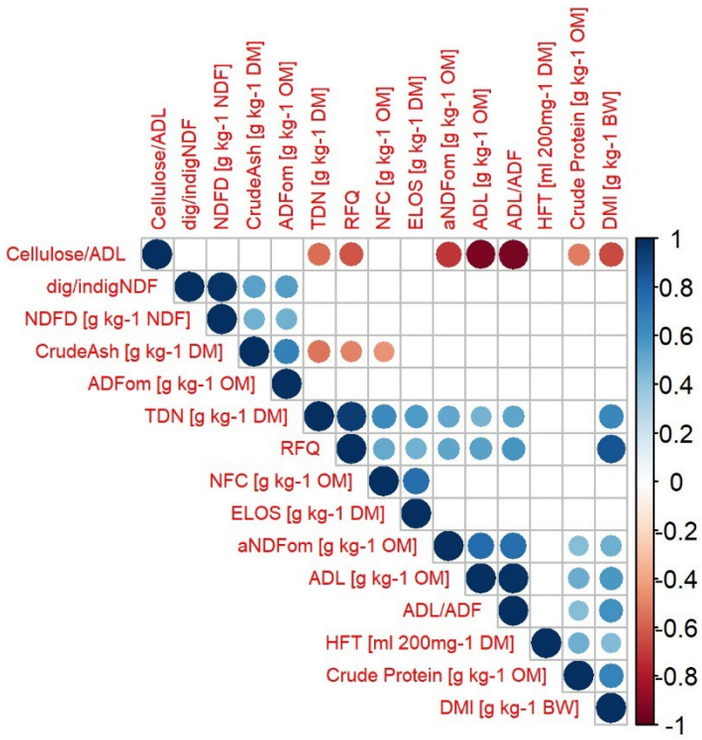

Fig. A1. Correlation matrix (Trial 1 – NaOH). The bigger the circle the higher the correlation. Blue – positive, red - negative correlation. aNDFom: neutral detergent fibre analysed with heat-stable amylase and expressed without residual ash; ADFom: acid detergent fibre expressed without residual ash; ADL: acid detergent lignin; NFC: non-fibre carbohydrates; NDFD: aNDFom *in vitro* digestibility in 30 h; HFT: Hohenheim Feed value Test; ELOS: enzymatically soluble organic substance; TDN: total digestible nutrients; RFQ: relative forage quality; DMI: dry matter intake

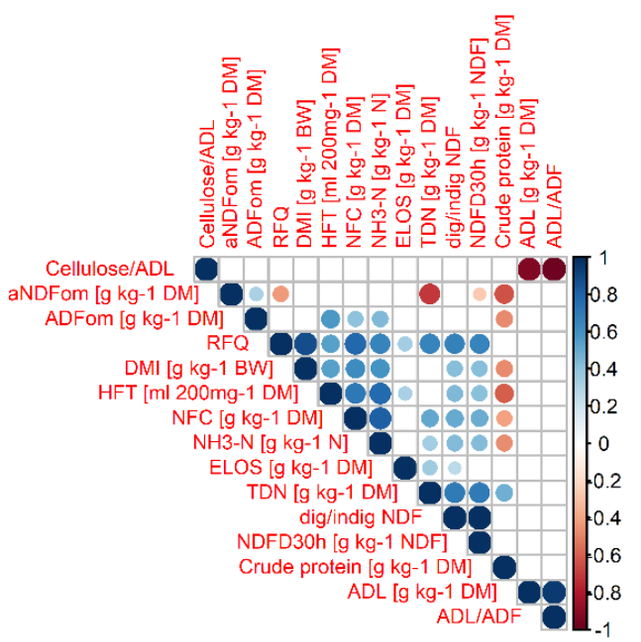

Fig. A2. Correlation matrix (Trial 3 – Urea). The bigger the circle the higher the correlation. Blue – positive, red - negative correlation. aNDFom: neutral detergent fibre analysed with heat-stable amylase and expressed without residual ash; ADFom: acid detergent fibre expressed without residual ash; ADL: acid detergent lignin; NFC: non-fibre carbohydrates; NDFD: aNDFom in vitro digestibility in 30 h; HFT: Hohenheim Feed value Test; ELOS: enzymatically soluble organic substance; TDN: total digestible nutrients; RFQ: relative forage quality; DMI: dry matter intake

Appendix

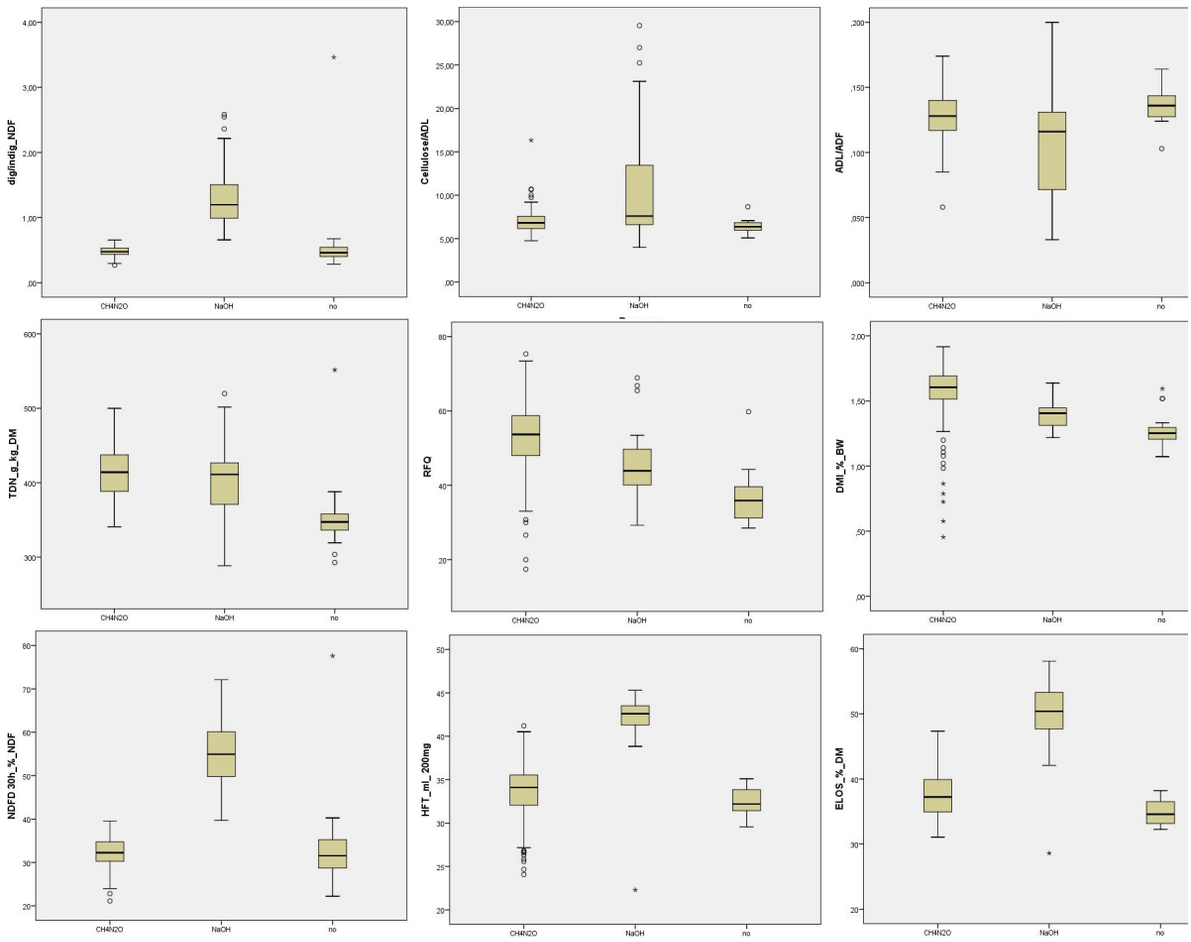

Fig. A3. Box plots showing the range of different digestibility indicators for the three treatment groups (urea, NaOH, untreated). Top: ratio of digestible/indigestible aNDFom, cellulose/ADL, ADL/ADFom. Middle line: TDN total digestible nutrients, RFQ relative forage quality, DMI dry matter intake (% of body weight). Bottom: $NDFD_{30h}$ aNDFom digestibility (%); HFT Hohenheim feed value test (ml 200 mg^{-1} DM); ELOS enzymatically soluble organic substance (% of DM)