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# Proportions of protein and concentrate in diets for buffaloes and cows affect neutral detergent fibre degradability

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## Abstract

The study was designed to compare low and high levels of protein, namely 90 and 147 g/kg of dietary dry matter, and to evaluate the effect of concentrate proportions on the in situ digestion kinetics of neutral detergent fibre in buffaloes and cows fed a low protein diet at maintenance intake level. In the first experiment, heifers and lactating females were offered a high protein diet. In the second, the performances of buffaloes and cows were compared when fed diets with low and high proportions of concentrate at low dietary protein level. At higher protein supply, the heifers showed a 6% unit increase in neutral detergent fibre degradability (NDFD) compared with lactating animals. Similarly, at a higher level of concentrate proportion, an 8% unit increase was observed in NDFD. In both experiments the comparison of buffaloes and cows was non-significant for NDFD. Those data that were pooled against the stage of development of both experiments for protein levels depicted a 13% unit increase in NDFD at high protein level compared with low level. At maintenance intake level, a high dietary crude protein or concentrate supply improved the in situ NDFD of tropical forages in buffaloes and cows, owing to the enhanced intake of NDF from concentrate and better synchronization of protein and energy availability in the rumen.

**Keywords:** buffalo, low dietary protein, maintenance intake level, NDF degradability
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## Introduction

Buffaloes are the second largest source of milk in the world after cows, with a total of 100 billion tons of milk production, contributing about 11% of the world's milk production annually (Wahid & Rosnina, 2016). They are unique in the superior quality of their milk, in their ability to withstand hot and humid climates, and in their proficiency in utilizing highly fibrous feeds (Paul, 2011). They are an important contributor to the socio-economic history of Asian countries through milk and meat production, their use as cart and ploughing animals, and their role in ritual celebrations. Buffaloes consume less dry matter (DM) per unit of bodyweight and have greater protein and energy efficiencies than cows (Paul *et al.*, 2003). Many studies determined the energy and protein requirements of buffaloes (Paul *et al.*, 2003; Paul, 2011). However, many aspects of their nutrient requirements have yet to be evaluated (Haque *et al.*, 2018).

Neutral detergent fibre (NDF) is a measure of cellulose, hemicellulose and lignin. Ruminant performance in intake, diet digestibility, and production efficiency is determined by the dietary concentration and extent of degradation of NDF (Mertens, 1993). The properties of cell walls and their relationship with extrinsic dietary and animal factors affect the upper limits of fibre utilization in ruminants (Hatfield *et al.*,

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1999; Huhtanen *et al.*, 2006). The structural and bonding interactions between cell wall components are controlled by the composition and arrangement of individual cell wall components. These bonding interactions control the integrity and expansion of cells during plant growth, and are important in establishing the rate and extent of degradation of cell wall components (Hatfield *et al.,* 1999).

Neutral detergent fibre digestion and passage kinetics have been studied extensively in sheep and cows (Huhtanen *et al.,* 2006; 2007). The in vivo technique is the standard procedure for quantifying the compartmental degradation of fibre, but in situ (Åkerlind *et al.*, 2011) and in vitro methods have also been used (Krizsan *et al.*, 2013). Factors such as the level of feeding, coupled with diet composition, have variable effects on NDF degradability (NDFD) in ruminants. A meta-analysis of the factors affecting feed digestion in cows reported that fibre digestibility was reduced as daily intake (Huhtanen *et al.*, 2009) and the amount of concentrate supplementation (CN) increased at production intake level (Nousiainen *et al.*, 2009). Increased fibre digestibility was reported to be associated with increased dietary and concentrate crude protein (CP) levels (Broderick *et al.*, 2003; Huhtanen *et al.*, 2009; Nousiainen *et al.*, 2009). The classical studies reporting the digestibility in sheep or cows were conducted at maintenance intake level (Tyrrel & Moe, 1975; Colucci *et al.*, 1982; 1990; Yan *et al.*, 2002) fed typical European or American diets with high dietary CP (usually greater than 140 g/kg diet DM) and overall high CN proportions (greater than 200 g/kg diet DM) (NRC, 2001). The maintenance intake level eliminated the changes in potential digestibility associated with reduced digesta rumen retention time arising from increased intakes (Huhtanen *et al.*, 2006). Further, the effects of the dietary CP and CN supply on the digestion kinetics of fibre at maintenance intake level have not yet been studied in buffaloes. These observations led to the question of the ways in which the digestion kinetics of fibre varied in buffaloes when the amounts of concentrate supplementation and dietary CP level were modified at maintenance intake level. The use of cows as an experimental model for dairy buffaloes assumed that dietary changes and the type of diet result in similar changes in the digestion kinetics in the two species (Sarwar *et al.,* 1998; Tahir *et al.*, 2019). Furthermore, animal-related factors such as developmental stage (Linden *et al.*, 2014) affected the degradation of forage.

The objectives of this research were i) to describe changes in NDF digestion associated with dietary CP levels and the amount of CN supplementation at low CP levels in ruminants fed at a maintenance intake level, and ii) to assess NDF digestion in buffaloes compared with cows at two developmental stages.

## Materials and Methods

All experiments were conducted according to the criteria of The Islamia University's Animal Care and Management Committee (IUB, 2015). This study was conducted at The Islamia University of Bahawalpur (29.39 °N, 71.68 °E), Bahawalpur, Pakistan. Ten forage species were studied, including six cereals, namely maize *(Zea mays),* millet *(Pennisetum glaucum),* sorghum *(Sorghum bicolor),* barley *(Hordeum vulgare)*, oats *(Avena sativa),* and wheat *(Triticum aestivum),* and four legumes, lucerne *(Medicago sativa)*, jantar *(Sesbania bispinosa),* berseem *(Trifolium alexandrinum)*, and mustard *(Brassica napus).* The detailed chemical composition of forages, their growing locations and conditions, and their transportation and handling of collected samples were described in Tahir *et al.* (2019). The feed samples were stored in airtight jars at 25 ºC and re-analysed chemically prior to use.

Experiment 1 used eight rumen-cannulated (Bar Diamond, Parma, ID, USA) animals, including two lactating Nili-Ravi buffalo cows (mean live weight (LW) = 509 ± 43.4 kg, milk yield = 5.63 ± 0.207 kg/day, days in milk (DIM) = 240 ± 20, age = 2225 ± 49.5 days, parity no. = 3.5), two Nili-Ravi buffalo heifers (LW = 531 ± 48.8 kg, age = 1913 ± 123.7 days), two lactating Cholistani cows (LW = 289 ± 29.4 kg, milk yield = 3.34 ± 0.271 kg/day, DIM = 235 ± 18, age = 1815 ± 21.9 days, parity no. = 3.0), and two Cholistani heifers (LW = 312 ± 35.4 kg, age = 1147 ± 64.3 days) for in situ incubations in a 2 × 2 × 2 split-plot design. The factors examined were animal species (buffaloes and cows) and their developmental stage (lactating animals and heifers). All animals were given an adjustment period of seven days in both experiments. The animals were offered a standard diet containing 147 g CP and 450 g NDF/kg diet DM with a forage to concentrate ratio of 8 to 20 on a DM basis. This diet was typical of one that is provided to dairy cows in developed countries (NRC, 2001). The animals' intake was similar to a maintenance intake level per NorFor guidelines (Volden, 2011). Ingredients, chemical composition, and feed intake of the diets are shown in Table 1. The animals were fed at 06h00 and 18h00, were confined to individual stalls (1.5 × 2.5 m), and had free access to drinking water individually.

**Table 1** Ingredients, chemical composition and intake of diets offered to rumen-cannulated heifers and lactating females

| | Lactating | | Heifer | |
|---|---|---|---|---|
| Item | Buffalo | Cow | Buffalo | Cow |
| Ingredients (g/kg as fed basis) | | | | |
| Sorghum fodder | 865 | 859 | 868 | 872 |
| Concentrate[1] | 72 | 74 | 65 | 65 |
| Cotton seed cake | 63 | 67 | 67 | 63 |
| Concentrate in total | 210 | 210 | 195 | 195 |
| Intake, % of BW | 1.70 | 1.70 | 1.70 | 1.70 |
| Intake, % of $BW^{0.75}$ | 8.07 | 7.01 | 8.16 | 7.14 |
| Chemical composition (g/kg diet dry matter) | | | | |
| Dry matter (as fed) | 481 | 489 | 476 | 470 |
| Crude protein | 146 | 147 | 147 | 147 |
| Ether extract | 26 | 26 | 26 | 26 |
| Neutral detergent fibre | 450 | 450 | 453 | 453 |
| Non-fibre carbohydrates | 195 | 195 | 196 | 196 |
| Ash | 183 | 182 | 178 | 179 |
| Intake (g/d) | | | | |
| Sorghum fodder | 5750 | 3200 | 5720 | 3150 |
| Concentrate mixture | 2000 | 1100 | 1800 | 1000 |
| Cotton seed cake | 1600 | 1000 | 1700 | 1000 |
| Dry matter | 9350 | 5300 | 9220 | 5150 |
| Crude protein | 1370 | 780 | 1360 | 760 |
| Ether extract | 240 | 140 | 240 | 140 |
| Neutral detergent fibre | 4210 | 2390 | 4180 | 2330 |
| Non-fibre carbohydrates | 1830 | 1040 | 1800 | 1010 |
| Ash | 1710 | 950 | 1640 | 910 |

BW: bodyweight
[1]Ground maize: 451 g/kg, soybean meal: 35.9 g/kg, corn gluten meal: 149.5 g/kg, rice bran: 134.2 g/kg, wheat bran: 132 g/kg, molasses: 76.4 g/kg, sodium chloride: 5.4 g/kg, lime 5.4 g/kg, di-calcium phosphate 5.2 g/kg, vitamin mineral premix: 5 g/kg

The same rumen-cannulated animals (n = 8) were used in Experiment 2. The forage samples were incubated in a 2 × 2 × 2 split-plot design. The factors examined were animal species, dietary CN proportions, (200 and 300 g/kg DM), and replicate. The CP and NDF levels of the diets were approximately 87 and 93 g/kg DM, and 540 and 520 g/kg DM for the high and low forage diets, respectively. This diet was typical of one offered to dairy animals in South Asian countries (Haque *et al.*, 2018). The diets were offered twice a day (at 06h00 and 18h00) at maintenance intake level throughout the experiment (Volden, 2011). Ingredients, their mean chemical composition, and feed intakes are shown in Table 2.

**Table 2** Ingredients, chemical composition and intake of the diets offered to rumen-cannulated animals in Experiment 2

| Item | Low concentrate | | High concentrate | |
|---|---|---|---|---|
| | Buffalo | Cow | Buffalo | Cow |
| Ingredients (g/kg as fed basis) | | | | |
| Sorghum fodder | 872 | 882 | 833 | 822 |
| Concentrate[1] | 60 | 59 | 104 | 110 |
| Wheat straw | 67 | 59 | 63 | 69 |
| Concentrate in total | 180 | 180 | 280 | 280 |
| Intake, % of BW | 1.70 | 1.70 | 1.70 | 1.70 |
| Chemical composition (g/kg dry matter) | | | | |
| Dry matter (as fed) | 506 | 490 | 559 | 574 |
| Crude protein | 87 | 88 | 93 | 93 |
| Ether extract | 27 | 28 | 29 | 29 |
| Neutral detergent fibre | 545 | 540 | 522 | 524 |
| Non-fibre carbohydrates | 226 | 229 | 243 | 242 |
| Ash | 115 | 116 | 112 | 111 |
| Intake (g/d) | | | | |
| Sorghum | 5720 | 3500 | 5280 | 3000 |
| Concentrate mixture | 1670 | 1000 | 2790 | 1600 |
| Wheat straw | 1940 | 1000 | 1750 | 1000 |
| Dry matter (as fed) | 9340 | 5500 | 9810 | 5600 |
| Crude protein | 810 | 490 | 910 | 520 |
| Ether extract | 250 | 150 | 280 | 160 |
| Neutral detergent fibre | 5080 | 2960 | 5120 | 2920 |
| Non-fibre carbohydrates | 2100 | 1270 | 2380 | 1360 |
| Ash | 1070 | 640 | 1100 | 630 |

[1]Ground maize: 451 g/kg, soybean meal: 35.9 g/kg, corn gluten meal: 149.5 g/kg, rice bran: 134.2 g/kg, wheat bran: 132 g/kg, molasses: 76.4 g/kg, sodium chloride: 5.4 g/kg, lime 5.4 g/kg, di-calcium phosphate 5.2 g/kg, vitamin mineral premix: 5 g/kg

The NDF degradation profiles were assessed according to NorFor standards (Volden, 2011) in both experiments. The in situ incubations were initiated from November 2016 to March 2017 for Experiment 1 and from June to October 2017 for Experiment 2 in batches (each batch consisted of seven days and five feeds) with a one-week interval between batches. Fodder samples were air-dried and ground through a 2-mm screen (POLYMIX PX-MFC, Kinematica AG, Germany). There was one sample per incubation period and animal and thus two experimental replicates per treatment and eight experimental replicates per feed. The fodder samples were sewn and glued into polyester bags (11 × 8.5 cm (10 × 7.5 effective size), with a pore-size of 33 µm and allowing for 25% free space in the bags (Sefar AG, Heiden, Switzerland). One-g feed samples (15 mg of feed sample for each $cm^2$ of the bag surface) were incubated inside the rumen of each cannulated animal for 0, 4, 8, 16, 24, 48, 96, and 168 hours. Samples were placed in the rumen using an all-in system and removed according to the pre-determined incubation period. When the incubation period ended, bags were retrieved, washed, and stored at –18 °C. At the end of incubation, all frozen bags were thawed and washed with tap water at room temperature (20 $^{o}$C). The amylase-treated NDF in the residues was analysed using the technique of Van Soest *et al.* (1991), modified by Mertens *et al.* (2002).

Fresh forage and dry feeds offered to the animals were sampled fortnightly during the study. Samples of rumen fluids were taken on the last two days of every batch (days 6–7). Approximately 200 mL of rumen fluid was collected from varioius spots in the rumen (reticulum, dorsal, and ventral sac) at six-hour intervals (07h00, 13h00, and 19h00). The pH of samples was determined immediately with a pH meter (Starter 2100 pH Bench,Ohaus Corporation, Parsippany, NJ, USA).

Fresh chopped forage and the dry feed DM value were calculated at 60 °C for 48 hours and 105 °C for 16 hours (AOAC method 7.003). Ash (AOAC method 923.03), CP (6.25 × N) (AOAC method 7.015), ether extract (AOAC method 7.062) and NDF (Van Soest *et al.,* 1991; Mertens *et al.*, 2002) were assessed.

The in situ degradation data were categorized as particle loss or washable fraction (*a*, 0 hour values for washed samples) and non-washable fraction. The non-washable fraction was further divided into potentially degradable (*b*) and indigestible fractions, which corresponded to the degradation and residue at the final incubation interval. A first-order kinetic model with intercept and lag (Dhanoa, 1988; Ørskov *et al.*, 1979) was fitted to the degradation data:

$$Yt = a + b \times (1 - \exp(-Kd(t - L)))$$

where $Y_t$ denotes the degraded fraction at a given time *t*, $K_d$ denotes the fractional degradation rate of fraction *b, L* denotes the lag time (h) for *t* > *L*, and *t* denotes the time of incubation (h). Table Curve 2D, version 5.0 (Systat Software, Inc., San Jose, CA, USA) was used for model fitting. Effective ruminal predicted NDFD was calculated as:

$$NDFD = a + [b \times Kd/(Kd + Kp)]$$

assuming the fractional rate of passage ($K_p$) to be 0.028/h (a 36-hour rumen retention time) according to a 2-pool model for forage NDF (Krämer *et al.*, 2013).

The GLM procedure of Minitab, version 16.1.1.0 (Minitab Ltd., Coventry, UK) was used to analyse the data statistically. Each cow/heifer was considered an experimental replicate. To determine the effect of the dietary CP level, data from Experiment 1 and Experiment 2 were combined and analysed using the following model:

$$Y_{ijkl} = \mu + A_i + CP_j + F_k + (AxCP)_{ij} + (AxF)_{ik} + (CPxF)_{jk} + (AxCPxF)_{ijk} + e_{ijkl}$$

and data on in situ parameters recorded in Experiment 1 were analysed using the following model:

$$Y_{ijkl} = \mu + A_i + D_j + F_k + (AxD)_{ij} + (AxF)_{ik} + (DxF)_{jk} + (AxDxF)_{ijk} + e_{ijkl}$$

Data from Experiment 2 were analysed using the model:

$$Y_{ijkl} = \mu + A_i + C_j + F_k + (AxC)_{ij} + (AxF)_{ik} + (CxF)_{jk} + (AxCxF)_{ijk} + e_{ijkl}$$

where $Y_{ijkl}$ is the dependent variable, $\mu$ is the overall mean, $A_i$ is the *ith* animal species ($_{i =}$ 1–2), $CP_j$ is the *jth* dietary crude protein level ($_{j =}$ 1–2), $D_j$ is the *jth* developmental stage of the animal (*jj* $_{=}$ 1–2), $C_j$ is the *jth* concentrate proportion in the diet (j $_{=}$ 1–2), $F_k$ is the *kth* family of forage incubated (k $_{=}$ 1–2), and $e_{ijkl}$ is the residual error. The results were considered significant when *P* <0.05. Trends were observed when 0.05 ≤ *P* ≤ 0.10.

## Results and Discussion

Although there was a slight difference in the proportion of concentrate of the diet between lactating animals and heifers, the composition of dietary nutrients was similar across treatments in Experiment 1. The degradation parameters described in this study followed the model that was presented by Ørskov & McDonald (1979). Effective NDFD was calculated assuming the fractional rate of passage ($K_p$) to be 0.028/h according to a 2-pool model with 36-hour rumen retention for forage NDF (Krämer *et al.,* 2013). The effects of the dietary CP levels on NDF degradation kinetics and NDFD are shown in Figures 1 and 2, and in Table 3. The NDF degradation curves at different dietary CP levels are shown in Figure 1. All degradation fractions and NDFD expressed on an NDF or DM basis increased linearly (*P* <0.05) with the dietary CP level. All interaction effects between the main effects were not significant, with the exception of that between dietary CP level and forage type for $K_d$ (*P* <0.001). Overall, the NDFD responses at longer incubation times were better for diets with low dietary CP levels. The regression equations were pdNDF = 0.234 + 0.113 (CP), pdNDF = 0.298 + 0.109 (CP) and pdNDF = 0.374, + 0.100 (CP) at 24, 48 and 168 hours of incubation, respectively (*P* <0.001, n = 80).

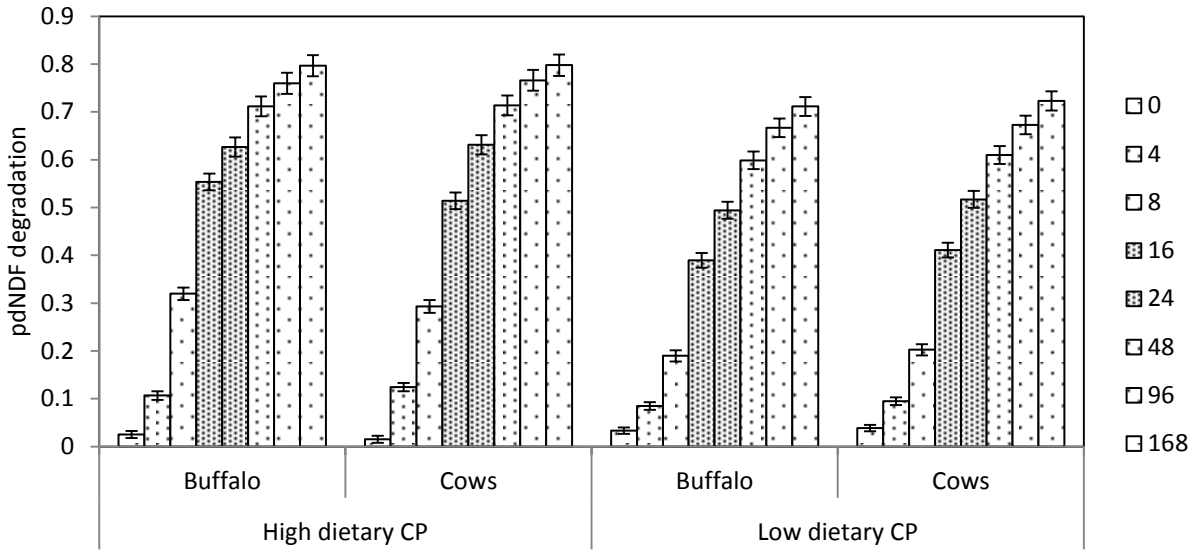


**Figure 1** Degradation curves of potentially degradable neutral detergent fibre of buffaloes and cows (species $P$ >0.10; time of incubation $P$ <0.05) at low (90) and high dietary crude protein (147 g/kg dry matter) levels in the diet for 0, 4, 8, 16, 24, 48, 96 and 168 hours after incubation

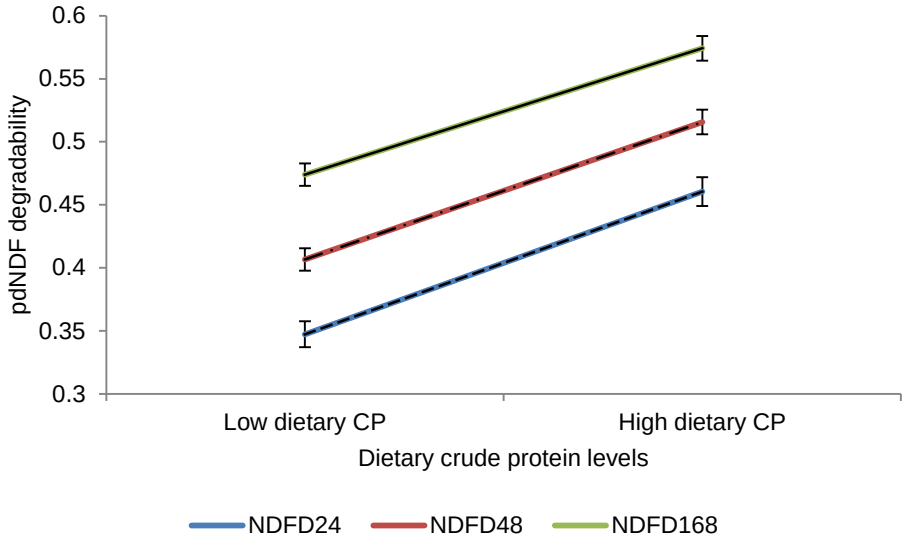


**Figure 2** Potentially degradable neutral detergent fibre degradability at 24, 48 and 168 hours of incubation (time × crude protein level interaction) at low (90 g/kg DM) and high (147 g/kg DM) dietary crude protein levels in the diet

**Table 3** Probability levels for effects of animal and diet factors on in situ neutral detergent fibre degradation kinetics and effective degradability of cereal and legume fodder

| | | *P-values* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Item | Animal species (A) | Crude protein level (CP) | Forage (F) | Incubation time | A × CP | A × F | CP × F | A × CP × F |
| Degradation parameter | *a* | 0.720 | 0.043 | <0.001 | | 0.290 | 0.987 | 0.445 | 0.985 |
| | *b* | 0.592 | <0.001 | <0.001 | | 0.736 | 0.763 | 0.036 | 0.555 |
| | $K_d$ | 0.377 | <0.001 | <0.001 | | 0.168 | 0.468 | 0.009 | 0.701 |
| | L | 0.644 | <0.001 | <0.001 | | 0.565 | 0.933 | 0.169 | 0.955 |
| Effective degradability | NDFD24 | 0.794 | <0.001 | <0.001 | | 0.169 | 0.466 | 0.165 | 0.550 |
| | NDFD48 | 0.980 | <0.001 | <0.001 | 0.001 | 0.166 | 0.657 | 0.366 | 0.724 |
| | NDFD168 | 0.956 | <0.001 | <0.001 | 0.001 | 0.104 | 0.857 | 0.049 | 0.505 |
| | NDFD168DM | 0.779 | <0.001 | 0.089 | | 0.505 | 0.857 | 0.001 | 0.588 |

*a:* washable fraction representing the portion of neutral detergent fibre (NDF) that disappeared at time 0; *b*: potentially degradable NDF fraction; $K_d$: fractional rate of degradation of fraction *b*' L: lag time; NDFD24, NDFD48 and NDFD168: NDF degradability at 24, 48, and 168 hours of incubation, respectively; NDFD168DM: NDF degradability at 168 hours expressed relative to DM

There was no effect of animal species ($P$ >0.10) on NDF degradation parameters and NDFD values in either of the experiments (Tables 4 and 5). However, slightly greater values were observed for NDFD at all three incubation intervals in buffaloes compared with cows. Comparison of the NDFD values at various incubation intervals revealed a relative increase of 26% in NDFD for both species in Experiment 1 when the incubation time was extended from 24 to 168 hours ($P$ <0.001). Similarly, a relative increase of 34% in NDFD was observed for both species in Experiment 2 over the same incubation interval ($P$ <0.001). The interaction of animal species and time of incubation was not significant ($P$ >0.10).

The developmental stage (Table 4) affected the *b* fraction ($P$ <0.10) and the NDFD ($P$ <0.01), but not the degradation parameters and NDFD expressed on a DM basis ($P$ >0.10). Heifers could utilize the feed materials in the nylon bags in the rumen more effectively than lactating animals. However, the corresponding rumen undegradable values were reduced for heifers. The interaction effects between animal species and developmental stage on these parameters were significant ($P$ <0.05), and lactating cows had greater NDFD values than lactating buffalos. A positive effect ($P$ <0.001) and significant interaction (P <0.001) were observed between developmental stage and incubation time on effective degradability, which was shown by a 5% unit relative increase in NDFD in cows compared with heifers.

In this study (Table 5), rumen pH was relatively consistent across all treatments with means ranging from 6.95 to 6.99 and with non-significant effects ($P$ >0.05) of CN in the diet. However, pH remained higher ($P$ <0.001) than 7.0 before the morning feeding, reduced to 6.8 around 12h00 almost 5 hours after the morning feeding, and increased to 6.9 at 18h00. Increasing concentrate proportion in the diet affected all degradation fractions and effective degradability. Increasing the CN in the diet tended to increase ($P$ <0.10) $K_d$ and NDFD at 24 hours (NDFD24) and 168 hours (NDFD168) of incubation. A positive and significant ($P$ <0.01) interaction between CN and forage family also increased degradation fractions such as *a* and *b* and NDFD values for cereals at higher concentrate levels, but the effect of CN and forage family was non-significant ($P$ >0.10) for $K_d$. NDFD was also affected by incubation time, and greater NDFD values were observed for longer incubation intervals ($P$ <0.001). A low concentrate proportion × higher time of incubation interaction increased NDFD by 40% compared with a high concentrate × higher time of incubation interaction (29%). The incremental increase in NDFD was greater for low CN diets compared with high CN diets (4.6 vs. 3.8 g/10 g/kg concentrate DM increase) when expressed on a CN basis.

The present findings were consistent with those of Broderick (2003), who found a positive relationship between the CP content of the diet, DM intake, and NDFD. Furthermore, they showed that acid detergent fibre digestibility and NDFD rose in dairy cows as the dietary CP level increased. The meta-analysis of Nousiainen *et al.* (2009) showed that supplementation with high dietary CP levels improved potential organic matter digestibility in dairy cows. This increase in the potential organic matter digestibility was associated with an improvement in CP and NDF digestibility owing to the increased digestion rates and nutrient supply to fibre-degrading bacteria, which were stimulated by the ruminal degradation of protein. Luc *et al.* (2009) suggested that organic matter and NDF digestibility for tropical grasses containing a low amount of protein increased with dietary protein level, and these digestibilities were also affected by the dietary protein source. Shabi *et al.* (1998) suggested that increased NDFD with higher dietary CP level could result from the improved synchronization of protein and energy availability in the rumen, which would provide an environment that was more conducive to microbial growth, and thus facilitate the utilization of dietary carbohydrates and proteins.

In the present study, a 1.68 g incremental increase in NDFD was observed for every gram increase in the dietary CP level from 90 to 145 g/kg DM (two protein concentrations, under the constraints of maintenance intake level). This increase was relatively lower at longer incubation periods (1.7 vs. 1.6 g/g/kg CP in the diet). In the previous studies, the relative incremental increase was 0.77 and 1.15 g for every g/kg increase in the dietary CP level from 111 to 229 g/kg DM for grass silage-based diets (Nousiainen *et al.*, 2009). The greater increase in digestibility in the current research may be associated with the increased digestion rates of NDF, a less acidic rumen environment, and the increased supply of amino acids to the rumen microbes (Broderick, 2003; Huhtanen *et al.,* 2009; Nousiainen *et al.*, 2009). The relationship between the ruminant host animal and microbes in its rumen is synergistic with the host providing heat, moisture and food, while the microorganisms produce protein and volatile fatty acids for use by the host (Hungate, 1966). This may explain in part the profound effect of protein source on NDFD in addition to increased dietary protein supply.

**Table 4** Effects of animal factors on in situ neutral detergent fibre degradation kinetics and effective degradability of diets containing 147 g crude protein and 450 g neutral detergent fibre /kg diet dry matter with a forage to concentrate ratio of 80:20 on a dry matter basis

| | Items | Animal species (A) | | | Developmental stage (D) | | | *P*-values | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Buffalo | Cow | SE | Heifer | Lactating | SE | A | D | F | I | A × D | A × F | D × F | A × D × F |
| Degradation parameter | a | 0.045 | 0.050 | 0.0068 | 0.043 | 0.053 | 0.0068 | 0.599 | 0.304 | <0.001 | --- | 0.631 | 0.510 | 0.534 | 0.732 |
| | b | 0.574 | 0.573 | 0.0376 | 0.449 | 0.697 | 0.0376 | 0.979 | 0.064 | <0.001 | --- | 0.965 | 0.883 | 0.242 | 0.972 |
| | $K_d$ | 0.059 | 0.056 | 0.0041 | 0.046 | 0.069 | 0.0041 | 0.579 | 0.953 | <0.001 | --- | 0.853 | 0.997 | 0.549 | 0.815 |
| | L | 0.790 | 0.790 | 0.0530 | 0.660 | 0.960 | 0.053 | 0.980 | 0.825 | <0.001 | --- | 0.747 | 0.859 | 0.127 | 0.948 |
| Effective degradability | NDFD24 | 0.451 | 0.444 | 0.0137 | 0.478 | 0.416 | 0.0121 | 0.655 | 0.002 | 0.002 | --- | 0.025 | 0.753 | 0.347 | 0.920 |
| | NDFD48 | 0.507 | 0.499 | 0.0130 | 0.535 | 0.471 | 0.0114 | 0.659 | 0.001 | <0.001 | 0.001 | 0.012 | 0.774 | 0.230 | 0.911 |
| | NDFD168 | 0.568 | 0.561 | 0.0122 | 0.595 | 0.534 | 0.0107 | 0.722 | 0.001 | <0.001 | 0.001 | 0.014 | 0.896 | 0.130 | 0.933 |
| | NDFD168DM | 0.779 | 0.776 | 0.0076 | 0.788 | 0.766 | 0.0067 | 0.664 | 0.049 | 0.229 | --- | 0.941 | 0.998 | 0.221 | 0.840 |

*a:* washable fraction representing the portion of the neutral detergent fibre (NDF) that disappeared at time 0, *b*: potentially degradable NDF fraction, $K_d$: fractional rate of degradation of fraction *b*, F: forage, L: lag time, NDFD24, NDFD48 and NDFD168: NDF degradability at 24, 48, and 168 hours of incubation, respectively, NDFD168DM: NDF degradability at 168 hours expressed relative to DM

**Table 5** Effect of animal and diet factors on *in situ* neutral detergent fibre degradation kinetics and effective degradability of cereal and legume fodder in diets containing 87 and 93 g crude protein and 540 and 520 g NDF/kg diet DM for the high and low forage diets, respectively

| Items | Animal species (A) | | | Concentrate proportions (CP) | | | *P*-values | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Buffalo | Cow | SE | 20% | 30% | SE | A | C | F | I | A × C | A × F | C × F | A × C × F |
| *pH* | 6.98 | 6.97 | 0.045 | 6.95 | 6.99 | 0.045 | 0.846 | 0.543 | | <0.001 | 0.776 | | | |
| *a* | 0.008 | 0.010 | 0.0063 | 0.001 | 0.019 | 0.0070 | 0.860 | 0.021 | <0.001 | | 0.907 | 0.376 | 0.021 | 0.907 |
| *b* | 0.666 | 0.661 | 0.0087 | 0.654 | 0.673 | 0.0097 | 0.727 | 0.117 | <0.001 | | 0.706 | 0.765 | 0.001 | 0.866 |
| $K_d$ | 0.051 | 0.048 | 0.0019 | 0.047 | 0.052 | 0.0021 | 0.270 | 0.048 | 0.387 | | 0.179 | 0.194 | 0.798 | 0.608 |
| L | -0.79 | -1.08 | 0.288 | 1.03 | -2.89 | 0.322 | 0.483 | <0.001 | <0.001 | | 0.473 | 0.457 | <0.001 | 0.440 |
| NDFD24 | 0.373 | 0.378 | 0.0180 | 0.313 | 0.398 | 0.0201 | 0.874 | 0.078 | 0.003 | | 0.251 | 0.47 | 0.001 | 0.952 |
| NDFD168 | 0.499 | 0.505 | 0.0160 | 0.443 | 0.521 | 0.0179 | 0.789 | 0.094 | <0.001 | <0.001 | 0.277 | 0.612 | 0.001 | 0.878 |
| NDFD168DM | 0.684 | 0.683 | 0.0069 | 0.697 | 0.670 | 0.0077 | 0.974 | 0.094 | 0.008 | | 0.341 | 0.551 | <0.001 | 0.754 |

*a:* washable fraction representing the portion of neutral detergent fibre that had disappeared at time 0, *b:* potentially degradable NDF fraction, $K_d$: fractional rate of degradation of fraction *b*; L: lag time, NDF: neutral detergent fibre; NDFD24 and NDFD168: NDF degradability at 24 and 168 hours of incubation; NDFD168DM: NDF degradability at 168 hours of incubation expressed on a dry matter basis, A: animal species, C: concentrate proportions, F: forage family, I: incubation time

Neutral detergent fibre degradation parameters and NDFD values obtained in this study were similar to those obtained in previous studies and were consistent with degradability observed in dairy cows (Lopes *et al.*, 2015). In previous studies which were conducted at a maintenance intake level , DMD and NDFD of selected desert grasses did not significantly differ between buffaloes and cows ((Tahir *et al.*, 2019 & 2020). These data indicated that these feedstuffs were nutritionally equivalent for different species of ruminants. In other studies, which were conducted at production intake level, researchers generally observed greater NDFD values in buffaloes compared with cows. Sarwar *et al.* (1998) compared the digestibility properties of various forage plants and agro-industrial by-products in buffaloes and cows using an in situ method. They found higher ruminal NDFD and DMD and $K_d$ of grasses in buffaloes than in cows after 48 hours of in situ incubation. However, no differences were reported among the species in the rate and extent of in situ NDFD and DMD for agricultural by-products and leguminous forages. Bhatia *et al.* (1995) found greater in situ $K_d$ for NDF and DM for berseem hay in buffaloes compared with cows. However, the effective degradability remained the same within animal species. Terramoccia *et al.* (2000) found that buffaloes showed greater values of DMD and $K_d$ for protein and protein-free ruminant feedstuffs than cows. Aside from finding that $K_d$ values were lower in cows than in buffaloes, Franzolin & Dehority (1999) studied the in situ degradation of DM and NDF for cows and buffaloes fed tropical forage grasses and obtained similar results for the two species. Nandra et al. (2000) studied the DM degradation parameters for forages and concentrates in cows and sheep and detected no significant effect of species. Furthermore, no interaction effect was observed of animal species with study duration or experimental diet. Nandra et al. (2000) proposed that a single ruminal degradation curve for each experimental feed in sheep and cows could be used to represent DMD in the rumen. Huntington & Givens (1997) found no effect of host animal species on the DMD of soybean meal, hay, and fish meal using the in situ digestibility technique. Yan *et al.* (2002) found that digestibility coefficients were similar in sheep and cows, although sheep had a greater proportion of hindgut digestion. This difference in energy and protein demand might stem from the body mass of these animals. Therefore, more details about feed utilization and production variables should be integrated with in situ degradation parameters to analyse the efficiency of feed use among species.

The energy and protein demand of lactating animals differs from that of heifers (NRC, 2001). The authors hypothesized that lactating animals use feed material efficiently depending on their nutritional requirements for milk and their enhanced capacity to extract nutrients from the digesta of the rumen (Johnson *et al.*, 2003). Although the present findings do not support this hypothesis, the results are consistent with the conclusions of previous studies (Varel & Kreikemeier, 1999; Johnson *et al.*, 2003), which showed that heifers have 5% greater OM digestibility than lactating multiparous cows when consuming a similar amount of dry matter per unit of bodyweight. Linden *et al.* (2014) suggested that lactation does not affect diet digestibility, even though heifers in their experiment tended to have greater dry matter intake as a percentage of bodyweight than cows. Conversely, Colucci *et al.* (1982) reported that mature dairy cows experienced a postpartum decrease in DM digestibility as dry matter intake increased.

A meta-analysis based on a large dataset that included studies in which dairy cows were fed at production intake level revealed that NDFD decreased as the amount of concentrate increased in the diet (Huhtanen *et al.*, 2009; Nousiainen *et al.*, 2009). Furthermore, the decrease in NDFD was more pronounced for concentrates with greater organic matter digestibility. Hetta *et al.* (2010) found that the amount of starch in concentrate affected the digestibility and substitution of forage, and the forage substitution rate was greater at higher starch levels regardless of the type of forage. They also found that an increase in the dietary CN decreased the NDFD regardless of the forage composition. Initially, this decrease in NDF digestibility was associated with the increased passage of the fibre particles from the rumen (Nousiainen *et al.*, 2009) and not with the changes occurring in rumen pH or in the level of microbial activity. Concentrate fibres contain less lignin, and their particle size can be easily reduced by rumen microbes. This makes them pass quickly from the age-dependent to age-independent ruminal compartment compared with forage fibres (Wylie *et al.*, 2000), which resulted in the passage of both concentrates and forage fibres. The improvement in NDFD with increased concentrate supply may be explained by the studies of Colucci *et al.* (1989; 1990). They found a strong correlation ($R^2$ = 0.86) between the decrease in ruminal retention time and decrease in digestibility in sheep and cows that were offered different feeds. The rumen residence time is much longer for high-concentrate diets when fed at maintenance intake level (Colucci *et al.*, 1982; 1990), which permits compensation for the reduced NDF digestion rates with high-concentrate diets at low levels of feeding. However, when the feeding level increased, the rumen residence time decreased more with high-concentrate than with low-concentrate diets (Colucci *et al.*, 1989; 1990), thus precluding compensation for the effects of a reduced digestion rate on NDFD. The improvement in

NDFD may also be attributed to the improved balance between energy and protein components at the rumen level, which resulted in enhanced microbial production (Granja-Salcedo *et al.*, 2016), and greater concentrate NDF supply, which is more digestible than forage NDF. Wanapat *et al.* (2013) suggested that only the high concentrates levels in the diets caused a serious decline in ruminal pH, ultimately reducing fibre degradability.

## Conclusions

Higher dietary protein levels and an increased concentrate supply enhanced the in situ NDF degradability of selected tropical forages in buffaloes and cows at maintenance intake level. This enhanced degradability resulted from better synchronization of protein and energy availability in the rumen. The degradability parameters were similar in buffaloes and cows. However, the heifers showed greater degradability values than lactating animals.

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**Authors' contributions**

MNT conceived and designed the study. ZK and MNT carried out the experiments. MNT and MNH analysed the data. MNT, MNH, and RR wrote the first draft of the manuscript. AJ and SG analysed the rumen fluid samples for microbial analyses. MZI, NZ, MNH, SM, TNP, and JA revised various drafts of the manuscript.

**Conflict of interest**

No conflict of interest is declared.

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