Evaluation of buffer solutions and urea addition for estimating the in vitro digestibility of feeds

Larissa F. Camacho, Tadeu E. Silva, Malber N. N. Palma, Amanda S. Assunção, João P. Rodrigues, Luiz Fernando Costa e Silva, and Edenio Detmann[1]

Departamento de Zootecnia, Universidade Federal de Viçosa, Campus Universitário, Viçosa, Minas Gerais 36570-900, Brazil

ABSTRACT: The aim of this study was to compare the in vitro digestibility of dry matter (**IVDMD**) and neutral detergent fiber (**IVNDFD**) using 2 buffer solutions with or without urea addition. The study was comprised of 2 separate experiments. In both experiments, the treatments were composed of Kansas or McDougall's buffer solutions with or without urea addition, according to a 2 × 2 factorial arrangement. In Exp. I, the IVDMD and IVNDFD of 25 forages and 25 concentrates were evaluated. Samples were incubated for 48 h using an artificial fermenter and nonwoven textile filter bags (100 g/m^2). In this experiment, the repeatability and discriminatory power among samples were calculated within forage or concentrate samples, for each treatment. In Exp. II, Tifton hay and ground corn samples were incubated for 48 h. The pH and ammonia nitrogen (**NH_3-N**) concentration were measured after 0, 3, 6, 12, 18, 24, and 48 h of incubation. In Exp. I, the interaction between buffer solution and urea addition impacted the IVDMD and IVNDFD of forages ($P < 0.05$), with greater values being exhibited when using McDougall's buffer with urea ($P < 0.05$). For concentrates, the effect of buffer and urea interaction did not affect IVDMD and IVNDFD ($P > 0.05$). However, greater IVDMD and IVNDFD were observed for McDougall's buffer ($P < 0.05$), while urea addition increased IVDMD and IVFDFD estimates ($P < 0.05$) regardless of buffer solution used. In general, repeatability of the digestibility was better using McDougall's buffer and improved when urea was added. Urea addition also decreased the discriminatory power among samples for both buffers. In Exp. II, a buffer solution × urea addition × incubation time interaction was detected ($P < 0.05$) for pH and NH_3-N in both Tifton hay and ground corn. Kansas buffer exhibited lower pH values with a greater decrease observed throughout incubation time when compared to McDougall's buffer. The use of Kansas buffer with urea addition was the only treatment exhibiting NH_3-N accumulation throughout incubation. In conclusion, McDougall's buffer provides both better conditions for in vitro fiber digestion and greater precision in digestibility estimates, and is recommended over Kansas buffer. In spite of urea addition increases the precision of in vitro digestibility estimates, it decreases discriminatory power among samples.

Key words: ammonia nitrogen, buffering capacity, Kansas buffer, McDougall's buffer, neutral detergent fiber, repeatability

© The Author(s) 2018. Published by Oxford University Press on behalf of the American Society of Animal Science. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com.

J. Anim. Sci. 2019.97:922–931
doi: 10.1093/jas/sky464

INTRODUCTION

Buffers of variable chemical compositions are available to perform rumen in vitro digestibility assays. However, differences in chemical compositions may influence the longevity of the buffer (Herod et al., 1978) and affect the

[1]Corresponding author: detmann@ufv.br

Received September 27, 2018.
Accepted December 3, 2018.

fermentation rate of substrate (Mould et al., 2005b). McDougall's buffer is primarily composed of sodium bicarbonate as the buffering compound (McDougall, 1948). However, this buffer requires CO_2 bubbling to achieve and maintain an adequate pH, which makes its utilization more expensive and laborious compared to other buffers. Kansas buffer is based on potassium phosphate as the main buffering compound and the pH adequacy is achieved by mixing only 2 solutions (Marten and Barnes, 1980). This simpler procedure for pH adjustment highlights Kansas buffer as a viable alternative that reduces costs and laboratory manipulation. Existing in vitro studies estimating the digestibility of feeds for ruminants and nonruminant herbivores have used either McDougall's or Kansas buffer (Adesogan, 2005; Earing et al., 2010; Roth et al., 2016). Moreover, the addition of urea in the fermentation medium aims to provide nitrogen (**N**) for microbial inoculum, which, in turn, could decrease the influence of the donor animal's diet on in vitro digestibility estimates (Marten and Barnes, 1980). However, N addition may decrease the discrimination power among samples in in vitro digestibility assays, due to a more prominent increase in the digestibility of less-digestible feeds. Thus, we hypothesized that using different buffer solutions alters the estimates of in vitro rumen digestibility and that addition of N from urea to the medium may decrease the discrimination among samples with regards to digestibility. Our objective was to evaluate the discrimination power among samples for in vitro ruminal digestibility, as well as to evaluate the changes in the fermentation medium during the in vitro incubations using Kansas or McDougall's buffer solutions with or without urea addition.

MATERIALS AND METHODS

The study was carried out in the Animal Nutrition Laboratory of the Animal Science Department at the Universidade Federal de Viçosa, Viçosa, MG, Brazil. All procedures were approved by the Ethics Committee in Animal Use and Care at the Universidade Federal de Viçosa (CEUAP, protocol no. 061/2016).

Experiment I—Obtaining Estimates of In Vitro Digestibility

Experiment I was designed to evaluate the in vitro digestibility of dry matter (**IVDMD**) and neutral detergent fiber (**IVNDFD**) of forage and concentrate feeds using Kansas or McDougall's buffer solutions with or without urea addition.

Samples. A total of 25 samples were evaluated in the forage group: 19 fresh [*Panicum maximum* cv. Mombaça (*n* = 1), *Saccharum* sp. (*n* = 4), *Brachiaria* sp. (*n* = 10), *Cajanus cajan* (*n* = 1), *Calopogonium mucunoides* (*n* = 1), *Leucaena lecocephala* (*n* = 1), and *Neonotonia wightii* (*n* = 1)], 5 hay [*Cynodon* sp. (*n* = 3), *Stylosanthes guianensis* cv. Campo Grande (*n* = 1), and *Medicago sativa* (*n* = 1)], and 1 silage (*Arachis pintoi*). Fresh forages and silage samples were oven-dried at 55 °C for 72 h. Also, a total of 25 samples were evaluated in the concentrate group: soybean hulls (*n* = 2), ground flint corn (*n* = 5), corn gluten meal (*n* = 1), citrus pulp (*n* = 1), rice bran (*n* = 1), sunflower meal (*n* = 1), soybean meal (*n* = 2), wheat bran (*n* = 1), ground sorghum (*n* = 2), and commercial concentrate mixtures (*n* = 9).

All samples were milled through a 1-mm screen sieve and stored until chemical analysis and in vitro incubations. Sample dry matter (**DM**; method INCT-CA G-003/1), crude protein (**CP**; method INCT-CA N-001/1), and neutral detergent fiber (**aNDF**; method INCT-CA F-002/1) contents were analyzed according to the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (Detmann et al., 2012; Table 1). The NDF was analyzed using heat-stable amylase (Termamyl 2X, Novozymes, São Paulo, Brazil), omitting sodium sulfite and expressed inclusive of residual ash. More details about individual samples are presented as an e-supplement.

Treatments. Treatments included combinations of McDougall's (McDougall, 1948) or Kansas (Marten and Barnes, 1980; Silva and Queiroz, 2002) buffer solutions, with or without urea addition, according to a 2 × 2 factorial arrangement. Buffer solutions were prepared as described by McDougall (1948) and Silva and Queiroz (2002) (Table 2). In brief, for McDougall's buffer solution, pH adjustment was performed by bubbling CO_2 until the buffer pH reached 6.8. For Kansas buffer solution, which was composed by 2 solutions (A and B), pH was adjusted to 6.8 by mixing the 2 solutions at ratios of 5:1 and 7.7:1, with and without urea addition, respectively (Table 2). For McDougall's buffer, urea addition was conducted by adding 5 mL of urea solution (55 g/liter) in 300 mL buffer solution prior to pH adjustment (Silva and Queiroz, 2002). For Kansas buffer, urea was added (0.5 g/liter) during

solution A preparation (Silva and Queiroz, 2002). All procedures were completed 24 h prior to each in vitro incubation. The buffer solutions were stored into a climate-controlled room (39 °C) until incubation began.

In vitro incubations and digestibility calculations. Six 48-h in vitro incubations were conducted, with the first and last 3 incubations assigned for forage and concentrate evaluations, respectively. In vitro digestibility assays were conducted using a TE-150 artificial fermenter (Tecnal Equipamentos Científicos, Piracicaba, SP, Brazil) (Silva et al., 2017). Samples were incubated using nonwoven textile filter bags (100 g/m^2) of 4 × 4.5 cm (Valente et al., 2011). In each incubation period, four 500-mg aliquots per sample were weighed and stored in heat-sealed filter bags.

To reduce variation among incubations, only 1 animal was used as a rumen inoculum donor. A rumen cannulated heifer, fed a sugarcane and concentrate (220 g CP/kg DM)-based diet with a forage-to-concentrate ratio of 80:20 was used. The heifer had free access to water and mineral mixture (90 g/kg of phosphorus), and was adapted to the diet for 14 d prior to rumen inoculum collections (Machado et al., 2016). The ruminal inoculum (liquid and solid) collections were performed at several points in the rumen shortly before the beginning of each incubation. Ruminal inoculum was stored in preheated (39 °C) thermal bottles and then mixed for a few seconds using a blender to homogenize liquid and solid phases. The fluid was then filtered through 4 layers of cheesecloth. The steps from rumen inoculum collection and incubation onset were conducted within 20 min in a climate-controlled room (39 °C).

Table 1. Descriptive statistics of crude protein (CP) and neutral detergent fiber (NDF) concentrations (% dry matter) of forage and concentrate samples used in the Exp. I

	Statistics			
Group	Minimum	Maximum	Mean	Standard deviation
Forages (*n* = 25)				
CP	2.08	20.1	7.02	4.05
NDF	24.7	71.0	58.3	11.4
Concentrates (*n* = 25)				
CP	4.87	59.0	17.3	13.2
NDF	10.3	62.6	23.2	11.3

The artificial fermenter possessed 4 jars (3,200 mL), and each jar randomly received 1 treatment. Moreover, each jar received 25 filter bags with samples and 1 blank filter bag. In each jar, 400 mL of ruminal inoculum and 1,600 mL of buffer solution were added. Carbon dioxide was flushed into the head space of each jar, which was closed and placed into the preheated (39 °C) artificial fermenter. After 48 h of incubation, the filter bags were washed using hot distilled water (90 °C) until the water became clear, and bags were gently pressed to remove gases. Then, to estimate the apparently undigested DM residue, filter bags were oven-dried (55 °C/24 h and 105 °C/16 h, sequentially), placed in a desiccator, and weighed.

For the IVNDFD evaluations, filter bags containing the incubation residues were placed into polypropylene screw-capped flasks (120 mL; autoclavable universal collection vial, Bioplast 2605, Porto Alegre, RS, Brazil) with 80 mL of neutral

Table 2. Final composition of the evaluated buffer solutions with or without urea addition

	Buffer solution			
	McDougall		Kansas[1]	
Component, g/liter	Without urea	With urea	Without urea	With urea
$NaHCO_3$	9.80	9.80	–	–
Anhydrous Na_2HPO_4	3.71	3.71	–	–
KCl	0.57	0.57	–	–
NaCl	0.47	0.47	0.42	0.44
Heptahydrate $MgSO_4$	0.12	0.12	0.42	0.44
Dihydrate $CaCl_2$	0.05	0.05	0.08	0.09
Anhydrous KH_2PO_4	–	–	8.33	8.85
Na_2CO_3	–	–	2.50	1.72
Nonahydrate Na_2S	–	–	0.17	0.11
Urea	–	0.90	–	0.50

[1]Reported values are final concentrations. Kansas buffer solution was composed by mixture of 2 solutions (A and B). Solution A: anhydrous KH_2PO_4 (10.0 g/liter), heptahydrate $MgSO_4$ (0.5 g/liter), NaCl (0.5 g/liter), and dihydrate $CaCl_2$ (0.1 g/liter). Solution B: Na_2CO_3 (15.0 g/liter), and nonahydrate Na_2S (1 g/liter). In the treatment with urea addition, 0.5 g/liter of urea was added in the solution A. Differences in composition with or without urea are due to different proportions of solutions A and B (5:1 and 7.7:1, respectively) for the adequate pH adjustment.

detergent solution and 500 µL of a heat-stable amylase (Termamyl 2X, Novozymes, São Paulo, Brazil). Flasks containing the filter bags were closed and autoclaved for 1 h at 105 °C (Barbosa et al., 2015). After that, the filter bags were washed using hot distilled water (90 °C) and acetone. The drying and weighing procedures were performed as previously described.

The IVDMD and IVNDFD (*D*, g/kg) were calculated as:

$$D = \frac{M - (R - B)}{M} \times 1{,}000 \quad (1)$$

where *M* is the incubated mass of DM or aNDF (g); *R* is the undigested residue of DM or NDF (g); and *B* is the DM or aNDF residue in blank filter bags (g).

Experiment II—Monitoring Buffering Capacity

Experiment II was designed to evaluate the buffering capacity of McDougall's and Kansas buffer solutions with or without urea addition.

Samples. Samples of Tifton 85 (*Cynodon* sp.) hay (113 g CP and 687 g NDF/kg DM) and ground flint corn (49.1 g CP and 197 g NDF/kg DM) were used. Both samples were chosen for being feeds with high aNDF and starch content, respectively.

Treatments. As described for Exp. I, the treatments involved combinations of McDougall's or Kansas buffer solutions, with or without urea addition, according to a 2 × 2 factorial arrangement (Table 2).

In vitro incubations and measurements. One in vitro incubation was performed for 48 h according to the recommendations of Tilley and Terry (1963), with some adaptations. In brief, bottles were sealed and gas was released at 3, 6, 9, 12, 18, 24, 30, 36, and 42 h of incubation with needle instead of Bunsen valves. For each treatment, twenty-one 250-mg aliquots per sample were weighed into 60 mL penicillin bottles. Then, 10 mL of ruminal inoculum and 40 mL of buffer solution were added into each bottle. The head space was then flushed with CO_2 and bottles were sealed with rubber caps and aluminum seals. The bottles were kept on a stirring table (40 rpm) in a climate-controlled room (39 °C).

The pH and ammonia-N (**NH_3-N**) concentrations were measured at 0, 3, 6, 12, 18, 24, and 48 h of incubation. Each time, 3 bottles from each treatment and sample were randomly selected. The content of each bottle was transferred to 50-mL conical polypropylene screw top tubes, and pH was measured using a digital potentiometer (Quimis, São Paulo, Brazil). Then, 1 mL of a H_2SO_4 solution (500 mL/liter) was added into each tube, and all tubes were kept at 4 °C until NH_3-N analysis. Ammonia N was estimated by colorimetric reactions catalyzed by indophenol (method INCT-CA N-006/1; Detmann et al., 2012).

Statistical Analysis

For Exp. I, statistical analysis was performed separately for forages and concentrates using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC), according to the model:

$$Y_{ijklm} = \mu + A_i + B_j + T_k + U_l + TU_{kl} + \varepsilon_{ijklm}, \quad (2)$$

where µ is the general constant; A_i is the random effect of sample *i*; B_j is the random effect of incubation *j*; T_k is the fixed effect of buffer solution *k*; U_l is the fixed effect of urea addition *l*; TU_{kl} is the fixed effect of interaction between buffer solution and urea addition; and ε_{ijklm} is the random error.

For outlier evaluation, the INFLUENCE statement of MIXED procedure was used and residuals (observations) with restricted likelihood distance higher than 1 were removed. Then, another variance analysis was performed following the previously described model.

To evaluate specific variances of IVDMD and IVNDFD results, the estimates obtained for forages and concentrates were analyzed separately for each treatment, following the model:

$$Y_{ijk} = \mu + A_i + B_j + \varepsilon_{ijk}, \quad (3)$$

where µ is the general constant; A_i is the random effect of sample *i*; B_j is the random effect of incubation *j*; and ε_{ijk} is the random error.

Estimates of the residual variance ($\hat{\sigma}^2_\varepsilon$) and the variance among samples ($\hat{\sigma}^2_a$) were obtained by the restricted maximum likelihood method. The standardized repeatability (***r***, lower is better) was calculated as:

$$r = \frac{\sqrt{\hat{\sigma}^2_\varepsilon}}{\bar{Y}} \times 100, \quad (4)$$

where *r* is the standardized repeatability (%); $\hat{\sigma}^2_\varepsilon$ is the residual variance; and $\bar{Y}$ is the average digestibility.

The relative standard deviation among samples (**SDas**) was used as an indicator of the power

of discrimination among samples (higher is better) within treatments (or methods), which was calculated as:

$$\text{SD as} = \frac{\sqrt{\hat{\sigma}_a^2}}{\bar{Y}} \times 100, \quad (5)$$

where SDas is the relative standard deviation among samples (%); $\hat{\sigma}_a^2$ is the variance among samples; and $\bar{Y}$ is the average digestibility.

For pH values and NH_3-N concentration in Exp. II, an analysis of variance was performed for each feed using the MIXED procedure of SAS 9.4, according the model:

$$Y_{ijkl} = \mu + T_i + U_j + TU_{ij} + t_k + tT_{ki} + tU_{kj} + tTU_{kij} + \varepsilon_{ijkl}, \quad (6)$$

where μ is the general constant; T_i is the fixed effect of buffer solution i; U_j is the fixed effect of urea addition j; t_k is the fixed effect of incubation time k; TU_{ij}, tT_{ki}, tU_{kj}, and tTU_{kij} are the fixed effects of interactions between buffer, urea addition, and time; and ε_{ijkl} is the random error.

When a significant interaction was detected, the results were analyzed using the SLICE statement of the MIXED procedure. In all procedures, 0.05 was used as the critical level for type I error occurrence.

RESULTS

Experiment I—In Vitro Digestibility, Repeatability, and Discrimination Among Samples

There was an interaction between buffer solution and urea addition on forage IVDMD ($P < 0.001$) and IVNDFD ($P = 0.037$), in which urea addition increased both IVDMD and IVNDFD when McDougall's solution was used, while no effects of urea were observed with Kansas buffer (Fig. 1). For concentrate samples, the interaction between buffer and urea addition was not observed for either IVDMD ($P = 0.109$) or IVNDFD ($P = 0.493$). McDougall's buffer presented greater ($P < 0.01$) IVDMD and IVNDFD when compared to Kansas buffer (Fig. 1). On the other hand, urea addition increased ($P < 0.01$) both IVDMD and IVNDFD in concentrate samples (Fig. 1). There was no effect of the different incubations ($P > 0.05$) on IVDMD and IVNDFD for both forages and concentrates.

Values for the r and SDas of forages and concentrates are presented in Fig. 2. Generally, for both forage and concentrate samples, McDougall's solution exhibited better repeatability values for IVDMD and IVNDFD. Better r values for forage IVDMD and IVNDFD were observed by adding urea to both buffers. For concentrate, urea addition decreased r in all cases except IVDMD using McDougall's buffer. The SDas for forages were similar among treatments for IVDMD and IVNDFD. For concentrates, SDas were quite similar among treatments for IVDMD, with higher values for IVNDFD using Kansas buffer. Generally, urea addition decreased SDas for IVNDFD.

Experiment II—Buffering Capacity and Ammonia Nitrogen Concentration

A buffer solution × urea addition × incubation time interaction was detected ($P \leq 0.04$) on pH and NH_3-N (Fig. 3) for both Tifton hay and ground corn. For both feeds, Kansas buffer had lowest pH values ($P < 0.05$). The addition of urea only increased pH ($P < 0.05$) in Kansas buffer. Regardless of feed, decreases in pH were more profound during incubation in Kansas than McDougall's buffer ($P < 0.01$). After 3 h of incubation, the NH_3-N concentrations were greater for Kansas buffer with added urea, reaching 29.6 ± 1.69 mg/dL at 48 h of incubation. For the other treatments, NH_3-N accumulation was not observed for either Tifton hay or ground corn, and NH_3-N values at 48 h of incubation were lower than 15 mg/dL.

DISCUSSION

Digestibility Estimates and Buffers Characteristics

The significant effect of interaction between buffer solution and urea addition on IVDMD and IVNDFD for forage samples reinforces the interference of fermentation medium conditions in digestibility (Mould et al., 2005a; Detmann et al., 2008). However, this interaction did not affect IVDMD and IVNDFD for concentrate samples. The N and carbohydrates availability for microbial growth are the main factors affecting the in vitro digestibility estimates (Mould et al., 2005b). Under in vitro conditions, these substrates are primarily derived from the incubated diet or feed and buffered rumen fluid. Moreover, forages typically present lower nutrient availability than concentrates, making microbes more dependent on buffered rumen fluid substrates. Since NH_3-N is the primary N substrate for rumen fibrolytic bacteria (Russell, 2002), the added urea likely provided greater NH_3-N availability for fiber digestion (Costa et al., 2008; Detmann et al., 2009).

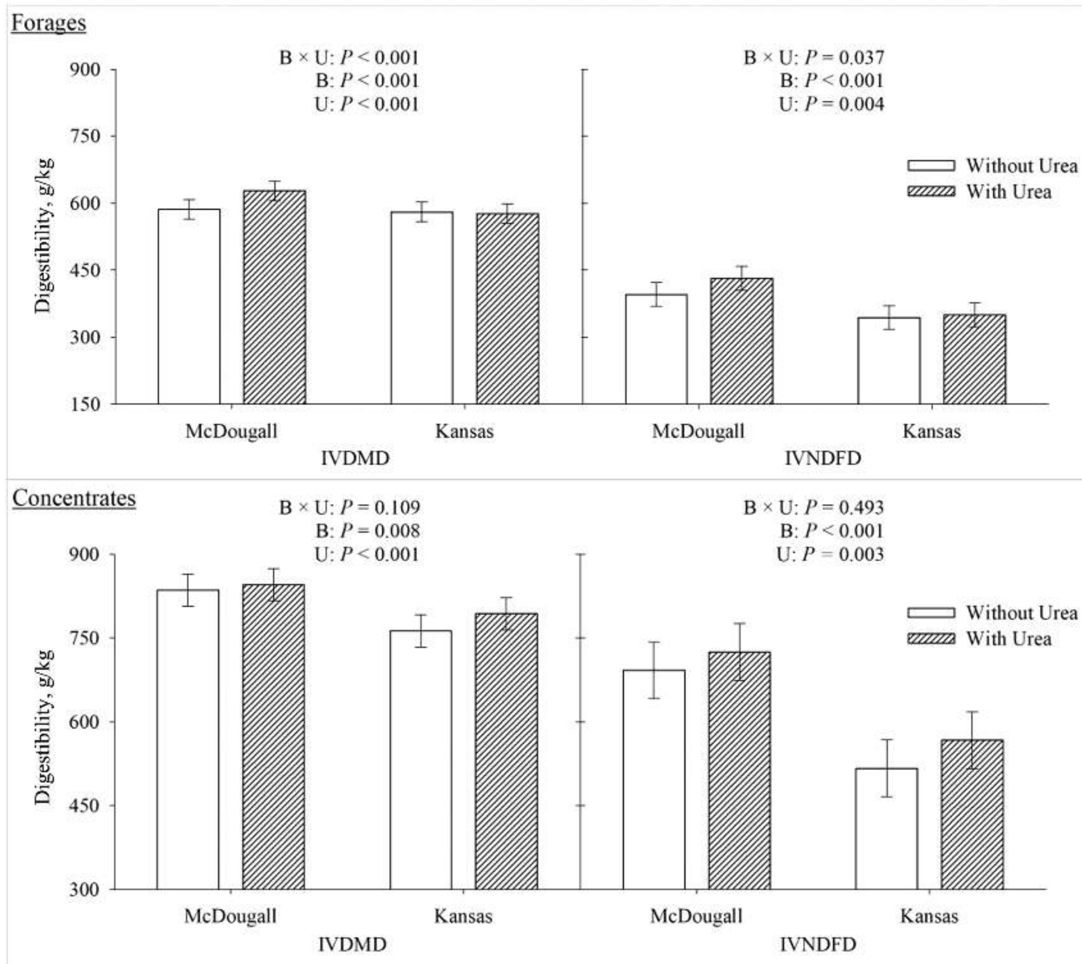

Figure 1. In vitro digestibility of dry matter (IVDMD) and neutral detergent fiber (IVNDFD) of forages or concentrates using Kansas or McDougall's buffer solutions with or without urea addition.

The absence of an increase in forage IVNDFD when adding urea to Kansas buffer seems to be a consequence of its lower buffering capacity and pH values (Fig. 3). Although urea resulted in increased pH, likely due to the production of ammonia bicarbonate from CO_2 in the fermentation medium, its addition did not avoid a greater pH decrease along incubation times when included in Kansas buffer. For concentrates, the greater allowance of fermentable substrates from the feed, coupled with a greater N availability, can lead to a greater microbial activity, which explains the increased IVDMD and IVNDFD observed upon adding urea to both buffer solutions.

Overall, the results demonstrate greater IVDMD and IVNDFD for McDougall's solution than for Kansas buffer, which indicates greater microbial activity when using McDougall's buffer. Moreover, those effects were more prominent for IVNDFD than IVDMD, allows us to infer that differences in digestibility estimates are more related to constraints on fiber digestion. Furthermore, Kansas buffer exhibited lower pH than McDougall's buffer, which can significantly impair fiber digestion. Although the majority of pH values were above 6.0, which represents a critical low limit recommended for proper fibrolytic activity (Hoover, 1986), the Kansas buffer experienced a greater pH decrease over time, resulting in values below or approaching 6.0 after 24 h of incubation for both Tifton hay and ground corn. Congruent with the results of the present study, previous in vitro studies have demonstrated the lower buffering capacity of phosphate (e.g., Kansas) over bicarbonate-based buffers (e.g., McDougall) (Turner and Hodgetts, 1955; Herod et al., 1978; Kohn and Dunlap, 1998). It should be also emphasized the McDougall's buffer is more similar to a in vivo environment than Kansas buffer, as it is based on the chemical composition of ruminant saliva (McDougall, 1948).

Within the observed pH range, 2 additional buffer characteristics that could affect pH and buffering capacity should be also considered. First, the effect of CO_2 bubbling for pH adjustment in

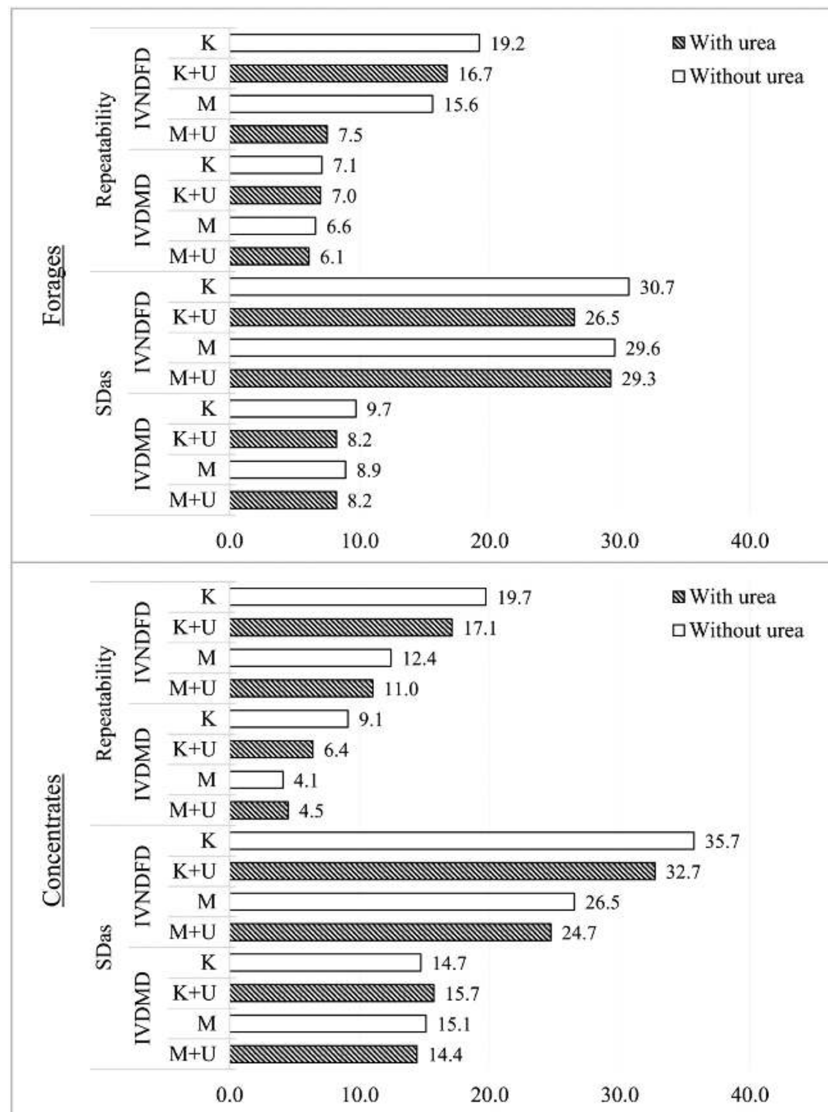

Figure 2. Standardized repeatability and relative standard deviation among samples (SDas) of in vitro digestibility of dry matter (IVDMD) and neutral detergent fiber (IVNDFD) in forages or concentrates using Kansas (K) or McDougall's (M) buffer solutions with (U) or without urea addition.

McDougall's buffer is important, as carbon dioxide bubbling is a common step in buffer solution preparation (McDougall, 1948; Pittman and Bryant, 1964; Menke and Steingass, 1988). This is important not only for pH adequacy (McDougall, 1948; Van Soest and Robertson, 1985), but also for the removal of dissolved oxygen (Bryant, 1972), which could increase redox potential of buffered rumen fluid and compromise microbial growth (Van Soest, 1994). Moreover, rumen microbes cannot grow without CO_2, which is important for carboxylation reactions of amino acid synthesis (Van Soest and Robertson, 1985). Therefore, the absence of CO_2 bubbling in Kansas buffer can be an additional limitation. Second, the presence of sodium sulfide in Kansas buffer, which has a strong redox power, could negatively affect anaerobic microbial growth. This argument is supported by the formation of toxic intermediate compounds (e.g., sulfur compounds) and the precipitation of metal ions (Fukushima et al., 2003).

For forages, urea increased digestibility, particularly for IVNDFD when using McDougall's buffer. However, NH_3-N accumulated over time when urea was added to the Kansas buffer, which was not observed for McDougall's buffer. Such a pattern indicates a limited NH_3-N uptake by microbes when Kansas buffer was used. In other words, urea hydrolysis did occur. However, N assimilation by microorganisms was limited, which reflected no increase in microbial activity and IVNDFD when using Kansas buffer.

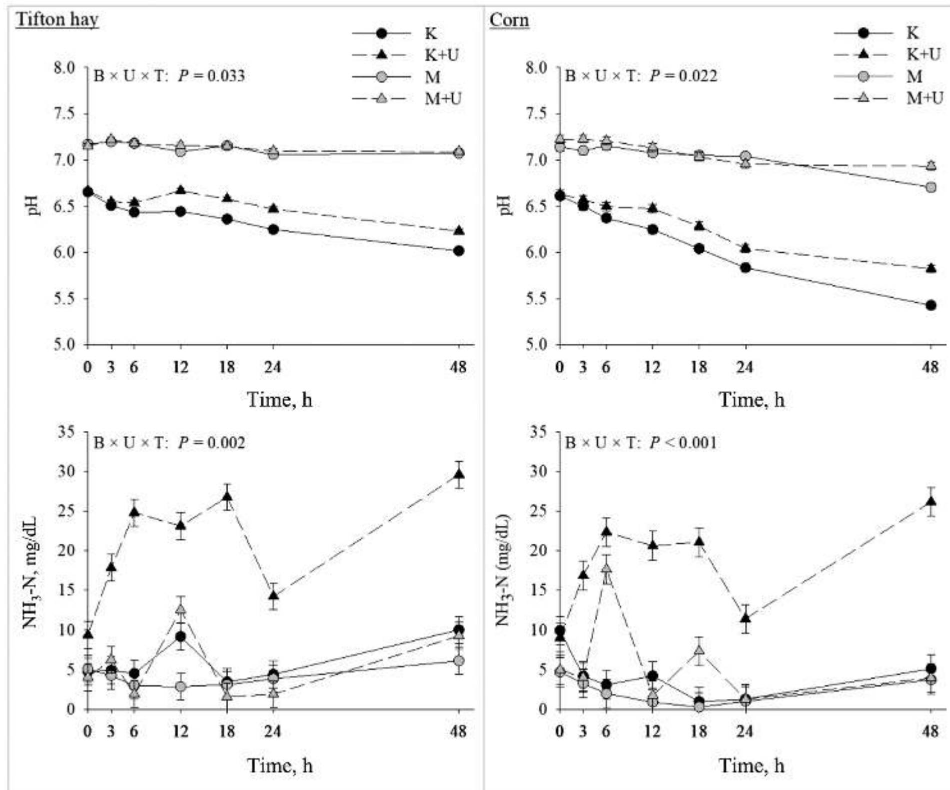

Figure 3. In vitro pH and ammonia nitrogen (NH_3-N) concentration at different incubation times of Tifton hay or ground corn incubated using Kansas (K) or McDougall's (M) buffer solutions with (U) or without urea addition (SEM for pH: 0.0258 for Tifton hay, and 0.0434 for ground corn).

Standardized Repeatability and Relative Standard Deviation Among Samples

An adequate buffer solution should provide lower *r* values and greater SDas, which indicates greater precision and discrimination power among samples, respectively. In this sense, lower overall *r* values for McDougall's solution over Kansas buffer, and similar SDas between them, highlight McDougall's solution as the best option. The only notable exception was the greater SDas for concentrate IVNDFD when using Kansas buffer; however, this could be the result of its limited buffering capacity, mainly with concentrates.

The observed reduction in *r* could be viewed as a positive effect of urea in laboratory procedures. In this sense, the use of McDougall's buffer with urea addition had predominantly lower *r* values, indicating that random variations among replicates decreased, leading to more precise IVDMD and IVNDFD estimates. However, urea addition predominantly decreased SDas for both buffers. In spite of addition of N compounds to in vitro fermentation medium has been suggested (Pittman and Bryant, 1964; Silva and Queiroz, 2002), some authors have pointed out a proper rumen inoculum should provide sufficient N compounds for microbial growth (Tilley and Terry, 1963), and N addition should not be recommended for estimating the in vitro digestion kinetics of sole feeds diets (Mertens, 2005). In this way, the donor animal diet cannot have limited N concentration in the diet. As observed in the present study, N addition to in vitro assays could reduce discriminatory power among samples. This is most likely due to increased digestibility estimates of lower-quality feeds, and a consequent decrease in the differences between high and low digestibility feeds. This can be a negative effect, primarily when in vitro assays aim to compare feeds with regards of IVDMD and IVNDFD. Thus, urea addition may represent an adverse factor for this type of in vitro study.

CONCLUSIONS

McDougall's buffer provides a better in vitro environment for fiber digestion and is recommended over Kansas buffer for more precise estimation of feed digestibility. Moreover, urea addition increases the precision of in vitro digestibility estimates, but decreases the discriminatory power among samples.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Animal Science* online.

ACKNOWLEDGMENTS

This project was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Instituto Nacional de Ciência e Tecnologia de Ciência Animal. The authors gratefully acknowledge Prof. Stefanie A. Santos (Universidade Federal da Bahia, Brazil) for the advice during the manuscript preparation.

LITERATURE CITED

Adesogan, A. T. 2005. Effect of bag type on the apparent digestibility of feeds in ANKOM DaisyII incubators. Anim. Feed Sci. Technol. 119:333–344. doi:10.1016/j.anifeedsci.2004.09.012

Barbosa, M. M., E. Detmann, G. C. Rocha, M. O. Franco, and S. C. Valadares Filho. 2015. Evaluation of laboratory procedures to quantify the neutral detergent fiber content in forage, concentrate and ruminant feces. J. AOAC Int. 98:883–889. doi:10.5740/jaoacint.14-156

Bryant, M. P. 1972. Commentary on the hungate technique for culture of anaerobic bacteria. Am. J. Clin. Nutr. 25:1324–1328. doi:10.1093/ajcn/25.12.1324

Costa, V. A. C., E. Detmann, S. C. Valadares Filho, M. F. Paulino, L. T. Henriques, and H. C. Mantovani. 2008. In vitro degradation of low-quality tropical forage neutral detergent fiber according to protein and (or) carbohydrates supplementation. Rev. Bras. Zootec. 37:494–503. doi:10.1590/S1516-35982008000300015

Detmann, E., M. F. Paulino, H. C. Mantovani, S. C. Valadares Filho, C. B. Sampaio, M. A. Souza, I. Lazzarini, and K. S. C. Detmann. 2009. Parameterization of ruminal fibre degradation in low-quality tropical forage using Michaelis–Menten kinetics. Livest. Sci. 126:136–146. doi:10.1016/j.livsci.2009.06.013

Detmann, E., M. F. Paulino, and S. C. Valadares Filho. 2008. Avaliação nutricional de alimentos ou de dietas? Uma abordagem conceitual. In: Proceedings of 2nd International Symposium on Beef Cattle Production, Viçosa, Brazil. p. 21–52.

Detmann, E., M. A. Souza, S. C. Valadares Filho, A. C. Queiroz, T. T. Berchielli, E. O. Saliba, L. S. Cabral, D. S. Pina, M. M. Ladeira, and J. A. G. Azevedo. 2012. Métodos para análise de alimentos. 1st ed. Suprema, Visconde do Rio Branco, Brazil.

Earing, J. E., B. D. Cassill, S. H. Hayes, E. S. Vanzant, and L. M. Lawrence. 2010. Comparison of in vitro digestibility estimates using the daisyii incubator with in vivo digestibility estimates in horses. J. Anim. Sci. 88:3954–3963. doi:10.2527/jas.2010-2989

Fukushima, R. S., P. J. Weimer, and D. A. Kunz. 2003. Use of photocatalytic reduction to hasten preparation of culture media for saccharolytic *Clostridium* species. Braz. J. Microbiol. 34:22–26. doi:10.1590/S1517-83822003000100006

Herod, E. L., R. M. Bechtle, E. E. Bartley, and A. D. Dayton. 1978. Buffering ability of several compounds in vitro and the effect of a selected buffer combination on ruminal acid production in vivo. J. Dairy Sci. 61:1114–1122. doi:10.3168/jds.S0022-0302(78)83695-9

Hoover, W. H. 1986. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci. 69:2755–2766. doi:10.3168/jds.S0022-0302(86)80724-X.

Kohn, R. A., and T. F. Dunlap. 1998. Calculation of the buffering capacity of bicarbonate in the rumen and in vitro. J. Anim. Sci. 76:1702–1709. doi:10.2527/1998.7661702x

Machado, M. G., E. Detmann, H. C. Mantovani, S. C. Valadares Filho, C. B. P. Bento, M. I. Marcondes, and A. S. Assunção. 2016. Evaluation of the length of adaptation period for changeover and crossover nutritional experiments with cattle fed tropical forage-based diets. Anim. Feed Sci. Technol. 222:132–148. doi:10.1016/j.anifeedsci.2016.10.009

Marten, G. C., and R. F. Barnes. 1980. Prediction of energy digestibility of forages with in vitro rumen fermentation and fungal enzyme systems, In: W. J. Pigden, C. C. Balch, and M. Graham, editors, Standardization of analytical methodology for feeds. IDRC, Ottawa, Canada. p. 61–71.

McDougall, E. I. 1948. The composition and output of sheep's saliva. Biochem. J. 43:99–109. doi:10.1042/bj0430099

Menke, K. H., and H. Steingass. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev. 28:7–55.

Mertens, D. R. 2005. Rate and extent of digestion. In: J. Dijkstra, J. M. Forbes, and J. France, editors, Quantitative aspects of ruminant digestion and metabolism. CABI, Wallingford, UK. p. 13–47. doi:10.1079/9780851998145.0013

Mould, F. L., K. E. Kliem, R. Morgan, and R. M. Mauricio. 2005a. In vitro microbial inoculum: a review of its function and properties. Anim. Feed Sci. Technol. 123–124:31–50. doi:10.1016/j.anifeedsci.2005.04.028

Mould, F. L., R. Morgan, K. E. Kliem, and E. Krystallidou. 2005b. A review and simplification of the in vitro incubation medium. Anim. Feed Sci. Technol. 123–124:155–172. doi:10.1016/j.anifeedsci.2005.05.002

Pittman, K. A., and M. P. Bryant. 1964. Peptides and other nitrogen sources for growth of *Bacteroides ruminicola*. J. Bacteriol. 88:401–410.

Roth, A. P. T. P., G. R. Siqueira, C. H. S. Rabelo, C. J. Härter, F. C. Basso, T. T. Berchielli, and R. A. Reis. 2016. Impact of days post-burning and lime as an additive to reduce fermentative losses of burned sugarcane silages. Anim. Feed Sci. Technol. 216:68–80. doi:10.1016/j.anifeedsci.2016.03.010

Russell, J. B. 2002. Rumen microbiology and its role in ruminant nutrition. James B. Russell, Ithaca, NY.

Silva, T. E., E. Detmann, L. F. Camacho, E. O. S. Saliba, M. N. Palma, and S. C. Valadares Filho. 2017. Comparison of in vitro methods to quantify the dry matter and neutral detergent fiber digestibility of forages and concentrates. Arq. Bras. Med. Vet. Zootec. 69:1635–1644. doi:10.1590/1678-4162-9096

Silva, D. J., and A. C. Queiroz. 2002. Análise de alimentos: Métodos químicos e biológicos. 3rd ed. UFV, Viçosa, Brazil.

Tilley, J. M. A., and R. A. Terry. 1963. A two-stage technique for the in vitro digestion of forage crops. Grass Forage Sci. 18:104–111. doi:10.1111/j.1365-2494.1963.tb00335.x

Turner, A., and V. Hodgetts. 1955. Buffer systems in the rumen of sheep. II. Buffering properties in relationship to composition. Aust. J. Agric. Res. 6:125. doi:10.1071/AR9550125

Valente, T. N. P., E. Detmann, S. C. Valadares Filho, M. Cunha, A. C. Queiroz, and C. B. Sampaio. 2011. In situ estimation of indigestible compounds contents in cattle feed and feces using bags made from different textiles. R. Bras. Zootec. 40:666–675. doi:10.1590/S1516-35982011000300027

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. 2nd ed. Cornell University Press, Ithaca, NY.

Van Soest, P. J., and J. B. Robertson. 1985. Analysis of forages and fibrous foods. Cornell University Press, Ithaca, NY.