Improved prediction of dietary protein use and nitrogen excretion in tropical dairy cattle

Dissertation to obtain the doctoral degree of Agricultural Sciences
(Dr. sc. agr.)

Faculty of Agricultural Sciences
University of Hohenheim

Institute of Agricultural Sciences in the Tropics (Hans-Ruthenberg Institute)
Animal Nutrition and Rangeland Management in the Tropics and Subtropics

submitted by
Khaterine Cinthia Salazar Cubillas

Born in Lima, Peru

2022

The present thesis was accepted on 20st June 2023 as a doctoral dissertation in fulfilment of the requirements to obtain the degree “Doktor der Agrarwissenschaften” (Dr. sc. agr.) from the Faculty of Agricultural Sciences at the University of Hohenheim, Stuttgart, Germany.

Date of examination

July 12th, 2023

Examination committee

Supervisor and reviewer: Prof. Dr. Uta Dickhöfer

Co-reviewer and examiner: Prof. Dr. Karl-Heinz Südekum

Additional examiner: Prof. Dr. Georg Cadisch

Head of the examination committee: Prof. Dr. Jörn Bennewitz

To my beloved mother

This project was financially supported by the Program of Accompanying Research for Agricultural Innovation (PARI) of the Federal Ministry for Economic Cooperation and Development (BMZ, Germany, grant number 2014.0690.9) and the National Fund for Scientific, Technological Development and Technological Innovation (FONDECYT, Peru, grant number 129-2016).

Summary

The inefficient utilization of nitrogen (N) by dairy cows leads to a substantial release of N into the environment, causing pollution. This issue is particularly pronounced in tropical regions where most dairy cattle are located, resulting in higher pollution levels compared to temperate regions. To address this problem, one potential solution involves enhancing N use efficiency by aligning N supply with N requirements of dairy cows. However, the effectiveness of this approach faces challenges due to limited availability of detailed information on the dietary composition of tropical dairy cattle, as well as the partial incorporation of differences in digestion process efficiencies between tropical and temperate cattle into available feeding recommendations. Consequently, existing laboratory methodologies and modeling tools, originally designed for temperate regions, have been adopted for tropical regions due to the limited information available. Therefore, the overall objective of the present doctoral thesis was to evaluate the adequacy (i.e., accuracy and precision) of existing laboratory methodologies and modeling tools, originally designed for temperate systems, in predicting the N supply and excretion of cattle in tropical husbandry systems. It was hypothesized that the adoption of laboratory methodologies and modeling tools from temperate systems without validating and adapting them for tropical systems may result in inaccurate estimations of N supply, utilization, and excretion, which will hamper the assessment of N use efficiency.

An *in vitro* study was conducted to evaluate the adequacy of the chemical method (Sniffen et al., 1992) to predict rumen-undegraded crude protein (RUP) of tropical forages grasses and legumes. The equations developed by Kirchhof et al. (2010) and Valdés et al. (2011) to predict RUP proportions of temperate forages as a function of chemical crude protein (i.e., concentration and proportion of crude protein fractions A, B_1, B_2, B_3, and C) and fiber fractions (i.e., concentration of neutral and acid detergent fiber) were selected. These equations were then used to calculate RUP proportions of forages commonly used as feed for ruminants in the (Sub-) Tropics (i.e., 23 forage grasses and 15 forage legumes). The adequacy of the predictions was assessed by comparing them with RUP proportions measured *in situ* at rumen passage rates of 2, 5, and 8% per hour. Results showed that the RUP of tropical forages estimated with the *in situ* method can be predicted using the chemical method. However, regression equations developed for temperate forages were not adequate enough to predict RUP proportions of tropical forages consistently for all rumen passage rates. Instead, developed equations in the present thesis can be used to predict RUP proportion of tropical forages with a similar chemical composition than the reference forage sample set.

A second *in vitro* study was conducted to evaluate the adequacy of the chemical (Sniffen et al., 1992) and *in vitro* methods (Steingaß et al., 2001) to predict post-ruminal crude protein (PRCP) supply of tropical forages (i.e., 23 forage grasses and 15 forage legumes). The equation developed by Zhao and Cao (2004) to predict PRCP supply of temperate forages as a function of chemical crude protein fractions (i.e., concentration of crude protein protein fractions A, B_1, B_2, B_3, and C) was selected. The adequacy of the PRCP supply with the chemical and *in vitro* methods were tested against PRCP supply estimated from *in situ* measurements at rumen passage rates of 2, 5, and 8% per hour and digested organic matter concentration determined from the gas production of the Hohenheim gas test. Results showed that the *in vitro* method can be used as an alternative method to estimate PRCP supply in tropical forages at moderate to fast rumen passage rate but not at slow rumen passage rate. Available regression equations developed for temperate forages were not adequate enough to predict the PRCP supply of tropical forages from concentrations of chemical crude protein fractions. Instead, developed equations in the present thesis can be used to estimate PRCP supply of tropical forages with a similar chemical composition than the reference forage sample set.

Following the improvement of the prediction of N supply to the animal (in the form of RUP and PRCP), a third study was conducted to assess the adequacy of modeling tools to predict N excretion of cattle in tropical husbandry systems. Three semi-mechanistic models were chosen for this purpose. These models, namely model A (built upon British ruminant feeding recommendations), model G (based on German ruminant feeding recommendations), and model I (developed by the French ruminant feeding recommendations), were selected to predict fecal N (FN), urine N (UN), and total N (TN) excretion as well as FN fractions of dairy cows, heifers, and steers kept under typical tropical husbandry conditions. The adequacy of the model predictions was assessed against reference values of UN (total collection and creatinine method) and FN excretion (total collection, internal and external markers) (n = 392 observations). Adjustments were made to the models with the greatest potential to predict N excretion. The adjustments were focused on the input variables driving the variability in N excretion predictions, identified through a sensitivity analysis. None of the tested models predicts adequately the excretion of UN, FN, and of different FN fractions of individual cattle kept under tropical conditions. Instead, model I in the present thesis, adjusted for increased efficiency of rumen microbial crude protein synthesis and reduced intercept of FN prediction, can be used to estimate FN and TN excretion of individual cattle kept under tropical conditions.

The findings from the present thesis partially support the hypothesis that the adoption of laboratory methodologies and modeling tools from temperate systems without validating and adjusting them for tropical systems results in inadequate estimates of N supply and excretion of cattle in tropical husbandry systems, which hampers the assessment of N use efficiency and the adjustment of nutrient supply to the actual requirements of the animal. The adjustment of laboratory methodologies, such as the chemical method used to estimate the protein value of temperate forages, to tropical forages, results in more adequate estimates of the proportion of RUP and PRCP supply of tropical forages. Model I is, therefore, able to predict the N excretion of cattle more adequately in tropical husbandry systems, because it is sensitive to differences in the RUP proportion and PRCP supply. In addition to increasing the adequacy of these input variables, adjustments made to the microbial protein synthesis and intercept of the FN excretion of model I results in a more adequate prediction of N excretion by cattle in tropical husbandry systems. However, not all adjustments to laboratory methodologies and modeling tools from temperate systems yielded adequate predictions for the protein value of tropical forages and cattle N excretion in tropical husbandry systems. Specifically, challenges remained in predicting RUP proportion and PRCP supply for tropical forage legume with slow rumen passage rates, as well as urinary N excretion in cattle with low N intakes. Consequently, further research is required to identify the factors contributing to their poor adequacy.

Zusammenfassung

Die ineffiziente Verwertung von Stickstoff (N) durch Milchkühe trägt dazu bei, dass große Mengen an umweltschädlichem N freigesetzt werden. Da die meisten Milchkühe in tropischen Regionen leben, ist dort der Grad der Emissionen höher als in gemäßigten Regionen. Eine mögliche Lösung zur Verringerung der Umweltbelastungen durch N ist die Verbesserung der N-Nutzung durch Anpassung der N-Zufuhr an den Bedarf der Milchkühe. Der Mangel an Informationen über die Nährstoffzusammensetzung des Futters und die Stoffwechselprozesse bei der Verdauung erschwert dies jedoch. Aufgrund der unzureichenden Informationslage wurde in der Forschung für tropische Systeme vermehrt die für gemäßigten Regionen entwickelten Labormethoden und Modellierungsinstrumente angewendet. Das übergeordnete Ziel der vorliegenden Doktorarbeit bestand daher darin, die Genauigkeit und Präzision der vorhandenen Labormethoden und Modellierungsinstrumente für die Vorhersage der N-Zufuhr und -ausscheidung von Rindern in tropischen Haltungssystemen zu bewerten. Es wurde die Hypothese aufgestellt, dass die Anwendung von Labormethoden und Modellierungsinstrumenten aus gemäßigten Breiten ohne deren Validierung und Anpassung an tropische Systeme zu ungenauen Schätzungen der N-Zufuhr, -verwendung und -ausscheidung führen kann, was die Bewertung der N-Verwertungseffizienz erschwert.

In einer *In-vitro*-Studie wurde die Eignung der chemischen Methode (Sniffen et al., 1992) zur Vorhersage des im Pansen nicht abbaubaren Rohproteins (RUP) tropischer Futtermittel untersucht. Die Gleichungen von Kirchhof et al. (2010) und Valdés et al. (2011) wurden ausgewählt, um die Anteile an RUP bei gemäßigten Futtermitteln vorherzusagen. Diese Gleichungen basieren auf dem chemischen Rohproteingehalt (d.h. Konzentration und Anteil der Rohproteinfraktionen A, B_1, B_2, B_3 und C) sowie den Faserfraktionen (d.h. Konzentration von neutralen und sauren Detergenzienfasern). Diese Gleichungen wurden dann zur Berechnung der RUP-Anteile von Futtermitteln verwendet, die üblicherweise als Futter für Wiederkäuer in den (Sub-)Tropen verwendet werden (d.h. 23 Futtergräser und 15 Futterleguminosen). Die Genauigkeit und Präzision der Vorhersagen wurde anhand der *in situ* gemessenen RUP-Anteile bei Pansenpassageraten von 2, 5 und 8 % pro Stunde bewertet. Die Ergebnisse zeigten, dass der mit der *In-situ*-Methode geschätzte RUP-Anteil tropischer Futtermittel mit der chemischen Methode vorhergesagt werden kann. Die für gemäßigten Futtermittel entwickelten Regressionsgleichungen reichten jedoch nicht aus, um die RUP-Anteile tropischer Futtermittel für alle Pansenpassageraten korrekt vorherzusagen. Stattdessen können die in dieser Arbeit entwickelten Gleichungen zur Schätzung des RUP-Anteils tropischer Futtermittel verwendet werden, die eine ähnliche chemische Zusammensetzung aufweisen wie die Referenz-Futtermittelproben.

Eine zweite *In-vitro*-Studie wurde durchgeführt, um die Eignung der chemischen Analysemethode (Sniffen et al., 1992) und der *In-vitro*-Methode (Steingaß et al., 2001) zur Vorhersage des postruminalen Rohproteinanteils (PRCP) von tropischen Futtermitteln zu bewerten (d.h. 23 Futtergräser und 15 Futterleguminosen). Die von Zhao und Cao (2004) entwickelte Gleichung zur Vorhersage des PRCP-Angebots gemäßigter Futtermittel als Funktion der chemischen Rohproteinfraktionen (d. h. der Konzentration der Rohproteinfraktionen A, B_1, B_2, B_3 und C) wurde ausgewählt. Die Genauigkeit und Präzision der PRCP-Schätzungen mit chemischen und *In-vitro*-Methoden wurde mit der PRCP-Versorgung verglichen, die anhand von *In-situ*-Messungen (Pansenpassage von 2, 5 und 8 %pro Stunde) und der Konzentration an verdauter organischer Substanz geschätzt wurde (Gasproduktion des Hohenheimer Gastests bestimmt). Die Ergebnisse zeigten, dass die *In-vitro*-Methode als alternative Methode zur Schätzung der PRCP-Versorgung in tropischen Futtermitteln bei mäßiger bis schneller Pansenpassage, nicht aber bei langsamer Passagerate, verwendet werden kann. Verfügbare Regressionsgleichungen, entwickelt für Futtermittel aus gemäßigten Zonen, waren nicht ausreichend, um die PRCP-Versorgung tropischer Futtermittel aus den Konzentrationen der chemischen Rohproteinfraktionen vorherzusagen. Stattdessen ermöglichen die in der vorliegenden Studie entwickelten Gleichungen eine Schätzung des PRCP-Angebots tropischer Futtermittel aus Faser- und Rohproteinfraktionen mit ähnlicher chemischer Zusammensetzung wie die in der vorliegenden Arbeit einbezogenen Proben.

Nach der Verbesserung der Vorhersage der N-Versorgung des Tieres (in Form von RUP und PRCP) wurde eine dritte Studie durchgeführt, um die Eignung von Modellierungsinstrumenten zur Vorhersage der N-Ausscheidung von Rindern in tropischer Haltung zu bewerten. Diese Modelle, nämlich Modell A (auf der Grundlage der britischen Wiederkäuer-fütterungsempfehlungen), Modell G (auf der Grundlage der deutschen Wiederkäuerfütterungsempfehlungen) und Modell I (entwickelt auf der Grundlage der französischen Wiederkäuerfütterungsempfehlungen), wurden ausgewählt, um die Ausscheidung von N im Kot (KN), N im Urin (UN) und Gesamt-N (GN) sowie die KN-Fraktionen von Milchkühen, Färsen und Ochsen, die unter typischen tropischen Haltungsbedingungen gehalten werden, vorherzusagen. Die Genauigkeit und Präzision der Modellvorhersagen wurde anhand von Referenzwerten für UN (Gesamtsammlung, Kreatininmethode und N-Bilanz) und KN (Gesamtsammlung, interne und externe Marker) bewertet (n = 440 Beobachtungen). An den Modellen mit dem größten Potenzial zur Vorhersage der N-Ausscheidung wurden Anpassungen vorgenommen. Die Anpassungen konzentrierten sich auf die Inputvariablen, die die größten Abweichungen bei der Vorhersage der N-Ausscheidung verursachten. Um diese Inputvariablen zu identifizieren, wurde eine Sensitivitätsanalyse

durchgeführt. Keines der getesteten Modelle liefert adäquate Vorhersagen für die Ausscheidung von UN, KN und verschiedener KN-Fraktionen einzelner Rinder, die unter tropischen Bedingungen gehalten wurden. Stattdessen kann das Modell I in der vorliegenden Arbeit, angepasst durch erhöhte Effizienz der mikrobiellen Synthese von Rohprotein im Pansen und den reduzierten Intercept der KN-Vorhersage, zur Schätzung der KN- und GN-Ausscheidung einzelner Rinder in tropischer Haltung verwendet werden.

Die Ergebnisse der vorliegenden Arbeit stützen die Hypothese, dass die Anwendung von Labormethoden und Modellierungsinstrumenten aus gemäßigten Systemen zu unzureichenden Schätzungen der Proteinversorgung und N-Ausscheidung von Rindern in tropischen Haltungssystemen führt, ohne deren Validierung und Anpassung an tropische Systeme. Die Bewertung der N-Nutzungseffizienz und die Anpassung der Nährstoffversorgung an den tatsächlichen Bedarf des Tieres wird dadurch erschwert. Die Anpassung von Labormethoden an tropische Futtermittel, wie z. B. der chemischen Methode zur Schätzung des Proteinwertes von Futtermitteln aus gemäßigten Zonen, führt zu genaueren und präziseren Schätzungen des Anteils der RUP- und PRCP-Versorgung mit tropischen Futtermitteln. Das Modell I ist daher in der Lage, die N-Ausscheidung von Rindern in tropischen Haltungssystemen besser vorherzusagen, da es auf Unterschiede im RUP-Anteil und PRCP-Angebot reagiert. Zusätzlich zur Erhöhung die Genauigkeit und Präzision dieser Inputvariablen führen die Anpassungen der mikrobiellen Proteinsynthese und des Schnittpunkts der KN-Ausscheidung des Modells I zu einer angemesseneren Vorhersage der N-Ausscheidung von Rindern in tropischen Haltungssystemen. Jedoch führten nicht alle Anpassungen der Labormethoden und Modellierungswerkzeuge aus gemäßigten Systemen zu angemessenen Vorhersagen für den Proteingehalt von tropischem Futter und die Stickstoffausscheidung bei Rindern in tropischen Haltungssystemen. Herausforderungen bleiben bestehen, insbesondere bei der präzisen Vorhersage des Anteils an RUP und PRCP für tropisches Leguminosen mit langsamer Pansenpassage, sowie bei der Harnstickstoffausscheidung bei Rindern mit geringer Stickstoffaufnahme in tropischen Haltungssystemen. Daher ist weitere Forschung erforderlich, um die Faktoren zu identifizieren, die zu einer fehlerhaften Vorhersage beitragen.

Chapter 1

1. General introduction

Inefficient utilization of nitrogen (N) by dairy cows contributes to a large amount of N being released into the environment, resulting in pollution (Powell et al., 2013). This issue is particularly pronounced in tropical regions where most dairy cattle are located, resulting in higher pollution levels compared to temperate regions (Reid et al., 2004). In addition, cattle in tropical husbandry systems tend to exhibit lower yields and reduced efficiency in N utilization, leading to heightened N emissions for each unit of milk produced (Reid et al., 2004). Dairy cattle in tropical husbandry systems show room for improvement but face several challenges such as heat stress, limited feed availability, and low nutritional quality of feedstuffs (Hernández-Castellano et al., 2019). Furthermore, there is a lack of information on dietary composition of tropical dairy cattle, coupled with an insufficient integration of variations in digestion process efficiencies between tropical and temperate cattle into available feeding recommendations (Mottet et al., 2017; Pica-Ciamarra et al., 2014). As a result, researchers have adopted for tropical regions the laboratory methodologies and feeding recommendations developed for temperate regions (Bateki, 2020; Hernández-Castellano et al., 2019). The adoption of laboratory methodologies, modeling tools, and feeding recommendations from temperate systems without validating and adapting them for tropical systems may result in inaccurate estimations of N supply, N utilization, and N excretion (Figure 1.1), which hampers the assessment of N use efficiency (section 1.1).

Though measurements of N supply and N use can be feasibly obtained under tropical conditions, N excretion is challenging to obtain, due to the laborious and impractical nature of on-farm measurements of total urine N (UN) and fecal N (FN) excretions (Hristov et al., 2019). Instead, mathematical N partitioning's models can be used to predict N excretion. Several semi-mechanistic models have been developed to simulate the N partitioning in dairy cattle in tropical husbandry systems such as the Dijkstra et al. (1996)'s digestion model, the animal module of the ANORAC model (Thorne et al., 2001), the Ruminant model (Herrero et al., 2002), the LIVSIM model (Rufino et al., 2009), and FN and UN excretion's equations from INRA (2019). Yet, these models predict the N excretion of dairy cattle in tropical husbandry systems by relying on feeding recommendations designed for dairy cattle in temperate regions (AFRC, 1993; GfE, 2001; INRA, 2019; Sniffen et al., 1992). This approach may potentially result in inaccurate estimations of N excretion by dairy cattle in tropical husbandry systems. Therefore, there is a need to evaluate existing models and their assumptions for their ability to predict N excretion by dairy cattle in tropical husbandry systems and identify the most accurate (section 1.2).

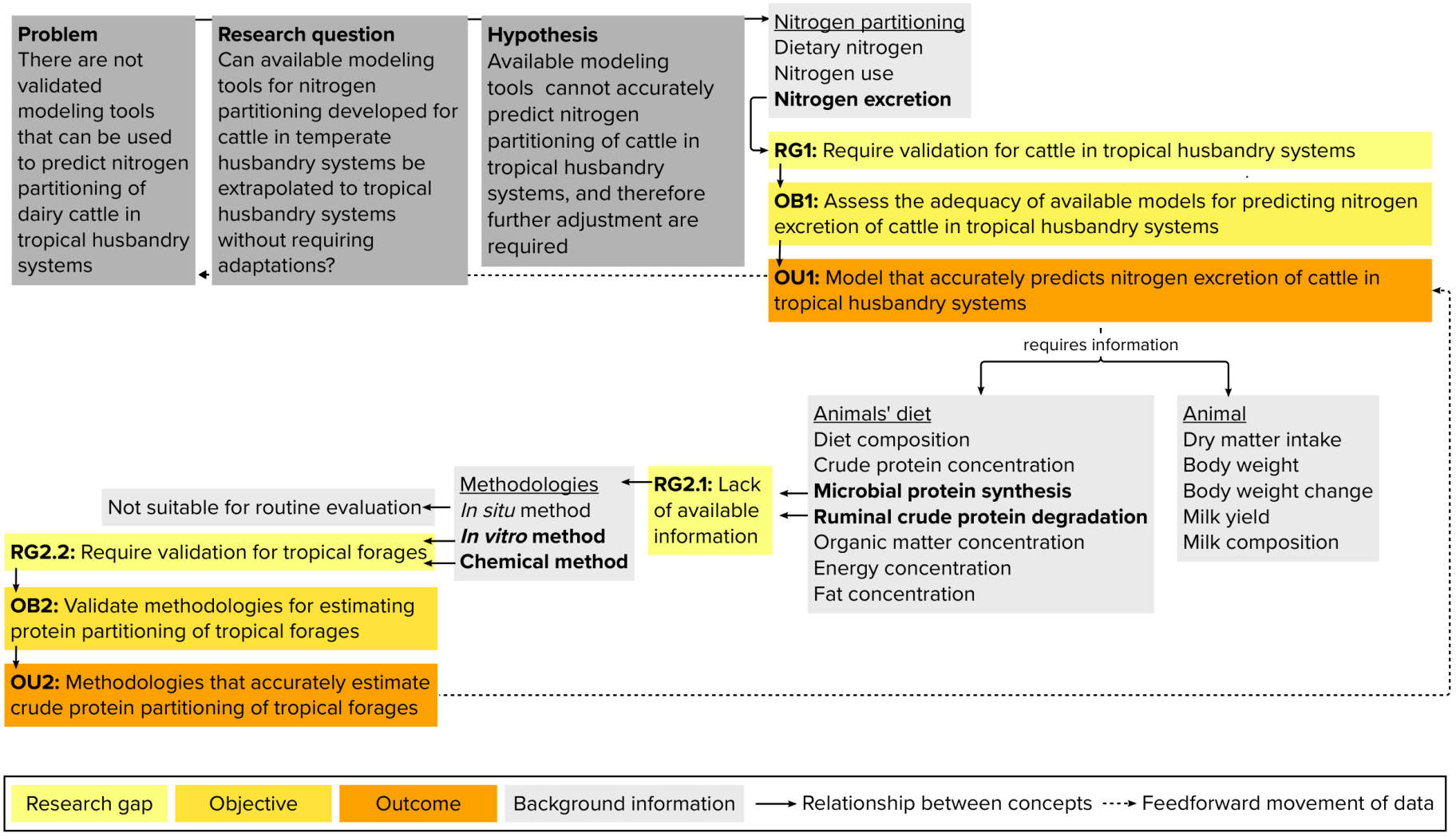


Figure 1.1

Flow chart of the research process

Predicting N excretion with the use of N partitioning's models requires information from the animal (e.g., dry matter intake, body weight, body weight change, milk yield, and milk composition) as well as information on the animal's diet (e.g., diet composition and concentration in the diet of protein, organic matter, energy, and fat). Most of these input variables are known for cattle in tropical husbandry systems, but information regarding the partitioning of the feed protein into rumen-degraded and rumen-undegraded (RUP) crude protein is still lacking, particularly for tropical forages. This partitioning is required by the dairy cattle feeding recommendations to estimate the protein supply at the duodenum (PRCP) and metabolizable protein supply to the animal (Figure 1.2).

The *in situ* method is the reference method used for estimating the proportion of feed rumen-degraded crude protein and RUP. However, it is laborious, time-consuming, and expensive (Madsen and Hvelplund, 1994), making it unsuitable for routine evaluation. There are alternative methods as well as related algorithms available for predicting the proportion of feed rumen-degraded crude protein and RUP and PRCP supply such as the *in vitro* (Steingaß et al., 2001) and chemical methods (Kirchhof et al., 2010; Shannak et al., 2000; Valdés et al., 2011). Nevertheless, these methods were developed primarily for temperate feedstuffs and therefore may not be suitable for tropical forages. Hence, these methods and their logarithms need to be validated and adapted to accurately predict the N supply to the animal (section 1.3).

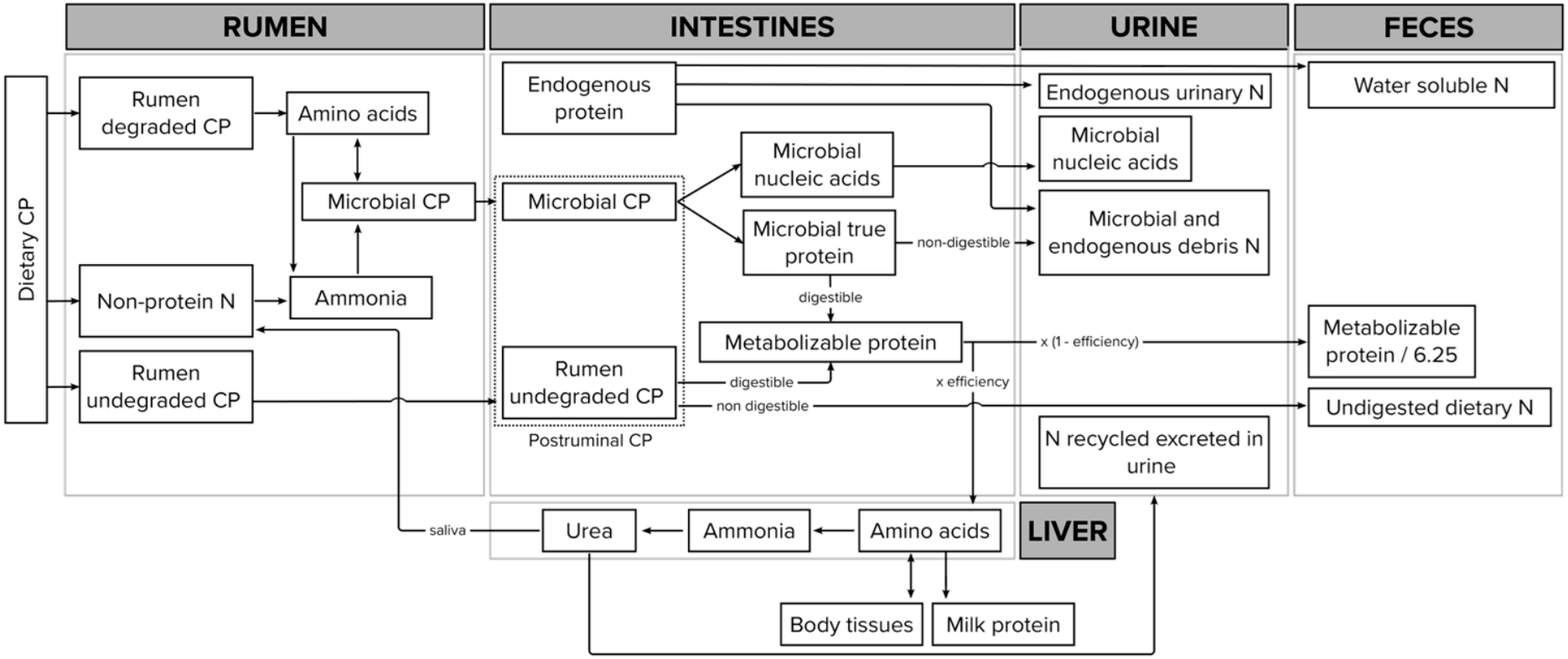


Figure 1.2

Nitrogen sources of urine and fecal nitrogen excretion (CP, crude protein; N, nitrogen)

1.1 Nitrogen use efficiency of dairy cattle in tropical husbandry systems

It is predicted that the demand for dairy products will continue to grow due to an increasing human population and dietary patterns that favor animal-based products consumption (OECD, 2023). Therefore, dairy cattle farming needs to increase to meet the growing global milk demand. To achieve this growth sustainably, the dairy cattle industry must prioritize enhancing nutrient use efficiency (Alexandratos and Bruinsma, 2012). Over the past few years, there has been an increased focus on improving the nutrient use efficiency of dairy cattle, particularly for N (Bergen, 2007; Schwab and Broderick, 2017). This is due to increased public concern about the environmental impact of N release to the environment by dairy cattle farming and pressure on dairy producers to reduce their production costs (Lapierre et al., 2005).

Dairy cattle are inefficient N users, with an average global N use efficiency in milk (NUE-milk; g of milk per 100 g of N intake) of 16 g/100g N intake, with the remaining 84 g/100g N intake being excreted primarily in the form of UN and FN (Powell et al., 2013). The effects of FN and UN excretion on air, soil, and water pollution vary considerably with the major contaminants being ammonia, nitrous oxide, and N oxide in the atmosphere and nitrate in the soil and groundwater (Diaz and Rosenberg, 2008; Tamminga, 1992).

Globally, the efficiency of N use in milk of dairy cattle varies widely, ranging from 1.8 to 32.5 g/100g N intake (Powell et al., 2013). Approximately 37% of dairy cattle worldwide have an NUE-milk lower than 10 and account for 10% of global milk production and 33% of the excreted manure N worldwide. About 92% of dairy cattle in Africa (average NUE-milk 5.3, from 1.8 to 23.6), 15% of dairy cattle in Asia (average NUE-milk 14, from 2.6 to 36.9), and 82% of dairy cattle in Central and South America (average NUE-milk 10.2, from 2.5 to 23.1) have a NUE-milk below 10 (Powell et al., 2013). Conversely, about 35% of dairy cattle worldwide have NUE-milk greater than 20 and account for 60% of global milk production and 40% of excreted manure N globally (Powell et al., 2013). Close to 70% of these high production cows are found in Europe, North America, and Oceania (average NUE- milk 23.8, ranging from 18.1 to 32.5). Hence, attributable to their diminished N utilization efficiency and the significant number of dairy cattle in tropical regions (comprising approximately one-third of the global dairy cattle population), cattle situated in tropical regions generate more N emissions per unit of milk produced than dairy cattle in temperate regions (Reid et al., 2004). Thus, there is huge interest in increasing N use efficiency and reducing N emissions per unit of milk produced from dairy cattle in tropical husbandry systems.

Productivity of dairy cattle in tropical husbandry systems has great room for improvement. This is due to lower producing cows (representing 57% of global lactating cows population) achieve a greater increase in NUE-milk relative to their original NUE-milk levels, in comparison to high-producing cows (Powell et al., 2013). However, the improvement of dairy cattle productivity in tropical systems faces several challenges. These challenges include heat stress, low availability and low nutritional quality of tropical feedstuffs (Hernández-Castellano et al., 2019). Moreover, the pursuit of improved productivity is hindered by the lack of information regarding the nutritional composition of diets offered as well as the metabolic processes associated with digestion (Mottet et al., 2017; Pica-Ciamarra et al., 2014).

Due to the scarcity of available information, researchers have extended the application of laboratory methodologies and feeding recommendations designed for temperate regions to tropical regions (Bateki, 2020; Hernández-Castellano et al., 2019) without prior evaluation and adaptation. Given the distinctions between tropical and temperate dairy systems (Oliveira, 2015; Wassie et al., 2019), along with variations in tropical and temperate forages (Minson and Wilson, 1980; Van Soest, 1994), this approach may lead to imprecise assessments of nutrient supply and requirements. For example:

(1) In temperate grasses, there is a strong inverse relationship between the fiber concentration and dry matter apparent digestibility (Butterworth and Diaz, 1970; Reid et al., 1988). In contrast, in tropical grasses this relationship is weak (Van Soest, 1994), attributed to differences in both the quantity and digestibility of fiber components between tropical and temperate grasses (Minson and Wilson, 1980; Van Soest, 1994). Thus, predicting the total tract apparent dry matter digestibility of tropical grasses based on fiber components will result in inaccurate predictions.

(2) In heifers, there is a lower efficiency of microbial protein synthesis in tropical husbandry systems compared to temperate husbandry systems (Wassie et al., 2019). This is due to the lower feed intake levels and poor nutritional quality of feedstuffs in tropical than in temperate cattle systems, which results in slower fermentation rates and a higher abundance of slow growth rate microbes (Russell et al., 1992).

(3) *Bos taurus* x *Bos indicus* crossbreed dairy cows have a lower metabolizable energy for maintenance and lower net energetic efficiency for milk production than *Bos taurus* dairy cows (Oliveira, 2015). This is due to *Bos taurus* x *Bos indicus* crossbreed dairy cows have a lower viscera size and activity, lower rate of body protein turnover, lower internal fat and/or lower body heat loss under tropical climate than *Bos taurus* dairy cows (Koong et al., 1985; Marcondes et al., 2013; Solis et al., 1988).

Consequently, inaccurate estimates of nutrient supply, nutrient use efficiency, and nutrient requirements hamper the adjustment of nutrient supply to the actual requirements of the animal. Therefore, the adoption of laboratory methodologies, modeling tools, and feeding recommendations from temperate systems without validating and adapting them for tropical systems may result in inaccurate estimations of N supply, N utilization, and N excretion, which will hamper the assessment of N use efficiency.

1.2 Modeling tools for predicting nitrogen partitioning of dairy cattle in tropical husbandry systems

To optimize N use efficiency and reduce environmental impact of dairy cattle in tropical husbandry systems, accurate estimates of N consumed, utilized, and excreted are necessary. Under tropical conditions, measuring N supply and utilization is feasible, while determining N excretion is challenging due to the impracticality of directly measuring total UN and FN excretion on-farm (Hristov et al., 2019). Instead, mathematical N partitioning's models can be used to predict N excretion. A mathematical model is a representation of a system that is based on mathematical language and concepts (Tedeschi, 2019). Its main objective is to translate real-life situations into mathematical formulations that allow for describing existing patterns or predicting future behaviors in real-life situations (Tedeschi, 2019). Mathematical models have been used in dairy cattle nutrition for a variety of purposes including predicting feed N degradation (Dhanoa et al., 1999), excreta N composition (Dijkstra et al., 2018), N use efficiency (Foskolos and Moorby, 2018), and impact of N excretion on the environment (Kebreab et al., 2002).

Currently, advanced models and logarithms exist that predict N excretion of dairy cattle based on empirical, mechanistic, or semi-mechanistic approaches. Excretion of N can be predicted using empirical equations (e.g., Garg et al., 2016; Johnson et al., 2016; Reed et al., 2015; Zahra et al., 2020), where N excretion is predicted by identifying relationships between measured N excretion and independent variables such as dietary N intake (Castillo et al., 2000; Huhtanen et al., 2008; Jonker et al., 1998; Reed et al., 2015), metabolizable energy of the diet (Kebreab et al., 2010; Reed et al., 2015), milk N concentration (Jonker et al., 1998), and feed fiber concentration (Reed et al., 2015). One of the limitations of empirical equations is their reliance on mathematical relationships without necessarily being grounded in established biological theories (France and Dijkstra, 2006). Consequently, their robustness is compromised, as they cannot predict N excretion adequately in conditions other than those used to fit the model (Johnson et al., 2016).

Mechanistic models, on the other hand, are based on processes and seek to understand causality, where N excretion is predicted based on the interaction of its individual components (France and Dijkstra, 2006). Mechanistic models have a greater level of robustness than empirical equations and therefore can be applied in a wide range of conditions (France and Dijkstra, 2006). This approach, however, requires a greater degree of parametrization and input parameters than empirical equations. Semi-mechanistic models offer an alternative to both previous approaches, because they have greater robustness than empirical equations, require fewer parameters, and are built with simpler equations than mechanistic models (Haddon, 2011).

Several semi-mechanistic models have been developed to predict the nutrient dynamics in dairy cattle in tropical husbandry systems (Thorne et al., 2001; INRA, 2019). These models, however, predict N excretion of dairy cattle in tropical husbandry systems based on feeding recommendations for dairy cattle in temperate regions (AFRC, 1993; GfE, 2001; INRA, 2019; Sniffen et al., 1992). For example: LIVSIM (Rufino et al., 2009) is a herd model for Sub-Saharan Africa, in which N requirements and excretion were drawn from the AFRC (1993) and later replaced by the GfE (2001). The Ruminant model (Herrero et al., 2002) predicts the potential intake, digestion, and animal performance of individual ruminants consuming tropical and temperate diets, in which N excretion is predicted based on the main flows of carbohydrates and N derived from the Cornell Net Carbohydrate and Protein system (CNCPS; Sniffen et al., 1992) and the AFRC (1993), respectively. The animal module of the ANORAC model (Thorne et al., 2001) describes the effects of different animal feeding and management strategies on N excretion, in which N excretion was drawn from the AFRC (1993). Based on the feeding recommendations of INRA (2019), the INRA predicts N excretion for temperate systems based on metabolizable protein supply, N expenditures, and metabolizable protein use efficiency, with some adaptations for ruminant livestock in warm climates.

The AFRC (1993), CNCPS (Sniffen et al., 1992), GfE (2001), and INRA (2019) are the ruminant feeding recommendation systems that have been used to predict the N excretion of dairy cattle in tropical husbandry systems. All feeding systems follow a similar approach to predict N excretion; however, they differ in their complexity, the level of characterization of the feedstuffs and the animal, and the magnitude of different parameters in the regulation of biological processes. Since there is a limited amount of information regarding the nutritional composition of the feedstuffs offered to dairy cattle in tropical husbandry systems, the feeding recommendation system selected to predict N excretion should be based on input variables that are readily available in tropical dairy cattle farming systems. Under this condition, the CNCPS might not be suitable because it requires a higher level of characterization

from feedstuffs, such as concentration of soluble carbohydrates and rumen-undegradable neutral detergent fiber. In the context of tropical dairy cattle farming systems, such detailed feed information is often lacking (Castro-Montoya and Dickhoefer, 2020).

Considering the differences between tropical and temperate husbandry systems, using feeding recommendations for dairy cattle in temperate regions might lead to inaccurate estimates of N excretion in tropical husbandry systems. Therefore, there is a need to evaluate existing models and its assumptions for their ability to predict N excretion by dairy cattle in tropical husbandry systems and identify the most accurate.

1.3 Feed protein partitioning into rumen-degraded and rumen-undegraded crude protein

The approach utilized by the feeding recommendations of the AFRC (1993), GfE (2001), and INRA (2019), predicts UN excretion by summing up the supply of endogenous urinary N, N recycled and excreted as urine, microbial nucleic acids excreted in urine, and inefficiencies in metabolizable protein use (Figure 1.2). The FN excretion is predicted by summing up fecal bacterial and endogenous debris N excretion, fecal undigested dietary N excretion, and fecal water-soluble N excretion (Figure 1.2). The prediction of UN and FN excretion based on the approaches of the AFRC (1993), GfE (2001), and INRA (2019) requires information from the animal as well as information on the animal's diet (Table 1.1). Most of these input variables are known for tropical dairy cattle, but information regarding the proportion of RUP is still lacking, particularly for tropical forages. The proportion of RUP is essential for protein evaluation because it is required for calculating PRCP supply (GfE, 2001) as well as metabolizable protein available for the animal (AFRC, 1993; INRA, 2019) (Figure 1.2).

Currently, advanced lab techniques exist to estimate feed RUP proportion, and PRCP supply including the nylon bag technique (*in situ* method; Madsen and Hvelplund, 1994), the modified Hohenheim gas test (*in vitro* method; Steingaß et al., 2001), and the CNCPS chemical fractionation (Sniffen et al., 1992). The *in-situ* method measures the rate and extent of nutrient degradation in the rumen using a standardized procedure (Madsen and Hvelplund, 1994). The procedure involves incubating feed samples for up to 72 hours in the rumen of fistulated animals. Then, the nutrient composition of the residues after incubation is analyzed to determine the rate and extent of nutrient degradation (GfE, 2022; NRC, 2001). The *in situ* method is widely used for measuring crude protein degradation in the rumen in several systems (e.g., AFRC, 1993; INRA, 2019; NorFor, 2011; NRC, 2001),

and is the reference method for comparison with new methods that aim at estimating crude protein degradation in the rumen. However, the *in situ* method is time-consuming, labor-intensive, and requires rumen-fistulated animals (Edmunds et al., 2012; Hvelplund and Weisbjerg, 1998; Stern et al., 1997), making it unsuitable for routine evaluations of temperate and tropical forages.

Table 1.1
Input variables required by models A, G, and I.

Variables	Model A	Model G	Model I
Variables related to the animals			
Body weight	■	■	■
Body weight change		■	■
Dry matter intake	■	■	■
Milk yield		■	■
Milk protein		■	■
Milk fat			■
Milk lactose			■
Variables related to the animals' diet			
Proportion of concentrate in the diet			■
Organic matter			■
Crude fat	■		
Rumen-degraded crude protein	■	■	■
Rumen-undegraded crude protein	■	■	■
Acid-detergent insoluble nitrogen	■		
Metabolizable energy	■		■

The *in vitro* method provides an estimate of the sum of the microbial crude protein and RUP based on changes in the ammonia-N concentration in buffered rumen fluid during *in vitro* incubation (Steingaß et al., 2001). This method has the advantage of being less time-consuming and costly, and it allows for processing a larger number of samples than the *in situ method*. Furthermore, the *in vitro* method allows for the estimation of effective PRCP as well as the analysis of individual feedstuffs and mixed rations (Edmunds et al., 2012). However, it presents certain limitations. These include the need for rumen-fistulated animals and the consideration of rumen passage rate only in terms of solid passage rates. Furthermore, the *in vitro* method functions within a closed system, disregarding the ongoing inflow of nutrients and saliva (Edmunds et al., 2012). This constraint restricts the incorporation of new carbohydrates and recycled nitrogen, processes that occur under normal *in vivo* and *in situ* conditions. The *in vitro* method has been used to predict the PRCP supply of a variety of feed types, including concentrate compounds (Leberl et al., 2007; Rupp et al., 2021;

Westreicher-Kristen et al., 2015), concentrate mixtures (Zhao and Lebzien, 2000; Rupp et al., 2021), and temperate forage grasses and legumes (Edmunds et al., 2012; Rupp et al., 2021). The accuracy and precision of the *in vitro* method vary notably across studies. The root means square error ranges from 8 to 99 g/kg dry matter or 5 to 44% of the observed mean and the determination coefficient varies between 0.19 and 0.87 (Edmunds et al., 2012; Leberl et al., 2007; Rupp et al., 2021; Westreicher-Kristen et al., 2015; Zhao and Lebzien, 2000; Table 1.2). These differences are attributed to factors like feed type. For instance, the coefficient of determination (R^2) for concentrates and mixtures ranges from 0.75 to 0.87, contrasting with forages at 0.71 (Zhao and Lebzien, 2000). Variations persist even within the same feed type across studies, as shown by Leberl et al. (2007; determination coefficient from 0.21 to 0.31) and Westreicher-Kristen et al. (2015; determination coefficient from 0.19 to 0.70) and between rumen passages rates (Edmunds et al., 2012; R^2 of 0.19, 0.56, and 0.67 at rumen passage rates of 2, 4, and 6%/hour) (Westreicher-Kristen et al., 2015; R^2 of 0.19, 0.54, and 0.70 at rumen passage rates of 2, 5, and 8%/hour). Given the substantial variability across feed types and rumen passages rates, evaluating the *in vitro* method's accuracy for estimating PRCP supply in tropical forage grasses and legumes becomes imperative.

In the original chemical method, the feed RUP proportion was estimated based on concentrations of crude protein fractions (Licitra et al., 1996) and the rate of degradation of these fractions in the rumen (Sniffen et al., 1992). Because of the low reproducibility of degradation rates of individual crude protein fractions, the approach of the chemical method was modified. The modified version estimates the feed RUP proportion and PRCP supply based on the concentrations of crude protein, concentrations and proportions of crude protein fractions A, B_1, B_2, B_3, and C, neutral detergent fiber, and neutral detergent insoluble protein (Table 1.3).

The modified chemical method has the advantage that crude protein fractions can be easily analyzed in most laboratories (Zhao and Cao, 2004), there is no need for rumen-fistulated animals (Zhao and Cao, 2004), and a greater number of samples can be processed than the *in situ* method. Although there are some disadvantages, for example, some crude protein fractions have low reproducibility (Bovera et al., 2003) and the logarithms developed are only applicable to samples having similar chemical compositions to those used in the development of the equations. Several equations are available to estimate the RUP proportion of temperate concentrates (Shannak et al., 2000; R^2 from 0.87 to 0.94), byproducts (Westreicher-Kristen et al., 2012; R^2 from 0.51 to 0.94) tropical concentrates (Mondal et al., 2008; R^2 from 0.90 to 0.93), and forages from temperate pastures (Kirchhof et al., 2010, R^2 from 0.51 to 0.56; Valdés et al., 2011, R^2 from 0.59 to 0.69), as well as the PRCP supply of energy and protein

concentrates, corn and soybean by-products, and temperate forages (Zhao and Cao, 2004; R^2 from 0.95 to 0.97) and byproducts (Westreicher-Kristen et al., 2015; R^2 from 0.75 to 0.95) (Table 1.3). These equations have been successfully applied to specific sample sets based on the assumption that the proportions of different crude protein fractions are strongly correlated with the proportions of soluble crude protein and potentially degraded crude protein determined *in situ* (Chrenková et al., 2014; Lanzas et al., 2008).

In summary, the *in vitro* and chemical methods can be used as alternatives to the *in situ* method for routine evaluation of feed RUP proportion, and PRCP supply. However, these methods were developed primarily for temperate feedstuffs and therefore may not be suitable for tropical forages. Hence, these methods and their logarithms need to be validated and adapted to accurately predict feed RUP and PRCP supply of tropical forages.

Table 1.2
Summary of studies reporting the comparison between the *in vitro* method (alternative method) against reference values for estimating the post-ruminal crude protein supply (PRCP) of several feedstuffs.

Reference method (M1)	Alternative method (M2)	Type of sample	Mean ± SD[1] (g/kg DM[2])	RMSE (g/kg DM)[3]	R^2 (0 to 1)[4]	Author
PRCP supply (GfE, 2001) from estimates of digested organic matter and RUP[5] estimated *in vivo* Kp[6] Not specified	First stage of the *in vitro* digestion technique (Tilley and Terry, 1963) Incubation times M2.1 = 24h M2.2 = 30h Kp Not specified	Concentrates (n = 14), concentrate mixtures (n = 7), dry grass (n = 1), grass silage (n = 2), and straw (n = 1)	Concentrates M1 = 184.7 ± 56 M2.1 = 187.5 ± 58 M2.2 = 188.9 ± 54 Mixtures M1 = 177.9 ± 26 M2.1 = 202.1 ± 38 M2.2 = 193.3 ± 53 Forages M1 = 121.5 ± 31 M2.1 = 132.8 ± 46 M2.2 = 126.8 ± 26 All feedstuffs M1 = 172.7 ± 50 M2.1 = 182.8 ± 55 M2.2 = 180.2 ± 54	Concentrates M1 vs M2.1 = 20.1 M1 vs M2.2 = 26.8 Mixtures M1 vs M2.1 = 30.7 M1 vs M2.2 = 45.8 Forages M1 vs M2.1 = 26.6 M1 vs M2.2 = 15.2 All feedstuffs M1 vs M2.1 = 24.6 M1 vs M2.2 = 32.1	Concentrates M1 vs M2.1 = 0.87 M1 vs M2.2 = 0.77 Mixtures M1 vs M2.1 = 0.75 M1 vs M2.2 = 0.22 Forages M1 vs M2.1 = 0.65 M1 vs M2.2 = 0.71 All feedstuffs M1 vs M2.1 = 0.84 M1 vs M2.2 = 0.74	Zhao and Lebzien, 2000

*Statistical parameters were calculated without including corn gluten sample because PRCP supply predicted with method 1 gave illogical results (Kp 5%hour = -842 g/kg DM, Kp 8%/hour = -640 g/kg DM).
[1] SD, standard deviation.
[2] DM, dry matter.
[3] RMSE, root mean square error.
[4] R^2, coefficient of determination.
[5] RUP, rumen-undegraded crude protein.
[6] Kp, rumen passage rate.
[7] PRCP supply expressed in g/kg organic matter.

(Table 1.2 Continued)

Reference method (M1)	Alternative method (M2)	Type of sample	Mean ± SD[1] (g/kg DM[2])	RMSE (g/kg DM)[3]	R^2 (0 to 1)[4]	Author
PRCP supply (GfE, 2001) from estimates of digested organic matter and RUP estimated based on crude protein fractions and Shannak et al. (2000) equation Kp 5 and 8%/hour	Modified Hohenheim gas test (Steingaß et al., 2001) Incubation times M2.1 = 8/24h M2.2 = 8/48h Kp 5 and 8%/hour	Cereals (n = 9), energy rich by-products (n = 4), legumes (n = 2), oilseeds (n = 3), extraction meal (n = 6), oilcake and expeller (n = 3), and protein-rich by-products (n = 5)	All feedstuffs Kp 5%/hour M1 = 217.8 ± 105 M2.1 = 211.7 ± 64 M2.2 = 190.5 ± 52 All feedstuffs Kp 8%/hour M1 = 241.4 ± 119 M2.1 = 235.8 ± 76 M2.2 = 225.3 ± 72	All feedstuffs Kp 5%/hour M1 vs M2.1 = 92.3 M1 vs M2.2 = 95.5 All feedstuffs Kp 8%/hour M1 vs M2.1 = 97.8 M1 vs M2.2 = 98.6	All feedstuffs Kp 5%/hour M1 vs M2.1 = 0.22 M1 vs M2.2 = 0.21 All feedstuffs Kp 8%/hour M1 vs M2.1 = 0.31 M1 vs M2.2 = 0.31	Leberl et al., 2007*
PRCP supply (GfE, 2001) from estimates of metabolizable energy and RUP estimated *in situ* Kp 2, 4, and 6%/hour	Modified Hohenheim gas test (Steingaß et al., 2001) Incubation times M2 = 8 and 48h Kp 2, 4, and 6%/hour	Fresh grasses and legumes (n = 12), hay grass (n = 1), grass silage (n = 6), and artificial dried grasses and legumes (n = 4)	Kp 2%/hour M1 = 139.1 ± 9 M2 = 165.7 ± 17 Kp 4%/hour M1 = 145.5 ± 11 M2 = 143.2 ± 15 Kp 6%/hour M1 = 151.0 ± 13 M2 = 133.3 ± 15	Kp 2%/hour M1 vs M2 = 8.4 Kp 4%/hour M1 vs M2 = 7.6 Kp 6%/hour M1 vs M2 = 7.6	Kp 2%/hour M1 vs M2 = 0.19 Kp 4%/hour M1 vs M2 = 0.56 Kp 6%/hour M1 vs M2 = 0.67	Edmunds et al., 2012

*Statistical parameters were calculated without including corn gluten sample because PRCP supply predicted with method 1 gave illogical results (Kp 5%hour = -842 g/kg DM, Kp 8%/hour = -640 g/kg DM).

[1] SD, standard deviation. [2] DM, dry matter. [3] RMSE, root mean square error. [4] R^2, coefficient of determination. [5] RUP, rumen-undegraded crude protein. [6] Kp, rumen passage rate. [7] PRCP supply expressed in g/kg organic matter.

(Table 1.2 Continued)

Reference method (M1)	Alternative method (M2)	Type of sample	Mean ± SD[1] (g/kg DM[2])	RMSE (g/kg DM)[3]	R^2 (0 to 1)[4]	Author
PRCP supply (GfE, 2001) from estimates of metabolizable energy and RUP estimated *in situ* Kp 2, 5 and 8%/hour	Modified Hohenheim gas test (Steingaß et al., 2001) Incubation times M2 = 8, 24h, and 48h Kp 2, 5 and 8%/hour	Dried distillers' grain obtained from the processing of wheat (n = 5), maize (n = 3), barley (n = 1), and blends (n = 4)	Kp 2%/hour M1 = 195.0 ± 24 M2 = 187.0 ± 36 Kp 5%/hour M1 = 216.0 ± 36 M2 = 252.0 ± 28 Kp 8%/hour M1 = 229.0 ± 42 M2 = 285.0 ± 28	Kp 2%/hour M1 vs M2 = 22.2 Kp 5%/hour M1 vs M2 = 25.5 Kp 8%/hour M1 vs M2 = 24.4	Kp 2%/hour M1 vs M2 = 0.19 Kp 5%/hour M1 vs M2 = 0.54 Kp 8%/hour M1 vs M2 = 0.70	Westreicher-Kristen et al., 2015

*Statistical parameters were calculated without including corn gluten sample because PRCP supply predicted with method 1 gave illogical results (Kp 5%hour = -842 g/kg DM, Kp 8%/hour = -640 g/kg DM).

[1] SD, standard deviation.

[2] DM, dry matter.

[3] RMSE, root mean square error.

[4] R^2, coefficient of determination.

[5] RUP, rumen-undegraded crude protein.

[6] Kp, rumen passage rate.

[7] PRCP supply expressed in g/kg organic matter.

(Table 1.2 Continued)

Reference method (M1)	Alternative method (M2)	Type of sample	Mean ± SD[1] (g/kg DM[2])	RMSE (g/kg DM)[3]	R^2 (0 to 1)[4]	Author
PRCP supply[7] comprised *in situ* estimated RUP and microbial crude synthesis at a rate of 181 g/kg of fermented organic matter (Lebzien and Voigt, 1999) <u>Kp</u> 5 and 8%/hour	Modified Hohenheim gas test (Steingass and Südekum, 2013) <u>Incubation times</u> M2 = 8, and 24h <u>Kp</u> 5 and 8%/hour	Grass/maize silage (n = 2), grass hay (n = 1), wheat straw (n = 1), maize grain (n = 1), dried distillers' grains (n = 1), solvent-extracted oilseed meals (n = 3), feed mixtures (n = 13), and commercial feed mixtures (n = 12)	Individual feeds Kp 5%/hour M1 = 173.7 ± 76 M2 = 181.7 ± 55 Kp 8%/hour M1 = 182.0 ± 94 M2 = 198.1 ± 76 Feed mixtures Kp 5%/hour M1 = 147.9 ± 10 M2 = 184.3 ± 9 Kp 8%/hour M1 = 147.4 ±12 M2 = 192.3 ±12 All samples Kp 5%/hour M1 = 154.7 ± 40 M2 = 183.6 ± 28 Kp 8%/hour M1 = 156.6 ± 50 M2 = 193.8 ± 39	Individual feeds Kp 5%/hour M1 vs M2 = 36.8 Kp 8%/hour M1 vs M2 = 42.0 Feed mixtures Kp 5%/hour M1 vs M2 = 37.0 Kp 8%/hour M1 vs M2 = 45.4 All samples Kp 5%/hour M1 vs M2 = 37.0 Kp 8%/hour M1 vs M2 = 44.5	Individual feeds Kp 5%/hour M1 vs M2 = 0.78 Kp 8%/hour M1 vs M2 = 0.71 Feed mixtures Kp 5%/hour M1 vs M2 = 0.57 Kp 8%/hour M1 vs M2 = 0.70 All samples Kp 5%/hour M1 vs M2 = 0.68 Kp 8%/hour M1 vs M2 = 0.76	Rupp et al., 2021

*Statistical parameters were calculated without including corn gluten sample because PRCP supply predicted with method 1 gave illogical results (Kp 5%hour = -842 g/kg DM, Kp 8%/hour = -640 g/kg DM).

[1] SD, standard deviation. [2] DM, dry matter. [3] RMSE, root mean square error. [4] R^2, coefficient of determination. [5] RUP, rumen-undegraded crude protein. [6] Kp, rumen passage rate. [7] PRCP supply expressed in g/kg organic matter.

Table 1.3
Available equations developed to predict reference values of rumen-undegraded crude protein (RUP; g/kg dry matter) and post-ruminal crude protein (PRCP; g/kg dry matter) supply from crude protein fractions, proximal analysis, fiber fractions, and *in vitro* fermentation parameters.

Reference values	Type of sample	Mean ± Standard deviation	Equation[1]	RMSE (g/kg DM)[2]	R^2 (0 to 1)[3]	Author
Rumen-undegraded crude protein (RUP)						
RUP estimated *in situ*	Grass silage (n = 3), maize gluten feed (n = 2), palm kernel meal (n = 2), rapeseed products (n = 4), soybean products (n = 7), sunflower seed meal (n = 1), and commercial dairy compound feeds (n = 11)	RUP Kp 2%/h = 56.8 ± 43	RUP Kp 2%/h = -243.58 + -299.84 x (CP / NDFp) + 0.0028 x (CP x B_2) + -0.0315 x (CP x C) + 0.0039 x CP x (A + B_1) + 0.0002 x CP x C^2 + -0.0017 x NDFp x B_1 + 0.0036 x B_2 x (B_3 +C)	11.9	0.87	Shannak et al., 2000*
		RUP Kp 5%/h = 87.7 ± 67	RUP Kp 5%/h = -189.68 + -304.72 x (CP / NDFp) + 0.0030 x (CP x B_2) + -0.0263 x (CP x C) + 0.0038 x CP x (A + B_1) + 0.0002 x CP x C^2 + -0.0022 x NDFp x B_1 + 0.0038 x B_2 x (B_3 +C)	11.6	0.93	
		RUP Kp 8%/h = 107.6 ± 74	RUP Kp 8%/h = -98.66 + -275.13 x (CP / NDFp) + 0.0028 x (CP x B_2) + -0.0220 x (CP x C) + 0.0032 x CP x (A + B_1) + 0.0002 x CP x C^2 + -0.0020 x NDFp x B_1 + 0.0035 x B_2 x (B_3 +C)	12.0	0.94	

*Crude protein fractions A, B_1, B_2, B_3, C are in g/kg crude protein; **Crude protein fractions A, B_1, B_2, B_3, C are in g/kg dry matter; ***Crude protein fractions A, B_1, B_2, B_3, C are in % of dry matter; ****Crude protein fractions A, B_1, B_2, B_3, C are in % crude protein.
[1] A, crude protein soluble in the borate-phosphate buffer and tungstic acid solution; B_1, true protein soluble in buffer solution and precipitated by the tungstic solution; B_2, true protein insoluble in buffer solution but soluble in neutral-detergent solution; B_3, true protein soluble in acid detergent solution but insoluble in neutral-detergent solution; C, true protein insoluble in the acid-detergent solution; CP, crude protein (g/kg dry matter); Kp, rumen passage rate (%/h); NDFp, neutral detergent fiber assayed using heat-stable amylase and without the use of sodium sulfite using the crude protein fractionation method and expressed inclusive ash (g/kg dry matter); NDIP, neutral detergent insoluble protein (g/kg dry matter).
[2] RMSE, root mean square error. [3] R^2, coefficient of determination.

(Table 1.3 Continued)

Reference values	Type of sample	Mean ± Standard deviation	Equation[1]	RMSE (g/kg DM)[2]	R^2 (0 to 1)[3]	Author
RUP estimated *in situ*	Maize products (n = 4), wheat products (n = 2), barley (n = 1), soybean meal (n = 1), sunflower meal (n = 1), deoiled coconut cake (n = 1), cottonseed cake (n = 1), groundnut cake (n = 1), mustard cake (n = 1), fish meal (n = 1), guar chuni (n = 1), and *Leucaena leucocephala* (n = 1)	RUP Kp 2%/h = 108.3 ± 111	RUP Kp 2%/h = [100 – [19.07 + 2.10 x $(A + B_1)$ + -0.016 x $(A + B_1)^2$ + -0.004 x B_3^2]] x CP / 100	13.4	0.90	Mondal et al., 2008 ****
		RUP Kp 4%/h = 141.9 ± 129	RUP Kp 4%/h = [100 – [10.41 + 1.43 x $(A + B_1)$ + -0.009 x $(A + B_1)^2$ + 1.18 x B_3 + -0.024 x B_3^2 + -0.104 x C^2]] x CP / 100	9.0	0.93	
		RUP Kp 6%/h = 161.9 ± 138	RUP Kp 6%/h = [100 – [5.93 + 1.21 x $(A + B_1)$ + -0.006 x $(A + B_1)^2$ + 1.348 x B_3 + -0.027 x B_3^2 + -0.115 x C^2]] x CP / 100	8.8	0.93	
RUP estimated *in situ*	Grassland forages (n = 47), hay (n = 7), and maize silage (n = 7)	RUP Kp 2%/h = 27.7 ± 6	RUP Kp 2%/hour = [204.32 + (1.08 x C) + (0.0014 x (CP x $(A + B_1)$))] x CP / 1000	27.6	0.51	Kirchhof et al., 2010*
		RUP Kp 5%/h = 44.3 ± 12	RUP Kp 5%/hour = [321.90 + (0.17 x NDFp) + (0.0022 x (CP x $(A + B_1)$)) + (0.0001 x (CP x C^2))] x CP / 1000	37.8	0.52	
		RUP Kp 8%/h = 55.6 ± 17	RUP Kp 8%/hour = [285.55 + (1.21 x C) + (0.0005 x (NDFp x B_2)) + (110·17 x (($A + B_1$) / NDFp))] x CP / 1000	47.5	0.56	

*Crude protein fractions A, B_1, B_2, B_3, C are in g/kg crude protein; **Crude protein fractions A, B_1, B_2, B_3, C are in g/kg dry matter; ***Crude protein fractions A, B_1, B_2, B_3, C are in % of dry matter; ****Crude protein fractions A, B_1, B_2, B_3, C are in % crude protein.

[1] A, crude protein soluble in the borate-phosphate buffer and tungstic acid solution; B_1, true protein soluble in buffer solution and precipitated by the tungstic solution; B_2, true protein insoluble in buffer solution but soluble in neutral-detergent solution; B_3, true protein soluble in acid detergent solution but insoluble in neutral-detergent solution; C, true protein insoluble in the acid-detergent solution; CP, crude protein (g/kg dry matter); Kp, rumen passage rate (%/h); NDFp, neutral detergent fiber assayed using heat-stable amylase and without the use of sodium sulfite using the crude protein fractionation method and expressed inclusive ash (g/kg dry matter); NDIP, neutral detergent insoluble protein (g/kg dry matter).

[2] RMSE, root mean square error. [3] R^2, coefficient of determination.

(Table 1.3 Continued)

Reference values	Type of sample	Mean ± Standard deviation	Equation[1]	RMSE (g/kg DM)[2]	R^2 (0 to 1)[3]	Author
RUP estimated *in situ*	Grassland herbs (n = 29)	RUP Kp 2%/h = 29.2 ± 4	RUP Kp 2%/hour = 13.57 + 0.16 x B_2 + 0.19 x B_3 + 0.17 x C	-	0.56	Valdés et al., 2011**
		RUP Kp 4%/h = 40.1 ± 6	RUP Kp 4%/hour = 15.14 + 0.21 x B_2 + 0.30 x B_3 + 0.32 x C	-	0.65	
		RUP Kp 6%/h = 48.0 ± 7	RUP Kp 6%hour = 16.16 + 0.24 x B_2 + 0.39 x B_3 + 0.42 x C	-	0.67	
		RUP Kp 8%/h = 54.0 ± 8	RUP Kp 8%/hour = 16.55 + 0.26 x B_2 + 0.46 x B_3 + 0.51 x C	-	0.69	
RUP estimated *in situ*	Dried distillers' grain obtained from the processing of wheat (n = 5), maize (n = 3), barley (n = 1), and blends (n = 4)	RUP Kp 8%/h = 122.4 ± 55	RUP Kp 8%/hour = 221.7 + 6.82 x EE – 17.67 x CF	42.4	0.51	Westreicher-Kristen et al., 2012*
			RUP Kp 8%/hour = 235.6 – 0.62 x A – 0.20 x B_2 + 0.28 x B_3	19.5	0.91	
			RUP Kp 8%/hour = -383.6 + 0.45 x B_2 + 0.89 x B_3 + 0.65 x C	16.3	0.93	
			RUP Kp 8%/hour = 48.01 – 0.0003 x (B_3 + C) x A + (120.31 x NDIP / (A + B_1))	14.6	0.94	

*Crude protein fractions A, B_1, B_2, B_3, C are in g/kg crude protein; **Crude protein fractions A, B_1, B_2, B_3, C are in g/kg dry matter; ***Crude protein fractions A, B_1, B_2, B_3, C are in % of dry matter; ****Crude protein fractions A, B_1, B_2, B_3, C are in % crude protein.

[1] A, crude protein soluble in the borate-phosphate buffer and tungstic acid solution; B_1, true protein soluble in buffer solution and precipitated by the tungstic solution; B_2, true protein insoluble in buffer solution but soluble in neutral-detergent solution; B_3, true protein soluble in acid detergent solution but insoluble in neutral-detergent solution; C, true protein insoluble in the acid-detergent solution; CP, crude protein (g/kg dry matter); Kp, rumen passage rate (%/h); NDFp, neutral detergent fiber assayed using heat-stable amylase and without the use of sodium sulfite using the crude protein fractionation method and expressed inclusive ash (g/kg dry matter); NDIP, neutral detergent insoluble protein (g/kg dry matter).

[2] RMSE, root mean square error. [3] R^2, coefficient of determination.

(Table 1.3 Continued)

Reference values	Type of sample	Mean ± Standard deviation	Equation[1]	RMSE (g/kg DM)[2]	R^2 (0 to 1)[3]	Author
Post-ruminal crude protein supply (PRCP; g/kg DM)						
First stage of the *in vitro* digestion technique (Tilley and Terry, 1963)	Energy and protein concentrates (n = 16)	PRCP 24h = 239.0 ± 113	PRCP 24h incubation = 11.67 x A + 4.09 x B_1 + 6.31 x B_2 + 5.17 x B_3 + 18.66 x C + 60.59	18.9	0.97	Zhao and Cao, 2004***
	Temperate forages, corn, and soybean by-products (n = 14)	PRCP 24h = 216.0 ± 120	PRCP 24h incubation = 8.78 x A + 15.69 x B_1 + 12.36 x B_2 + 11.83 x B_3 + 6.99 x PC + 52.39	26.9	0.95	
	All feedstuffs (n = 30)	PRCP 24h = 228.0 ± 115	PRCP 24h incubation = 9.95 x A + 2.92 x B_1 + 7.24 x B_2 + 8.20 x B_3 + 17.67 x PC + 63.26	35.5	0.90	

*Crude protein fractions A, B_1, B_2, B_3, C are in g/kg crude protein; **Crude protein fractions A, B_1, B_2, B_3, C are in g/kg dry matter; ***Crude protein fractions A, B_1, B_2, B_3, C are in % of dry matter; ****Crude protein fractions A, B_1, B_2, B_3, C are in % crude protein.

[1] A, crude protein soluble in the borate-phosphate buffer and tungstic acid solution; B_1, true protein soluble in buffer solution and precipitated by the tungstic solution; B_2, true protein insoluble in buffer solution but soluble in neutral-detergent solution; B_3, true protein soluble in acid detergent solution but insoluble in neutral-detergent solution; C, true protein insoluble in the acid-detergent solution; CP, crude protein (g/kg dry matter); Kp, rumen passage rate (%/h); NDFp, neutral detergent fiber assayed using heat-stable amylase and without the use of sodium sulfite using the crude protein fractionation method and expressed inclusive ash (g/kg dry matter); NDIP, neutral detergent insoluble protein (g/kg dry matter).

[2] RMSE, root mean square error. [3] R^2, coefficient of determination.

(Table 1.3 Continued)

Reference values	Type of sample	Mean ± Standard deviation	Equation[1]	RMSE (g/kg DM)[2]	R^2 (0 to 1)[3]	Author
PRCP supply (GfE, 2001) from estimates of metabolizable energy and RUP estimated *in situ*	Dried distillers' grain obtained from the processing of wheat (n = 5), maize (n = 3), barley (n = 1), and blends (n = 4)	PRCP Kp 2%/h = 195.0 ± 24	PRCP Kp 2%/h = 298 – 0.31 x A – 0.13 x B_2	12.8	0.75	Westreicher-Kristen et al., 2015*
			PRCP Kp 2%/h = 231 – 23 x [(A + B_1) / 6.25) x NDIP / 6.25]	5.19	0.83	
			PRCP Kp 2%/h = 124 + 33 x [NDIP / (A+B_1)] + 0.0009 x NDIP x B_2	9.23	0.87	
			PRCP Kp 2%/h = 90 + 0.002 x [NDIP x B_2] + 0.0003 x CP x C	8.17	0.90	
		PRCP Kp 5%/h = 216.0 ± 36	PRCP Kp 5%/h = 367 – 0.48 x A – 0.18 x B_2	19.5	0.76	
			PRCP Kp 5%/h = 118 – 0.25 x (A+B_1) + 0.58 x modHGT uCP5	18.5	0.78	
			PRCP Kp 5%/h = 146 + 86 x [NDIP / (A+B_1)]	14.5	0.85	
			PRCP Kp 5%/h = 106 + 55 x [NDIP / (A+B_1)] + 0.001 x NDIP x B_2	11.4	0.92	
			PRCP Kp 5%/h = 141 + 125 x [NDIP / (A+B_1)] + 0.0006 x CP x B_2] – 0.0006 x [(B_3+C) x B_2]	9.9	0.94	
			PRCP Kp 5%/h = 125 – 0.20 x B_2 + 80 x [NDIP / (A+B_1)] + 0.0008 x CP x B_2	9.31	0.95	
		PRCP Kp 8%/h = 229.0 ± 42	PRCP Kp 8%/h = 398 – 0.55 x A – 0.20 x B_2	23.5	0.74	
			PRCP Kp 8%/h = 10.6 – 0.23 x A + 0.90 x modHGT uCP8	18.3	0.84	
			PRCP Kp 8%/h = 148 + 99 x [NDIP / (A+B_1)]	18.4	0.83	
			PRCP Kp 8%/h = 96 + 0.58 x [NDIP / (A+B_1)] + 0.002 x NDIP x B_2	13.9	0.91	
			PRCP Kp 8%/h = 139 + 147 x [NDIP / (A+B_1)] + 0.0008 x CP x B_2 – 0.0008 x [(B_3 + C) x B_2]	5.23	0.94	

*Crude protein fractions A, B_1, B_2, B_3, C are in g/kg crude protein; **Crude protein fractions A, B_1, B_2, B_3, C are in g/kg dry matter; ***Crude protein fractions A, B_1, B_2, B_3, C are in % of dry matter; ****Crude protein fractions A, B_1, B_2, B_3, C are in % crude protein.

[1] A, crude protein soluble in the borate-phosphate buffer and tungstic acid solution; B_1, true protein soluble in buffer solution and precipitated by the tungstic solution; B_2, true protein insoluble in buffer solution but soluble in neutral-detergent solution; B_3, true protein soluble in acid detergent solution but insoluble in neutral-detergent solution; C, true protein insoluble in the acid-detergent solution; CP, crude protein (g/kg dry matter); Kp, rumen passage rate (%/h); NDFp, neutral detergent fiber assayed using heat-stable amylase and without the use of sodium sulfite using the crude protein fractionation method and expressed inclusive ash (g/kg dry matter); NDIP, neutral detergent insoluble protein (g/kg dry matter).

[2] RMSE, root mean square error.[3] R^2, coefficient of determination.

1.4 Objectives, research hypotheses, and thesis outline

There is a lack of information regarding the nutritional composition of diets offered as well as the metabolic processes associated with digestion for cattle in tropical husbandry systems (Mottet et al., 2017; Pica-Ciamarra et al., 2014). As a result, researchers have adopted for tropical regions the laboratory methodologies and feeding recommendations developed for temperate regions (Bateki, 2020; Hernández-Castellano et al., 2019). Therefore, the overall objective of the present doctoral thesis was to evaluate the accuracy of existing laboratory methodologies and modeling tools in predicting the N supply and excretion of dairy cattle in tropical husbandry systems. It was hypothesized that the adoption of laboratory methodologies, modeling tools, and feeding recommendations from temperate systems without validating and adapting them for tropical systems may result in inaccurate estimations of N supply, utilization, and excretion, which will hamper the assessment of N use efficiency.

This research resulted in three publications presented in chapter 2, 3, and 4.

In chapter 2, equations developed to predict RUP proportions of temperate feedstuffs as a function of chemical crude protein and fiber fractions were selected. These equations were then used to calculate RUP proportions of forages commonly used as feed for ruminants in the (Sub-) Tropics. Samples were collected from Brazil, Costa Rica, El Salvador, Ethiopia, Kenya, and Peru (n = 38 samples). The accuracy and precision of the predictions was tested against RUP proportions measured *in situ*. A set of equations was also proposed for predicting the RUP proportions of the evaluated tropical forages based on chemical crude protein and fiber fractions.

In chapter 3, an equation developed to predict the PRCP supply of temperate feedstuffs from chemical crude protein fractions was chosen as well as the *in vitro* method to estimate PRCP supply for forages commonly used as feed for ruminants in the (Sub-) Tropics. The accuracy of the PRCP estimations with the chemical and *in vitro* methods were tested against PRCP supply estimated from *in situ* measurements and digested organic matter concentration determined from the gas production of the Hohenheim gas test. Samples were collected from Brazil, Costa Rica, El Salvador, Ethiopia, Kenya, and Peru (n = 38 samples). A set of equations was also developed for predicting the PRCP supply of the evaluated tropical forages based on *in vitro* parameters, chemical composition, and fiber fractions.

In chapter 4, three semi-mechanistic models built based on ruminant feeding recommendations of the British, German, and French systems were selected to predict FN, UN, and total N (TN) excretion as well as FN fractions of dairy cows, heifers, and steers kept under typical (sub-) tropical husbandry conditions. The accuracy and precision of the model

predictions was assessed against reference values of UN (total collection, creatinine method, and N balance) and FN excretion (total collection, internal and external markers) from eight N balance trials in El Salvador, Kenya, and Peru (n = 392 observations). To improve the accuracy and precision of models' prediction, potential model improvements were recommended.

In chapter 5, the contributions of the present thesis to reliably estimate N use efficiency in the Tropics are discussed (section 5.1) as well as the main assumptions and hypotheses that influenced the development of the thesis (sections 5.2 – 5.3). Furthermore, the challenges and limitations (section 5.4) of the present thesis are discussed as well as suggestions for future research (section 5.5). In chapter 6, conclusions are drawn based on the findings of chapters 2 - 5.

1.5 References

AFRC (Agricultural and Food Research Council). 1993. Energy and protein requirements of ruminants: An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. CAB International, Wallingford, UK.

Alexandratos, N., Bruinsma, J. 2012. World agriculture towards 2030/2050: the 2012 revision, ESA Working Papers 12–03. The Food and Agriculture Organization of the United Nations, Rome, Italy.

Bateki, C. A. 2020. Adaptation of herd simulation models to predict the efficiency of the use of resources in tropical ruminant productions systems [Dissertation to obtain the doctoral degree of Agricultural Sciences]. University of Hohenheim.

Bergen, W. G. 2007. Contribution of research with farm animals to protein metabolism concepts:a historical perspective. J. Nutr. 137, 706–710. https://doi.org/10.1093/jn/137.3.706

Bovera, F., Spanghero, M., Galassi, G., Masoero, F., Buccioni, A. 2003. Repeatability and reproducibility of the Cornell Net Carbohydrate and Protein System analytical determinations. Ital. J. Anim. Sci. 2, 41–50. https://doi.org/10.4081/ijas.2003.41

Butterworth, M. H., Diaz L. J. A. 1970. Use of equations to predict the nutritive value of tropical grasses. J. Range Manag. 23, 55–8. https://doi.org/10.2307/3896009

Castillo, A. R., Kebreab, E., Beever, D. E., France, J. 2000. A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. J. Anim. Feed Sci. 9, 1–32. https://doi.org/10.22358/jafs/68025/2000

Castro-Montoya, J. M., Dickhoefer, U. 2020. The nutritional value of tropical legume forages fed to ruminants as affected by their growth habit and fed form: A systematic review. Anim. Feed Sci. Technol. 269, 114641. https://doi.org/10.1016/j.anifeedsci.2020.114641

Chrenková, M., Čerešňáková, Z., Weisbjerg, M., Formelová, Z., Poláčiková, M., Vondráková, M. 2014. Characterization of proteins in feeds according to the CNCPS and comparison to *in situ* parameters. Czech J. Anim. Sci. 59, 288–295. https://doi.org/10.17221/7499-CJAS

Dhanoa, M. S., France, J., López, S., Dijkstra, J., Lister, S. J., Davies, D. R., Bannink, A. 1999. Correcting the calculation of extent of degradation to account for particulate matter loss at zero time when applying the polyester bag method. J. Anim. Sci. 77, 3385–3391. https://doi.org/10.2527/1999.77123385x

Diaz, R. J., Rosenberg, R. 2008. Spreading dead zones and consequences for Marine ecosystems. Science 321, 926–929. https://doi.org/10.1126/science.1156401

Dijkstra, J., Bannink, A., Bosma, P. M., Lantinga, E. A., Reijs, J. W. 2018. Modeling the effect of nutritional strategies for dairy cows on the composition of excreta nitrogen. Front. Sustain. Food Syst. 2:63. https://doi.org/10.3389/fsufs.2018.00063

Dijkstra, J., France, J., Assis, A. G., Neal, H. D. S. C., Campos, O. F., Aroeira, L. J. M. 1996. Simulation of digestion in cattle fed sugarcane: prediction of nutrient supply for milk production with locally available supplements. J. Agric. Sci. 127, 247–260. https://doi.org/10.1017/S0021859600078023

Edmunds, B., Südekum, K.-H., Spiekers, H., Schuster, M., Schwarz, F. J. 2012. Estimating utilisable crude protein at the duodenum, a precursor to metabolisable protein for ruminants, from forages using a modified gas test. Anim. Feed Sci. Technol. 175, 106–113. https://doi.org/10.1016/j.anifeedsci.2012.05.003

Foskolos, A., Moorby, J. M. 2018. Evaluating lifetime nitrogen use efficiency of dairy cattle: A modelling approach. PLOS ONE 13, e0201638. https://doi.org/10.1371/journal.pone.0201638

France, J., Dijkstra, J. 2006. Scientific progress and mathematical modelling: different approaches to modelling animal systems, in: Mechanistic Modelling in Pig and Poultry Production. CAB International, Wallingford, UK.

Garg, M. R., Sherasia, P. L., Bhanderi, B. M., Makkar, H. P. S. 2016. Nitrogen use efficiency for milk production on feeding a balanced ration and predicting manure nitrogen excretion in lactating cows and buffaloes under tropical conditions. Anim. Nutr. Feed Tech. 16, 1. https://doi.org/10.5958/0974-181X.2016.00001.9

GfE (Gesellschaft für Ernährungsphysiologie). 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt, Germany.

GfE (Gesellschaft für Ernährungsphysiologie). 2022. Recommended protocol for the determination of nutrient disappearance *in situ* for estimation of ruminal degradation. Proc. Soc. Nutr. Physiol. 31, 177–189.

Haddon, M. 2011. Modelling and quantitative methods in Fisheries. Editorial Chapman and Hall/CRC, Boca Raton, USA.

Hernández-Castellano, L. E., Nally, J. E., Lindahl, J., Wanapat, M., Alhidary, I. A., Fangueiro, D., Grace, D., Ratto, M., Bambou, J. C., de Almeida, A. M. 2019. Dairy science and health in the tropics: challenges and opportunities for the next decades. Trop. Anim. Health Prod. 51, 1009–1017. https://doi.org/10.1007/s11250-019-01866-6

Herrero, M., Fawcett, R., Jessop, N. 2002. Predicting intake and nutrient supply of tropical and temperate diets for ruminants using a simple dynamic model of digestion. Institute of Ecology and Resource Management, University of Edinburgh, UK.

Hristov, A. N., Bannink, A., Crompton, L. A., Huhtanen, P., Kreuzer, M., McGee, M., Nozière, P., Reynolds, C. K., Bayat, A. R., Yáñez-Ruiz, D. R., Dijkstra, J., Kebreab, E., Schwarm, A., Shingfield, K. J., Yu, Z. 2019. Invited review: Nitrogen in ruminant nutrition: A review of measurement techniques. J. Dairy Sci. 102, 5811–5852. https://doi.org/10.3168/jds.2018-15829

Huhtanen, P., Nousiainen, J. I., Rinne, M., Kytölä, K., Khalili, H. 2008. Utilization and partition of dietary nitrogen in dairy cows fed grass silage-based diets. J. Dairy Sci. 91, 3589–3599. https://doi.org/10.3168/jds.2008-1181

Hvelplund, T., Weisbjerg, M. R. 1998. *In vitro* techniques to replace *in vivo* methods for estimating amino acid supply. BSAP Occasional Publication 22, 131–144. https://doi.org/10.1017/S0263967X00032419

INRA (Institut National de la Recherche Agronomique). 2019. INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, Netherlands.

Johnson, A. C. B., Reed, K. F., Kebreab, E. 2016. Short communication: Evaluation of nitrogen excretion equations from cattle. J. Dairy Sci. 99, 7669–7678. https://doi.org/10.3168/jds.2015-10730

Jonker, J. S., Kohn, R. A., Erdman, R. A. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J. Dairy Sci. 81, 2681–2692. https://doi.org/10.3168/jds.S0022-0302(98)75825-4

Kebreab, E., France, J., Mills, J. A. N., Allison, R., Dijkstra, J. 2002. A dynamic model of N metabolism in the lactating dairy cow and an assessment of impact of N excretion on the environment. J. Anim. Sci. 80, 248–259. https://doi.org/10.2527/2002.801248x

Kebreab, E., Strathe, A. B., Dijkstra, J., Mills, J. A. N., Reynolds, C., Crompton, L. A., Yan, T., France, J. 2010. Energy and protein interactions and their effect on nitrogen excretion in dairy cows. Presented at the EAAP Scientific Series, pp. 417–426.

Kirchhof, S., Eisner, I., Gierus, M., Südekum, K.-H. 2010. Variation in the contents of crude protein fractions of different forage legumes during the spring growth. Grass Forage Sci. 65, 376–382. https://doi.org/10.1111/j.1365-2494.2010.00756.x

Koong, L. J., Ferrell, C. L., Nienaber, J. A. 1985. Assessment of interrelationships among levels of intake and production, organ size and fasting heat production in growing animals. J. Nutr. 115, 1383–1390. https://doi.org/10.1093/jn/115.10.1383

Lanzas, C., Broderick, G. A., Fox, D. G. 2008. Improved feed protein fractionation schemes for formulating rations with the Cornell net carbohydrate and protein system. J. Dairy Sci. 91, 4881–4891. https://doi.org/10.3168/jds.2008-1440

Lapierre, H., Berthiaume, R., Raggio, G., Thivierge, M. C., Doepel, L., Pacheco, D., Dubreuil, P., Lobley, G.E. 2005. The route of absorbed nitrogen into milk protein. Anim. Sci. 80, 11–22. https://doi.org/10.1079/ASC41330011

Leberl, P., Gruber, L., Steingass, H., Schenkel, H. 2007. Comparison of the methods modified Hohenheimer Futter-Werttest (moHFT) and Cornell system for determination of nXP-content of concentrates. Presented at the 16 International Science Symposium on Nutrition of Domestic Animals, pp. 171–176.

Lebzien, P., Voigt, J. 1999. Calculation of utilizable crude protein at the duodenum of cattle by two different approaches. Archiv für Tierernaehrung 52, 363–369. 10.1080/17450399909386174.

Licitra, G., Hernandez, T. M., Van Soest, P. J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347–358. https://doi.org/10.1016/0377-8401(95)00837-3

Madsen, J., Hvelplund, T. 1994. Prediction of *in situ* protein degradability in the rumen: Results of a European ringtest. Livest. Prod. Sci. 39, 201–212. https://doi.org/10.1016/0301-6226(94)90185-6

Marcondes, M. I., Tedeschi, L. O., Valadares Filho, S. C., Gionbelli, M. P. 2013. Predicting efficiency of use of metabolizable energy to net energy for gain and maintenance of Nellore cattle. J. Anim. Sci. 91, 4887–4898. https://doi.org/10.2527/jas.2011-4051

Minson, D. J., Wilson, J.R. 1980. Comparative digestibility of tropical and temperate forage - a contrast between grasses and legumes. Aust. J. Agric. Res. 46, 247–249.

Mondal, G., Walli, T. K., Patra, A. K. 2008. *In vitro* and *in sacco* ruminal protein degradability of common Indian feed ingredients. Livest. Res. Rural Dev. 20. http://www.lrrd.org/lrrd20/4/mond20063.htm

Mottet, A., de Haan, C., Falcucci, A., Tempio, G., Opio, C., Gerber, P. 2017. Livestock: On our plates or eating at our table? A new analysis of the feed/food debate. Global Food Security, Food Security Governance in Latin America 14, 1–8. https://doi.org/10.1016/j.gfs.2017.01.001

NorFor (Nordic Feed Evaluation System). 2011. The Nordic feed evaluation system. Wageningen Academic Publishers, Wageningen, Netherlands.

NRC (National Research Council). 2001. Nutrient Requirements of Dairy Cattle. The National Academies Press, Washington DC, USA. https://doi.org/10.17226/9825

OECD (Organisation for Economic Co-operation and Development). 2023. OECD-FAO Agricultural Outlook 2020-2029, OECD Publishing, Paris/FAO, Rome, https://doi.org/10.1787/1112c23b-en

Oliveira, A. S. 2015. Meta-analysis of feeding trials to estimate energy requirements of dairy cows under tropical conditions. Anim. Feed Sci. Technol. 210, 94–103. https://doi.org/10.1016/j.anifeedsci.2015.10.006

Pica-Ciamarra, U., Baker, D., Morgan, N., Zezza, A., Azzarri, C., Ly, C., Nsiima, L., Nouala, S., Okello, P., Sserugga, J. 2014. Investing in the livestock sector: why good numbers matter, a sourcebook for decision makers on how to improve livestock data. World Bank, Washington, DC, USA.

Powell, J. M., MacLeod, M., Vellinga, T. V., Opio, C., Falcucci, A., Tempio, G., Steinfeld, H., Gerber, P. 2013. Feed-milk-manure nitrogen relationships in global dairy production systems. Livest. Sci. 152, 261–272. https://doi.org/10.1016/j.livsci.2013.01.001

Reed, K. F., Moraes, L. E., Casper, D. P., Kebreab, E. 2015. Predicting nitrogen excretion from cattle. J. Dairy Sci. 98, 3025–3035. https://doi.org/10.3168/jds.2014-8397

Reid, R. L., Jung, G. A., Thayne, W. V. 1988. Relationships between nutritive quality and fiber components of cool season and warm season forages: a retrospective study. J. Anim. Sci. 66, 1275–1291. https://doi.org/10.2527/jas1988.6651275x

Reid, R. S., Thornton, P. K., McCrabb, G. J., Kruska, R. L., Atieno, F., Jones, P. G. 2004. Is it possible to mitigate greenhouse gas emissions in pastoral ecosystems of the tropics? Environ. Dev. Sustain. 6, 91. https://doi.org/10.1023/B:ENVI.0000003631.43271.6b

Rufino, M., Herrero, M., Van Wijk, M., Hemerik, L., Ridder, N., Giller, K. 2009. Lifetime productivity of dairy cows in smallholder farming systems of the Central highlands of Kenya. Animal 3, 1044–56. https://doi.org/10.1017/S1751731109004248

Rupp, C., Westreicher-Kristen, E., Susenbeth, A. 2021. *In situ* and *in vitro* determination of the protein value of feeds for ruminants. Arch. Anim. Nutr. 75, 329–344. https://doi.org/10.1080/1745039X.2021.1962149

Russell, J. B., O'Connor, J. D., Fox, D. G., Van Soest, P. J., Sniffen, C. J. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim. Sci. 70, 3551–3561. https://doi.org/10.2527/1992.70113551x

Schwab, C. G., Broderick, G. A. 2017. A 100-Year Review: Protein and amino acid nutrition in dairy cows. J. Dairy Sci. 100, 10094–10112. https://doi.org/10.3168/jds.2017-13320

Shannak, S., Südekum, K.-H., Susenbeth, A. 2000. Estimating ruminal crude protein degradation with *in situ* and chemical fractionation procedures. Anim. Feed Sci. Technol. 85, 195–214. https://doi.org/10.1016/S0377-8401(00)00146-2

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., Russell, J. B. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 70, 3562– 3577. https://doi.org/10.2527/1992.70113562x

Solis, J. C., Byers, F. M., Schelling, G. T., Long, C. R., Greene, L. W. 1988. Maintenance requirements and energetic efficiency of cows of different breed types. J. Anim. Sci. 66, 764–773. https://doi.org/10.2527/jas1988.663764x

Steingaß, H., Nibbe, D., Südekum, K.-H., Lebzien, P., Spiekers, H. 2001. Schätzung des nXP-Gehaltes mit Hilfe des modifizierten Hohenheimer Futterwerttests und dessen Anwendung zur Bewertung von Raps- und Sojaextraktionsschroten. Presented at the Congress VDLUFA 113, p. 114.

Stern, M. D., Bach, A., Calsamiglia, S. 1997. Alternative techniques for measuring nutrient digestion in ruminants. J. Anim. Sci. 75, 2256– 276. https://doi.org/10.2527/1997.7582256x

Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci. 75, 345–357. https://doi.org/10.3168/jds.S0022-0302(92)77770-4

Tedeschi, L. O. 2019. Mathematical modeling in ruminant nutrition: approaches and paradigms, extant models, and thoughts for upcoming predictive analytics. J. Anim. Sci. 97, 1921–1944. https://doi.org/10.1093/jas/skz092

Thorne, P., Delve, R., Cadisch, G., Tanner, J., Giller, K., Kimani, S., Thorpe, W. 2001. R6283: Implications of livestock feeding management for long-term soil fertility in smallholder mixed farming systems. A Final technical report on a research project funded by the Department for International Development's Livestock Production Research Programme.

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

Valdés, C., Andrés, S., Giráldez, F. J., García, R., Peláez, R., Calleja, A. 2011. Estimación de la proteína no degradable en el rumen de pastos de la Montaña Leonesa empleando el CNCPS (Cornell). Pastos, paisajes culturales entre tradición y nuevos paradigmas del siglo XXI. Sociedad Española para el Estudio de los Pastos, pp. 401–406.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Cornell University Press, New York, USA.

Wassie, S. E., Ali, A. I. M., Korir, D., Butterbach-Bahl, K., Goopy, J., Merbold, L., Schlecht, E., Dickhoefer, U. 2019. Effects of feed intake level on efficiency of microbial protein synthesis and nitrogen balance in Boran steers consuming tropical poor-quality forage. Arch. Anim. Nutr. 73, 140–157. https://doi.org/10.1080/1745039X.2019.1572343

Westreicher-Kristen, E., Steingass, H., Rodehutscord, M. 2015. Estimation of utilisable crude protein at the duodenum of dried distillers' grains with solubles using a modified gas test. Arch. Anim. Nutr. 69, 351–365. https://doi.org/10.1080/1745039X.2015.1073058

Westreicher-Kristen, E., Steingass, H., Rodehutscord, M. 2012. Variations in chemical composition and *in vitro* and *in situ* ruminal degradation characteristics of dried distillers' grains with solubles from European ethanol plants. Arch. Anim. Nutr. 66, 458–472. https://doi.org/10.1080/1745039X.2012.740310

Zahra, W. A., Middelaar, C. E. van, de Boer, I. J. M., Oosting, S. J. 2020. Predicting nutrient excretion from dairy cows on smallholder farms in Indonesia using readily available farm data. Asian- Australas J. Anim. Sci. 33, 2039– 049. https://doi.org/10.5713/ajas. 20.0089

Zhao, G. Y., Cao, J. E. 2004. Relationship between the *in vitro*-estimated utilizable crude protein and the Cornell Net Carbohydrate and Protein System crude protein fractions in feeds for ruminants. J. Anim. Physiol. Anim. Nutr. (Berl) 88, 301–310. https://doi.org/10.1111/j.1439-0396.2004.00485.x

Zhao, G. Y., Lebzien, P. 2000. Development of an *in vitro* incubation technique for the estimation of the utilizable crude protein (uCP) in feeds for cattle. Arch. Tierernahr. 53, 293–302. https://doi.org/10.1080/17450390009381953

Chapter 2

2. Estimating the proportion of *in situ* rumen-undegraded crude protein from chemical crude protein and fiber fractions in tropical forage grasses and legumes

This chapter was published as:

Salazar-Cubillas, K., Dickhoefer, U., 2021. Estimating the proportion of *in situ* rumen-undegraded crude protein from chemical crude protein and fiber fractions in tropical forage grasses and legumes. Animal Feed Science and Technology 282, 115122. https://doi.org/10.1016/j.anifeedsci.2021.115122

Abstract

Advanced methods exist to estimate dietary crude protein (CP) degradation in the rumen from the concentrations and/or proportions of chemical nutrient fractions. These methods have been developed primarily for feeds in temperate climates. Therefore, their accuracy and precision to predict proportions of rumen-undegraded CP (RUP) in tropical forages is uncertain. The objectives of the present study were (1) to assess the accuracy and precision of the estimates of *in situ* RUP proportions from CP fractions using established algorithms, and (2) to identify chemical nutrient fractions that accurately and precisely predict RUP proportions of tropical forages. The proximate nutrients, CP and fiber fractions of 23 unconserved forage grasses and 15 forage legumes commonly used as feeds for ruminants in the Tropics and Subtropics were analyzed. Their RUP proportions were determined at ruminal passage rates (Kp) of 2, 5, and 8%/hour using the *in situ* method (i. e., reference) and were also estimated from the analysis of the CP and fiber fractions using established equations. The RUP proportions determined with the *in situ* and the chemical method were evaluated using error index and dimensionless parameters. The nutritional composition variables that can predict RUP proportions of tropical forages (proximate nutrients, fiber, and CP fractions and their ratios) were selected using stepwise multiple linear regression models. The CP concentrations of our sample set ranged from 46 to 212 g/kg dry matter with *in situ* RUP proportions from 73 to 596 g/kg CP across all Kp. Previously established equations could not predict accurately and precisely RUP proportions of all forages. The concentrations of CP and fiber fractions and Kp were able to predict the proportion of RUP estimated *in situ* for tropical forages at Kp of 2, 5, and 8%/hour with an adjusted coefficient of determination ranging from 0.79 to 0.82 and a root mean square error ranging from 13 to 15% of the mean *in situ* RUP proportion. While equations established for temperate forages either over- or underestimate RUP proportions, algorithms developed in the present study might allow for an accurate and precise prediction of RUP proportions in forages commonly used in ruminant feeding in the Tropics and Subtropics.

Keywords: feed evaluation, protein metabolism, tropical forages, protein fractionation, ruminants.

2.1 Introduction

Dietary rumen-degraded crude protein (CP) and rumen-undegraded CP (RUP) proportion (g/kg CP) of individual feeds is required in most feed evaluation systems for domestic ruminants (e. g., AFRC, 1993; NRC, 2001; NorFor, 2011; INRA, 2018) to estimate the CP supply to rumen microbes and their host. Moreover, accurate and precise predictions of dietary rumen-degraded CP and RUP proportions are needed to optimize animal performance and minimize losses of excess nitrogen (N) (Lanzas et al., 2008).
Forages commonly account for the majority of CP and RUP supply in feeds for domestic cattle in the Tropics and Subtropics. The nutritive value of tropical forages greatly varies with forage species and varieties, environment, and climatic conditions (Detmann et al., 2010), as well as the geographical zones (Onyango et al., 2019), fertilization, crop management strategies, and plant growth rates (Tran et al., 2009).

The proportion of RUP in feeds has often been estimated by the *in situ* method, which has been widely adopted as the best reference method (Hvelplund and Weisbjerg, 2000). However, this method is costly and time-consuming, and the requirement of rumen-cannulated animals may have animal welfare inflections (Stern et al., 1997; Edmunds et al., 2012), thereby making it unsuitable for routine evaluation of tropical ruminant feedstuffs. Alternative RUP methods have been used that allow for the analysis of a large number of samples, and do not require the use of rumen-cannulated animals. One approach, which has also been adopted by the Cornell Net Carbohydrate and Protein System (CNCPS), estimates the RUP proportion in ruminant feedstuffs from the concentrations of various chemical CP fractions and their rate of degradation in the rumen (Sniffen et al., 1992).

The reproducibility of the degradation rates of individual CP fractions is low and a main source of error, in turn greatly influencing the RUP estimates using the original method (Edmunds et al., 2012). An attempt to bypass the need to estimate the degradation rates of the CP fractions, Shannak et al. (2000) and Mondal et al. (2008) developed equations to predict the proportion of RUP and the concentration of rumen-degraded CP as determined with the *in situ* method of temperate and tropical concentrates, respectively, from analyzed CP and fiber fractions. Kirchhof (2007) and Valdés et al. (2011) further developed similar equations to predict RUP proportions and concentrations, respectively, of forages from temperate pastures. These equations were successfully applied to specific sample sets based on the assumption that the proportions of the different CP fractions are strongly correlated with the proportions of soluble CP and potentially degraded CP determined *in situ* (Lanzas et al., 2008; Chrenková et al., 2014).

Forages grown in tropical regions differ in their composition from temperate forages with generally greater concentrations of total fiber and lignin, lower concentrations of CP, and less readily digestible CP than in temperate forages (Lee, 2018). The CP fractions B_3 and C (i. e., cell-wall-bound protein) are the most important fractions for estimating RUP (Fox et al., 2004; Schroeder, 2004; Tran et al., 2009). However, the proportions of cell-wall-bound protein are greater and more variable in tropical than in temperate forages (Tran et al., 2009; Lowe et al., 2011), and in tropical forage legumes than in tropical forage grasses (Lowe et al., 2011). Thus, it appears likely that the cell-wall-bound protein will have a greater impact on proportions of RUP in tropical than in temperate forages and in tropical forage legumes than tropical forage grasses.

In the present study, it was hypothesized that equations developed for temperate forages to predict RUP proportions only from CP fractions and chemical composition variables will not be accurate and precise to predict RUP proportions of tropical forage grasses and legumes as determined *in situ*. Furthermore, a single equation may not be feasible to predict accurately and precisely the RUP proportion of tropical forage grasses and legumes. The objectives of the present study were (1) to assess the accuracy and precision of the estimates of their *in situ* RUP proportions from chemical CP fractions using established algorithms; and (2) to identify nutritional composition variables (e. g., proximate nutrients, fiber fractions and chemical CP fractions) that are most appropriate to predict RUP proportion of tropical forage grasses and legumes.

2.2 Materials and methods

2.2.1 Feed sample material

The forages commonly used for cattle were identified in literature review of trials performed in the Tropics and Subtropics. Most frequently mentioned forages were selected, and samples were collected, where possible, from research stations in Brazil, Costa Rica, El Salvador, Ethiopia, Kenya, and Peru where the forages had been grown under controlled field conditions (Appendix 2.1). Some additional country-specific forages used in cattle feeding were also obtained. All forage samples (23 forage grasses and 15 forage legumes) were freshly harvested from May to September 2017 and neither ensiled nor dried as hay.

Forage samples were oven-dried at 45°C for 48 hours. One subsample was milled (Culatti Typ MFC, Kleinfeld Labortechnik GmbH, Hanover, Germany) through a 1-mm screen for analysis of proximate nutrients, fiber fractions, and chemical CP fractions, and one subsample was milled through a 2-mm screen for *in situ* incubations.

2.2.2 Proximate nutrient and fiber analysis

The samples were then analyzed in duplicate according to the German Handbook of Agricultural Experimental and Analytical Techniques (VDLUFA, 2012) for dry matter (DM; method 3.1), ash (method 8.1), CP (Kjeldahl; method 4.1.1), neutral-detergent fiber assayed with a heat-stable amylase and sodium sulfite and expressed inclusive of residual ash (aNDF; method 6.5.1), acid-detergent fiber expressed inclusive of residual ash (ADF; method 6.5.2), and acid-detergent lignin assayed with the use of sulfuric acid (Lignin $_{(sa)}$; method 6.4.1).

2.2.3 Rumen-undegraded crude protein estimated by *in situ* incubation

All animal handling and procedures were performed following the Animal Welfare Legislation approved by the Government Presidium of Stuttgart, Germany (approval code V319/14 TE).

The *in situ* CP degradability in the rumen was measured by incubating the feed samples in polyester bags following the protocol described by Madsen and Hvelplund (1994) with some modifications. The *in situ* CP degradability in the rumen was analyzed in two periods, because all samples could not be handled in one period. Three 10-year-old (SD 0.6) Jersey cows fitted with rumen cannulae were used in the first period. The cows weighed on average 523 kg liveweight (SD 11) with an average of 287 days (SD 104) in lactation and produced on average 16.1 kg (SD 5.4) of milk per cow each day. Cows were fed a total mixed ration with a forage to concentrate ratio of 68 to 32 containing (on DM basis) a concentrate mixture (251 g/kg), maize silage (243 g/kg), grass silage (243 g/kg), grass hay (170 g/kg), rapeseed meal (52 g/kg), barley straw (22 g/kg), and a mineral-amino-acid-vitamin mixture (19 g/kg). The diet contained 6.2 MJ/kg DM of net energy for lactation and had a CP concentration of 134 g/kg DM. The CP degradation kinetics of 26 feed samples were determined in this period.

The CP degradation kinetics of the remaining 12 feed samples were determined during the second period. Additionally, two feed samples from the first period were also incubated in the second period (i. e., *G. max* and *D. lablab*) to quantify differences in CP degradability between periods. Three non-lactating 8-year-old (SD 2.9) Jersey cows were used, including two that were also used in the first period. The cows weighed on average 529 kg (SD 77) and were fed a total mixed ration with a forage to concentrate ratio of 98 to 2 containing (on DM basis) maize silage (325 g/kg), grass silage (325 g/kg), grass hay (226 g/kg), barley straw (108 g/kg), a concentrate mixture (10 g/kg), and a mineral-amino-acid-vitamin mixture (6 g/kg). The diet contained 5.4 MJ/kg DM of net energy for lactation and had

a CP concentration of 93 g/kg DM. Feed and drinking water were offered for consumption *ad libitum* during both periods.

Polyester bags had a pore size of 50±10 µm (R1020, Ankom Technology, NY, USA) and a feed sample surface area of 15 mg/cm^2. Bags with feed sample material were sealed with a plastic cable tie and attached to a 900-g-plastic weight. Before incubation, the bags were soaked in warm water (~ 39°C) for 1 minute. Feed samples were incubated in duplicate in the rumen of each of the three cows. A maximum of 25 bags with feed samples was inserted into the ventral rumen sac of each cow at 08:00 hours before morning feeding and incubated for 2, 4, 8, 16, 24, 48, and 72 hours. Immediately after removal, the bags with residual substrate were immersed in ice water to inhibit further microbial action. Then, the bags with residual substrate were washed by hand until the water remained clean and then washed once in a washing machine (WM14A160, Siemens GmbH, Munich, Germany) for 17 minutes using cold water with two water changes and without soap or spinning. Bags with residual substrate were then oven-dried (F720, Binder GmbH, Tuttlingen, Germany) at 60°C for at least 3 hours and weighed. The residual substrate after incubation was pooled for each cow and incubation time. The DM (method 3.1) and CP (method 4.1.2) concentration of the pooled residual substrate was done according to VDLUFA (2012).

The CP disappearance at each hour was corrected for losses of water-soluble feed CP and of water-insoluble feed CP escaping the bag in form of small particles (Weisbjerg et al., 1990) as:

$$CDi = Di - WISP \times (1 - (Di - (WISP + WSP))) / (1 - (WISP + WSP));$$

where: CDi was corrected CP disappearance at the ith hour (i = 2, 4, 8, 16, 24, 48, and 72 hours; g/kg CP); Di was the CP disappearance at the ith hour (i = 2, 4, 8, 16, 24, 48, and 72 hours; g/kg CP); WISP was the water-insoluble feed CP escaping the bag in form of small particles (g/kg CP); and WSP was the water-soluble feed CP (g/kg CP).

The WSP proportion was estimated in duplicate by washing the feed samples with 39°C distilled water through a 125-mm diameter cellulose-filter paper (N°5951/2, GE Healthcare Life Sciences, Darmstadt, Germany). The WISP proportion was estimated by subtracting WSP from zero-time disappearance proportion. Zero-time disappearance proportion (g/kg CP) was determined in duplicate by washing bags with feed sample in a washing machine as described above. Then, CP disappearance concentration at each incubation hour was corrected for microbial attachment to undegraded particles (Krawielitzki et al., 2006) as:

$MA = Amax \times (1 - e^{-R \times t})$;

where: MA was the microbial attachment to undegraded particles (mg CP/g residual CP); Amax was the maximum extent of microbial attachment at time ≈ ∞ (mg/g residual CP); R was the fractional rate of colonization (/hour); and t was the incubation time (hour). The maximum extent of microbial colonization (i. e., Amax) was estimated in duplicate according to Mass et al. (1999). For this, the residual substrate after incubation (incubation time > 16 hours) was weighed in an ANKOM filter bag (F57, ANKOM Technology, NY, USA) and boiled with neutral-detergent solution (Van Soest et al., 1991) in a ANKOM fiber analyzer (A200, ANKOM Technology, NY, USA) for 1 hour. The N concentration of the neutral-detergent-insoluble fraction of the residual substrate was determined by the Kjeldahl method (B324, Büchi Labortechnik GmbH, Essen, Germany) and converted to CP (VDLUFA, 2012; method 4.1.1). The CP concentration of the neutral-detergent-soluble fraction of the residual substrate was calculated as the difference between CP of original feed sample material and CP of the neutral-detergent-insoluble fraction of the residual substrate. The mean N concentration of the neutral-detergent-soluble fraction of 16, 24, 48, and 72 hours represented Amax. The fractional rate of microbial colonization was calculated (Krawielitzki et al., 2006) as:

$R = (133.0 + 0.1 \times aNDF - 0.4 \times CP) / 1000$;

where: R was the fractional rate of microbial colonization (/hour); aNDF was the neutral-detergent fiber of original feed sample (g/kg DM); and CP was the CP of original feed sample (g/kg DM). Then, effective degradability of CP was calculated (Dhanoa et al., 1999) as:

$ED = a + (b \times c / (c + Kp)) \times e^{-(Kp \times L)}$;

where: ED was the effective degradability (g/kg CP); a represents the soluble protein (g/kg CP); b represents the insoluble but potentially degradable protein (g/kg CP); c represents the fractional degradation rate of b (/hour); Kp was the passage rate through the rumen (%/hour); and L was the lag phase (hour). Parameters a, b, c, and L were estimated using an iterative least square procedure using SAS software (version 9.4, SAS Institute Inc., NC, USA). The effective CP degradability was estimated at assumed Kp of 2, 5, and 8%/hour. Those Kp were chosen in the present study, because they were within the range of Kp that can be found in the Tropics and Subtropics (Salazar-Cubillas and Dickhoefer, 2019).

2.2.4 Rumen-undegraded crude protein estimated with the chemical method

The chemical CP fractions of all feedstuffs was analyzed as described by Licitra et al. (1996) and partitioned into four fractions composed of soluble protein (SP), non-protein N, neutral-detergent-insoluble protein (NDIP), and acid-detergent-insoluble protein (ADIP). The N fractions were determined in duplicate using the Kjeldahl method (B324, Büchi 194 Labortechnik GmbH, Essen, Germany) and converted to CP (VDLUFA, 2012; method 4.1.1). In addition to aNDF (VDLUFA, 2012; method 6.5.1) and ADF (VDLUFA, 2012; method 6.5.2) analyses, concentrations of aNDF and ADF estimated from the residue after boiling in the respective solution according to Licitra et al. (1996) were also determined, herein referred to as NDFp and ADFp, respectively (i. e., NDF and ADF determined with the chemical CP fractionation method).

The concentrations of the different CP fractions as defined by CNCPS were then calculated according to Sniffen et al. (1992): fraction A is the concentration of CP soluble in the borate-phosphate buffer and tungstic acid solution; fraction B_1 is the concentration of true protein soluble in buffer solution and precipitated by the tungstic solution; fraction B_2 is the concentration of true protein insoluble in buffer solution but soluble in the neutral-detergent solution; fraction B_3 is the concentration of true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution; and fraction C is the concentration of true protein insoluble in the acid-detergent solution. The RUP proportions were then calculated (Kirchhof, 2007) as follows:

$$RUP\ Kp\ 2\%/hour = 204.32 + (1.08 \times C) + (-0.0014 \times (CP \times (A + B_1)))$$

$$RUP\ Kp\ 5\%/hour = 321.90 + (0.17 \times ADFp) + (-0.0022 \times (CP \times (A + B_1))) + (0.0001 \times (CP \times C^2))$$

$$RUP\ Kp\ 8\%/hour = 285.55 + (1.21 \times C) + (0.0005 \times (NDFp \times B_2)) + (-110.17 \times ((A + B_1)/NDFp))$$

where: RUP was the feed rumen-undegraded CP proportion (g/kg CP); Kp was the passage rate through the rumen (%/hour); A was CP soluble in the borate-phosphate buffer and tungstic acid solution (g/kg CP); B_1 was true protein soluble in buffer solution and precipitated by the tungstic solution (g/kg CP); B_2 was true protein insoluble in buffer solution but soluble in the neutral-detergent solution (g/kg CP); C was true protein insoluble in the acid-detergent solution (g/kg CP); CP was feed CP (g/kg DM); ADFp was acid-detergent fiber estimated using the CP fractionation method and expressed inclusive ash (g/kg DM); and NDFp was neutral-detergent fiber assayed using heat-stable amylase and without the use of sodium sulfite using the CP fractionation method and expressed inclusive ash (g/kg DM).

The RUP concentration of each feedstuff predicted with the equations of Valdés et al. (2011) was estimated from the concentrations of the CP fractions B_2, B_3, and C at assumed Kp of 2 and 8%/hour, whereas the RUP concentration at Kp of 5%/hour was estimated as the average between the RUP concentration at Kp of 4 and 6%/hour:

$$RUP\ Kp\ 2\%/hour = 13.57 + 0.16 \times B_2 + 0.19 \times B_3 + 0.17 \times C$$
$$RUP\ Kp\ 4\%/hour = 15.14 + 0.21 \times B_2 + 0.30 \times B_3 + 0.32 \times C$$
$$RUP\ Kp\ 6\%hour = 16.16 + 0.24 \times B_2 + 0.39 \times B_3 + 0.42 \times C$$
$$RUP\ Kp\ 8\%/hour = 16.55 + 0.26 \times B_2 + 0.46 \times B_3 + 0.51 \times C$$

where: RUP was feed rumen-undegraded CP concentration (g/kg DM); Kp was passage rate through the rumen (%/hour); B_2 was true protein insoluble in buffer solution but soluble in the neutral-detergent solution (g/kg DM); and B_3 was true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution (g/kg DM); C was true protein insoluble in the acid-detergent solution (g/kg DM). The RUP proportion in CP (g/kg CP) predicted with the equations of Valdés et al. (2011) was calculated from the ratio between the concentration of RUP (g/kg DM) and CP (g/kg DM) multiplied by 1000 (i. e., transforming the unit to g/kg CP).

In addition, the concentrations of cell-wall-bound protein (i. e., sum of CP fractions B_3 and C), CP not bound to the cell wall (i. e., sum of CP fractions A, B_1, and B_2), soluble CP (i. e., sum of CP fractions A and B_1), insoluble CP (i. e., sum of CP fractions B_2, B_3, and C), and true protein (i. e., sum of CP fractions B_1, B_2, B_3, and C) were calculated.

2.2.5 Statistical analyses and development of regression models

All statistical analyses were conducted using R statistical software version 3.6.1 (R Core Team, Vienna, Austria). Chemical composition and RUP proportions as estimated with the *in situ* method and from different chemical CP fractions of forage grasses (n = 23) and legumes (n = 15) were characterized using descriptive statistics including measures of central tendency (i. e., mean and median) and measures of variability and dispersion (i. e., minimum, maximum, and SD).

A Grubbs outlier test (Grubbs, 1969) was performed on the proportions of RUP estimated with the *in situ* and chemical method using the equations of Kirchhof (2007) and Valdés et al. (2011) at Kp of 2, 5, and 8%/hour. The outlier test identified three outliers in the proportion of RUP estimated with the chemical method using Kirchhof (2007) equation at Kp of 5%/hour: *M. pruriens* (1,107 g/kg CP), *H. rufa* (1,726 g/kg CP), and *S. guianensis*

(889 g/kg CP). The RUP at Kp of 5%/hour of these three samples were removed from Kirchhof (2007), Valdés et al. (2011), and our sample set. The remaining sample set for the accuracy and precision measurements were 38 feed samples at Kp of 2 and 8%/hour and 35 feed samples at Kp of 5%/hour.

The accuracy and precision of the estimates of RUP proportions of forages determined with the *in situ* method and those estimated with the chemical method were compared using error index parameters (i. e., root mean square error (RMSE) and mean absolute percentage error (MAPE)). Ratio between RMSE and SD (RSR), and concordance correlation coefficient (CCC, Lin, 1989) were also calculated. The CCC provides a joint measure of accuracy and precision, and was partitioned into a correlation coefficient (ρ, i. e., precision) and a bias correction factor (Cb, i. e., accuracy).

The most accurate and precise predictions were considered those with lower RMSE, MAPE, RSR, and greater CCC. The scale of McBride (2005) was used to assess the degree of agreement between a new laboratory method and a reference method and to classify the CCC as very strong (CCC ≥ 0.90), strong (CCC ≥ 0.80 < 0.90), moderate (CCC > 0.65 - 0.80), and poor (CCC ≤ 0.65). Given the low reproducibility of the *in situ* RUP estimates and the CP fractions (see section: sources of error in the measurements), estimates of RUP proportions were considered accurate and precise enough in the present study, when the CCC of the relationship between RUP estimates determined *in situ* and those predicted from chemical CP fractions using existing equations were ≥ 0.80, indicating a strong degree of agreement (McBride, 2005). In addition, the scale of Evans (1996) was used to classify the level of ρ, which classifies the ρ as greater (ρ ≥ 0.80), strong (ρ ≥ 0.60 - < 0.80), moderate (ρ ≥ 0.40 - <0.60), weak (ρ > 0.20 - < 0.40), and very weak (ρ ≤ 0.20) (objective 1).

Based on previous studies, concentrations (g/kg DM) of proximate nutrients (i. e., ash, and CP), fiber fractions (i. e., aNDF, ADF, NDFp, ADFp, and $Lignin_{(sa)}$), CP fractions (i. e., A, B_1, B_2, B_3, and C), soluble CP (i. e., sum of CP fractions A and B_1), insoluble CP (i. e., sum of CP fractions B_2, B_3, and C), true protein (i. e., sum of CP fractions B_1, B_2, B_3, and C), cell-wall-bound protein (i. e., sum of CP fractions B_3 and C), and CP not bound to the cell wall (i. e., sum of CP fractions A, B_1, and B_2) as well as the ratios between concentrations of chemical CP fractions (e. g., soluble CP/insoluble CP, true protein/ non-protein N, cell-wall-bound protein/CP not bound to the cell wall) were selected as a set of independent variables that can predict RUP proportion of common feedstuffs used in tropical ruminant husbandry systems.

Models were developed using a multiple linear regression with forward and backward stepwise approach, where predictor variables were added or removed based on Akaike Information Criteria (AIC). The multiple linear regression was performed using independent variables (i. e., chemical composition, fiber fractions, CP fractions, chemical CP fractions and their ratios) and dependent variables (i. e., RUP estimated with the *in situ* method) expressed as a concentration (g/kg DM) in order to maintain a unit consistency between independent and dependent variables following the approach of Valdés et al. (2011) study.

As several models per feed type and Kp were obtained with the stepwise multiple linear regression, the selection of the best model by feed type and Kp was based on Bayesian Information Criterion (BIC) where models with low BIC were selected. Then, multicollinearity of the selected models was performed using Variance Inflation Factor (VIF). Independent variables with VIF greater than 10 were removed from the model until the VIF of the remaining variables were lower than 10 (Hair et al., 1995). Thereafter, the DAAG package (Maindonald and Braun, 2010) was used to perform k-fold cross-validation (k = 3; seed = 11235) and minimize over-fitting of the developed equations. In the case of overfitting, an additional forward stepwise procedure was performed to the developed equations in the previous step. Then, the selected models with low AIC, low BIC, VIF lower than 10, and similar RMSE in the three fold of the cross-validation assessment were reported in Tables 2.4 and 2.5, including information on standard error of the mean, P value, determination coefficients adjusted by the number of predictors in the model (adjusted R^2), RMSE, and MAPE calculated from the relationship between observed (i. e., RUP proportions estimated with the *in situ* method) and predicted RUP (i. e., RUP estimated with the chemical method using the equations of Valdés et al. (2011) or Kirchhof (2007)) proportions for each feed type and Kp (objective 2). The boxplot of the residuals (i. e., observed – predicted) between RUP proportion measured *in situ* (i. e., observed) and the RUP proportion predicted with the regression model and the cross-validation model of forage grasses and legumes at Kp of 2, 5, and 8%/hour are reported in Appendices 2.3 and 2.4.

2.3 Results

2.3.1 Nutritional characteristics of forages

The concentrations of proximate nutrients, fiber fractions, as well as RUP proportion estimated *in situ* of forages grasses and legumes are presented in Table 2.1. The proportions of true protein, insoluble CP, and CP not bound to the cell wall were greater than the proportions of non-protein N, SP, and cell-wall-bound protein, respectively, in most forage samples (Table 2.1). Proportions of CP fractions B_1 ($P = 0.09$) and C ($P = 0.31$) were similar between forage grasses and legumes, whereas the proportions of CP fractions A ($P < 0.01$) and B_3 ($P = 0.04$) were greater, and of CP fraction B_2 ($P < 0.01$) lower in forage grasses than in forage legumes.

2.3.2 Accuracy and precision of existing equations to predict RUP proportions in forages

Relationships between the RUP proportion of forages estimated with the *in situ* method and those estimated with the chemical method (Figure 2.1, Appendix 2.2) showed that estimates derived using the equation of Valdés et al. (2011) gave better predictions than those determined using the equation of Kirchhof (2007) with lower RMSE, MAPE, and RSR and a greater CCC (Table 2.3).

An exception was the RUP proportion of forage grasses at Kp of 2%/hour, which was better predicted by the equation of Kirchhof (2007) (CCC = 0.83) than that of Valdés et al. (2011) (CCC = 0.54). The RUP proportions at all Kp were better predicted (i. e., lower RMSE, MAPE, and RSR, and greater CCC) for forage grasses than legumes, irrespective of whether the equations of Kirchhof (2007) or of Valdés et al. (2011) were used.

2.3.3 Multivariate regressions to predict RUP proportions in forages

The variables retained in the multiple regression model for predicting RUP proportions of forage grasses were, from highest to lowest variance explanation, the concentrations of CP fraction C, Kp, ADFp and of the sum of CP fractions B_3 and C (i. e., CP with slow rumen degradation rate). The variables retained for predicting RUP proportions of forage legumes were Kp as well as the concentrations of NDFp, ADFp and $Lignin_{(sa)}$ (Table 2.4). The adjusted R^2 and RMSE values ranged from 0.79 to 0.82, and 13 to 15% of the mean *in situ* RUP proportion, respectively (Table 2.5). The RMSE derived from the cross-validation of the equations developed in the present study for the forage grasses was 16.6%, whereas

that for the forage legumes was 16.7% (Table 2.5). The residual plots showed no clear patterns and revealed a similar distribution of plotted points around the line at 0 for all the equations developed in the present study to predict RUP proportions of forage grasses and legumes at Kp of 2, 5, and 8%/hour (Appendix 2.3). In general, equations to predict RUP proportions of forage grasses had greater adjusted R^2 and lower RMSE than those to predict RUP proportions of forage legumes. Moreover, the distribution of the cross-validation residuals (i. e., difference between the RUP proportion measured *in situ* and the RUP proportion predicted with the cross-validation) was slightly greater than the distribution of the regression residuals (i. e., difference between the RUP proportion measured *in situ* and the RUP proportion predicted with the regression model) for both, tropical forages and forage legumes (Appendix 2.4).

2.4 Discussion

2.4.1 Sources of error in the measurements

The number of samples (n = 38) used to evaluate RUP proportions, in the present study, was greater than those in studies of Shannak et al. (2000) (n = 32), Valdés et al. (2011) (n = 29), and Mondal et al. (2008) (n = 16), although smaller than used by Kirchhof (2007) (n = 61). Per feed type, fewer samples were available than in the studies mentioned above, but a greater number of plant species was included in our sample set, covering a wide range of nutritional quality. Moreover, samples were obtained from crops grown under field conditions. Hence, our sample set thus appears to allow for the development of robust equations for a wide range of feeding situations of domestic cattle in the Tropics and Subtropics.

The *in situ* method used as reference method has several sources of variation, reducing the repeatability and reproducibility of its results (van der Koelen et al., 1992; Mathis et al., 2001). The standardized procedure proposed by Madsen and Hvelplund (1994) was followed in the present study. Samples were incubated *in situ* in two different batches with cows fed different diets, because lactating cows were used in the first period and dry cows in the second period. Two forage samples (i. e., *G. max* and *D. lablab*) were incubated in both periods to examine any differences in *in situ* CP degradation between periods. The coefficients of variation of the CP degradability of the two forages were 1.14% and 3.40% (% of the mean CP degradation across all incubation times), respectively and both coefficients of variation were lower than the coefficient of variation calculated between cows within the same period (3.70%).

Similar to the *in situ* determination of CP degradation, analysis of CP fractions using the chemical method has been criticized for its low reproducibility (Bovera et al., 2003). In the present study, the standardized procedures of Licitra et al. (1996) were followed and results of the concentrations of the different CP fractions (Sniffen et al., 1992) of six forage samples (i. e., dried forages and silages) were compared with values obtained by an official laboratory for feed analysis (data not shown).

The relative reproducibility (i. e., SD expressed as a percentage of the average obtained from different laboratories) of the concentrations of the different CP fractions (ranging from 11 to 26%) was greater than the one obtained by Bovera et al. (2003) (ranging from 4 to 45%), but not acceptable, with an even lower reproducibility (i. e., greater SD) for CP fraction A (26%) than fractions B_1 to B_3 (11 - 20%) and C (15%).

2.4.2 Chemical composition, *in situ* rumen-undegraded crude protein proportion, and chemical crude protein fractions of forages

Comparisons of the chemical composition and RUP proportions estimated *in situ* with values in the literature are hampered by mentioned methodological constraints. Moreover, factors such as crop species and variety, climate, soil fertility, crop management, post-harvest feed handling, and storage affect the nutritional composition of forages and thus their nutrient digestibility and rumen CP degradability. Yet, nutrient concentrations and rumen CP degradability of most forage species (Table 2.1) were within the range of values reported for the respective species in the literature (e. g., Khandaker et al., 1996; Khamseekhiew et al., 2001; Ramírez Lozano et al., 2002; Tedeschi et al., 2002; Mupangwa et al., 2003; La O et al., 2006; Valarini and Possenti, 2006; Ajayi et al., 2007; Bowen et al., 2008; Singh et al., 2012; Evitayani et al., 2014; Wigati et al., 2014; Melesse et al., 2017; INRA, 2018; Juárez et al., 2018). Hence, samples in the present study appear representative for forage grasses and legumes used for domestic cattle feeding in the Tropics and Subtropics.

Nevertheless, proportions of different CP fractions, of soluble or insoluble CP, of true protein or non-protein N, and of protein bound or not bound to the cell wall were highly variable amongst the forage grasses and legumes (Table 2.2). Such variation in the composition of CP hampers the prediction of the concentrations of different CP fractions in feeds (Valdés et al., 2006; Juárez et al., 2018; Salazar-Cubillas and Dickhoefer, 2018), particularly of CP fractions B_1 and B_2, because they can only poorly be predicted by near-infrared reflectance spectroscopy (Valdés et al., 2006), and of CP fractions B_1 and C, because they cannot be estimated from concentrations of proximate nutrient and fiber fractions (Salazar-Cubillas

and Dickhoefer, 2018). Therefore, chemical analysis of CP fractions B_1, B_2, and C appears necessary.

2.4.3 Accuracy and precision of estimated rumen-undegraded crude protein proportions of forages

The equations developed by Kirchhof (2007), and Valdés et al. (2011) could not predict accurately and precisely the RUP proportion determined *in situ* across all forage grasses and legumes and Kp (CCC < 0.80), which confirms our hypothesis that equations used to predict RUP proportions from CP fractions developed for temperate forages are not valid for tropical forage grasses and legumes. The exception was the equation of Kirchhof (2007), which predicted accurately and precisely the RUP proportion of forage grasses at Kp of 2%/hour (CCC = 0.83) (Table 2.3). Similarly, Edmunds et al. (2012) found a lower accuracy of RUP predictions at fast (RMSE = 37.94 g/kg DM) than at slow Kp (RMSE = 19.03) when using the equations of Kirchhof (2007), although the precision was similar at all Kp in their study (ρ from 0.71 to 0.73). At slow Kp, overall degradability rather than the rate of degradation total CP is decisive, resulting in more similar RUP proportions across a range of forages (Klopfenstein et al., 2001), explaining the greater accuracy and precision at slow than fast Kp. Nevertheless, RUP proportions estimated with the equations of Kirchhof (2007) did not increase with increasing Kp but were greater at Kp of 5%/hour than at 8%/hour, which was unexpected and does not correspond to actual differences between Kp in RUP proportions as determined *in situ*. In contrast, estimates of RUP proportions using the equations of Valdés et al. (2011) were greater at fast than at slow Kp and the accuracy (Cb from 0.79 to 0.85) and precision (ρ from 0.68 to 0.81) of predicted RUP proportions was similarly across all three Kp (Table 2.3). The sample set of Valdés et al. (2011) had similar concentrations of CP fraction C and only slightly lower or slightly greater concentrations of CP fractions B_2, and B_3 (i. e., independent variables in the equation of Valdés et al. (2011)) than the forages in the present study. Hence, compared to algorithms proposed by Kirchhof (2007) (CCC from 0.23 to 0.58), the equations of Valdés et al. (2011) (CCC from 0.58 to 0.64) better predicted the RUP proportion of tropical forages across different Kp; however, their accuracy and precision were still poor (CCC ≤ 0.65) (Table 2.3).

Table 2.1
Descriptive statistics of tropical forage grasses (n = 23) and legumes (n = 15) on concentrations of chemical composition and proportions of feed rumen-undegraded protein at rumen passage rate of 2%, 5%, and 8%/hour estimated with the *in situ* method.

	Forage grasses					Forage legumes				
	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max
Chemical composition (g/kg dry matter)										
Ash	123	119	29	76	178	74	70	16	45	99
Crude protein	117	119	34	46	201	177	174	25	135	212
Neutral-detergent fiber[1]	576	573	41	481	654	448	460	69	328	586
Acid-detergent fiber[2]	308	304	33	220	359	313	320	59	201	414
Acid-detergent lignin[3]	33	30	20	6	93	69	69	19	46	125
NDFp[4]	677	678	40	592	758	477	459	65	382	585
ADFp[5]	357	363	33	278	421	356	340	62	269	486
Feed rumen-undegraded protein (g/kg crude protein)[6]										
2%/hour	256	266	76	95	418	170	166	61	73	289
5%/hour	333	318	97	119	538	226	226	69	112	353
8%/hour	373	377	106	135	596	267	260	76	141	405

[1] Neutral-detergent fiber determined using heat-stable amylase and sodium sulfite and expressed inclusive of residual ash.
[2] Acid-detergent fiber expressed inclusive of residual ash.
[3] Acid-detergent lignin determined by solubilization of cellulose with sulfuric acid and expressed inclusive residual ash.
[4] NDFp, neutral-detergent fiber assayed with heat-stable amylase without the use of sodium sulfite using the chemical crude protein fractions method according to Licitra et al. (1996) expressed inclusive of residual ash.
[5] ADFp, acid-detergent fiber estimated using the chemical crude protein fractions method according to Licitra et al. (1996) expressed inclusive of residual ash.
[6] Rumen-undegraded crude protein estimated with the *in situ* method following the protocol of Madsen and Hvelplund (1994) with some modifications at passage rates of 2, 5, and 8%/hour.

Table 2.2
Descriptive statistics of tropical forage grasses (n = 23) and legumes (n = 15) on proportions of chemical crude protein fractions estimated according to Licitra et al. (1996) and Cornell Net Carbohydrate Protein System (CNCPS) fractions estimated according to Sniffen et al. (1992).

	Forage grasses					Forage legumes				
	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max
Chemical crude protein fractions (g/kg crude protein)[1]										
Non-protein nitrogen	367	392	85	206	521	274	273	65	160	380
Soluble protein	398	427	87	227	524	312	318	68	218	438
NDIP[2]	393	409	101	250	586	328	351	138	108	619
ADIP[3]	125	114	56	44	282	117	105	59	51	300
CNCPS crude protein fractions (g/kg crude protein) [4]										
A	367	392	85	206	521	274	273	65	160	380
B_1	31	29	19	2	72	38	32	29	1	85
B_2	209	212	53	122	338	360	360	92	155	553
B_3	268	271	77	132	422	211	247	96	46	344
C	125	114	56	44	282	117	105	59	51	300

[1] Crude protein fractions analyzed following procedure of Licitra et al. (1996).
[2] NDIP, neutral-detergent insoluble protein.
[3] ADIP, acid-detergent insoluble protein.
[4] Crude protein fractions calculated according to Sniffen et al. (1992): A, crude protein soluble in the borate-phosphate buffer and tungstic acid solution; B_1, true protein soluble in buffer solution and precipitated by the tungstic solution; B_2, true protein insoluble in buffer solution but soluble in the neutral-detergent solution; B_3, true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution; and C, true protein insoluble in the acid-detergent solution.

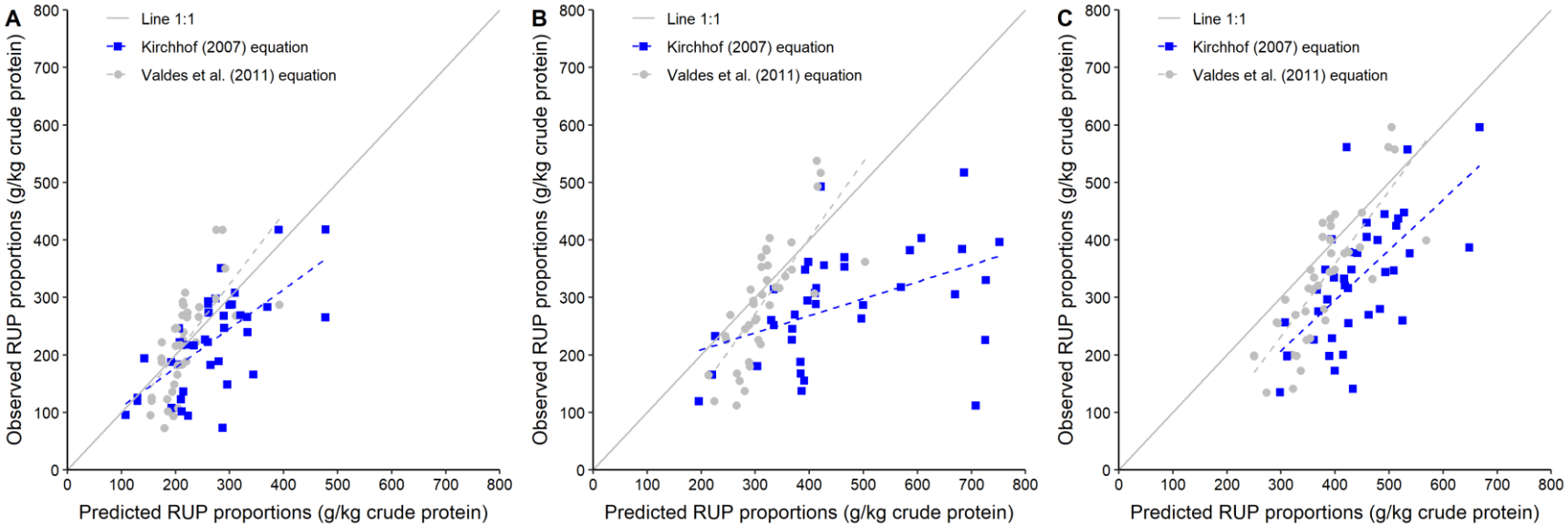


Figure 2.1
Rumen-undegraded crude protein (RUP) proportions of 23 forage grasses and 15 forage legumes that are commonly used in domestic cattle feeding in the Tropics and Subtropics evaluated at Kp of 2%/hour (A), 5%/hour (B), and 8%/hour (C) using the reference *in situ* method (i. e., observed RUP) or the chemical method (i. e., predicted RUP) and the equations of Kirchhof (2007) and Valdés et al. (2011).

Table 2.3
Predictions of the rumen-undegraded crude protein proportions as estimated with the *in situ* method and chemical method using the equations of Kirchhof (2007) and Valdés et al. (2011) at passage rate of 2%/hour (n = 38), 5%/hour (n = 35), and 8%/hour (n = 38; g/kg crude protein) of tropical forage grasses and legumes.

Kp[1]	RUP method[2]	Mean	Error index[3]		Dimensionless[4]			
			RMSE	MAPE	RSR	Concordance correlation coefficient		
			(% mean *in situ*	(% mean *in*	(0 to ∞)	Coefficient	ρ	Cb
(%/hour)			RUP)	*situ* RUP)		(-1 to 1)	(-1 to 1)	(-1 to 1)
All forages								
2	*in situ* RUP	220						
	chemical RUP_K	264	36	37	0.44	0.58	0.67	0.87
	chemical RUP_V	217	28	29	0.33	0.58	0.68	0.85
5	*in situ* RUP	281						
	chemical RUP_K	452	78	76	0.80	0.23	0.47	0.48
	chemical RUP_V	310	25	27	0.26	0.63	0.74	0.84
8	*in situ* RUP	328						
	chemical RUP_K	441	43	47	0.39	0.37	0.65	0.57
	chemical RUP_V	377	25	27	0.23	0.64	0.81	0.79
Forage grasses								
2	*in situ* RUP	256						
	chemical RUP_K	273	18	16	0.23	0.83	0.85	0.98
	chemical RUP_V	234	25	22	0.32	0.54	0.61	0.88
5	*in situ* RUP	324						
	chemical RUP_K	454	53	43	0.59	0.40	0.70	0.57
	chemical RUP_V	330	20	18	0.22	0.66	0.70	0.94
8	*in situ* RUP	373						
	chemical RUP_K	442	25	27	0.23	0.62	0.79	0.78
	chemical RUP_V	400	19	19	0.18	0.70	0.78	0.89

[1] Kp, passage rates through the rumen. [2] Feed rumen-undegraded crude protein (RUP) methods: *in situ* RUP, *in situ* RUP proportion estimated following the protocol of Madsen and Hvelplund (1994) with some modifications; chemical RUP_K, chemical RUP proportion estimated from the proportions of different crude protein and fiber fractions using the equations of Kirchhof (2007); chemical RUP_V, chemical RUP proportion estimated from the concentrations of different crude protein fractions using the equations of Valdés et al. (2011). [3] Error index includes measures of root mean square error (RMSE) and mean absolute percentage error (MAPE). [4] Dimensionless includes measures of RSR as the ratio between RMSE and SD; concordance correlation coefficient and its partitioning into correlation coefficient (ρ, i. e., precision) and bias correction factor (Cb; i. e., accuracy).

(Table 1.3 Continued)

Kp[1] (%/hour)	RUP method[2]	Mean	Error index[3]		Dimensionless[4]			
			RMSE (% mean *in situ* RUP)	MAPE (% mean *in situ* RUP)	RSR (0 to ∞)	Concordance correlation coefficient		
						Coefficient (-1 to 1)	ρ (-1 to 1)	Cb (-1 to 1)
Forage legumes								
2	*in situ* RUP	170						
	chemical RUP_K	252	65	65	1.03	0.28	0.48	0.59
	chemical RUP_V	195	33	38	0.52	0.34	0.67	0.51
5	*in situ* RUP	219						
	chemical RUP_K	450	125	125	1.79	0.07	0.25	0.26
	chemical RUP_V	280	37	40	0.52	0.33	0.67	0.49
8	*in situ* RUP	267						
	chemical RUP_K	439	69	74	0.88	0.18	0.62	0.29
	chemical RUP_V	347	36	39	0.46	0.39	0.73	0.53

[1] Kp, passage rates through the rumen.
[2] Feed rumen-undegraded crude protein (RUP) methods: *in situ* RUP, *in situ* RUP proportion estimated following the protocol of Madsen and Hvelplund (1994) with some modifications; chemical RUP_K, chemical RUP proportion estimated from the proportions of different crude protein and fiber fractions using the equations of Kirchhof (2007); chemical RUP_V, chemical RUP proportion estimated from the concentrations of different crude protein fractions using the equations of Valdés et al. (2011).
[3] Error index includes measures of root mean square error (RMSE) and mean absolute percentage error (MAPE).
[4] Dimensionless includes measures of RSR as the ratio between RMSE and SD; concordance correlation coefficient and its partitioning into correlation coefficient (ρ, i. e., precision) and bias correction factor (Cb; i. e., accuracy).

Table 2.4
Multivariate regression models developed to estimate the proportions (g/kg crude protein (CP)) of rumen-undegraded crude protein (RUP) of tropical forage grasses and legumes.

Tropical forage grasses (n = 23)[1]	$[\beta_0 + \beta_1 \times C + \beta_2 \times Kp - \beta_3 \times ADFp + \beta_4 \times (B_3 + C)] \times 1000 / CP$
Tropical forage legumes (n = 15)[2]	$[\beta_5 + \beta_6 \times Kp + \beta_7 \times NDFp + \beta_8 \times ADFp + \beta_9 \times Lignin_{(sa)}] \times 1000 / CP$

All independent variables are expressed in g/kg dry matter; β_0-β_9, paramater estimates.
[1] C, protein bound to the acid-detergent fiber; Kp, rumen passage rate (2, 5, or 8%/hour); ADFp, acid-detergent fiber estimated using the chemical CP fractions method; Sum of CP fraction B_3 and C represents the concentration of the cell-wall-bound protein.
[2] NDFp, neutral-detergent fiber assayed with heat-stable amylase and without the use of sodium sulfite using the chemical CP fractions method according to Licitra et al. (1996) expressed inclusive of residual ash; $Lignin_{(sa)}$, acid-detergent lignin assayed with the use of sulfuric acid.

Table 2.5
Statistical parameters of multivariate regression and cross-validation (CV) analyses of the models developed to estimate the proportions (g/kg crude protein (CP)) of rumen-undegraded crude protein (RUP) of tropical forage grasses and legumes.

	Tropical forage grasses				Tropical forage legumes		
	Parameter estimate	SE	*P*-value		Parameter estimate	SE	*P*-value
β_0	21.43	8.01	< 0.01	β_5	-25.09	6.98	< 0.01
β_1	1.07	0.15	< 0.01	β_6	2.88	0.36	< 0.01
β_2	2.23	0.25	< 0.01	β_7	0.26	0.03	< 0.01
β_3	-0.06	0.02	< 0.01	β_8	-0.25	0.03	< 0.01
β_4	0.25	0.06	< 0.01	β_9	0.18	0.07	< 0.01
Adj. R^2[1]	0.82			Adj. R^2	0.79		
RMSE[2]	13.86			RMSE	15.07		
MAPE[3]	13.36			MAPE	13.29		
CV–RMSE	16.60			CV–RMSE	16.70		

[1] Adj. R^2, determination coefficient adjusted by the number of predictors in the model.
[2] RMSE, root mean square error expressed as a percentage of the observed mean.
[3] MAPE, mean absolute percentage error expressed as a percentage of the observed mean.

The poor prediction of the RUP proportions by the equations of Kirchhof (2007) and Valdés et al. (2011) were related to their low accuracy rather than a poor precision (Table 2.3). The precision measured as ρ (i. e., moderate to strong precision using Evans (1996) scale) was similar to the one reported by Edmunds et al. (2012) (i. e., strong precision), Kirchhof (2007) (i. e., strong precision), and Valdés et al. (2011) (i. e., strong to very strong precision), even though both, Kirchhof (2007) and Valdés et al. (2011), evaluated their estimates using the same dataset as for developing their own equations. Nevertheless, the RMSE of the relationships between the RUP proportions determined *in situ* or using the equations of Kirchhof (2007) in the present study were greater than the RMSE reported by Kirchhof (2007) and Edmunds et al. (2012). The RMSE could not be calculated for the equation of Valdés et al. (2011), because RUP proportions of their individual feed samples were not provided. Hence, the chemical method could be used to predict the RUP proportions in tropical forages due to its good precision; however, its accuracy needs to be improved.

The accuracy and precision of predicted RUP proportions using the equations of Kirchhof (2007), and Valdés et al. (2011) were greater for forage grasses than forage legumes (i. e., lower RMSE, MAPE, RSR, and greater CCC) (Table 2.3). This result confirms our hypothesis that a single equation will not be feasible to predict the RUP proportion across both, tropical forage grasses and legumes. Similarly, the RUP proportion of temperate legumes were poorly predicted by the equations of Kirchhof (2007) in the study of Edmunds et al. (2012). The lower accuracy and precision for the forage legumes (Cb from 0.26 to 0.59 and ρ from 0.25 to 0.73) than for forage grasses (Cb from 0.57 to 0.98 and ρ from 0.61 to 0.85) determined in the present study might be due to the small proportion of forage legumes and the limited number of different legume species in the sample sets of Kirchhof (2007) and Valdés et al. (2011). Thus, the equations of Kirchhof (2007) and Valdés et al. (2011) might not be very robust, be highly specific to their sample sets (Edmunds et al., 2012) and be more appropriate for grasses than for legumes (Appendix 2.4).

2.4.4 Prediction of rumen-undegraded crude protein proportions of forages

Since available equations could not predict accurately and precisely RUP proportions in forage grasses and legumes across all Kp, new equations for each feed type were developed in the present study (Tables 2.4 and 2.5). The concentrations of fraction C, ADFp, and cell-wall-bound protein (i. e., sum of the fractions B_3 and C) as well as Kp were selected to predict the RUP proportion of the forage grasses. These independent variables were also retained in the equations of Valdés et al. (2011) (i. e., B_2, B_3, and C) and Kirchhof (2007) (i. e., CP, fractions A, B_1, B_2, C, NDFp, and ADFp). The CP concentration was not retained in

any of the models in the present study, probably because it was highly correlated with the concentration of CP not bound to the cell wall (ρ = 0.91) in the forage grasses.

The concentration of fraction C explained the greatest proportion of the variance in the RUP proportions of the forage grasses as estimated with the *in situ* method, which agrees with previous findings that fractions B_3 and C are the most important predictors of RUP proportions in ruminant forages (Fox et al., 2004; Schroeder, 2004; Tran et al., 2009), mainly because most fiber-bound-protein is slowly degradable in the rumen, thus accounting for the majority of RUP (Sniffen et al., 1992). The low reproducibility of fraction C when determined wet-chemically (Bovera et al., 2003) may hamper prediction of RUP proportions by our equations. Standardizing the procedures to analyze the CP fractions could improve the precision of RUP predictions of forage grasses.

The independent variables selected to predict the RUP proportion of forage legumes were Kp as well as the concentrations of NDFp, ADFp, and $Lignin_{(sa)}$. The concentrations of CP fractions were not selected, in contrast to the equations developed by Kirchhof (2007) and Valdés et al. (2011). Yet, NDFp concentration comprises aNDF, NDIP (i. e., fractions B_3 and C), and ash, whereas the ADFp concentrations includes ADF, ADIP (i. e., fraction C), and ash. Thus, the fractions B_3 and C were also indirectly considered in our equations for forage legumes.

The Kp explained the greatest proportion of the variance in the RUP proportion of forage legumes as estimated with the *in situ* method, followed by the concentrations of NDFp and ADFp. Contrary to NDFp and ADFp analyses (Licitra et al., 1996), sodium sulfite is used for NDF analysis (VDLUFA, 2007) to reduce contamination of the residue with protein (Mertens, 2002), yielding lower estimates of NDF and ADF in particular in forage legumes (Gomes et al., 2012). Hence, concentrations of NDFp and ADFp should not be replaced by those of aNDF and ADF in the equations proposed here.

The RMSE from the multiple linear regression (RMSE from 13 to 15%) and the k-fold cross-validation of the developed equations (RMSE from 13.3 to 13.4%) (Table 2.5) suggest that RUP proportions of tropical forage grasses and legumes can be predicted with acceptable validity. Nevertheless, an independent larger dataset is required to further validate the prediction of the RUP proportion of tropical forages with the chemical method.

2.5 Conclusions

The RUP of tropical forage grasses and legumes estimated with the *in situ* method can be predicted using the chemical method. However, regression equations based on concentrations and/or proportions of total CP as well as concentrations of CP and fiber fractions developed for temperate forages were not accurate and precise enough to predict RUP proportions of tropical forages using one single equation for each Kp. Instead, our proposed equations by feed type can be used to estimate RUP proportion of tropical forage grasses and legumes with a similar chemical composition than the forage samples included in the present study. However, further research is needed to clarify reasons for such differences in the accuracy and precision of the chemical method between tropical forage grasses and legumes across Kp.

2.6 Acknowledgments

We appreciate the technical assistance of Mr. H. Baumgaertner, Dr. J. Castro-Montoya, companion doctoral students of the working group of Animal Nutrition and Rangeland Management in the Tropics and Subtropics, University of Hohenheim, and M.S. Felipe de Mendiburu, National Agrarian University La Molina, during laboratory and statistical analysis. The supply of forage samples by the research institutions in Brazil, Costa Rica, El Salvador, Ethiopia, Kenya, and Peru, as well as the supply of rumen fluid and the support on the *in situ* trial by the Institute of Animal Science of the University of Hohenheim are much appreciated.

Appendix 2.1

Concentrations of crude protein and fiber fractions (g/kg dry matter) of common feedstuffs used in tropical husbandry systems at rumen passage rates of 2, 5, and 8%/hour.

Forage species	Origin[1]	Season[2]	Period[3]	CP[4]	NDFp[5]	ADFp[6]	Lignin$_{(sa)}$[7]
Tropical forage grasses (n = 23)							
Andropogon gayanus Kunth	ES	RS	2	81	677	370	14
Andropogon gayanus Kunth	KY	DS	1	85	686	363	32
Brachiaria brizantha (Hochst. ex A. Rich.) Stapf	PE	RS	1	46	690	324	22
Brachiaria brizantha x *Brachiaria ruziziensis* R. Germ. and C.M. Evrard	KY	DS	2	110	648	363	64
Cenchrus ciliaris L.	KY	DS	2	140	688	383	27
Chloris gayana Kunth	KY	DS	2	133	713	349	48
Cynodon dactylon (L.) Pers.	KY	DS	2	139	758	331	39
Cynodon nlemfuensis Vanderyst	KY	DS	2	150	727	347	30
Digitaria decumbens Stent	PE	RS	2	75	626	330	35
Digitaria eriantha Steud.	KY	DS	2	111	656	368	36
Eragrostis echinochloidea Stapf	KY	DS	2	120	722	354	30
Hyparrhenia rufa (Nees) Stapf	ET	RS	2	116	686	343	16
Hyparrhenia rufa (Nees) Stapf	KY	DS	1	96	740	412	52
Melinis minutiflora P. Beauv.	KY	DS	2	112	678	382	93
Panicum coloratum L.	ET	RS	2	150	602	278	8
Panicum coloratum L.	KY	DS	1	140	669	318	36
Panicum maximum Jacq.	PE	RS	2	60	653	421	29
Pennisetum clandestinum Hochst. Ex Chiov.	ET	RS	2	201	592	366	6
Pennisetum clandestinum Hochst. Ex Chiov.	KY	DS	1	146	707	315	64
Pennisetum pedicellatum Trin.	ET	RS	1	125	666	336	6
Pennisetum purpureum Schumach.	ET	RS	2	119	637	395	12
Pennisetum purpureum Schumach.	KY	DS	1	92	671	392	29
Tripsacum andersonii J. R. Gray	KY	DS	2	143	681	373	27

[1] BR, Brazil; CR, Costa Rica; ES, El Salvador; ET, Ethiopia; KE, Kenya; PE, Peru. [2] DS, dry season; RS, rainy season. [3] 1, feed sample incubated during the first period; 2, feed sample incubated during the second period. [4] CP, crude protein. [5] NDFp, neutral-detergent fiber assayed with heat-stable amylase and without the use of sodium sulfite using the chemical CP fractions method according to Licitra et al. (1996) and expressed inclusive of residual ash. [6] ADFp, acid-detergent fiber estimated using the chemical CP fractions method according to Licitra et al. (1996) expressed inclusive of residual ash. [7] Lignin$_{(sa)}$, acid-detergent lignin determined by solubilization of cellulose with sulfuric acid expressed inclusive residual ash.

(Appendix 2.1 Continued)

Forage species	Origin[1]	Season[2]	Period[3]	CP[4]	NDFp[5]	ADFp[6]	Lignin$_{(sa)}$[7]
Tropical forage legumes (n = 15)							
Arachis glabrata Benth.	ES	RS	2	152	412	269	46
Arachis pintoi Krapov. & W. C. Greg.	BR	DS	1	174	382	340	65
Calopogonium mucunoides Desv.	BR	DS	1	194	472	329	61
Canavalia ensiformis (L.) DC.	ES	RS	1	185	450	367	64
Centrosema (DC.) Benth.	BR	DS	1	204	558	369	93
Crotalaria longirostrata Hook. and Arn.	ES	RS	1	135	560	454	75
Dolichos lablab L.	ES	RS	1	154	522	370	69
Dolichos lablab L.	ES	RS	2	154	522	370	69
Glycine max (L.) Merr.	ES	RS	1	211	388	277	50
Glycine max (L.) Merr.	ES	RS	2	211	388	277	50
Glyricidia sepium (Jacq.) Kunth.	ES	RS	1	212	459	303	71
Macroptilium atropurpureum (DC.) Urb.	PE	RS	1	198	445	333	74
Mucuna pruriens (L.) DC.	ES	RS	1	157	560	433	125
Pueraria phaseoloides (Roxb.) Benth.	BR	DS	1	194	530	436	68
Stylosanthes guianensis (Aubl.) Sw.	PE	RS	2	158	442	315	69
Stylosanthes guianensis (Aubl.) Sw.	CR	RS	1	154	585	486	84
Vigna sinensis (L.) Savi ex Hassk.	ES	RS	1	165	440	330	47

[1] BR, Brazil; CR, Costa Rica; ES, El Salvador; ET, Ethiopia; KE, Kenya; PE, Peru.
[2] DS, dry season; RS, rainy season.
[3] 1, feed sample incubated during the first period; 2, feed sample incubated during the second period.
[4] CP, crude protein.
[5] NDFp, neutral-detergent fiber assayed with heat-stable amylase and without the use of sodium sulfite using the chemical CP fractions method according to Licitra et al. (1996) and expressed inclusive of residual ash.
[6] ADFp, acid-detergent fiber estimated using the chemical CP fractions method according to Licitra et al. (1996) expressed inclusive of residual ash.
[7] Lignin$_{(sa)}$, acid-detergent lignin determined by solubilization of cellulose with sulfuric acid expressed inclusive residual ash.

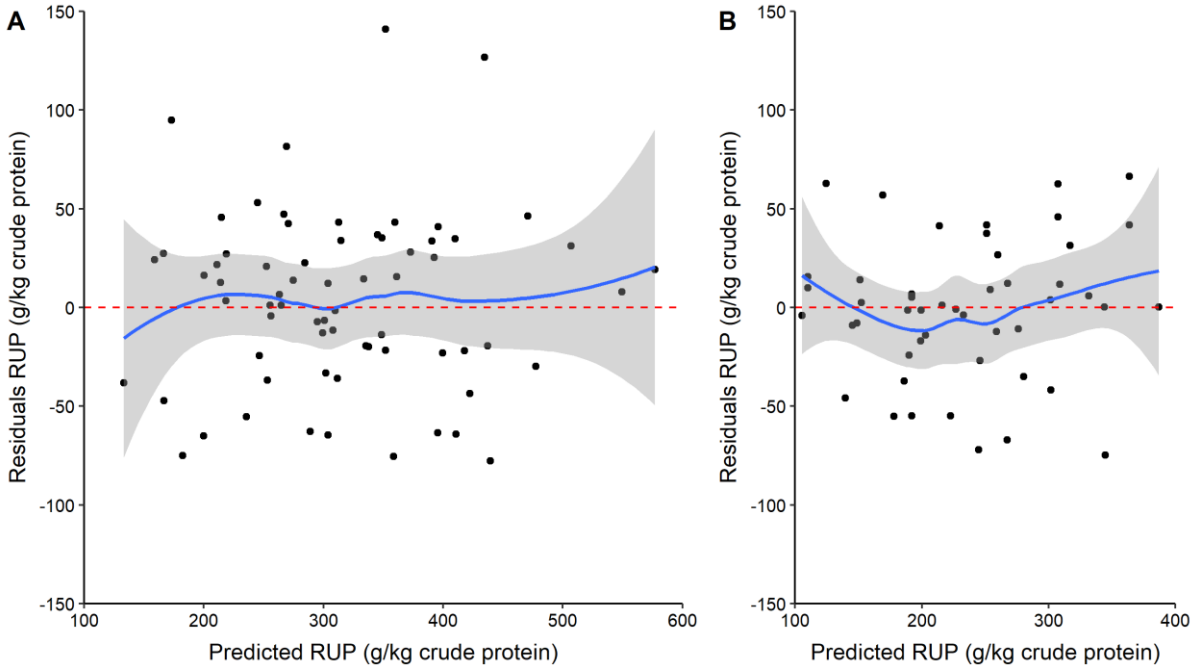
Appendix 2.2

Rumen-undegraded crude protein proportion (RUP; g/kg crude protein) estimated *in situ* and predicted with Kirchhof (2007; RUP_K), Valdés et al. (2011; RUP_V), and the equations developed in the present study (RUP_S; expressed as a difference (g/kg crude protein) from the RUP estimated *in situ* and the RUP predicted) of tropical forage grasses and legumes at a rumen passage rate (Kp) of 2, 5, and 8%/hour.

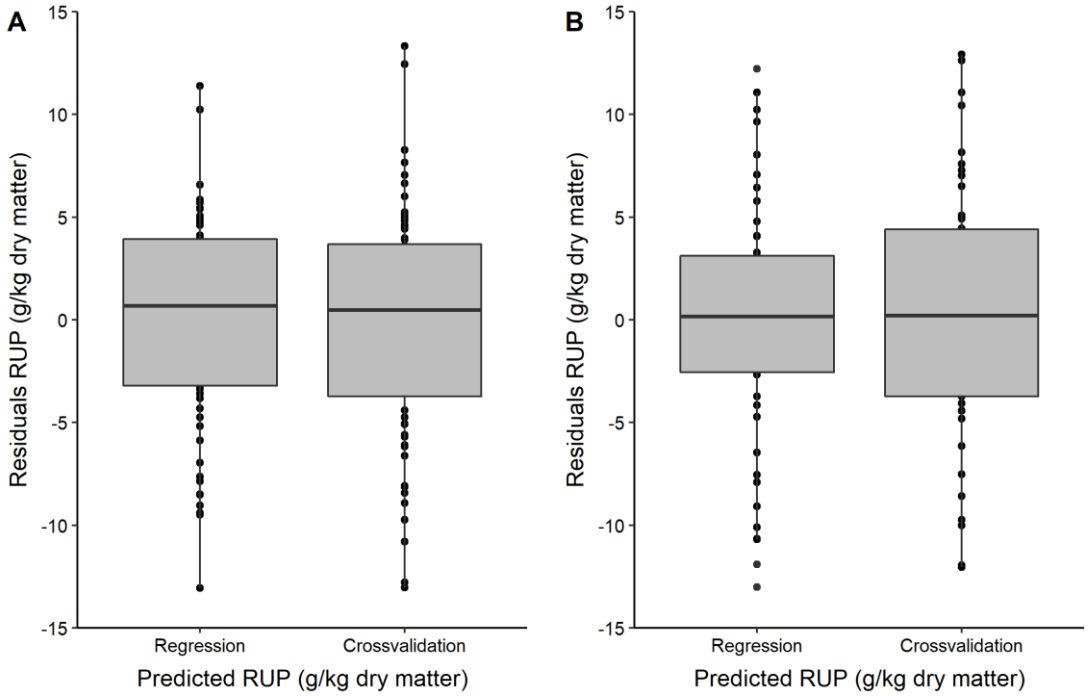
Feedstuffs	RUP at Kp 2%/hour				RUP at Kp 5%/hour				RUP at Kp 8%/hour			
	RUP	RUP_K	RUP_V	RUP_S	RUP	RUP_K	RUP_V	RUP_S	RUP	RUP_K	RUP_V	RUP_S
Tropical forage grasses (n = 23)												
Andropogon gayanus (Kunth)	351	66	59	81	493	72	78	141	561	140	63	127
Andropogon gayanus (Kunth)	418	27	131	25	517	-169	97	46	558	23	48	8
Brachiaria brizantha (Hochst. ex A. Rich.) Stapf	288	-17	-105	-7	362	-37	-141	-78	400	-79	-169	-185
Brachiaria brizantha x Brachiaria ruziziensis R. Germ. and C.M. Evrard	227	-28	13	13	288	-124	-8	14	316	-108	-35	-19
Cenchrus ciliaris L.	239	-94	24	-65	330	-396	9	-22	377	-162	-16	-23
Chloris gayana Kunth	308	-2	90	-2	403	-204	77	43	445	-47	45	35
Cynodon dactylon (L.) Pers.	246	40	47	27	314	-20	23	47	348	-34	-6	34
Cynodon nlemfuensis Vanderyst	222	14	48	3	270	-103	16	7	296	-89	-11	-12
Digitaria decumbens Stent	298	24	24	53	348	-44	-19	14	379	-47	-47	-44
Digitaria eriantha Steud.	273	12	51	21	356	-71	33	43	401	7	10	28
Eragrostis echinochloidea Stapf	217	5	14	16	252	-82	-36	-4	276	-93	-69	-36
Hyparrhenia rufa (Nees) Stapf	222	-38	-16	-24	316	-97	-29	12	377	-65	-40	16
Hyparrhenia rufa (Nees) Stapf	418	-60	142	-19	538	-569	125	31	596	-71	92	19
Melinis minutiflora P. Beauv.	283	-87	39	-75	396	-356	29	-22	448	-80	-2	-30
Panicum coloratum L.	194	52	20	27	233	7	-12	22	256	-51	-36	1
Panicum coloratum L.	216	-18	16	-37	294	-102	-2	-7	335	-63	-27	-14
Panicum maximum Jacq.	268	-21	-43	95	307	-103	-102	23	332	-85	-137	-63
Pennisetum clandestinum Hochst. Ex Chiov.	95	-12	-58	-38	119	-76	-105	-47	135	-164	-139	-65
Pennisetum clandestinum Hochst. Ex Chiov.	287	-13	72	-13	382	-204	61	37	425	-89	32	34
Pennisetum pedicellatum Trin.	107	-85	-98	-75	180	-123	-110	-55	226	-135	-121	-63
Pennisetum purpureum Schumach.	183	-22	-29	24	260	-69	-39	46	313	-53	-45	42
Pennisetum purpureum Schumach.	266	-67	24	1	318	-252	-19	-20	347	-162	-50	-64
Tripsacum andersonii J. R. Gray	269	-51	56	-33	384	-298	65	35	437	-80	45	41

(Appendix 2.2 Continued)

Feedstuffs	*RUP at Kp 2%/hour*				*RUP at Kp 5%/hour*				*RUP at Kp 8%/hour*			
	RUP	*RUPK*	*RUPV*	*RUPS*	*RUP*	*RUPK*	*RUPV*	*RUPS*	*RUP*	*RUPK*	*RUPV*	*RUPS*
Tropical forage legumes (n = 15)												
Arachis glabrata Benth.	189	-91	-31	-14	287	-213	-40	27	348	-82	-51	32
Arachis pintoi Krapov. & W. C. Greg.	73	-214	-107	24	112	-596	-154	13	141	-292	-181	-8
Calopogonium mucunoides Desv.	123	-87	-61	-55	168	-217	-98	-55	200	-215	-121	-67
Canavalia ensiformis (L.) DC.	102	-110	-84	-4	155	-235	-116	3	198	-191	-131	-1
Centrosema (DC.) Benth.	247	-43	44	-12	305	-365	-8	4	344	-149	-45	0
Crotalaria longirostrata Hook. and Arn.	217	-2	8	1	245	-124	-36	-35	270	-193	-57	-75
Dolichos lablab L.	289	28	75	38	353	-112	42	46	405	-53	29	42
Dolichos lablab L.	293	32	79	42	370	-95	59	63	430	-29	53	66
Glycine max (L.) Merr.	126	-4	-30	16	165	-55	-48	14	197	-114	-53	5
Glycine max (L.) Merr.	120	-10	-35	10	165	-55	-48	14	199	-112	-51	7
Glyricidia sepium (Jacq.) Kunth.	149	-147	-49	-37	226	-500	-79	-1	280	-203	-100	12
Macroptilium atropurpureum (DC.) Urb.	136	-78	-58	-9	188	-196	-101	-1	229	-166	-125	-4
Mucuna pruriens (L.) DC.	265	-212	44	-11	337	-1389	-18	6	387	-262	-59	0
Pueraria phaseoloides (Roxb.) Benth.	187	-5	13	63	226	-142	-20	57	255	-169	-39	41
Stylosanthes guianensis (Aubl.) Sw.	182	-83	-22	-17	263	-233	-39	9	321	-98	-48	12
Stylosanthes guianensis (Aubl.) Sw.	166	-179	-37	-24	219	-670	-91	-27	260	-265	-122	-42
Vigna sinensis (L.) Savi ex Hassk.	94	-129	-102	-46	137	-248	-143	-55	173	-227	-164	-72



Appendix 2.3
Residuals rumen-undegraded crude protein proportion (RUP) proportion (RUP measured *in situ* – RUP predicted with the developed model) versus predicted RUP proportion of tropical forage grasses (A) and legumes (B).



Appendix 2.4
Boxplot of the residuals (observed – predicted) between rumen-undegraded crude protein proportion (RUP) measured *in situ* (observed) and the RUP proportion predicted with the regression model (R) and the cross-validation (CV) model of tropical forage grasses (A) and forage legumes (B).

2.7 References

AFRC (Agricultural and Food Research Council). 1993. Energy and protein requirements of ruminants: An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. CAB International, Wallingford, UK.

Ajayi, F. T., Babayemi, O. J., Taiwo, A. A. 2007. Effects of *Stylosanthes guianensis* and *Aeschynomene histrix* on the yield, proximate composition and *in-situ* dry matter and crude protein degradation of *Panicum maximum* (Ntchisi). Livest. Res. Rural 19, 32.

Bovera, F., Spanghero, M., Galassi, G., Masoero, F., Buccioni, A. 2003. Repeatability and reproducibility of the Cornell Net Carbohydrate and Protein System analytical determinations. Ital. J. Anim. Sci. 2, 41–50. https://doi.org/10.4081/ijas.2003.41

Bowen, M. K., Poppi, D. P., McLennan, S. R. 2008. Ruminal protein degradability of a range of tropical pastures. Aust. J. Exp. Agric., 48, 806–810. https://doi.org/10.1071/EA07414

Chrenková, M., Čerešňáková, Z., Weisbjerg, M., Formelová, Z., Poláčiková, M., Vondráková, M. 2014. Characterization of proteins in feeds according to the CNCPS and comparison to *in situ* parameters. Czech J. Anim. Sci. 59, 288–295. https://doi.org/10.17221/7499-CJAS

Detmann, E., Paulino, M. F., Valadares Filho, S. C. 2010. Otimização do uso de recursos forrageiros basais. Presented at the 3rd International Symposium on Beef Cattle Production, pp. 191–240.

Dhanoa, M. S., France, J., López, S., Dijkstra, J., Lister, S. J., Davies, D. R., Bannink, A. 1999. Correcting the calculation of extent of degradation to account for particulate matter loss at zero time when applying the polyester bag method. J. Anim. Sci. 77, 3385–3391. https://doi.org/10.2527/1999.77123385x

Edmunds, B., Südekum, K.-H., Spiekers, H., Schuster, M., Schwarz, F. J. 2012. Estimating utilisable crude protein at the duodenum, a precursor to metabolisable protein for ruminants, from forages using a modified gas test. Anim. Feed Sci. Technol. 175, 106–113. https://doi.org/10.1016/j.anifeedsci.2012.05.003

Evans, J. D. 1996. Straightforward statistics for the behavioral sciences. Thomson Brooks/Cole Publishing Company, Pacific Grove, USA.

Evitayani, Warly, L., Fariani, A., Ichinohe, T., Fujihara, T. 2004. Study on nutritive value of tropical forages in North Sumatra, Indonesia. Asian-Australas. J. Anim. Sci. 17, 1518–1523. https://doi.org/10.5713/ajas.2004.1518

Fox, D. G., Tedeschi, L. O., Tylutki, T. P, Russell, J. B., Van Amburgh, M. E., Chase, L. E., Pell, A. N., Overton, T. R., 2004. The Net Carbohydrate and Protein System for evaluating herd nutrition and nutrient excretion: model documentation. Anim. Feed Sci. Technol. 112, 29–78. https://doi.org/10.1016/j.anifeedsci.2003.10.006

Gomes, D. I., Detmann, E., Valadares Filho, S. C., Mezzomo, R., Krish de Paiva Souza, N., de Queiroz, A. C., da Silva, K., Detmann, C. 2012. Technical note: Evaluation of sodium sulfite and protein correction in analyses of fibrous compounds in tropical forages. R. Bras. Zootec. 41, 225 –231. https://doi.org/10.1590/S1516-35982012000100032

Grubb, F. 1969. Procedures for detecting outlying observations in samples. Technometrics 11, 1–21.

Hair, J. F., Anderson, R. E., Tatham, R. L., Black, W. C. 1995. Multivariate data analysis. New York Macmillan, USA.

Hvelplund, T., Weisbjerg, M. 2000. *In situ* techniques for the estimation of protein degradability. In: Givens, D.I., Owen, E., Axford, R.F.E., Omed, H.M. 2000. Forage evaluation in ruminant nutrition, CABI Publishing, New York, USA, pp. 233–258.

INRA (Institut National de la Recherche Agronomique). 2018. INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, Netherlands.

Juárez, F.I., Pell, A. N., Blake, R. W., Montero Lagunes, M., Pinos Rodriguez, J. M. 2018. *In vitro* ruminal degradation of neutral detergent fiber insoluble protein from tropical pastures fertilized with nitrogen. Rev. Mex. Cienc. Pecu. 9, 588–600. https://doi.org/10.22319/rmcp.v9i3.4490

Khamseekhiew, B., Liang, J. B., Wong, C. C., Jalan, Z. A. 2001. Ruminal and intestinal digestibility of some tropical legume forages. Asian-Australas. J. Anim. Sci. 14, 321–325. https://doi.org/10.5713/ajas.2001.321

Khandaker, Z. H., Tareque, A. M. M. 1996. Studies on protein degradabilities of feedstuffs in Bangladesh. Asian-Australas. J. Anim. Sci. 9, 637–642. https://doi.org/10.5713/ajas.1996.637

Kirchhof, S. 2007. Untersuchungen zur Kinetik des ruminalen *in situ*-Nährstoffabbaus von Grünlandaufwüchsen des Alpenraumes unterschiedlicher Vegetationsstadien sowie von Maissilagen und Heu - ein Beitrag zur Weiterentwicklung der Rationsgestaltung für Milchkühe. [Dissertation to obtain the doctoral degree of Agricultural Sciences]. University of Kiel.

Klopfenstein, T. J., Mass, R. A., Reighton, K. W., Patterson, H. H. 2001. Estimating forage protein degradation in the rumen. J. Anim. Sci. 79, 208–217. https://doi.org/10.2527/jas2001.79E-SupplE208x

Krawielitzki, K., Schmidt, T., Voigt, J., Kowalczyk, J., Gabel, M. 2006. Dynamics of microbial contamination of protein during ruminal *in situ* incubation of feedstuffs. Anim. Feed Sci. Technol. 15, 313–328. https://doi.org/10.22358/jafs/66903/2006

La O, O., Chongo, B., Delgado, D., Ruiz, T., Ruiz, O. 2006. Fraccionamiento proteico y digestión ruminal de nutrientes de siratro (*Macroptilium atropurpureum*). Rev. Cuba. Cienc. Agríc. 40, 315–320.

Lanzas, C., Broderick, G. A., Fox, D. G. 2008. Improved feed protein fractionation schemes for formulating rations with the Cornell net carbohydrate and protein system. J. Dairy Sci. 91, 4881–4891. https://doi.org/10.3168/jds.2008-1440

Lee, M. A. 2018. A global comparison of the nutritive values of forage plants grown in contrasting environments. J. Plant. Res. 131, 641–654. https://doi.org/10.1007/s10265-018-1024-y

Licitra, G., Hernandez, T. M., Van Soest, P. J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347–358. https://doi.org/10.1016/0377-8401(95)00837-3

Lin, L. I.-K. 1989. A Concordance correlation coefficient to evaluate reproducibility. Biometrics, 45(1), 255-268. PMID: 2720055.

Lowe, K. F., Hume, D. E., Fulkerson, W. J. 2011. Forage and Pastures: Perennial forage and pasture crops – species and varieties. In: Abbas, A., Abd El-Salam, M.H., Afonso, C.F., Aherne, S.A., Ahmadzadeh, A., Aitken, S.L., Akers, R.M., Akuzawa, R., Alhadrami, G.A., Almeida, R.A., Alvarez, V.B., Amigo, L., Anand, S., Anand, S.K., Andrén, A., Andrews, J., Antelli, K., Ardö, Y., Arora, S., Arve, A.M., Asakuma, S., Asperger, H., Atamer, Z., Auldist, M., Aureli, P., Auty, M., Avendano-Reyes, L., Averdunk, G. , Awad, S., Azaïs-Braesco. V. Encyclopedia of Dairy Sciences. Elsevier, Mississippi, USA, pp. 576–585.

Madsen, J., Hvelplund, T. 1994. Prediction of *in situ* protein degradability in the rumen: Results of a European ringtest. Livest. Prod. Sci. 39, 201–212. https://doi.org/10.1016/0301-6226(94)90185-6

Maindonald, J. H., Braun, W. J. 2010. Data analysis and graphics using R: an example-based approach. Cambridge University Press. Cambridge, UK.

Mass, R. A., Lardy, G. P., Grant, R. J., Klopfenstein, T. J., 1999. *In situ* neutral detergent insoluble nitrogen as a method for measuring forage protein degradability. J. Anim. Sci. 77, 1565–1571. 10.2527/1999.7761565x

Mathis, C. P., Cochran, R. C., Vanzant, E. S., Abdelgadir, I. E. O., Heldt, J. S., Olson, K. C., Johnson, D. E., Caton, J., Faulkner, D. B., Horn, G., Paisley, S., Mass, R., Moore, K., Halgerson, J. 2001. A collaborative study comparing an *in situ* protocol with single time-point enzyme assays for estimating ruminal protein degradability of different forages. Anim. Feed Sci. Technol. 93, 31–42. https://doi.org/10.1016/S0377-8401(01)00273-5

McBride, G. B. 2005. A proposal for strength-of-agreement criteria for Lin's concordance correlation coefficient. NIWA Client Report: HAM2005-062, Hamilton, New Zealand.

Melesse, A., Steingass, H., Schollenberger, M., Rodehutscord, M. 2017. Screening of common tropical grass and legume forages in Ethiopia for their nutrient composition and methane production profile *in vitro*. Trop. Grassl.- Forrajes Trop. 5, 63–175. https://doi.org/10.17138/tgft(5)163-175

Mertens, D. V. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. AOAC Int. 85 (6), 1217–1240. PMID: 12477183.

Mondal, G., Walli, T. K., Patra, A. K. 2008. *In vitro* and *in sacco* ruminal protein degradability of common Indian feed ingredients. Livest. Res. Rural Dev. 20. http://www.lrrd.org/lrrd20/4/mond20063.htm

Mupangwa, J. F., Ngongoni, N. T., Hamudikuwanda, H. 2003. Effects of stage of maturity and method of drying on *in situ* nitrogen degradability of fresh herbage of *Cassia rotundifolia*, *Lablab purpureus* and *Macroptilium atropurpureum*. Livest. Res. Rural 15, 35.

NorFor (Nordic Feed Evaluation System). 2011. The Nordic feed evaluation system. Wageningen Academic Publishers, Wageningen, Netherlands.

NRC (National Research Council). 2001. Nutrient Requirements of Dairy Cattle. The National Academies Press, Washington DC, USA. https://doi.org/10.17226/9825

Onyango, A. A., Dickhoefer, U., Rufino, M. C., Butterbach-Bahl, K., Goopy, J. P. 2019. Temporal and spatial variability in the nutritive value of pasture vegetation and supplement feedstuffs for domestic ruminants in Western Kenya. Asian-Australas. J. Anim. Sci. 32, 637–647. 10.5713/ajas.18.0114

Ramírez Lozano, R. G., González Rodríguez, H., García Dessommes, G. 2002. Chemical composition and rumen digestion of forage from Kleingrass (*Panicum coloratum*). Interciencia. 27, 705–709. ISSN: 0378-1844

Salazar-Cubillas, K., Dickhoefer, U. 2018. Estimation of CNCPS protein fractions from proximate nutrient and fiber concentration in tropical ruminant forages. Presented at Tropentag, p. 32.

Salazar-Cubillas, K., Dickhoefer, U. 2019. Evaluating the accuracy of conceptual models for predicting the nitrogen excretion of cattle in tropical environments. Presented at the International Symposium on Ruminant Physiology 2019, p. 487.

Schroeder, J. W. 2004. Forage Nutrition for Ruminants. NDSU Extension Service. North Dakota State University, Fargo, USA.

Shannak, S., Südekum, K.-H., Susenbeth, A. 2000. Estimating ruminal crude protein degradation with *in situ* and chemical fractionation procedures. Anim. Feed Sci. Technol. 85, 195–214. https://doi.org/10.1016/S0377-8401(00)00146-2

Singh, S., Kushwaha, B. P., Nag, S. K., Mishra, A. K., Singh, A., Anele, U. Y. 2012. *In vitro* ruminal fermentation, protein and carbohydrate fractionation, methane production and prediction of twelve commonly used Indian green forages. Anim. Feed Sci. Technol. 178, 2–11. https://doi.org/10.1016/j.anifeedsci.2012.08.019

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., Russell, J. B. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 70, 3562– 3577. https://doi.org/10.2527/1992.701135 62x

Stern, M. D., Bach, A., Calsamiglia, S. 1997. Alternative techniques for measuring nutrient digestion in ruminants. J. Anim. Sci. 75, 2256– 276. https://doi.org/10.2527/1997.758 2256x

Tedeschi, L. O., Fox, D. G., Pell, A. N., Lanna, D. P. D., Boin, C. 2002. Development and evaluation of a tropical feed library for the Cornell Net Carbohydrate and Protein System Model. Sci. Agr. 59, 1–18. https://doi.org/10.1590/S0103-90162002000100001

Tran, H., Salgado, P., Lecomte, P. 2009. Specie, climate, and fertilizer effects on grass fibre and protein in tropical environments. J. Agric. Sci. 147, 555–568. https://doi.org/10.1017/S0021859609008752

Valarini, M. J., Possenti, R. A. 2006. Research note: Nutritive value of a range of tropical forage legumes. Trop. Grassl. -Forrajes Trop. 40, 183–187.

Valdés, C., Andrés, S., Giráldez, F. J., García, R., Calleja, A. 2006. Potential use of visible and near infrared reflectance spectroscopy for the estimation of nitrogen fractions in forages harvested from permanent meadows. J. Sci. Food Agric. 86, 308–314. https://doi.org/10.1002/jsfa.2309

Valdés, C., Andrés, S., Giráldez, F. J., García, R., Peláez, R., Calleja, A. 2011. Estimación de la proteína no degradable en el rumen de pastos de la Montaña Leonesa empleando el CNCPS (Cornell). Pastos, paisajes culturales entre tradición y nuevos paradigmas del siglo XXI. Sociedad Española para el Estudio de los Pastos, pp. 401–406.

van der Koelen, C. J., Goedhart, P. W., van Vuuren, A. M., Savoini, G. 1992. Sources of variation of the *in situ* nylon bag technique. Anim. Feed Sci. Technol. 38, 35–42. https://doi.org/10.1016/0377-8401(92)90074-G

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. J. Dairy Sci. 74 (10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

VDLUFA (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten). 2012. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik: (Methodenbuch III). Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

Weisbjerg, M. R., Bhargava, P. K., Hvelplund, T., Madsen, J. 1990. Use of degradation curves in feed evaluation. Report from the National Institute of Animal Science, Denmark.

Wigati, S., Kustantinah, A., Wiyanto, E., Ørskov, E. R. 2014. *In sacco* degradability of six different tropical feedstuffs. Presented at the 16th AAAP Animal Science Congress, pp. 376–379.

Chapter 3

3. Evaluating the protein value of fresh tropical forage grasses and forage legumes using *in vitro* and chemical fractionation methods

This chapter was published as:

Salazar-Cubillas, K., Dickhoefer, U., 2021. Evaluating the Protein Value of Fresh Tropical Forage Grasses and Forage Legumes Using In Vitro and Chemical Fractionation Methods. Animals 2021 (11) 2853. https://doi.org/10.3390/ani11102853

Abstract

The objectives of the present study were (1) to assess the adequacy of the *in vitro* and chemical methods to predict post-ruminal crude protein supply (PRCP) from fresh tropical forage, and (2) to identify PRCP supply predictors. Twenty-three fresh forage grasses and 15 forage legumes commonly used in domestic cattle feeding in the tropics and subtropics were incubated in the rumen of cows to determine ruminal crude protein (CP) degradation. The PRCP supply was calculated from *in situ* rumen-undegraded CP and *in vitro* organic matter digestibility (i.e., reference method), from ammonia-nitrogen release during *in vitro* incubation (i.e., *in vitro* method), and from the concentrations of chemical CP fractions (i.e., chemical method). The adequacy was evaluated using error-index and dimensionless parameters, and stepwise regression was used to select PRCP predictors. Adequacy ranged from poor to moderate (0.53 to 0.74) for the *in vitro* method being lower for forage legumes at a slow rumen passage rate (0.20), and even poorer (0.02 to 0.13) for the chemical method. Hence, the *in vitro* method can estimate PRCP supply in tropical forages with moderate to high but not with slow passage rates. Equations developed in the present study appear to predict PRCP supply with reasonable adequacy.

Keywords: feed evaluation, post-ruminal protein, protein fractionation, tropical forages, ruminants.

3.1 Introduction

Freshly cultivated forages are a major source of protein for domestic ruminants, particularly in the tropics and subtropics. The amount of rumen-undegraded feed crude protein (RUP) and microbial crude protein (CP) leaving the rumen are key variables in assessing their protein value. According to the German feeding recommendation system (GfE, 2001), the sum of RUP and microbial CP at the duodenum of ruminants is defined as post-ruminal crude protein (PRCP; formerly referred to as utilizable CP).

The PRCP supply to the small intestine has been studied for temperate ruminant feedstuffs using in vivo and *in situ* methods; however, these methods are costly, time-consuming, require ruminally and duodenally fistulated animals, and thus compromise animal welfare (Stern et al., 1997; Edmunds et al., 2012), rendering these methods unsuitable for routine evaluation of ruminant feedstuffs in tropical husbandry systems. Alternative methods such as the *in vitro* method developed by Steingaβ et al. (2001) and the chemical method proposed by Zhao and Cao (2004) have been tested in a wide range of temperate ruminant feeds; however, the adequacy (i.e., accuracy and precision; Edmunds et al., 2012; Zhao and Cao, 2004; Gidlund et al., 2018) of these methods to predict the PRCP supply of common feedstuffs used in tropical husbandry systems is still questioned.

Forages grown in tropical regions differ in their chemical composition (Lee, 2018) and are characterized by a slower rate and lower extent of carbohydrate and CP degradation in the rumen than forages grown in temperate regions (Minson, 1990), which may hamper the estimation of PRCP supply with the *in vitro* and chemical methods. In the present study, it was therefore hypothesized that accuracy and precision of the in vitro method in estimating the PRCP supply from tropical forages might be poor due to early microbial lysis in the blank and higher rate of ammonium-nitrogen (NH3-N) uptake than release in the early stage of incubation of the feed samples (Gidlund et al., 2018), the latter being more pronounced in tropical than in temperate forages, because of their slow rate and low extent of carbohydrate and CP degradation in the rumen (Lee, 2018).

Moreover, it was hypothesized that the precision and accuracy of the PRCP supply predicted from the CP fractions using the only available equation of Zhao and Cao (2004) for dried forage grasses, a grass silage, a fresh forage legume, and corn and soybean by-products are most likely poor and lower than that of the *in vitro* method, because forage samples (i.e., forage grasses and forage legumes) were not representative of common forages used for domestic cattle feeding in the tropics and subtropics, and the relationships between

chemical CP fractions and PRCP supply might be different between tropical and temperate forages.

Therefore, the objectives of the present study were (1) to assess the adequacy of the PRCP supply of fresh tropical forage grasses and forage legumes estimated with the *in vitro* and chemical methods, and (2) to identify nutritional composition variables and develop specific algorithms that can be used to predict the PRCP supply of fresh tropical forages commonly used in domestic cattle feeding in the Tropics and Subtropics.

3.2 Materials and Methods

Detailed information on the collection and origin is described in Appendix 3.1. All animal handling and procedures were performed following the Animal Welfare Legislation approved by the Government Presidium of Stuttgart, Germany (approval code V319/14 TE).

3.2.1 Proximate nutrients and fiber analysis

The proximate nutrient and chemical fiber fractions of the forage samples were analyzed in duplicate according to the German Handbook of Agricultural Experimental and Analytical Techniques (VDLUFA, 2012) and then mean values of duplicate measurements were reported in Table 3.1. The dry matter (DM) concentration of the forage samples was determined by drying the forage samples in a forced-air oven (F115, Binder GmbH, Tuttlingen, Germany) at 103 °C until constant weight (Method 3.1). The remaining feed substrate after drying was weighed and incinerated in a muffle furnace (D-2804, Nabertherm GmbH, Bremen, Germany) at 550 °C for 5 h to determine the crude ash (CA) concentration (Method 8.1).

The nitrogen (N) concentration of the forage samples was determined by the Kjeldahl method using a distillation apparatus (B324, Büchi Labortechnik GmbH, Essen, Germany) and then converted to CP by multiplying it by 6.25 (method 4.1.1). The neutral-detergent fiber concentration assayed using heat-stable amylase and sodium sulfite and expressed inclusive of residual CA (aNDF) was determined in an ANKOM Fiber Analyzer (A200, ANKOM Technology, NY, USA; Method 6.5.1). The remaining substrate after aNDF analysis was treated with an acid-detergent solution in an ANKOM Fiber Analyzer to determine the acid-detergent fiber concentration expressed inclusive of residual CA (ADF; Method 6.5.2). Thereafter, the remaining substrate was rinsed with a sulfuric acid solution in a 500 mL beaker to determine the acid-detergent lignin (Lignin(sa)) concentration (Method 6.4.1).

3.2.2 Reference post-ruminal protein estimation

The reference PRCP supply was estimated using the equation N°11 of Lebzien et al. (1996) at rumen passage rate (Kp) of 2, 5, and 8%/hour taking into consideration that in tropical areas, animals with very low to low feed intake level and low-yielding (i.e., slow Kp), as well as high-yielding dairy cows can be found (i.e., fast Kp).

$$PRCP = [187.7 - (115.4 \times (RUP/CP))] \times DOM + 1.03 \times RUP; \quad (1)$$

where PRCP is the PRCP supply (g/kg DM) at Kp of 2, 5, and 8%/h; RUP is the RUP concentration (g/kg DM) estimated with the *in situ* method at Kp of 2, 5, and 8%/h; CP is the CP concentration of the original forage sample (g/kg DM); DOM is the digested organic matter concentration (g/1000 g DM).

The rumen *in situ* CP degradation kinetics were determined following the Madsen and Hvelplund (1994) protocol with incubation times of 2, 4, 8, 16, 24, 48, and 72 h during two periods with three cows per period. The CP disappearance at each incubation time was corrected for losses of water-soluble feed CP and water-insoluble feed CP escaping the bag in the form of small particles using the equation suggested by Weisbjerg et al. (1990). The CP disappearance at each incubation time was corrected for microbial attachment to undegraded feed particles using the equation of Krawielitzki et al. (2006). Then, CP degradability at Kp of 2, 5, and 8%/h was estimated using the equation of Dhanoa et al. (1999) and RUP was estimated as the concentration of CP minus the concentration of rumen-degraded CP.

The DOM (g/1000 g DM) was estimated by multiplying digested organic matter (dOM; g/1000 g organic matter) by the organic matter concentration (g/kg DM) of the forage sample and divided by 1000. The dOM was estimated using the equation N°43e of Menke and Steingass (1988).

$$dOM = (15.38 + 0.85 \times GP + 0.06 \times CP + 0.07 \times CA) \times 10; \quad (2)$$

where dOM is the dOM proportion (g/1000 g organic matter); GP is the net gas release after 24 h *in vitro* incubation (ml/200 mg DM of the original feed substrate); CP is the CP concentration of the original forage sample (g/kg DM); CA is the CA concentration of the original forage sample (g/kg DM).

Table 3.1
Descriptive statistics of the concentrations of proximate nutrients, chemical fiber fractions, crude protein fractions, feed fermentation parameters after 24 h *in vitro* incubation, as well as the post-ruminal crude protein supply at rumen passage rates of 2, 5, and 8%/hour of fresh tropical forage grasses and legumes.

	Tropical Forage Grasses (n = 23)					Tropical Forage Legumes (n = 15)				
	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max
Proximate nutrient and chemical fiber fractions (g/kg dry matter)										
Crude ash	123	119	29	76	178	74	70	16	45	99
Crude protein	117	119	34	46	201	177	174	25	135	212
Neutral-detergent fiber[1]	576	573	41	481	654	448	460	69	328	586
Acid-detergent fiber[2]	308	304	33	220	359	313	320	59	201	414
$Lignin_{(sa)}$[3]	33	30	20	6	93	69	69	19	46	125
NDFp[4]	677	678	40	592	758	477	459	65	382	585
ADFp[5]	357	363	33	278	421	356	340	62	269	486
Crude protein fractions (g/kg dry matter)[6]										
A	43.7	41.0	18.8	15.7	93.6	47.9	42.5	14.1	24.4	75.4
B_1	3.4	3.3	2.3	0.3	9.2	6.4	5.1	5.0	0.2	17.2
B_2	23.9	23.1	8.0	11.3	40.4	62.3	65.2	18.5	24.2	95.7
B_3	32.0	34.0	12.0	6.1	51.8	38.6	39.5	17.7	6.2	69.9
C	14.0	12.6	6.4	5.4	27.0	21.2	16.6	9.6	9.6	47.0
In vitro fermentation parameters (24 h)[7]										
GP (mL/200 mg dry matter)	29	29	3	24	34	34	33	6	25	43
DOM (g/g dry matter)	0.48	0.48	0.03	0.43	0.53	0.55	0.55	0.05	0.49	0.64
ME (MJ/kg dry matter)	6.73	6.73	0.43	5.81	7.62	8.02	7.97	0.89	6.81	10.01
Post-ruminal crude protein (g/kg dry matter)[8]										
2%/h	105	107	10	82	119	125	122	9	111	150
5%/h	110	113	12	81	127	132	128	11	117	162
8%/h	113	116	13	81	132	137	133	12	121	171

[1] Neutral-detergent fiber determined using heat-stable amylase and sodium sulfite and expressed inclusive of residual ash.
[2] Acid-detergent fiber expressed inclusive of residual ash.
[3] $Lignin_{(sa)}$, acid-detergent lignin assayed using sulfuric acid expressed inclusive ash.

[4] NDFp, neutral-detergent fiber assayed using heat-stable amylase and without the use of sodium sulfite using the crude protein fractionation method and expressed inclusive ash.
[5] ADFp, acid-detergent fiber estimated using the crude protein fractionation method and expressed inclusive ash.
[6] Crude protein fractions described by Sniffen et al. (1992) and analyzed following Licitra et al. (1996): A, crude protein soluble in the borate-phosphate buffer and tungstic acid solution; B_1, true protein soluble in buffer solution and precipitated by the tungstic solution; B_2, true protein insoluble in buffer solution but soluble in the neutral-detergent solution; B_3, true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution; and C, true protein insoluble in the acid-detergent solution.
[7] GP, gas production obtained from *in vitro* fermentation using the Hohenheim gas test; DOM, digested organic matter estimated using the equation N°43e (Menke and Steingass, 1988). The digested organic matter (g/g organic matter) was then multiplied by the organic matter concentration (g/kg dry matter) of the forage sample and divided by 1000 to obtain digested organic matter (g/g dry matter); ME, metabolizable energy estimated with the equation N°12f (Menke and Steingass, 1988).
[8] Post-ruminal supply determined at rumen passage rates of 2, 5, and 8%/h with the equation N°11 of Lebzien et al. (1996) using information on *in situ* rumen-undegraded crude protein at rumen passage rates of 2, 5, and 8%/h, crude protein, and digested organic matter concentration determined from *in vitro* gas production.

The GP was estimated following procedures of the regular Hohenheim gas test (Menke and Steingass, 1988).

$$GP24 = (V24 - V0 - GP0) \times 200 \times CF/W; \quad (3)$$

where GP24 is the net gas release after 24 h *in vitro* incubation of the original feed substrate (ml/200 mg DM of the original feed substrate); V24 is the position of the piston after 24 h *in vitro* incubation of the syringe containing feed substrate and inoculum (ml); V0 is the position of the piston at the beginning of the incubation of the syringe containing feed substrate and inoculum (ml); GP0 is the mean gas release after 24 h *in vitro* incubation of the three syringes containing only inoculum (ml; i.e., blanks); CF is the mean correction factor of the three syringes containing hay standard and the three syringes containing concentrate standard sample material (from 0 to 1; i.e., standard of the University of Hohenheim); W is the weight of the original feed substrate of the syringe containing feed substrate and inoculum (mg DM).

The GP24 of the hay and concentrate standards were used to correct the net gas release of each forage sample in the same incubation run. For this, the reference GP24 of the hay and concentrate standard was divided by the mean GP24 of the three syringes containing hay and concentrate standard sample material, respectively. Runs were repeated if these correction factors were <0.9 or >1.1. Three GP24 for each forage sample were calculated for each run. A maximum 10% coefficient of variation (CV; expressed as a percentage of the overall mean) was allowed in GP24 between and within runs. The mean of at least five repetitions of GP24 represented the GP24 of each forage sample.

3.2.3 Modified Hohenheim gas test

The PRCP supply of all feedstuffs was estimated in two or three runs with three repetitions per incubation time in each run. Incubation times were 8 and 48 h following the recommendations of Leberl et al. (2007). Rumen fluid was collected with a vacuum pump from two or three fistulated cows, including those used for the *in situ* incubation. The rumen fluid was extracted before morning feeding and transported to the laboratory in prewarmed thermal flasks, where it was first filtered through a cloth layer with a pore size of 100 µm. Of the filtered rumen fluid, 420.6 mL was taken and added to 841.1 mL of a prewarmed colorless incubation solution (~39 °C) to generate the inoculum for the *in vitro* incubations. The incubation solution was prepared following the procedure of the regular Hohenheim gas test (Menke and Steingass, 1988) with a chemical alteration of 2 g/L increase in ammonium bicarbonate and 2 g/L decrease in sodium bicarbonate. The incubation solution (841.1 mL) of the modified

Hohenheim gas test was prepared in the following order: 400 mL distilled water, 0.1 mL micromineral solution (13.2 g calcium chloride × 2 H2O, 10 g manganese chloride × 4 H2O, 1 g cobalt chloride × 6 H2O, 8 g ferric trichloride × 6 H2O, and made up to 100 mL with distilled water), 200 mL buffer solution (6 g ammonium bicarbonate, 33 g sodium bicarbonate, and made up to 1000 mL with distilled water), 200 mL macro-mineral solution (5.7 g disodium hydrogen phosphate, 6.2 g potassium dihydrogen phosphate, 0.6 g magnesium sulfate × 7 H2O, and made up to 1000 mL with distilled water), 1 mL resazurin solution (0.1%, 100 mg resazurin in 100 mL of distilled water), and 40 mL freshly prepared reduction solution (4 mL sodium hydroxide 1N, 625 mg sodium sulfide × 9 H2O, and 95 mL distilled water). The incubation solution and later the inoculum were stirred with a magnetic stir and kept under a continuous flux of carbon dioxide in a water bath at ~39 °C.

After 5 min of homogenization, 30 mL of the inoculum was added to each pre-warmed syringe (~39 °C) containing approximately 200 mg DM of forage sample material. Per incubation time, three syringes containing only inoculum (i.e., blanks) and three syringes containing a standard protein sample material (i.e., protein standard of the University of Hohenheim) were additionally included in each run. Syringes were randomly placed in a prewarmed water bath (~39 °C) and were shaken every hour during the first 6 h of incubation. Immediately after 8 and 48 h of incubation, all contents of the respective syringes were transferred to a 50 mL sterile plastic tube and stored (4 °C) until the next day for analysis. Then, two subsamples of 10 mL each of the content of each syringe were transferred into two Kjeldahl flasks and 10 mL of 0.25 M phosphate buffer with a pH of 11 was added to each flask to increase the pH of the sample solution. Immediately thereafter, the NH3-N release from the inoculum of the blanks and syringes containing forage samples or protein standard was then estimated with back titration using a 0.05 M sulfuric acid solution.

The mean NH3-N release from the two 10 mL aliquots for each syringe containing the blank, the forage sample, or the protein standard was multiplied by three to calculate the NH3-N release from 30 mL of inoculum. Each triplicate measurement of NH3-N release in 30 mL of the syringes containing the blank, the forage sample, or the protein standard after 8 and 48 h *in vitro* incubation was then used to calculate the PRCP supply after 8 and 48 h *in vitro* incubation using the equation of Steingaβ et al. 2001:

$$PRCP = ((N\ sample + NH3\text{-}N\ blank - NH3\text{-}N\ sample)/W) \times 1000 \times 6.25; \quad (4)$$

where PRCP is the PRCP supply of the forage samples or protein standard after 8 or 48 h *in vitro* incubation (g/kg DM); N sample is the N concentration of the original forage sample or

protein standard incubated in 30 mL of inoculum (mg/30 mL inoculum); NH3-N blank is the NH3-N release from the blank after 8 and 48 h *in vitro* incubation (mg/30 mL inoculum); NH3-N sample is the NH3-N release from the forage sample or protein standard after 8 or 48 h *in vitro* incubation (mg/30 mL inoculum); W is the initial weight of the original forage sample or protein standard incubated in 30 mL inoculum (mg DM/30 mL inoculum).

The PRCP supplies of the protein standard were used to correct the PRCP supply after 8 and 48 h *in vitro* incubation. For this, the reference PRCP supply of the protein standard at each *in vitro* incubation time was divided by the mean PRCP supply of the three syringes containing the protein standard after 8 and 48 h. Runs were repeated if these correction factors were <0.9 or >1.1. Then, each PRCP supply after 8 and 48 h *in vitro* incubation of the forage samples was multiplied by the respective correction factor. The PRCP supply at Kp of 2, 5, and 8%/h was obtained by plotting the log of the time of incubation (i.e., ln(8) and ln(48)) against PRCP supply after 8 and 48 h *in vitro* incubation, respectively. From the resulting non-linear regression equation, the intercept and slope were obtained. The PRCP supply was then calculated using the equation presented by Edmunds et al. (2012):

$$PRCP = a \times \ln(1/Kp) + b; \quad (5)$$

where PRCP is the PRCP supply at Kp of 2, 5, and 8%/h of the forage sample (g/kg DM); a is the slope (g/kg DM); Kp is the assumed Kp expressed as 2, 5, and 8%/h; b is the intercept (g/kg DM).

Three PRCP supplies for each Kp were calculated for each run. A maximum 10% CV (expressed as a percentage of the overall mean) was allowed in PRCP supplies at a given Kp between and within runs. The mean of at least five repetitions of PRCP supplies was calculated for each Kp, representing the PRCP supply at a given Kp of each forage sample.

3.2.4 Chemical crude protein fractionation

The non-protein N (NPN) concentration was determined in duplicate using the tungstic acid method (Greenberg et al., 1979). The forage sample material was weighed into a 100 mL flask, and then 50 mL of cold distilled water and 8 mL of a 0.3 M sodium tungstate solution were added. The forage sample material and solution were mixed for 30 min under continuous stirring before reducing the pH to 2.0 with a 0.5 M sulfuric acid solution. Flasks were then covered and kept at room temperature for 16 h. Then, the suspension was filtered through cellulose filter paper (Whatman paper N°54, GE Healthcare Life Sciences, Darmstadt,

Germany). Both the filter paper and residual substrate were washed once with 250 mL of cold distilled water before they were analyzed for N. Then, the NPN concentration was calculated by subtracting the N amount in the residual substrate and the N amount in the cellulose filter paper from the total N amount in the original forage sample material.

The soluble true protein (SP) concentration was determined in duplicate following Licitra et al. (1996) recommendations. Briefly, 50 mL of a borate-phosphate buffer (pH 6.7 - 6.8) (Krishnamoorthy et al., 1982) and 1 ml of freshly prepared sodium azide 1.5 M were added to a 100 mL flask containing forage sample material. Flasks were covered for 3 h before the mixture was filtered through cellulose filter paper. Both the filter paper and residual substrate were washed once with 250 mL of cold distilled water before both were analyzed for N. The SP concentration was calculated by subtracting the N amount in the residual substrate and the N amount in the cellulose filter paper from the total N amount in the original forage sample material.

The concentration of neutral-detergent-insoluble protein (NDIP) was determined in duplicate following the procedures of aNDF analysis without the use of sodium sulfite (Licitra et al., 1996). The forage sample material was boiled in a 500 mL beaker with 100 mL of neutral-detergent solution (van Soest and Mason, 1991) using a laboratory heater (EV1, Gerhardt GmbH & Co-erhardt, Königswinter, Germany). After the solution started boiling, 25 µL of alpha-amylase was added each at 1 min and 30 min. One hour after the solution started boiling, the mixture was filtered through cellulose filter paper. Both the filter paper and residual substrate were washed once with 250 mL of hot distilled water (~80 °C), rinsed twice with 5 mL of acetone, and dried at room temperature for 1 h before they were analyzed for N. The analysis of acid-detergent-insoluble protein (ADIP) followed the same procedure as NDIP, except that the neutral-detergent solution was substituted for an acid-detergent solution and alpha-amylase was not used.

In addition to CP fraction analyses, concentrations of aNDF and ADF estimated from the residue after boiling in the respective solution without the use of sodium sulfite according to Licitra et al. (1996) were also determined, herein referred to as NDFp and ADFp, respectively. The N concentrations of the residual substrate and cellulose filter after the chemical CP fractionation procedure were determined using method 4.1.1 (VDLUFA, 2012) as described in Section 2.1. The means of the duplicate measurements of the different chemical CP fractions of the forage samples were then used to calculate the CP fractions as described by Sniffen et al. (1992):

$A = NPN \times 6.25;$

$B_1 = SP - (NPN \times 6.25);$

$B_2 = IP - NDIP;$

$B_3 = NDIP - ADIP;$

$C = ADIP.$ (6)

where A is the concentration of CP soluble in the borate-phosphate buffer and tungstic acid solution (g/kg DM); NPN is the concentration of NPN-N (g/kg DM); B_1 is the concentration of true protein soluble in buffer solution and precipitated by the tungstic solution (g/kg DM); SP is the concentration of SP (i.e., sum of CP fractions A and B_1; g/kg DM); B_2 is the concentration of true protein insoluble in buffer solution but soluble in the neutral-detergent solution (g/kg DM); IP is the concentration of insoluble true protein estimated as the concentration of CP minus the concentration of SP (i.e., sum of true protein fractions B_2, B_3, and C; g/kg DM); NDIP is the concentration of NDIP known as cell-wall-bound true protein (i.e., sum of true protein fractions B_3 and C; g/kg DM); B_3 is the concentration of true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution (g/kg DM); ADIP is the concentration of ADIP (g/kg DM); C is the concentration of true protein insoluble in the acid-detergent solution (g/kg DM). The CP not bound to the cell wall (i.e., sum of CP fractions A, B_1, and B_2) and the true protein (i.e., sum of true protein fractions B_1, B_2, B_3, and C) were also calculated.

The PRCP supply was estimated from the concentrations of chemical CP fractions using the only available equation for dried forage grasses, a grass silage, a fresh forage legume, and corn and soybean by-products (Zhao and Cao, 2004):

$$PRCP = 8.78 \times A + 15.69 \times B_1 + 12.36 \times B_2 + 11.83 \times B_3 + 6.99 \times C \quad (7)$$

where PRCP is PRCP supply after 24 h *in vitro* incubation (g/kg DM); A is the concentration of CP soluble in the borate-phosphate buffer and tungstic acid solution (g/kg DM); B_1 is the concentration of true protein soluble in buffer solution and precipitated by the tungstic solution (g/kg DM); B_2 is the concentration of true protein insoluble in buffer solution but soluble in the neutral-detergent solution (g/kg DM); B_3 is the concentration of true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution (g/kg DM); C is the concentration of true protein insoluble in the acid-detergent solution (g/kg DM).

3.2.5 Statistical analyses

All statistical analyses were conducted using R statistical software version 3.6.1 (R Core Team, Vienna, Austria). The means of the duplicate measurements per sample of proximate nutrients, chemical fiber fractions, CP fractions, fermentation parameters after 24 h *in vitro* incubation, and PRCP supply as estimated with Lebzien et al. (1996) equation (i.e., reference method) of fresh tropical forage grasses (n = 23) and forage legumes (n = 15) were calculated and described using descriptive statistics including measures of central tendency (i.e., mean and median) and measures of variability and dispersion (i.e., minimum, maximum, and standard deviation).

Previous to the adequacy assessment, a Grubbs outlier test (Grubbs, 1969) was performed to identify illogical values in the sample set of PRCP supply as estimated with the *in vitro*, chemical method, and reference method. The outlier test identified one outlier in the PRCP supply estimated with the *in vitro* method at Kp of 2%/h: Centrosema sp (DC.) Benth (179 g/kg CP). However, the outlier was not removed from the sample set, because the identified outlier was not a common-sense outlier (i.e., illogical value).

To evaluate the adequacy of the predictions of the *in vitro* and chemical methods, the estimates of PRCP supply at Kp of 2, 5, and 8%/h from the *in vitro* method and chemical method at Kp of 5%/h were evaluated against values determined by the reference method using error-index and dimensionless parameters. The estimates of PRCP supply according to the chemical method were evaluated only at Kp of 5%/h because the equation of Zhao and Cao (2004) was developed to predict the PRCP supply after 24 h *in vitro* incubation, which resembles a PRCP supply at Kp of 5%/h.

The error-index parameters included the mean bias, root mean square error (RMSE), and mean absolute percentage error (MAPE), whereas dimensionless parameters included the RMSE to standard deviation ratio (i.e., RSR), and the concordance correlation coefficient (CCC). The CCC as a combined measure of accuracy and precision was calculated and partitioned into the correlation coefficient (i.e., precision; ρ) and a bias correction factor coefficient (i.e., accuracy; Cb) (Lin, 1989).

The scale of McBride (2005) was used to assess the degree of agreement between the alternative method and the reference method, which classifies the CCC as very strong (CCC ≥ 0.90), strong (CCC ≥ 0.80 - <0.90), moderate (CCC ≥ 0.65 - < 0.80), and poor (CCC < 0.65). A more accurate and precise prediction was considered to be the one with lower mean bias, RMSE, MAPE, RSR, and greater CCC. In the present study, an alternative method

was considered adequate enough to replace the reference method, if the CCC was ≥ 0.80 because CCC estimates between PRCP estimated with the *in vitro* and in vivo methods had ranged from 0.81 to 0.89 in a previous study (Zhao and Lebzien, 2000). In addition, the scale of Evans (1996) was used to classify the correlation as very strong (p ≥ 0.80), strong (p ≥ 0.60 - < 0.80), moderate (p ≥ 0.40 - < 0.60), weak (p > 0.20- < 0.40), and very weak (p ≤ 0.20; objective 1).

According to previous studies on CP degradation in the rumen, concentrations (g/kg DM) of proximate nutrients (i.e., CA and CP), chemical fiber fractions (i.e., aNDF, ADF, NDFp, ADFp, and Lignin(sa)), and CP fractions (i.e., A, B_1, B_2, B_3, C, SP, IP, true protein, NPN, cell-wall-bound protein and CP not bound to the cell wall), as well as the ratios between concentrations of chemical CP fractions (i.e., SP/IP, IP/SP, true protein/NPN, NPN/true protein, cell-wall-bound true protein/CP not bound to the cell wall and CP not bound to the cell wall/cell-wall-bound true protein), were selected as a set of independent variables that can predict the PRCP supply of tropical forage grasses and forage legumes.

An attempt was made to develop one equation per Kp (i.e., 2, 5, and 8%/h) and per forage type (i.e., forage grasses and forage legumes), but the PRCP supply was better predicted with a general equation across both forage types rather than for forage grasses and forage legumes separately. Therefore, three equations (i.e., one equation per Kp) were developed with independent and dependent variables expressed in g/kg DM using a multiple linear regression forward and backward stepwise approach with Akaike Information Criteria as model selection criteria. In the case that several models were obtained per Kp with the stepwise multiple linear regression approach, the model with the lowest Bayesian Information Criterion was selected. Finally, multicollinearity and independence of residuals of the selected model were evaluated using variance inflation factor and residual plots, respectively. Independent variables with variance inflation factor > 10 were removed from the model until the variance inflation factor of the remaining independent variables was <10 (Hair et al., 2013).

The standard error of the mean, p-value, determination coefficients adjusted by the number of predictors in the model (adjusted R^2), RMSE, and MAPE were calculated from the relationship between PRCP supply estimated with the reference method and those predicted with the developed equations in the present study for tropical forages at Kp of 2, 5, and 8%/h (objective 2).

3.3 Results

3.3.1 Nutritional characteristics of forages

Descriptive statistics of the chemical composition, CP fractions (i.e., A, B_1, B_2, B_3, and C), *in vitro* fermentation parameters, and PRCP supply as estimated with the reference method of fresh tropical forage grasses and forage legumes are presented in Table 3.1.

For fresh forage grasses, the concentrations of SP, IP, and true protein ranged from 19 to 103 g/kg DM, from 27 to 104 g/kg DM, and from 30 to 107 g/kg DM, respectively. The concentrations of cell-wall-bound true protein and CP not bound to the cell wall ranged from 12 to 64 g/kg DM and from 35 to 140 g/kg DM, respectively. For fresh forage legumes, the concentrations of SP, IP, and true protein ranged from 36 to 93 g/kg DM, from 90 to 164 g/kg DM, and from 92 to 168 g/kg DM, respectively. The concentrations of cell-wall-bound true protein and CP not bound to the cell wall ranged from 16 to 98 g/kg DM and from 60 to 188 g/kg DM, respectively.

3.3.2 Adequacy of the *in vitro* method to predict post-ruminal crude protein supply

For all comparisons, greater CCC estimates complied with lower RMSE, MAPE, and RSR. The PRCP supply of tropical forages was poorly predicted by the *in vitro* method at Kp of 2%/h (CCC = 0.53), but moderately predicted at Kp of 5%/h (CCC = 0.69) and 8%/h (CCC = 0.74; Table 3.2). The precision (ρ from 0.53 to 0.74) to predict reference PRCP supply of tropical forages by the *in vitro* method increased as Kp increased (Kp from 2 to 8%/h), whereas the accuracy was similar across Kp (Cb from 0.82 to 0.84).

The range of PRCP supply determined with the *in vitro* method was wider (81 to 171 g/kg DM) than that of values estimated with the equation from Lebzien et al. (1996; 39 to 185 g/kg DM) for our sample set. The PRCP supply of forage grasses was poorly predicted at Kp of 2%/h but moderately predicted at Kp of 5 and 8%/h, whereas the PRCP supply of tropical forage legumes was poorly predicted by the *in vitro* method for all Kp.

The PRCP supply estimated using the *in vitro* method slightly underestimated (mean bias from 5.90 to 8.61 g/kg DM) the PRCP supply determined with the reference method of tropical forage grasses for all Kp and of forage legumes at Kp of 2%/h (mean bias of 4.05 g/kg DM), whereas it slightly overestimated (mean bias of from −9.87 to −6.03 g/kg DM) the PRCP supply of tropical forage legumes at Kp of 5 and 8 %/h (Table 3.2).

3.3.3 Adequacy of the chemical method to predict post-ruminal crude protein supply and its comparison with the *in vitro* method

Greater CCC estimates resulted in lower mean bias, RMSE, MAPE, and RSR. Irrespective of the forage type, the PRCP supply at Kp of 5%/h determined by the reference method was poorly predicted with the chemical method (CCC ≤ 0.14) using the equation of Zhao and Cao (2004) (Table 3.2). The equation of Zhao and Cao (2004) greatly overestimated (i.e., negative mean bias from −138.79 to −14.63 g/kg DM) the PRCP supply according to the reference method (Table 3.2; Figure 3.1b). Irrespective of the forage type, the poor adequacy of the equation of Zhao and Cao (2004) was more related to its low accuracy (Cb from 0.05 to 0.16) and not a poor precision (ρ from 0.56 to 0.87; Table 3.2).

Irrespective of the forage type, the PRCP supply at Kp of 5%/h determined by the reference method was better predicted by the *in vitro* method than by the chemical method with lower mean bias, RMSE, MAPE, and RSR, as well as greater CCC (Table 3.2). Adequacy of the estimates of PRCP supply was overall greater (lower RMSE, MAPE, and RSR, and greater CCC) for forage grasses than for forage legumes for both the *in vitro* and the chemical method (Table 3.2).

3.3.4 Multivariate regressions to predict post-ruminal crude protein supply in tropical forages

One equation per Kp was developed to predict the PRCP supply of both, tropical forage grasses and forage legumes (Table 3.3). The variance inflation factor was lower than 2.4 for all independent variables and the residual plots of the developed equations showed no clear patterns and revealed a similar distribution of plotted points around the line at 0 (Appendix 3.2).

The variables retained to predict the PRCP supply of tropical forages were IP and ADF, irrespective of the Kp. The adjusted R^2, RMSE, and MAPE calculated from the relationship between PRCP supply according to the reference method and that predicted with the equations developed in the present study for tropical forages ranged from 0.80 to 0.85, 6.0 to 6.6 g/kg DM, and 4.3 to 4.4% of the reference PRCP supply, respectively.

Table 3.2
Predictions of the post-ruminal crude protein (PRCP) supply as estimated with the reference and *in vitro* methods at rumen passage rates of 2, 5, and 8%/h and as calculated with the chemical method using the equation of Zhao and Cao (2004) at rumen passage rate of 5%/h of fresh tropical forage grasses and legumes.

Kp[1]	PRCP method[2]	Mean	Error-Index[3]			Dimensionless[4]			
			Mean bias	RMSE	MAPE	RSR	Concordance Correlation Coefficient		
(%/h)		(g/kg dry matter)	(g/kg dry matter)	(% mean reference PRCP)	(% mean reference PRCP)	(0 to ∞)	Coefficient (−1 to 1)	ρ (−1 to 1)	Cb (0 to 1)
Fresh Tropical Forages (n = 38)									
2	Reference PRCP	113							
	In vitro PRCP	108	5.17	17	14	1.26	0.53	0.65	0.82
5	Reference PRCP	119							
	In vitro PRCP	117	2.83	16	13	0.99	0.69	0.84	0.83
	Chemical PRCP	200	−66.91	74	67	4.68	0.14	0.87	0.16
8	Reference PRCP	122							
	In vitro PRCP	123	1.17	15	13	0.86	0.74	0.88	0.84
Fresh Tropical Forage Grasses (n = 23)									
2	Reference PRCP	105							
	In vitro PRCP	100	5.90	16	13	1.60	0.53	0.75	0.71
5	Reference PRCP	110							
	In vitro PRCP	102	8.61	15	13	1.22	0.66	0.89	0.73
	Chemical PRCP	173	−56.04	62	56	5.05	0.13	0.83	0.16
8	Reference PRCP	113							
	In vitro PRCP	105	8.37	13	12	0.98	0.73	0.93	0.78
Fresh Tropical Forage Legumes (n = 15)									
2	Reference PRCP	125							
	In vitro PRCP	120	4.05	19	14	1.93	0.20	0.30	0.65
5	Reference PRCP	132							
	In vitro PRCP	140	−6.03	16	13	1.46	0.29	0.39	0.73
	Chemical PRCP	242	−83.59	85	84	7.62	0.03	0.56	0.05
8	Reference PRCP	137							
	In vitro PRCP	150	−9.87	17	15	1.33	0.30	0.44	0.68

[1] Kp, passage rates through the rumen.

[2] PRCP methods: reference PRCP, PRCP supply determined at rumen passage rates of 2, 5, and 8%/h with the equation N°11 of Lebzien et al. (1996) using information on *in situ* rumen-undegraded crude protein at rumen passage rates of 2, 5 and 8%/h, crude protein, and digested organic matter concentration determined from *in vitro* gas production; *in vitro* PRCP, PRCP supply estimated with the *in vitro* method (Menke and Steingass, 1988); chemical PRCP, PRCP supply calculated from concentrations of crude protein fractions using the equation of Zhao and Cao (2004) for dried forage grasses, a grass silage, a fresh forage legume, and corn and soybean by-products. Results from the chemical method were only compared at a rumen passage rate of 5%/h, because the method was validated against a PRCP measurement after 24 h *in vitro* incubation, which resembles a PRCP supply at a rumen passage rate of 5%/h.

[3] Error-index measurements include measures on mean bias, root mean square error (RMSE), and mean absolute percentage error (MAPE).

[4] Dimensionless; includes measures such as the ratio between root mean square error and standard deviation (RSR), the concordance correlation coefficient (CCC), and its partitioning into correlation coefficient (ρ, i.e., precision) and bias correction factor (Cb; i.e., accuracy).

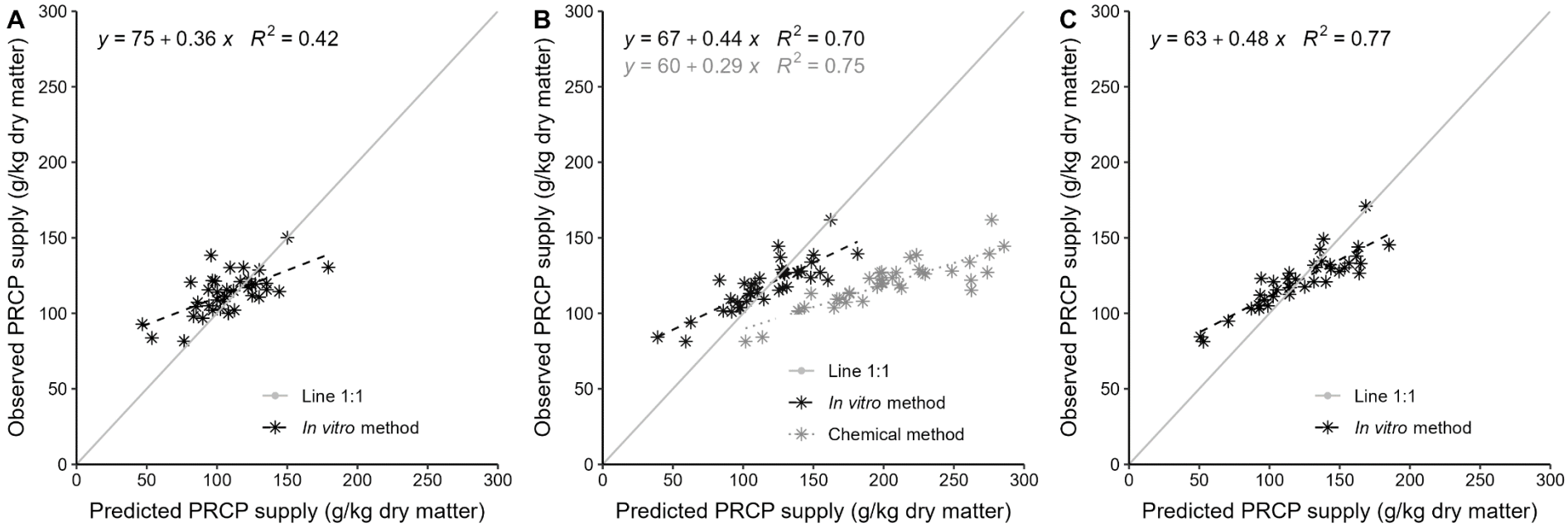


Figure 3.1
Relationship between post-ruminal protein (PRCP) supply of 23 fresh forage grasses and 15 fresh forage legumes that are commonly used in domestic cattle feeding in the tropics and subtropics estimated with a reference method (Lebzien et al., 1996; observed PRCP) and with an *in vitro* method (i.e., modified Hohenheim gas test; predicted PRCP) evaluated at Kp of 2%/h (A), 5%/h (B), and 8%/h (C), or with a chemical method (Zhao and Cao, 2004) at Kp of 5%/h (B).

Table 3.3
Statistical parameters of multivariate regression models developed to estimate post-ruminal crude protein (PRCP) supply at rumen passage rates of 2, 5, and 8%/h of fresh tropical forage grasses and legumes (n = 38).

Dependent variables[1] (g/kg dry matter)	Intercept and independent variables[2] (g/kg dry matter)	Parameters estimate	SEM	Value	Adjusted R^{2} [3]	RMSE[4] (% mean reference PRCP)	MAPE[5] (% mean reference PRCP)
PRCP	Intercept	94.96	8.23	<0.01	0.80	5.29	4.25
Kp 2%/h	B_2+B_3+C	0.36	0.03	<0.01			
	ADF	−0.05	0.02	0.05			
PRCP	Intercept	97.45	8.66	<0.01	0.82	5.31	4.37
Kp 5%/h	B_2+B_3+C	0.42	0.03	<0.01			
	ADF	−0.05	0.02	0.03			
PRCP	Intercept	97.52	9.07	<0.01	0.85	5.40	4.41
Kp 8%/h	B_2+B_3+C	0.47	0.04	<0.01			
	ADF	−0.06	0.02	0.03			

[1] PRCP supply determined at rumen passage rates of 2, 5, and 8%/h with the equation N°11 of Lebzien et al. (1996) using information on *in situ* rumen-undegraded crude protein at rumen passage rates of 2, 5, and 8%/h, crude protein, and digested organic matter concentration determined from *in vitro* gas production.
[2] ADF, acid-detergent fiber deter-mined in an ANKOM Fiber Analyzer and expressed inclusive ash; B_2, true protein insoluble in buffer solution but soluble in the neutral-detergent solution; B_3, true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution; and C, true protein insoluble in the acid-detergent solution.
[3] Adjusted R^2, coefficient of determination adjusted by the number of predictors in the model.
[4] RMSE, root mean square error.
[5] MAPE, mean absolute percentage error.

3.4 Discussion

The PRCP supply of 23 forage grasses and 15 forage legumes that are commonly used in domestic cattle feeding in the tropics and subtropics was estimated at Kp of 2, 5, and 8%/h using the modified Hohenheim gas test as *in vitro* method and predicted from chemical CP fractions using the equation of Zhao and Cao (2004) at Kp of 5%/h.

The present study aimed (i) at assessing the adequacy of these two approaches when compared to a reference method, for which RUP concentrations were determined *in situ*, and (ii) at identifying nutritional composition variables and develop specific algorithms for tropical forages to improve prediction of PRCP supply by the chemical method.

3.4.1 Experimental design and methods

Besides a low reproducibility of the concentrations of different CP fractions during the lab analysis, one limitation of the present study may be related to the choice of reference method and its robustness. Since cows equipped with both, ruminal and duodenal fistula, were not available, the PRCP supply was derived from the RUP concentration of the forages as determined *in situ*, while the microbial CP was estimated from the DOM using an efficiency of microbial CP synthesis adjusted for the availability of rumen-degraded CP (Lebzien et al., 1996).

The PRCP estimated with the equation of Lebzien et al. (Lebzien et al., 1996) was chosen as a reference because, to our knowledge, a specific equation for tropical forages to calculate PRCP supply from concentrations of RUP and DOM or metabolizable energy concentrations has not been published. The great number of observations used to develop the equation of Lebzien et al. (1996), the fact that the reference values were determined in in vivo studies using double-fistulated animals, and the strong relationship between dependent and independent variables (as indicated by high R^2 and low CV) indicate that the equation of Lebzien et al. (1996) might be able to predict with an acceptable margin of error the PRCP supply of diets and individual feedstuffs.

Although this equation has been developed for temperate diets and individual feedstuffs, their range of diets included those of only forages (e.g., forage to concentrate ratios from 100:0 to 30:70) and with low CP concentrations (e.g., grass hay). In addition, the CP and RUP concentrations of our forage sample set were within the range of those of the diets used to develop the equation of Lebzien et al. (1996). Moreover, the efficiency of rumen microbial CP synthesis calculated for the forage samples in the present study using the equation of

Lebzien et al. (1996) ranged from 119 to 179 g microbial CP/kg DOM, which is similar to the efficiency values estimated for cattle in tropical environments (111 to 201 g microbial CP/kg DOM; n = 444 individual observations) (Salazar-Cubillas and Dickhoefer 2019) using the equations proposed by INRA (2018). Therefore, we expect that the equation of Lebzien et al. (1996) can also adequately predict the PRCP supply of tropical forages.

The DOM was estimated from the GP during *in vitro* incubation of forage samples, according to Menke and Steingass (1988). Similarly, the equation used to predict dOM (Menke and Steingass, 1988) was developed using temperate feedstuffs, which might have affected estimates of the reference PRCP supply. However, the equation was developed based on in vivo digestibility data for a great variety of fresh and dry forages (n = 185). Furthermore, the CP concentration, *in vitro* GP, and thus dOM of our forage samples were within the range of those feedstuffs used to develop the equation of Menke and Steingass (1988), suggesting that it can also adequately predict the dOM of tropical forages.

The reference and *in vitro* PRCP methods were estimated at Kp of 2, 5, and 8%/h. Those Kp were chosen in the present study because they were considered appropriate to represent the range of Kp that can be found in the tropics and subtropics. This Kp range was also found in the dataset of Salazar and Dickhoefer (2019) that summarizes 444 individual observations of steers, heifers, and lactating cows under tropical conditions and includes animals with very low feed intake levels (e.g., during dry seasons; Kp < 5%/h), low-yielding animals, as well as high-yielding dairy cows (i.e., >30 kg milk/day; Kp > 5%/h).

3.4.2 Nutritional characteristic of forages

The concentrations of proximate nutrients, fiber fractions, CP fractions, and the *in vitro* fermentation parameters (i.e., GP, DOM, and metabolizable energy) of most analyzed forage species were within the range of values described for the respective species in the literature (INRA, 2018; Nogueira et al., 2000; Tedeschi et al., 2006; Fondevila et al., 2002; Osuga et al., 2006; Evitayani et al., 2004; Singh et al., 2012; Melesse et al., 2017; Juárez Lagunes et al., 2018). No published information was available on the PRCP supply from tropical forages; however, the RUP concentrations determined *in situ* were within the range of values found in previous studies for the respective forage species (INRA, 2018; Khandaker an Tareque, 1996; Kjamseekhiew et al., 2001; Ramírez Lozano et al., 2002; Tedeschi et al., 2002; Mupangwa et al., 2003; La O, 2006; Valarini and Possenti, 2006; Ajayi et al., 2007; Bowen et al., 2008; Wigati et al., 2014). Hence, in general, the forage samples included in the present study seem to be

representative of tropical forage grasses and legumes used in domestic cattle feeding in tropical and subtropical countries.

3.4.3 Adequacy of the estimates of post-ruminal crude protein supply using the *in vitro* method

It was expected that the *in vitro* method poorly predicts the PRCP supply from tropical forages as a result of its low accuracy and precision caused by the overestimation of NH3-N release of the blank and the underestimation of NH3-N release from the feed sample. The NH3-N release from the inoculum is overestimated because microbial lysis is greater in the blank than those syringes containing feed substrate due to a lack of fermentable substrates (Gidlund et al., 2018). It is also possible that NH3-N release of the feed sample in an *in vitro* system is underestimated because NH3-N release and NH3-N uptake by microorganisms occur simultaneously (Gidlund et al., 2018) with a higher rate of uptake than release in the early stage of incubation (Wallace and Lahlou-Kassi, 1995).

Our hypothesis was partly accepted. The overall mean bias was low (mean bias from 1.17 to 5.17 g/kg DM), indicating great compliance between the PRCP supply of tropical forages as estimated according to Lebzien et al. (1996) and with the *in vitro* method. Nevertheless, there were considerable and similar positive (from 0.16 to 53.60 g/kg DM) and negative biases (from −37.48 to −0.07g/kg DM) for individual forage samples, explaining the low mean bias. In this line relatively high RMSE (from 18.36 to 18.72 g/kg DM) and MAPE (from 15.12 to 15.99 g/kg DM) represent better the expected error of the *in vitro* method than the mean bias. Moreover, the *in vitro* method showed a poor to moderate agreement (CCC from 0.53 to 0.74), with lower CCC estimates at slow than at fast Kp and in tropical forage legumes than forage grasses. This poor to moderate level of agreement was related to a low precision (p = 0.65) rather than a low accuracy (Cb = 0.82). Hence, the precision of the *in vitro* method (p from 0.53 to 0.74) increased as Kp increased, whereas the accuracy was similar irrespective of the Kp (Cb from 0.82 to 0.84).

Similarly, a poor to moderate level of agreement was found in Edmunds et al. (2012) (n = 23 samples of fresh temperate and conserved forage grasses and legumes; CCC from 0.23 to 0.68) and Westreicher-Kristen et al. (2015) (n = 13 samples of dried distillers' grains with solubles; CCC from 0.35 to 0.44), between the PRCP supply estimated with the reference method (Lebzien et al., 1996) and the one derived with the *in vitro* method. In contrast thereto, predicted PRCP supply strongly agreed with the reference values in a study by Zhao and

Lebzien (2000) (n = 25 samples of conserved forages grasses, concentrates components, and concentrate mixtures; CCC from 0.81 to 0.89).

The wider PRCP supply of the sample set of Zhao and Lebzien (2000) (from 76 to 341 g/kg DM) and the in vivo reference PRCP method used (i.e., measured CP at the duodenum) could have contributed to reducing the uncertainty of the slope estimate (i.e., precision) (Salgueiro da Silva and Seixas, 2017) and might explain the greater adequacy of the *in vitro* method in their study. Additionally, Zhao and Lebzien (2000) also used the equation of Lebzien et al. (1996) as a reference method; however, their RUP concentration was estimated based on measured in vivo CP at the duodenum, measured microbial CP at the duodenum, and a fixed endogenous CP factor. Therefore, their reference PRCP supply was estimated indirectly from measured PRCP supply. The lower accuracy of predicting PRCP supply of forage legumes at fast Kp (i.e., short incubation time) than slow Kp (i.e., long incubation time) by the *in vitro* method can be explained by the prolonged lag phase presented in forage legumes (67 min) than in forage grasses (50 min) (Ibrahim et al., 1995).

The greater adequacy of the *in vitro* method in tropical forage grasses than forage legumes might be related to the fact that protein and carbohydrate degradation is more synchronous, both, in amount and time, in tropical forage grasses than forage legumes (i.e., high CP and low potentially digestible aNDF) (Nurdianti et al., 2019), allowing for an *in vitro* fermentation without at least temporal nutrient restrictions for microbial fermentation.

The PRCP supply estimated using the *in vitro* method slightly underestimated (mean bias from 5.90 to 8.61 g/kg DM) the PRCP supply determined with the reference method of tropical forage grasses for all Kp and of forage legumes at Kp of 2%/h (mean bias of 4.05 g/kg DM). In contrast, it slightly overestimated (mean bias of from −9.87 to −6.03 g/kg DM) the PRCP supply of tropical forage legumes at Kp of 5 and 8%/h. The underestimation of the PRCP supply of forage grasses for all Kp and forage legumes at slow Kp by the *in vitro* method can be explained by microbial lysis takes place in a close *in vitro* system, because rumen microbes lack sufficient substrate for continued fermentation. In the same line, overestimation of the PRCP supply from forage legumes at short incubation times (i.e., fast Kp) might be explained by early microbial lysis in the blank (Gidlund et al, 2018; Wallace and Lahlou-Kassi, 1995), which does not occur in the syringes filled with feed substrate.

In the present study, the adequacy of the *in vitro* method was considered unacceptable for tropical forages, because it could not reach a CCC of ≥ 0.80. Such a threshold to decide whether a method allows for predictions with acceptable accuracy and precision will certainly

depend on the purpose of its use. Moreover, the estimated CCC of the conjoint sample set was greater than 0.69 for Kp of 5 and 8%/h but not for Kp of 2%/h. These results suggest that the *in vitro* method can be used as an alternative method to estimate PRCP supply in diets with moderate to fast Kp (e.g., moderate to high feed intake levels) but not with very slow Kp.

3.4.4 Adequacy of the estimates of post-ruminal crude protein supply using the chemical method

In the present study, the CCC of the correlations between the PRCP supply at Kp of 5%/h from tropical forage grasses and forage legumes (n = 38) estimated with the equation of Lebzien et al. (1996) and those predicted with the chemical method using the equation of Zhao and Cao (2004) suggested a poor level of agreement (CCC from 0.03 to 0.14).

The equation of Zhao and Cao (2004) was used in the present study because their equation was developed to estimate the PRCP supply of dried forage grasses, a grass silage, a fresh forage legume, and corn and soybean by-products, whereas the equations of Westreicher-Kristen et al. (2015) were specifically developed to predict the PRCP supply of dried distillers' grains with solubles. Yet, this poor level of agreement was expected, mainly because only a few forage samples (n = 6) and of them only one fresh forage sample (i.e., Medicago sativa L.) was included in their sample set that was also used in domestic cattle feeding in the tropics and subtropics. Moreover, their mean MAPE was greater for forage samples (i.e., MAPE of 22%) than for by-product feeds (i. e, MAPE of 9%), which suggests that this equation may perform better for by-products than for forages.

The PRCP supply determined according to Zhao and Cao (2004) greatly overestimated the PRCP supply at Kp of 5%/h for both, forage grasses and legumes, and the low CCC was mainly due to a low accuracy (Cb from 0.05 to 0.16) rather than a poor precision (p from 0.56 to 0.87). Accordingly, Zhao and Lebzien (2000) concluded that the PRCP supply determined after 24 h of *in vitro* incubation, which was used as reference value by Zhao and Cao (2004), overestimates the PRCP supply of forages grasses, although the precision of the predicted PRCP supply of fresh tropical forages calculated with the same sample set used to develop their equations was high.

The low accuracy of the equation of Zhao and Cao (2004) might be due to the fact that tropical forages generally have a slower rate of ruminal CP degradation than temperate ones (Minson, 1990), which may alter the relationships between independent and dependent variables (i.e., coefficient values). Another possible explanation for the discrepancies between PRCP

supply predicted either by the equation of Lebzien et al. (1996) or of Zhao and Cao (2004) could be related to the fact that the latter equation was developed using the PRCP supply determined *in vitro* as a reference, which itself has its inherent errors (i.e., expected MAPE between measured and *in vitro* estimated PRCP from 12 to 17% depending on the Kp) as previously discussed in Section 4.3 of the present study.

In the present study, adequacy of the chemical method was considered unacceptable for tropical forages, because it could not reach a CCC of ≥ 0.80; however, as this low adequacy was mainly due to a low accuracy, specific equations for tropical forages will likely improve the prediction of PRCP supply from tropical forages using the chemical method.

3.4.5 Prediction of post-ruminal crude protein supply of tropical forages

An attempt was made to develop one equation per Kp and per forage type (i.e., forage grasses and forage legumes) to predict the PRCP supply from the concentrations of proximate nutrient, fiber, and CP fractions; nevertheless, the predictions were more accurate and precise with one general equation across both forage types than the separate specific equations. The poor prediction with specific equations by forage type could be due to the small sample size for either, the forage grasses (n = 23) or forage legumes (n = 15). Moreover, the chemical composition of the forages varies greatly amongst different species and varieties of tropical forages, even at the same PRCP supply, particularly in the forage legumes (Lee, 2018) as also shown by numerical differences in the present study, which hampers prediction of PRCP supply.

The independent variables selected in the present study to predict the PRCP supply of tropical forages at Kp of 2, 5 and 8%/h were the concentrations of IP (i.e., sum of true protein fractions B_2, B_3, and C) and of ADF. The same independent variables were retained in the equations of Westreicher-Kristen et al. (2015) developed to predict the PRCP supply of distillers' grains. Instead, the equation of Zhao and Cao (2004) only included concentrations of all CP fractions. The concentration of IP explained the greatest proportion of the variance in the PRCP supply of fresh tropical forages as estimated with the equation of Lebzien et al. (1996), which is likely related to the significant contribution of the CP fractions B_2, B_3, and C to total PRCP supply. The undegraded proportions of the true protein fractions B_2 and B_3, with variable rumen degradability, are a considerable part of the RUP (Zhao and Cao, 2004), and the true protein fraction C is assumed not to be degraded at all within the rumen (Sniffen et al., 1992).

Accordingly, the concentrations of the true protein fractions B_3 and C and their sum are the most important predictors of the RUP concentrations (Fox et al., 2004; Schroeder, 2004; Tran et al., 2009; Salazar-Cubillas and Dickhoefer, 2018) and thus PRCP supply (Westreicher-Kristen et al., 2015) in ruminant forages. The concentration of ADF is also a good predictor of the concentration of RUP (Kirchhof, 2007), with greater ADF concentrations resulting in greater RUP supply from ruminant feedstuffs. Nevertheless, the negative relationship observed between forage ADF concentrations and PRCP supply in the present study is likely related to the fact that greater ADF concentrations strongly reduce DOM, which is in turn highly correlated with microbial CP synthesis (Lebzien et al., 1996) as a major constituent of PRCP.

In the present study, an attempt was also made to develop an equation to predict the PRCP supply according to Lebzien et al. (1996) from the net NH3-N release after 8 and 48 h during *in vitro* incubation and PRCP supply at Kp of 2, 5, and 8%/h determined by the *in vitro* method by using linear regression. The NH3-N release after 8 h *in vitro* incubation explained better the variance in PRCP supply as estimated according to Lebzien et al. (1996) (R^2 from 0.64 to 0.71) than the NH3-N release after 48 h *in vitro* incubation (R^2 from 0.44 to 0.52), whereas the effective *in vitro* PRCP supply (i.e., PRCP supply at Kp of 2, 5, and 8%/h) determined with the *in vitro* method explained between 41 to 76% of the variance in PRCP supply (data not shown). Although variables obtained by the *in vitro* method explained a great proportion of the variance in our reference PRCP supply (R^2 from 0.41 to 0.76), the IP concentration, as determined by the chemical method, was yet a much better predictor of PRCP supply from tropical forages (R^2 from 0.78 to 0.83).

The RMSE (5.3 to 5.4%) and adjusted R^2 (0.80 to 0.85) of the equations developed for tropical forages in the present study, as measures of accuracy and precision, respectively, were lower than those of the equation proposed by Zhao and Cao (2004) (RMSE of 12.5% and adjusted R^2 of 0.95), but within the range of those reported by Westreicher-Kristen et al. (2015) (RMSE from 2.3 to 10.2% and adjusted R^2 from 0.75 to 0.95). These results show the significant relationship between CP fractions and PRCP supply irrespective of the forage type. Nevertheless, their validation using an independent larger dataset on the concentrations of different CP and fiber fractions in a range of tropical forage grasses and legumes and their PRCP supply determined in vivo is still needed.

3.5 Conclusions

The *in vitro* method can be used as an alternative method to estimate PRCP supply in tropical forages at moderate to fast Kp (e.g., moderate to high feed intake levels) but not at very slow Kp. A lower accuracy and precision of the PRCP supply should be expected in tropical forage legumes than forage grasses.

Moreover, available regression equations developed for temperate ruminant feedstuffs were not accurate and precise enough to predict the PRCP supply of fresh tropical forages from concentrations of chemical CP fractions. Instead, equations developed in the present study appear to allow for an estimation of the PRCP supply of tropical forage grasses and legumes from fiber and CP fractions with a similar chemical composition than the samples included in the present study with reasonable adequacy. Nevertheless, further research is required to validate these equations in diverse species, origins, and phenological stages of forages used in cattle feeding in the tropics and subtropics.

3.6 Acknowledgments

We appreciate the technical assistance of Mr. H. Baumgaertner and of companion doctoral students of the working group of Animal Nutrition and Rangeland Management in the Tropics and Subtropics, University of Hohenheim, during laboratory and statistical analysis. The supply of forage samples by the research institutions in Brazil, Costa Rica, El Salvador, Ethiopia, Kenya, and Peru, as well as the supply of rumen fluid and the support on the *in situ* trial by the Institute of Animal Science of the University of Hohenheim are much appreciated.

Appendix 3.1
Concentrations of crude protein fractions and post-ruminal crude protein supply determined using Lebzien et al. (1996) equation of forages commonly used in tropical and subtropical ruminant husbandry systems at rumen passage rates of 2, 5, and 8%/hour (all in g/kg dry matter).

Forage samples	Origin[1]	Season[2]	Crude protein fractions[3]					PRCP[4]		
			A	B_1	B_2	B_3	C	2%/hour	5%/hour	8%/hour
Fresh tropical forage grasses										
Andropogon gayanus Kunth	ES	RS	20.3	4.3	13.4	34.0	8.6	97	101	103
Andropogon gayanus Kunth	KY	DS	22.2	1.7	11.3	32.5	17.4	98	102	103
Brachiaria brizantha (Hochst. ex A. Rich.) Stapf	PE	RS	47.9	4.1	27.1	18.3	12.6	100	104	105
Brachiaria brizantha (Hochst. ex A. Rich.) Stapf *x* *Brachiaria ruziziensis* R. Germ. and C.M. Evrard	KY	DS	15.7	3.3	15.6	6.1	5.4	82	81	81
Cenchrus ciliaris L.	KY	DS	39.8	4.1	35.9	35.4	24.7	119	127	130
Chloris gayana Kunth	KY	DS	41.0	3.9	25.4	41.8	20.8	116	123	127
Cynodon dactylon (L.) Pers.	KY	DS	54.7	4.9	22.9	45.9	11.1	114	120	123
Cynodon nlemfuensis Vanderyst	KY	DS	77.8	0.5	21.3	34.2	15.7	116	120	123
Digitaria decumbens Stent	PE	RS	29.2	4.4	21.0	12.5	8.2	93	94	95
Digitaria eriantha Steud.	KY	DS	44.1	4.5	13.6	36.1	12.9	104	109	112
Eragrostis echinochloidea Stapf	KY	DS	57.6	1.4	19.1	31.7	10.0	105	107	109
Hyparrhenia rufa (Nees) Stapf	ET	RS	34.9	0.8	29.2	39.9	11.4	107	113	117
Hyparrhenia rufa (Nees) Stapf	KY	DS	19.7	2.0	18.8	28.2	27.0	108	113	116
Melinis minutiflora P. Beauv.	KY	DS	29.1	4.7	16.9	39.5	22.3	102	109	113
Panicum coloratum L.	ET	RS	69.2	8.9	31.8	33.7	6.6	115	119	121
Panicum coloratum L.	KY	DS	56.7	1.2	23.1	44.6	14.4	111	117	121
Panicum maximum Jacq.	PE	RS	26.9	3.0	13.3	9.8	7.1	84	84	84
Pennisetum clandestinum Hochst. ex Chiov.	ET	RS	93.6	9.2	37.1	51.8	8.8	112	115	118
Pennisetum clandestinum Hochst. ex Chiov.	KY	DS	40.2	2.5	40.4	42.1	21.1	114	124	128
Pennisetum pedicellatum Trin.	ET	RS	50.4	5.4	27.8	34.0	7.8	103	108	111
Pennisetum purpureum Schumach.	ET	RS	51.4	0.3	27.5	32.2	8.1	109	114	117
Pennisetum purpureum Schumach.	KY	DS	38.3	1.9	23.7	12.1	15.7	102	104	105
Tripsacum andersonii J. R. Gray	KY	DS	43.7	1.8	33.5	39.7	23.8	117	127	132

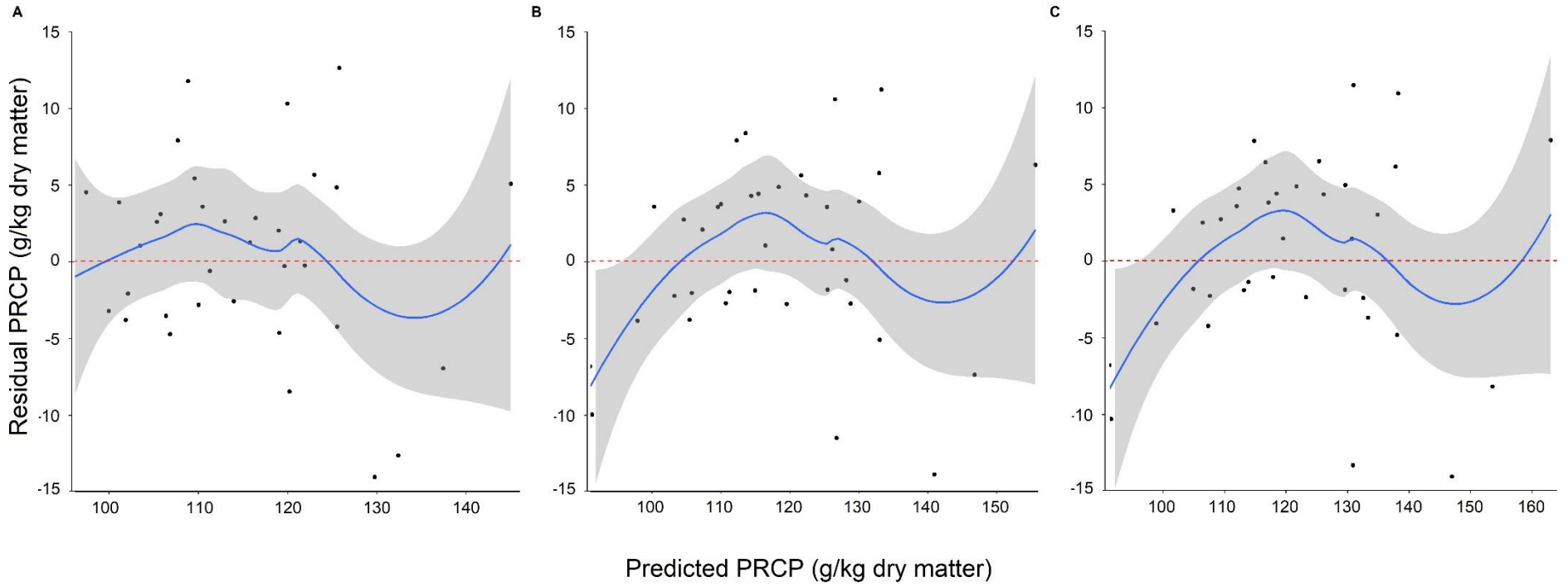
[1] BR, Brazil; CR, Costa Rica; ES, El Salvador; ET, Ethiopia; KE, Kenya; PE, Peru.
[2] DS, dry season; RS, rainy season.

[3] Crude protein fractions described by Sniffen et al. (1992) and analyzed following Licitra et al., 1996: A, crude protein soluble in the borate-phosphate buffer and tungstic acid solution; B_1, true protein soluble in buffer solution and precipitated by the tungstic solution; B_2, true protein insoluble in buffer solution but soluble in the neutral-detergent solution; B_3, true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution; C, true protein insoluble in the acid-detergent solution.
[4] Post-ruminal crude protein supply determined at rumen passage rates of 2, 5, and 8 %/h with the equation N°11 of Lebzien et al. (1996) using information on *in situ* rumen-undegraded crude protein at rumen passage rates of 2, 5, and 8 %/h, crude protein, and digested organic matter concentration determined from *in vitro* gas production.

(Appendix 3.1 Continued)

Forage samples	Origin[1]	Season[2]	Crude protein fractions[3]					PRCP[4]		
			A	B_1	B_2	B_3	C	2%/hour	5%/hour	8%/hour
Fresh tropical forage legumes										
Arachis glabrata Benth.	ES	RS	24.4	12.9	48.7	47.8	18.1	130	139	144
Arachis pintoi Krapov. and W. C. Greg.	BR	DS	66.2	2.2	53.8	23.0	28.9	123	127	130
Calopogonium mucunoides Desv.	BR	DS	57.4	4.3	81.6	33.9	16.6	116	122	126
Canavalia ensiformis (L.) DC.	ES	RS	61.0	1.8	65.2	40.4	16.4	121	128	133
Centrosema sp. (DC.) Benth.	BR	DS	35.7	8.6	61.4	69.9	27.9	130	139	145
Crotalaria longirostrata Hook. and Arn.	ES	RS	42.5	1.9	74.6	6.2	9.6	121	122	123
Dolichos lablab L.	ES	RS	38.6	1.7	59.6	38.0	16.2	130	137	142
Glycine max (L.) Merr.	ES	RS	75.4	17.2	95.7	12.1	10.8	138	144	149
Giricidia sepium (Jacq.) Kunth	ES	RS	47.6	0.2	67.4	65.5	31.1	150	162	171
Macroptilium atropurpureum (DC.) Urb.	ES	RS	42.3	11.0	71.1	57.8	15.6	120	127	133
Mucuna pruriens (L.) DC.	ES	RS	30.4	5.1	24.2	49.9	47.0	119	127	132
Pueraria phaseoloides (Roxb.) Benth.	PE	RS	65.4	6.1	85.2	21.5	15.9	129	134	138
Stylosanthes guianensis (Aubl.) Sw.	ES	RS	37.7	12.2	46.1	42.5	19.1	121	129	135
Stylosanthes guianensis (Aubl.) Sw.	BR	DS	42.2	9.2	33.1	39.5	30.4	111	117	121
Vigna sinensis (L.) Savi ex Hassk.	PE	RS	51.1	2.3	66.8	30.5	14.4	122	126	130

[1] BR, Brazil; CR, Costa Rica; ES, El Salvador; ET, Ethiopia; KE, Kenya; PE, Peru.
[2] DS, dry season; RS, rainy season.
[3] Crude protein fractions described by Sniffen et al. (1992) and analyzed following Licitra et al., 1996: A, crude protein soluble in the borate-phosphate buffer and tungstic acid solution; B_1, true protein soluble in buffer solution and precipitated by the tungstic solution; B_2, true protein insoluble in buffer solution but soluble in the neutral-detergent solution; B_3, true protein soluble in acid-detergent solution but insoluble in neutral-detergent solution; C, true protein insoluble in the acid-detergent solution.
[4] Post-ruminal crude protein supply determined at rumen passage rates of 2, 5, and 8 %/h with the equation N°11 of Lebzien et al. (1996) using information on *in situ* rumen-undegraded crude protein at rumen passage rates of 2, 5, and 8 %/h, crude protein, and digested organic matter concentration determined from *in vitro* gas production.



Appendix 3.2
Residuals between post-ruminal crude protein (PRCP) supply estimated with Lebzien et al. (1996) equation and PRCP predicted with the developed model versus predicted PRCP supply of tropical forage grasses and legumes at rumen passage rates (Kp) of 2 (A), 5 (B) and 8 %/h (C).

3.7 References

Ajayi, F. T., Babayemi, O. J., Taiwo, A. A. 2007. Effects of *Stylosanthes guianensis* and *Aeschynomene histrix* on the yield, proximate composition and *in situ* dry matter and crude protein degradation of *Panicum Maximum* (Ntchisi). Livest. Res. Rural. Dev. 19, 1–9.

Bowen, M. K., Poppi, D. P., McLennan, S. R. 2008. Ruminal protein degradability of a range of tropical pastures. Aust. J. Exp. Agric., 48, 806–810. https://doi.org/10.1071/EA07414

Dhanoa, M. S., France, J., López, S., Dijkstra, J., Lister, S. J., Davies, D. R., Bannink, A. 1999. Correcting the calculation of extent of degradation to account for particulate matter loss at zero time when applying the polyester bag method. J Anim Sci 77, 3385–3391. https://doi.org/10.2527/1999.77123385x

Edmunds, B., Südekum, K.-H., Spiekers, H., Schuster, M., Schwarz, F. J. 2012. Estimating utilisable crude protein at the duodenum, a precursor to metabolisable protein for ruminants, from forages using a modified gas test. Anim. Feed Sci. Technol. 175, 106–113. https://doi.org/10.1016/j.anifeedsci.2012.05.003

Evans, J. D. 1996. Straightforward statistics for the behavioral sciences. Thomson Brooks/Cole Publishing Company, Pacific Grove, USA.

Evitayani, Warly, L., Fariani, A., Ichinohe, T., Fujihara, T. 2004. Study on nutritive value of tropical forages in North Sumatra, Indonesia. Asian-Australas. J. Anim. Sci. 17, 1518–1523. https://doi.org/10.5713/ajas.2004.1518

Fondevila, M., Nogueira-Filho, J. C. M., Barrios-Urdaneta, A. 2002. *In vitro* microbial fermentation and protein utilisation of tropical forage legumes grown during the dry season. Anim. Feed Sci. Technol. 95, 1–14. https://doi.org/10.1016/S0377-8401(01)00315-7

Fox, D. G., Tedeschi, L. O., Tylutki, T. P, Russell, J. B., Van Amburgh, M. E., Chase, L. E., Pell, A. N., Overton, T. R., 2004. The Net Carbohydrate and Protein System for evaluating herd nutrition and nutrient excretion: model documentation. Anim. Feed Sci. Technol. 112, 29–78. https://doi.org/10.1016/j.anifeedsci.2003.10.006

GfE (Gesellschaft für Ernährungsphysiologie). 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder.DLG-Verlag, Frankfurt, Germany.

Greenberg, N. A., Shipe, W. F. 1979. Comparison of the abilities of trichloroacetic, picric, sulfosalicylic, and tungstic acids to precipitate protein hydrolysates and proteins. J. Food Sci. 44, 735–737.

Grubbs, F. E. 1969. Procedures for detecting outlying observations in samples. Technometrics 11, 1–21.

Gidlund, H., Vaga, M., Ahvenjarvi, S., Rinne, M., Ramin, M., Huhtanen, P. 2018. Predicting omasal flow of nonammonia n and milk protein yield from *in vitro*-determined utilizable crude protein at the duodenum. J. Dairy Sci. 101, 1164–1176. 10.3168/jds.2017-13422

Hair, J. F., Anderson, R. E., Tatham, R. L., Black, W. C. 1995. Multivariate data analysis. New York Macmillan, USA.

Ibrahim, M. N. M., Tamminga, S., Zemmelink, G. 1995. Degradation of tropical roughages and concentrate feeds in the rumen. Anim. Feed Sci. Technol. 54, 81–92. https://doi.org/10.1016/0377-8401(94)00758-2

INRA (Institut National de la Recherche Agronomique). 2019. INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, Netherlands.

Juárez, F.I., Pell, A. N., Blake, R. W., Montero Lagunes, M., Pinos Rodriguez, J. M. 2018. *In vitro* ruminal degradation of neutral detergent fiber insoluble protein from tropical pastures fertilized with nitrogen. Rev. Mex. Cienc. Pecu. 9, 588–600. https://doi.org/10.22319/rmcp.v9i3.4490

Khandaker, Z. H., Tareque, A. M. M. 1996. Studies on protein degradabilities of feedstuffs in Bangladesh. Asian-Australas. J. Anim. Sci. 9, 637–642. https://doi.org/10.5713/ajas.1996.637

Khamseekhiew, B., Liang, J. B., Wong, C. C., Jalan, Z. A. 2001. Ruminal and intestinal digestibility of some tropical legume forages. Asian-Australas. J. Anim. Sci. 14, 321–325. https://doi.org/10.5713/ajas.2001.321

Kirchhof, S. 2007. Untersuchungen zur Kinetik des ruminalen *in situ*-Nährstoffabbaus von Grünlandaufwüchsen des Alpenraumes unterschiedlicher Vegetationsstadien sowie von Maissilagen und Heu - ein Beitrag zur Weiterentwicklung der Rationsgestaltung für Milchkühe. [Dissertation to obtain the doctoral degree of Agricultural Sciences]. University of Kiel.

Krawielitzki, K., Schmidt, T., Voigt, J., Kowalczyk, J., Gabel, M. 2006. Dynamics of microbial contamination of protein during ruminal *in situ* incubation of feedstuffs. Anim. Feed Sci. Technol. 15, 313–328. https://doi.org/10.22358/jafs/66903/2006

Krishnamoorthy, U., Muscato, T. V., Sniffen, C. J., Van Soest, P. J. 1982. Nitrogen fractions in selected feedstuffs. J. Dairy Sci. 65, 217–225. https://doi.org/10.3168/jds.S0022-0302(82)82180-2

La O, O., Chongo, B., Delgado, D., Ruiz, T., Ruiz, O. 2006. Fraccionamiento proteico y digestión ruminal de nutrientes de siratro (*Macroptilium atropurpureum*). Rev. Cuba. Cienc. Agríc. 40, 315–320.

Lee, M. A. 2018. A global comparison of the nutritive values of forage plants grown in contrasting environments. J. Plant. Res. 131, 641–654. https://doi.org/10.1007/s10265-018-1024-y

Leberl, P., Gruber, L., Steingass, H., Schenkel, H. 2007. Comparison of the methods modified Hohenheimer Futter-Werttest (moHFT) and Cornell system for determination of nXP-content of concentrates. Presented at the 16 International Science Symposium on Nutrition of Domestic Animals, pp. 171–176.

Lebzien, P., Voigt, J. 1999. Calculation of utilizable crude protein at the duodenum of cattle by two different approaches. Archiv für Tierernaehrung 52, 363–369. 10.1080/17450399909386174.

Licitra, G., Hernandez, T. M., Van Soest, P. J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347–358. https://doi.org/10.1016/0377-8401(95)00837-3

Lin, L. I.-K. 1989. A Concordance correlation coefficient to evaluate reproducibility. Biometrics, 45(1), 255-268. PMID: 2720055.

Madsen, J., Hvelplund, T. 1994. Prediction of *in situ* protein degradability in the rumen: Results of a European ringtest. Livest. Prod. Sci. 39, 201–212. https://doi.org/10.1016/0301-6226(94)90185-6

McBride, G. B. 2005. A proposal for strength-of-agreement criteria for Lin's concordance correlation coefficient. NIWA Client Report: HAM2005-062, Hamilton, New Zealand.

Melesse, A., Steingass, H., Schollenberger, M., Rodehutscord, M. 2017. Screening of common tropical grass and legume forages in Ethiopia for their nutrient composition and methane production profile *in vitro*. Trop. Grassl.- Forrajes Trop. 5, 63–175. https://doi.org/10.17138/tgft(5)163-175

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

Minson, D. J., Wilson, J.R. 1980. Comparative digestibility of tropical and temperate forage - a contrast between grasses and legumes. Aust. J. Agric. Res. 46, 247–249.

Mupangwa, J. F., Ngongoni, N. T., Hamudikuwanda, H. 2003. Effects of stage of maturity and method of drying on *in situ* nitrogen degradability of fresh herbage of *Cassia rotundifolia*, *Lablab purpureus* and *Macroptilium atropurpureum*. Livest. Res. Rural 15, 35.

Nurdianti, R. R., Dickhoefer, U., Castro-Montoya, J. 2019. Evaluation of undigested and potential digestible fiber in tropical grasses and tropical legumes. Presented at Tropentag, p. 217.

Nogueira Filho, J. C. M., Fondevila, M., Urdaneta, A. B., Ronquillo, M. G. 2000. *In vitro* microbial fermentation of tropical grasses at an advanced maturity stage. Anim. Feed Sci. Technol. 83, 145–157. https://doi.org/10.1016/S0377-8401(99)00123-6

Osuga, I. M., Abdulrazak, S. A., Ichinohe, T., Fujihara, T. 2006. Rumen degradation and *in vitro* gas production parameters in some browse forages, grasses and maize stover from Kenya. J. Food Agric. Environ. 4, 60–64.

Ramírez Lozano, R. G., González Rodríguez, H., García Dessommes, G. 2002. Chemical composition and rumen digestion of forage from Kleingrass (*Panicum coloratum*). Interciencia. 27, 705–709. ISSN: 0378-1844

Salazar-Cubillas, K., Dickhoefer, U. 2019. Evaluating the accuracy of conceptual models for predicting the nitrogen excretion of cattle in tropical environments. Presented at the International Symposium on Ruminant Physiology 2019, p. 487.

Salazar-Cubillas, K., Dickhoefer, U. 2018. Validation of prediction equations to estimate rumen-undegradable crude protein in tropical feedstuffs using protein fractionation technique. Presented at the 10th International Symposium on the Nutrition of Herbivores, p. 526.

Salgueiro da Silva, M. A., Seixas, T. M. 2017. The role of data range in linear regression. Phys. Teach. 55, 371–372.

Schroeder, J. W. 2004. Forage Nutrition for Ruminants. NDSU Extension Service, North Dakota State University: Fargo, USA.

Singh, S., Kushwaha, B. P., Nag, S. K., Mishra, A.K., Singh, A., Anele, U. Y. 2012. *In vitro* ruminal fermentation, protein and carbohydrate fractionation, methane production and prediction of twelve commonly used Indian green forages. Anim. Feed Sci. Technol. 178, 2–11. https://doi.org/10.1016/j.anifeedsci.2012.08.019

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., Russell, J. B. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 70, 3562– 3577. https://doi.org/10.2527/1992.701135 62x

Steingaß, H., Nibbe, D., Südekum, K.-H., Lebzien, P., Spiekers, H. 2001. Schätzung des nXP-Gehaltes mit Hilfe des modifizierten Hohenheimer Futterwerttests und dessen Anwendung zur Bewertung von Raps- und Sojaextraktionsschroten. Presented at the Congress VDLUFA 113, p. 114.

Stern, M. D., Bach, A., Calsamiglia, S. 1997. Alternative techniques for measuring nutrient digestion in ruminants. J. Anim. Sci. 75, 2256– 276. https://doi.org/10.2527/1997.758 2256x

Tedeschi, L. O. 2006. Assessment of the adequacy of mathematical models. Agric. Syst. 89, 225–247. https://doi.org/10.1016/j.agsy.2005.11.004

Tedeschi, L. O., Fox, D. G., Pell, A. N., Lanna, D. P. D., Boin, C. 2002. Development and evaluation of a tropical feed library for the Cornell Net Carbohydrate and Protein System Model. Sci. Agr. 59, 1–18. https://doi.org/10.1590/S0103-90162002000100001

Tran, H., Salgado, P., Lecomte, P. 2009. Specie, climate, and fertilizer effects on grass fibre and protein in tropical environments. J. Agric. Sci. 147, 555–568. https://doi.org/10.1017/S0021859609008752

Valarini, M. J., Possenti, R. A. 2006. Research note: Nutritive value of a range of tropical forage legumes. Trop. Grassl. -Forrajes Trop. 40, 183–187.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Cornell University Press, New York, USA.

VDLUFA (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten). 2012. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik: (Methodenbuch III). Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

Wallace, R. J. Lahlou-Kassi, A. 1995. Rumen ecology research planning. Presented at the Workshop held at ILRI (International Livestock Research Institute), p. 270.

Westreicher-Kristen, E., Steingass, H., Rodehutscord, M. 2015. Estimation of utilisable crude protein at the duodenum of dried distillers' grains with solubles using a modified gas test. Arch. Anim. Nutr. 69, 351–365. https://doi.org/10.1080/1745039X.2015.1073058

Weisbjerg, M. R., Bhargava, P. K., Hvelplund, T., Madsen, J. 1990. Use of degradation curves in feed evaluation. Report from the National Institute of Animal Science, Denmark.

Wigati, S., Kustantinah, A., Wiyanto, E., Ørskov, E. R. 2014. *In sacco* degradability of six different tropical feedstuffs. Presented at the 16th AAAP Animal Science Congress, pp. 376–379.

Zhao, G. Y., Cao, J. E. 2004. Relationship between the *in vitro*-estimated utilizable crude protein and the Cornell Net Carbohydrate and Protein System crude protein fractions in feeds for ruminants. J. Anim. Physiol. Anim. Nutr. (Berl) 88, 301–310. https://doi.org/10.1111/j.1439-0396.2004.00485.x

Zhao, G. Y., Lebzien, P. 2000. Development of an *in vitro* incubation technique for the estimation of the utilizable crude protein (uCP) in feeds for cattle. Arch. Tierernahr. 53, 293–302. https://doi.org/10.1080/17450390009381953

Chapter 4

4. Predicting nitrogen excretion of cattle kept under tropical and subtropical conditions using semi-mechanistic models

This chapter was submitted as:

Salazar-Cubillas, K., Corea, E., Dickhoefer, U., 2023. Predicting nitrogen excretion of cattle kept under tropical and subtropical conditions using semi-mechanistic models. Journal of Animal Physiology and Animal Nutrition.

Abstract

The present study aims at evaluating whether current semi-mechanistic models developed for temperate cattle systems can be adopted for cattle under (sub-) tropical husbandry systems to adequately (accurately and precisely) predict total nitrogen (TN), urine nitrogen (UN), fecal nitrogen (FN) excretion, and its partition into different FN fractions. Selected models were built based on the feeding recommendations for ruminants of the British (model A), German (model G) French (INRA; model I) system. Model evaluation was conducted using eight nitrogen balance studies performed in El Salvador, Kenya, and Peru (n = 392 individual observations including lactating cows, heifers, and steers). Concordance correlation coefficient, root mean square errors (RMSE), and mean biases were estimated to evaluate the models' adequacy in predicting nitrogen excretion. Input variables causing greatest variation in nitrogen excretion prediction were identified by a sensitivity analysis and adjusted. None of the tested models was able to adequately (i.e., RMSE <25% of observed mean, systematic error <5% of mean square error) predict the excretion of TN, UN, FN, and of different FN fractions. Even after increasing duodenal microbial crude protein flow and changing efficiency of metabolizable protein use from a fixed to a variable factor, model A could not adequately predict TN (RMSE = 25%), UN (RMSE = 37%), FN (RMSE = 29%), and FN fractions (RMSE > 61%). Model I adequately predicted FN (RMSE = 18%) and TN (RMSE = 19%) excretion, when duodenal microbial crude protein flow was increased, and the intercept used to predict FN excretion was reduced from 4.30 to 3.82 g of nitrogen per kilogram of dry matter intake. These adjustments, however, were not sufficient to predict adequately UN excretion (RMSE = 33%) and individual FN fractions (RMSE > 55%) by model I.

Keywords: modeling, fecal nitrogen, urine nitrogen, feeding systems.

4.1 Introduction

Understanding protein metabolism is essential for matching nutrient supply to the requirements of cattle, and for thereby increasing animal performance and nitrogen (N) use efficiency (Arriaga et al., 2009; Rotz, 2004). Moreover, assessing partitioning of N excretion via urine (UN) and feces (FN) is crucial for estimating the environmental impact of N emissions from manure during storage and application (Johnson et al., 2016; Tamminga, 1992), as well as to evaluate the effectiveness of management and nutritional strategies (Dijkstra et al., 2013; Hristov et al., 2019). Further, different FN fractions, including microbial and endogenous debris N (BEDN), undigested dietary N (UDN), and water-soluble N (WSN) are important as well to determine N release in the soil and thus its availability for plant growth (Jost et al., 2013).

Direct measurements of UN, FN, and total N (TN) excretion in farms are challenging and impractical (Hristov et al., 2019). Therefore, several mathematical models have been developed to predict N excretion from lactating cattle, heifers, and steers (e.g., Dijkstra et al., 1996; Wilkerson et al., 1997; Herrero et al., 2002; Nennich et al., 2005; Johnson et al., 2016; Rufino et al., 2009; Bougouin et al., 2022). Most extant of these are empirical models that most likely cannot predict the partitioning of N excretion between feces and urine adequately under conditions different from those used for model fitting, hence their robustness is limited is low (Johnson et al., 2016). Semi-mechanistic models are an alternative to empirical models (Bateki and Dickhoefer, 2019) for estimating N excretion in cattle. Nevertheless, current semi-mechanistic models predict N excretion of cattle in tropical husbandry systems using feeding recommendations for cattle in temperate regions (AFRC, 1993; GfE, 2001; INRA, 2019; Sniffen et al., 1992) at a mean treatment level rather than individual observations. This might lead to inaccurate estimates of N excretion by cattle in (sub-) tropical husbandry systems as variations in the coefficients used in the models may not fully account for differences in feed quality, and other factors affecting nutrient utilization by cattle in (sub-) tropical husbandry systems.

The present study thus, aims at evaluating whether the approach used by available semi-mechanistic models and their assumptions could be adopted to adequately predict TN, UN, FN, and its partition into fecal BEDN, UDN, and WSN excretion of lactating cows, heifers, and steers in (sub-) tropical husbandry systems. It was hypothesized that semi-mechanistic models built based on feeding recommendations for cattle in temperate regions may result in inadequate estimates of TN, UN, and FN, as well as its partitioning into fecal BEDN, UDN, and WSN across different cattle production systems in the (Sub-) Tropics.

4.2 Materials and Methods

4.2.1 Identification and selection of models

Models were identified through online research (i.e., Google Scholar search engines), revising bibliographies of journal articles, conference proceedings, and feeding requirements for ruminants. The searches were conducted in English, German, Portuguese, and Spanish using the search terms: ruminant N excretion models; N excretion models for tropical cattle; UN excretion in tropical cattle; and FN excretion in tropical cattle. Models were selected if (1) N excretion was predicted following a semi-mechanistic approach, (2) N excretion was estimated for both, UN and FN, (3) model input variables were easily available in cattle farming systems in the (Sub-) Tropics (animal and feed input variables), and (4) models were developed using data from cattle farming systems in the (Sub-) Tropics or are currently used to predict N excretion for cattle in farming systems in the (Sub-) Tropics.

Three models were selected for evaluating their adequacy in predicting N excretion of cattle in (sub-) tropical husbandry systems. Two of the selected models were built based on the feeding recommendations for ruminants of the British (AFRC, 1993) (model A) and German (GfE, 2001) (model G) systems, and one model had been developed by INRA (2019) (model I) that includes modifications for cattle in warm areas (i.e., fermented organic matter, digestible dietary protein requirements for non-productive activities and growth).

4.2.2 Evaluation database and data sets

The database used for model evaluation was compiled from eight N balance studies performed in El Salvador, Kenya, and Peru by the Group of Animal Nutrition and Rangeland Management in the Tropics and Subtropics and local partners during the past eight years (Corea et al., 2017; Sainz-Sánchez, Rojas, Castro-Montoya et al., 2018; Ali et al., 2019a, 2019b; Castro-Montoya et al., 2019; Sainz-Sánchez et al., 2019; Corea et al., 2020; Aloba, 2022) (Table 1).

The evaluation database (n = 392 individual observations; Table 1 - 2) provided a reliable representation of the most common management systems (e.g., intensive and extensive husbandry systems), dietary forage to concentrate ratio (from 0.5 to 1), feed ingredients (e.g., fresh forages, silages, hays, energy and protein concentrates, and local agro-industrial by-products), feed origin (e.g., tropical and temperate origin), response to feed availability (fed above or below maintenance requirements), and nutrient balance (positive or negative rumen N balance).

The UN excretion (g/day) used as reference was measured using the total urine collection method (n = 144 observations) or estimated using the creatinine method (n = 248 observations) (Table 1). The reference FN excretion (g/day) was measured using the total fecal collection method (n = 176) or estimated using internal (i.e., acid insoluble ash) or external markers (i.e., titanium dioxide) (n = 216) (Table 1). The sum of UN and FN was considered TN excretion (g/day).

Each observation contained the same information, except for 95 observations (12 heifers and 83 steers; Table 3) that contained additional information on fecal BEDN, UDN, and WSN excretion (hereinafter referred to as data set 95; g/day). In data set 95, FN excretion was divided into three fractions according to Mason (1969): fecal BEDN, UDN, and WSN. The fecal BEDN excretion mainly contains indigestible microbial proteins from the rumen and the large intestine and some desquamated epithelial cells, fecal UDN contains neutral detergent fiber bound N, and fecal WSN mainly contains endogenous digestive secretions derived from the pancreas, bile, and the intestinal wall (Mason and Frederiksen, 1979).

4.2.3 Model input variables

The input variables required to run models A, G, and I (Table 4) were body-weight (BW), average daily BW change, age, breed, daily milk yield and its components (i.e., protein, fat, and lactose), daily dry matter (DM) intake, proportion of concentrate in the diet, and diet concentration of organic matter, crude fat, rumen degradable and undegradable crude protein (CP), acid-detergent-insoluble N, and metabolizable energy.

For all animal observations, BW was measured by using a cattle scale. The BW change was calculated as the difference between final and initial BW divided by the number of days between initial and final BW measurements. Milk yield was measured, and its components (i.e., protein, fat, and lactose) were analyzed with automatic milk analyzers. For concentrates, forages, and mixed rations, DM intake was calculated as the difference between offered and refused feeds while pasture DM intake was estimated using external markers (e.g., titanium dioxide).

The organic matter, crude fat, rumen degradable and undegradable CP, acid-detergent-insoluble N, and metabolizable energy concentrations of each diet were calculated by multiplying their concentration of each diet ingredient by its proportion in the diet. The organic matter concentration of the diet was calculated as the difference between the concentrations of DM (AOAC, 2005; method 934.01 or VDLUFA, 2012; method 3.1) and crude ash (AOAC, 2005; method 942.05 or VDLUFA, 2012; method 8.1). The crude fat concentration

(g/kg DM) of each diet ingredient was determined by ether extraction (VDLUFA, 2012; method 5.1.1) or obtained from the Feedipedia database (INRAE, CIRAD, AFZ, & FAO, 2012-2022) or Cornell-Penn-Miner dairy V3 program (CPM Dairy; Tedeschi et al., 2008).

The concentrations of rumen degradable and undegradable CP of each diet ingredient were obtained from the CPM Dairy (Tedeschi et al., 2008), Feedipedia database (INRAE et al., 2012-2022), or predicted from CP fractions by using the equations developed by Salazar-Cubillas and Dickhoefer (2021) (for forages), and Shannak et al. (2000) (for concentrate feeds) considering feeding level as digestive interaction. The concentration of acid-detergent-insoluble N of each diet ingredient was determined in the laboratory (Licitra et al., 1996), or obtained from the CPM Dairy (Tedeschi et al., 2008), Feedipedia database, (AFRC, 1993), or Salazar-Cubillas and Dickhoefer (2021) database. The metabolizable energy concentration in the diet was obtained from the CPM Dairy (Tedeschi et al., 2008), or estimated from crude nutrient concentrations and gas production release after 24 hours of in vitro incubation (Menke and Steingass, 1987), or digestible organic matter estimates (Aiple et al., 1992).

4.2.4 Statistical analysis

The R statistical software version 4.2.2 (R Core Team, Vienna, Austria) was used for constructing the structure of the models (Table 4), predicting N excretion, and statistical analysis. The input variables required to predict UN, FN, and TN excretion with models A, G, and I as well as reference values of UN, FN, TN (Table 2), fecal BEDN, UDN, and WSN excretions were characterized using descriptive statistics, including arithmetic mean, standard deviation (SD), minimum, and maximum (Table 2- 3).

The concordance correlation coefficient (CCC) (Lin, 1989), root mean square error (RMSE) (Bibby and Toutenburg, 1977), and mean bias (i.e., observed minus predicted estimates) were calculated to evaluate the adequacy of the predictions of UN, FN, and TN excretion with models A, G, and I (Table 5 - 6; Figure 1 - 2). The CCC as a combined measure of accuracy and precision ranges from -1 to 1 with a value of 1 indicating agreement between observed (i.e., measured or estimated UN, FN, and TN excretion) and predicted (i.e., predicted UN, FN, and TN excretion with models A, G, and I) N excretion (Lin, 1989; Tedeschi, 2006). The CCC was partitioned into a correlation coefficient that measures precision and a bias correction factor coefficient that measures accuracy (Lin, 1989). The RMSE (g/day and % of the observed mean) was calculated as the root square of the mean square error (MSE). The MSE was calculated and partitioned into overall bias, error due to regression, and error due to disturbance (expressed as a % of the MSE) (Bibby and

Toutenburg, 1977). In addition, RMSE was calculated after correcting for study bias. For this, linear regression models were fitted for each study to estimate the bias between observed and predicted values. The estimated bias for each study was then used to adjust the predicted values accordingly, and RMSE was computed between observed and adjusted predicted estimates. A more accurate and precise prediction was considered the one with greater CCC, RMSE not corrected for study bias < 25% of the observed mean (Reed et al., 2015), and systematic errors < 5% of the MSE (i.e., sum of overall bias and error due to regression) (Johnson et al., 2016).

Adjustments were made to the models with the greatest CCC to improve their adequacy. The adjustments were focused on the input variables that cause the greatest variation in N excretion prediction. To identify these input variables, a sensitivity analysis was conducted using a one-at-a-time approach in which input variables varied independently (±10%) while all other variables remained unchanged (Schouten et al., 2014). Adjustments were made based on the results presented in Tables 5 and 6. In brief, adjustment one involved replacing the approach of model I to predict duodenal microbial CP flow with that of model G. Adjustment two consisted of replacing the approach of model A to predict efficiency of MP with that of model I. In addition, the intercept of model I for predicting FN excretion was decreased by using a least squares iteration procedure. A detailed description and justification of these adjustments can be found in the results section.

4.3 Results

4.3.1 Prediction of urine nitrogen excretion

For the whole database, the CCC between observed and predicted UN excretion was greater for model I (0.89) than for models A (0.82) and G (0.85). None of the models had a RMSE of < 25% of the observed mean (from 35 to 45% of the observed mean). Systematic error of model A was lower than 5% of the MSE (< 4% of the MSE) but not for model G (23% of the MSE) and I (15% of the MSE). Model G overestimated (negative mean bias), whereas models A and I underestimated UN excretion (positive mean bias). Overestimation or underestimation by models A, G, and I were similar across different magnitudes of UN excretions (Figure 1). In addition, negative UN excretion were predicted by model I for animals with low N excretion (n = 10; -7 to -2 g/day), whereas no negative values were predicted by models A and G.

For the lactating cows' data set, the CCC between observed and predicted UN excretion were greater for model I (0.62) than for models A (0.36) and G (0.55). None of the models had a

RMSE of < 25% of the observed mean (from 27 to 35% of the observed mean) and systematic error of < 5% of the MSE (from 20 to 37% of the MSE) (Table 5). Similarly, for the heifers' and steers' data set, the CCC between observed and predicted UN excretion were greater for model I (0.67) than for models A (0.60) and G (0.44). None of the models had a RMSE of < 25% of the observed mean (from 64 to 102% of the observed mean) and a systematic error < 5% of the MSE (from 13 to 70% of the MSE) (Table 5).

Models I and A had a greater CCC than model G, therefore adjustments were made to improve their adequacy based on the results of the sensitivity analysis. The model I was more sensitive to changes in the efficiency of metabolizable protein (MP) use than to changes in MP supply, endogenous urinary N excretion, proportion of N recycled excreted in urine, microbial nucleic acids flow, and N balance (Table 4). The efficiency of MP use was more sensitive to changes in dietary rumen-undegraded CP concentrations and duodenal microbial CP flow than changes in milk protein yield, proportion of concentrate in the diet, and organic matter digestibility. As rumen-undegraded CP concentration of the diet could not be improved, because it was primarily obtained from tabulated values, adjustments were made to duodenal microbial CP flow. Despite the model being sensitive to changes in rumen-undegraded CP concentration, a greater RMSE was found in the group of observations where rumen-undegraded CP concentration of the diet was measured (n = 156), as opposed to when values were obtained from tabulated sources (n = 236) for UN (56 vs 28 % of the observed mean), FN (28 vs 16% of the observed mean), and TN excretion (32 vs 16% of the observed mean). This indicates that the adequacy of model I may be influenced by factors other than the reference method for estimating rumen-undegraded CP of the diet.

Based on the slightly better prediction of the BEDN fraction by model G (Table 6), the approach used to predict duodenal microbial CP flow by model I was replaced by the one in model G (adjustment one). As a result of adjustment one, RMSE was decreased from 35 to 33% of the observed mean, systematic error was reduced from 15 to 1% of the MSE, and CCC increased from 0.89 to 0.91.

In the same way as model I, model A was more sensitive to changes in the efficiency of MP use than changes in duodenal microbial CP flow, endogenous urinary N excretion, proportion of N recycled excreted in urine, and microbial nucleic acids flow (Table 4). Therefore, the approach of model A to predict efficiency of MP use was replaced by the approach of model I (adjustment two). Same to model I, the approach used to predict duodenal microbial CP flow by model A was replaced by the one in model G (adjustment one). As a result of adjustment one and two, RMSE decreased from 42 to 37 % of the observed mean, systematic error

decreased from 3 to 1% of the MSE, and CCC increased from 0.82 to 0.88. Adjustments one and two were not sufficient to reduce the RMSE to < 25% of the observed mean for UN prediction by models I and A, even when RMSE was corrected for study bias.

Apart from the adjustments, UN excretion was predicted with a specific equation for ruminants in warm areas (INRA, 2019; equation 22.23) and was applied only to animals with BW changes within the range used in the development of equation 22.23 (BW change from - 1 to 6 g/kg BW per day; RMSE of 0.034 g/kg BW per day). Results showed that model I and adjusted model I were more adequate (RMSE = 0.11 g/kg BW per day for both models; n = 368 individual observations) than the specific equation for ruminants in warm areas (RMSE = 0.21 g/kg BW per day; n = 368 individual observations).

4.3.2 Prediction of fecal nitrogen excretion

For the whole database, the CCC between observed and predicted FN excretion were greater for model I (0.96) than for models A (0.91) and G (0.85). The RMSE was < 25% of the observed mean for model I (19% of the observed mean) but not for model A (32% of the observed mean) and G (33% of the observed mean). None of the models had a systematic error < 5% of the MSE (from 9 to 68% of the MSE). Models A and I overestimated (negative mean bias) and model G underestimated FN excretion (positive mean bias). Overestimation of model I was similar across different magnitudes of FN excretions, whereas overestimation of model A and underestimation of model G increased as magnitudes of FN excretion increased (Figure1).

For the lactating cows' data set, the CCC between observed and predicted FN excretion was greater for model I (0.79) than for models A (0.58) and G (0.46). The RMSE was < 25% of the observed mean for model I (15% of the observed mean) but not for models A (26% of the observed mean) and G (28% of the observed mean). None of the models had a systematic error < 5% of the MSE (from 31 to 79% of the MSE) (Table 5).

For the heifers' and steers' data set, the CCC was greater for model G (0.81) than models I (0.64) and A (0.54). The RMSE was < 25% of the observed mean for model G (24% of the observed mean) but not for models A (52% of the observed mean) and I (41% of the observed mean). None of the models had a systematic error < 5% of the MSE (from 34 to 83% of the MSE) (Table 5).

For FN fractions, the CCC between observed and predicted FN fraction was greater for model G for BEDN (0.29) and WSN excretion (0.58) and model A for UDN excretion (0.45).

None of the models had a RMSE of < 25% of the observed mean (from 45 to 137% of the observed mean) and a systematic error < 5% of the MSE (from 47 to 86% of the MSE). Both fractions, BEDN and UDN, were overestimated by model A and underestimated by models G and I. Fraction WSN was overestimated by models A and G and underestimated by model I (Table 6; Figure 2).

In the case of model I, in addition to the adjustment one, the intercept used to predict FN excretion by model I was reduced from 4.30 to 3.82 g of N per kilogram of DM intake (i.e., reduction by 3 g of CP per kilogram of DM intake; adjustment three). As a result of adjustment one and three, RMSE decreased from 19 to 18% of the observed mean, systematic error decreased from 9 to 1% of the MSE, and CCC remained at 0.96. Therefore, adjustments one and three were sufficient to adequately predict FN excretion by model I.

In the case of model A, adjustments one and two resulted in a decrease in RMSE from 32 to 29% of the observed mean, an increase in systematic error from 68 to 71% of the MSE, and CCC remained at 0.91. Therefore, adjustments one and two in model A were not sufficient to adequately predict FN excretion.

4.3.3 Prediction of total nitrogen excretion

For the whole database, the CCC between observed and predicted TN excretion were greater for models I (0.96) and A (0.95) than for model G (0.93). The RMSE was < 25% of the observed mean for models A, G, and I (20 - 25% of the observed mean). Systematic error < 5% for model G (1% observed mean) but not for model A and I (8% of the MSE). Models A and G overestimated (negative mean bias), whereas models I underestimated TN excretion (positive mean bias) (Table 5; Figure 1).

For the lactating cows' data set, the CCC between observed and predicted TN excretion were greater for model I (0.81) than for models A (0.74) and G (0.75). The RMSE was < 25% of the observed mean for all models (from 16 to 20% the observed mean) but none of the models had a systematic error of < 5% of the MSE (Table 5).

For the heifers' and steers' data set, the CCC between observed and predicted TN excretion were greater for model I (0.81) than for models A (0.65) and G (0.67). None of the models had a RMSE of < 25% of the observed mean (from 35 to 52% of the observed mean) and a systematic error < 5% of the MSE (from 15 to 57% of the MSE) (Table 5).

As a result of adjustments one and three applied to FN excretion and adjustment one applied to UN excretion of model I, RMSE decreased from 20 to 19% of the observed mean, systematic error decreased from 8 to 1% of the MSE, and CCC remained at 0.96. Therefore, adjustments one and three applied to FN excretion and adjustment one applied to UN excretion were sufficient to adequately predict TN excretion by model I.

As a result of adjustments one and two applied to both FN and UN excretion of model A, RMSE increased from 23 to 25% of the observed mean, systematic error increased from 8 to 24% of the MSE and, CCC decreased from 0.95 to 0.94. Therefore, adjustments one and two applied to both FN and UN excretion of model A were not sufficient to adequately predict TN excretion.

Table 4.1.
Characteristics of the database used for model evaluation and reference values methodology per study.

Author[1]	N° observations	N° diets	Animal class	Breed	Diet's RUP[2]	Urine nitrogen excretion[3]	Fecal nitrogen excretion[4]
1	128	4	Lactating cows	Holstein	CPM Dairy Feedipedia	Creatinine	Acid insoluble ash
2	60	2	Lactating cows	Brown Swiss x Creole	Crude protein fractions	Creatinine	Titanium dioxide
3	28	4	Lactating cows	Brown Swiss x Creole	CPM Dairy	Creatinine	Titanium dioxide
4	32	4	Heifers	Holstein	CPM Dairy	Creatinine	Total collection
5	12	3	Heifers	Holstein x Boran	Crude protein fractions	Total collection	Total collection
6	48	4	Steers	Boran	Crude protein fractions	Total collection	Total collection
7	36	6	Steers	Boran	Crude protein fractions	Total collection	Total collection
8	48	4	Steers	Holstein x Cebu	CPM Dairy	Total collection	Total collection

[1] Authors, (1) Corea et al., 2017; (2) Sainz-Sánchez et al., 2018; (3) Castro-Montoya et al., 2019; (4) Corea et al., 2020; (5) Ali et al., 2019b; (6) Ali et al., 2019a; (7) Sainz-Sánchez et al., 2019; (8) Aloba, 2022.
[2] RUP, diet´s rumen undegraded crude protein; CPM Dairy, Cornell-Penn-Miner dairy V3 program (Tedeschi et al., 2008); Feedipedia database (INRAE et. al., 2012); Crude protein fractions, equations developed by Salazar-Cubillas and Dickhoefer (2021) for forages, and Shannak et al. (2000) for concentrate feeds.
[3] Creatinine (Valadares et al., 1999).
[4] Acid insoluble ash (Van Keulen and Young, 1977); titanium dioxide (Glindemann et al., 2009).

Table 4.2.
Means, standard deviations (SD), minimums (min), and maximums (max) of input variables required to run models A, G, and I, and observed nitrogen excretion (i.e., measured, or estimated reference values).

Variables[1]	Unit	Lactating cows (n = 216)				Heifers (n = 44)				Steers (n = 132)			
		Mean	SD	min	max	Mean	SD	min	max	Mean	SD	min	max
Variables related to the animals													
Body-weight	kg	501.6	65.0	371.0	703.2	202.6	34.1	125.7	252.5	211.0	35.0	123.5	318.0
Body-weight change	kg/day	0.2	0.2	-0.5	1.3	0.6	0.4	-0.1	1.2	0.3	0.5	-1.0	1.3
DM intake	kg DM/day	17.2	4.0	8.6	27.5	6.0	1.9	2.3	7.6	4.8	1.6	1.8	9.0
Milk yield	kg/day	22.7	8.9	5.7	45.5	-	-	-	-	-	-	-	-
Milk protein	g/100 g milk	3.3	0.4	2.7	4.7	-	-	-	-	-	-	-	-
Milk fat	g/100 g milk	3.4	0.6	2.2	5.9	-	-	-	-	-	-	-	-
Milk lactose	g/100 g milk	4.8	0.4	4.1	5.7	-	-	-	-	-	-	-	-
Variables related to the animals' diet													
PCO	g DM/g DM	0.4	0.1	0.1	0.5	0.3	0.2	0.0	0.4	0.2	0.1	0.0	0.3
Organic matter	g/kg DM	914.0	9.9	876.1	931.1	887.9	4.9	881.2	894.8	903.1	18.4	825.9	923.6
Crude fat	g/kg DM	4.0	1.3	1.7	5.1	3.0	1.0	1.7	4.5	1.9	1.1	0.7	9.0
RDP	g/kg DM	113.7	18.8	77.8	168.5	76.3	19.4	44.4	95.3	67.5	27.3	39.4	113.9
RUP	g/kg DM	50.0	7.0	36.5	57.7	36.7	7.4	26.9	46.2	29.6	12.1	19.6	53.9
ADIN	g/kg DM	1.5	0.4	1.1	2.5	1.1	0.5	0.7	2.0	1.5	0.2	1.2	2.1
Metabolizable energy	MJ/kg DM	10.3	0.9	8.1	11.2	8.5	0.5	7.7	9.1	7.6	1.2	5.7	9.2
DOM	g/kg DM	658.9	14.4	622.8	693.3	610.3	29.8	556.3	630.0	585.8	47.7	535.5	663.0
Nitrogen excretion													
Urine nitrogen	g/day	184.3	62.0	58.8	333.4	61.2	27.3	15.4	97.6	19.4	10.3	5.6	50.1
Fecal nitrogen	g/day	135.1	28.6	67.8	207.8	38.8	9.0	19.3	53.7	25.3	9.8	8.6	56.9
Total nitrogen	g/day	319.4	79.1	165.9	541.2	99.9	34.9	35.7	143.4	44.7	18.1	16.0	93.5

[1] ADIN, acid-detergent-insoluble nitrogen; DM, dry matter; DOM, dietary organic matter digestibility; PCO, proportion of concentrate in the diet; RDP, rumen-degraded crude protein; RUP, rumen-undegraded crude protein.

Table 4.3.
Means, standard deviations (SD), minimums (min), and maximums (max) of measured fecal nitrogen excretion and its fractions (g/day).

	Heifers (n = 12)				Steers (n = 83)				Mean	SD
	Mean	SD	min	max	Mean	SD	min	max		
Fecal nitrogen excretion	26.7	5.2	19.3	34.6	21.0	8.0	8.6	38.8	21.7	7.9
Microbial and endogenous debris nitrogen	14.5	3.1	11.2	19.7	9.0	3.0	4.0	16.5	9.7	3.5
Undigested dietary nitrogen	8.4	1.9	5.7	10.8	6.1	2.2	2.3	10.5	6.4	2.3
Water-soluble nitrogen	3.9	1.0	2.0	5.4	5.8	4.0	0.6	13.9	5.6	3.8

Table 4.4.
Equations used to predict urine and fecal nitrogen excretion by models A, G, and I.

Models	Equations	Equation N°[1]
A	$UNexcr\_A = (1 - MP_{I\ eff}) \times MP_A / 6.25 + EUN + N_{recycled\ A} + MNA_A$ $FNexcr\_A = BEDN_A + UDN_A + WSN_A$	
	$MP_{I\ eff}$ = 0.68 (for lactation); 0.59 (for growth)	page 19
	$MP_A = MCP_A \times 0.75 \times 0.85 + 0.90 \times (RUP - 6.25 \times ADIN) \times DMI$	equation 23
	if $MCP_{max} \geq RDP \times DMI$ then $MCP_A = RDP \times DMI$, otherwise $MCP_A = MCP_{max}$	equation 35
	$MCP_{max} = FME \times DMI \times MCP_{yield}$	equation 36
	$FME = ME - ME_{fat} - ME_{fermentable}$	equation 5
	ME_{fat} = 35 MJ/kg fat x fat	page 3
	$ME_{fermentable}$ = 0.90 x ME (for grass silages); 0.95 x ME (for brewery and distillers by-products)	equation 151
	$MCP_{yield} = 7 + 6 \times (1 - e^{(-0.35 \times DMI \times 100 / BW)})$	equation 34
	$EUN = (16.1 \times \ln(BW) - 42.2) / 6.25$	equation 84
	if $RDP \times DMI - MCP_A > 0$ then $N_{recycled\ A} = (RDP \times DMI - MCP_A) / 6.25$ otherwise $N_{recycled\ A} = 0$	page 20
	$MNA_A = 0.116 \times 0.80 \times 0.85 \times MCP_A / 6.25$	equation 7.7[2]
	$BEDN_A = (0.25 \times MCP_A + 0.15 \times 0.75 \times MCP_A) / 6.25$	page 20
	$UDN_A = (0.10 \times RUP / 6.25 + ADIN) \times DMI$	page 20
	$WSN_A = 0.35 \times BW^{0.75} - EUN$	equation 84
G	$UNexcr\_G = (PRCP / 6.25 + N_{BWC}) - (FNexcr\_G + N_{scurf} + PRCP_{req} / 6.25)$ $FNexcr\_G = BEDN_G + UDN_G + WSN_G$	
	$PRCP = [11.93 - (6.82 \times RUP / (RUP + RDP))] \times ME \times DMI / 1000 + 1.03 \times RUP \times DMI$	equation 9
	If $BWC \leq 0$ then $N_{BWC} = \lvert BWC \rvert \times PRCP_{req\ growth} / 6.25$ otherwise $N_{BWC} = 0$	page 26
	If $BWC \geq 0$ then $PRCP_{req\ growth} = BWC \times PRCP_{growth} / 6.25$ otherwise $PRCP_{req\ growth} = 0$ ($PRCP_{req\ growth}$ depending on BW gain and age)	page 26
	$PRCP_{req\ milk} = MY \times M_{protein} \times 1000$	page 39
	$N_{scurf} = 0.018 \times BW^{0.75}$	equation 2.1.3
	$BEDN_G = 0.15 \times (PRCP - RUP \times DMI) / 6.25$	page 39
	$UDN_G = 0.15 \times RUP \times DMI / 6.25$	page 39
	$WSN_G = 2.2 \times DMI$	equation 2.1.2

(Table 4.4 Continued)

Models	Equations	Equation N°[1]
I	$UNexcr\_I = (1 - MP_{I\ eff}) \times MP_I / 6.25 + EUN_I + N_{recycled\ I} + MNA_I + 0.47 \times N_{bal}$	equation 13.3
	$FNexcr\_I = BEDN_I + UDN_I + WSN_I + 4.30 \times DMI$	equation 13.1
	$MP_{I\ eff}$ = [67.5 – 0.52 x (MP_I/DMI – 100) + 0.014 x ((MY x $M_{protein}$ x 1000) – 1000)]/100 (for lactation); $MP_{I\ eff}$ = based on metabolic BW (for growth)	equation 7.22 page 131
	$MP_I = RUP \times DMI \times dr + MCP_I \times 0.80 \times 0.80$	equation 4.20
	$MCP_I = (41.7 + 71.9 \times 10^{-3} \times FOM + 8.4 \times PCO) \times DMI$	equation 4.15b
	$FOM = -63.34 + 0.971 \times (OMd \times 10 \times OM\ diet / 1000)$	equation 26.7
	$OMd = 40 + 35 \times [1 - \exp(-0.0083 \times (RUP + RDP)]$	equation 22.2
	$EUN_I = 0.05 \times BW$	equation 7.9
	$N_{recycled\ I} = RNB \times \alpha$; α = 0.79 (for cattle)	page 205
	$RNB = [-84.5 + 0.61 \times (RDP + RUP)] \times DMI / 6.25$	equation 4.11
	$MNA_I = MCP_I / 6.25 \times 0.116 \times 0.80 \times 0.85$	equation 7.7
	N_{bal} = [33 x (ME x DMI x 0.239 – NEmaint / kls – Nemilk / kls) x kls / 1.76] / 6.25 (lactation); N_{bal} = (MP_I – $MP_{req}NP$ – $MP_{req}GR$) / 6.25 (growth)	equations 6.12a and 6.12b
	$kls = 0.65 + 0.247 \times (q - 0.63)$	equation 6.11
	$q = -0.10 + 0.90 \times 0.01 \times [40 + 35 \times (1 - \exp(-0.0083 \times (RDP + RUP)))]$	equations 6.4 and 22.2
	$NE_{maint} = 93.6 \times BW^{0.75} / 1000$	page 99
	$NE_{milk} = 9.39 \times MY \times M_{fat} + 5.47 \times MY \times M_{protein} + 3.95 \times MY \times M_{lactose}$	page 98
	$MP_{req}NP = 1.71 \times BW^{0.897}$	equation 22.14b
	If BWC ≥ 0 then $MP_{req}GR = 0.75 + 289$ g/kg BWC x BWC otherwise $MP_{req}GR = 0$	equation 22.15
	$NDNDF = 591 – 6.09 \times (76.0 – (76.0 – OMd)$	equation 3.29
	$BEDN_I = 0.11 \times MCP_I / 6.25$	equation 13.1
	$UDN_I = 0.19 \times RUP \times DMI / 6.25$	equation 13.1
	$WSN_I = 0.02 \times NDNDF \times DMI / 6.25$	equation 13.1

[1] Number of the original equations of models A (AFRC, 1993), G (GfE, 2001), and I (INRA, 2018). The original equations and input variables have been renamed and restructured to provide the same structure and units across models. These changes, however, do not affect the original outcome of the equations.

[2] From INRA (2018)

α, proportion of rumen nitrogen balance recovered as urine (0 < α < 1)

ADIN, acid-detergent-insoluble nitrogen (g/kg DM)

BEDN, microbial and endogenous debris nitrogen (BEDNA, G, I = g/day)

BW, animal´s body-weight (kg)

BWC, animal´s body-weight change (kg/day)
DMI, dry matter intake (kg/day)
DOM, diet´s digested organic matter (g/kg DM)
dr, true intestinal digestibility of RUP (integration of equations 4.16, 4.19, and 4.18 yield in a constant value of 0.81)
EUN, endogenous urinary nitrogen (EUNA, I = g/day)
Fat, diet´s fat concentration (kg/kg DM)
FL, feeding level (kg DMI%BW)
FME, diet´s fermentable metabolizable energy (MJ/kg DM)
FNexcr_A, G or I, fecal nitrogen excretion predicted with model A, G, and I, respectively (g/day)
FOM, diet´s fermentable organic matter (g/kg DM)
kls, efficiency of metabolizable energy use for milk and maintenance (0 < kls < 1)
MCP, duodenal microbial crude protein flow (MCPA, I = g/day)
MCPyield, microbial protein yield (g/MJ FME)
MCPmax, maximum microbial protein synthesis (g/day)
ME, diet´s metabolizable energy (MJ/kg DM)
MEfat, diet´s metabolizable energy from total oils and fats (MJ/kg DM)
MEfermentable, diet´s metabolizable energy from fermentation acids (MJ/kg DM)
Mfat, lactose, protein, concentration of fat, lactose, and protein in milk, respectively (g/100g milk)
MNA, microbial nucleic acids (MNAA and I = g/day)
MP, metabolizable protein supply (MPA and I = g/day)
MPI eff, efficiency use of metabolizable protein (g/g)
MPreqNP, digestible dietary protein requirements for non-productive activities (g/day)
MPreqGR, digestible dietary protein requirements for growth (g/day)
MY, milk yield (kg/day)
Nbal, nitrogen balance (g/day)
NBWC, nitrogen resulting from a negative body-weight change (g/day)
NDNDF, non-digestible neutral detergent fiber (g/kg DM)
NEmaint, net energy requirement for maintenance (Mcal/day)
NEmilk, net energy requirement for lactation (Mcal/day)
Nrecycled, nitrogen recycled excreted in urine (Nrecycled A = g/day)
Nscurf, requirement for scurf nitrogen losses (g/day)
OMd, organic matter digestibility (% organic matter)
OMdiet, diet´s organic matter concentration (g/kg DM)
q, ratio between metabolizable and gross energy (from 0 to 1)
PCO, proportion of concentrate in the diet (g DM/g DM)

PRCP, post-ruminal crude protein intake (g/day)
PRCPreq milk, req growth, post-ruminal crude protein net requirements for lactation and growth, respectively (g/day)
PRCPgrowth, post-ruminal crude protein net requirements for growth (Bateki and Dickhoefer, 2019) (g/kg body-weight change)
RDP, diet´s rumen degradable crude protein (g/kg DM)
RNB, rumen nitrogen balance (g/day)
RUP, diet´s rumen undegraded crude protein (g/kg DM)
UDN, undigested dietary nitrogen (UDNA, G, I = g/day)
UNexcr_A, G or I, urine nitrogen excretion predicted with model A, G, and I, respectively (g/day)
WSN, water-soluble nitrogen (WSNA, G, I = g/day)

Table 4.5.
Observed (measured or estimated) urine, fecal, and total nitrogen (N) excretion and predicted with the models A, G, and I of lactating cows, heifers, and steers.

Variable estimated	Models[1]	Mean g/day	RMSE[2] g/day	RMSE[2] % mean	RMSE[3] % mean	MSE[2] ECT % MSE	MSE[2] ER % MSE	MSE[2] ED % MSE	CCC[2] CCC from -1 to 1	CCC[2] ρ from -1 to 1	CCC[2] Cb from -1 to 1
Lactating cows, heifers, and steers (n = 392)											
Urine N excretion	Observed	114.94									
	Model A	107.94	48.84	42.49	42.16	2.05	1.16	96.79	0.82	0.85	0.97
	Model G	136.67	51.41	44.72	40.61	17.86	5.50	76.64	0.85	0.87	0.97
	Model I	100.82	39.67	34.51	32.26	12.67	1.84	85.49	0.89	0.92	0.97
Fecal N excretion	Observed	87.31									
	Model A	107.38	27.66	31.68	22.89	52.67	15.29	32.04	0.91	0.96	0.94
	Model G	67.00	28.77	32.95	24.59	49.84	15.56	34.60	0.85	0.96	0.89
	Model I	92.20	16.99	19.46	19.32	8.26	0.27	91.47	0.96	0.96	1.00
Total N excretion	Observed	202.25									
	Model A	215.32	45.81	22.65	22.22	8.14	0.20	91.65	0.95	0.95	0.99
	Model G	203.67	50.22	24.83	25.11	0.08	0.05	99.87	0.93	0.94	1.00
	Model I	193.01	39.44	19.50	18.97	5.49	2.65	91.86	0.96	0.97	0.99

[1] Model A, G, and I, base-line models built based on ruminant´s feeding recommendations of the British (AFRC, 1993), German (GfE, 2001), and French (INRA, 2018) systems.
[2] CCC, Concordance Correlation Coefficient and its partitioning into correlation coefficient (ρ) and bias correction factor (Cb); MSE, Mean Square Prediction Error and its partitioning into error due to central tendency (i.e., overall bias; ECT), error due to regression (ER), and error due to disturbance (i.e., random error; ED); RMSE, Root Mean Square Error.
[3] RMSE, Root Mean Square Error corrected for study bias.

(Table 4.5 Continued)

Variable estimated	Models[1]	Mean g/day	RMSE[2] g/day	RMSE[2] % mean	RMSE[3] % mean	MSE[2] ECT % MSE	MSE[2] ER % MSE	MSE[2] ED % MSE	CCC[2] CCC from -1 to 1	CCC[2] ρ from -1 to 1	CCC[2] Cb from -1 to 1
Lactating cows (n = 216)											
Urine N excretion	Observed	184.29									
	Model A	164.30	63.14	34.26	33.29	10.02	9.75	80.23	0.36	0.41	0.89
	Model G	203.16	63.61	34.52	32.96	8.80	28.49	62.71	0.55	0.58	0.95
	Model I	161.80	50.59	27.45	24.91	19.76	1.26	78.98	0.62	0.69	0.90
Fecal N excretion	Observed	135.10									
	Model A	161.11	34.70	25.69	19.04	56.16	17.41	26.43	0.58	0.78	0.74
	Model G	101.41	38.28	28.34	15.33	77.44	1.52	21.04	0.46	0.79	0.58
	Model I	136.59	20.26	15.00	15.79	0.54	30.13	69.33	0.79	0.81	0.98
Total N excretion	Observed	319.39									
	Model A	325.41	55.24	17.30	17.62	1.19	6.79	92.03	0.74	0.74	0.99
	Model G	304.60	62.59	19.60	19.17	5.60	30.73	63.67	0.75	0.77	0.97
	Model I	298.39	49.72	15.57	14.51	17.84	7.06	75.10	0.81	0.84	0.97

[1] Model A, G, and I, base-line models built based on ruminant´s feeding recommendations of the British (AFRC, 1993), German (GfE, 2001), and French (INRA, 2018) systems.
[2] CCC, Concordance Correlation Coefficient and its partitioning into correlation coefficient (ρ) and bias correction factor (Cb); MSE, Mean Square Prediction Error and its partitioning into error due to central tendency (i.e., overall bias; ECT), error due to regression (ER), and error due to disturbance (i.e., random error; ED); RMSE, Root Mean Square Error.
[3] RMSE, Root Mean Square Error corrected for study bias.

(Table 4.5 Continued)

Variable estimated	Models[1]	Mean g/day	RMSE[2] g/day	RMSE[2] % mean	RMSE[3] % mean	MSE[2] ECT % MSE	MSE[2] ER % MSE	MSE[2] ED % MSE	CCC[2] CCC from -1 to 1	CCC[2] ρ from -1 to 1	CCC[2] Cb from -1 to 1
Heifers and steers (n = 176)											
Urine N excretion	Observed	29.83									
	Model A	38.77	20.52	68.80	82.93	18.99	3.27	77.74	0.60	0.67	0.91
	Model G	55.06	30.32	101.66	60.31	69.22	0.77	30.01	0.44	0.73	0.60
	Model I	25.97	19.09	63.99	73.13	4.08	8.83	87.09	0.67	0.68	0.98
Fecal N excretion	Observed	28.66									
	Model A	41.45	15.02	52.42	30.86	72.45	10.37	17.19	0.54	0.83	0.65
	Model G	24.76	6.71	23.42	78.08	33.69	0.17	66.14	0.81	0.87	0.92
	Model I	37.71	11.79	41.15	30.25	58.94	12.62	28.44	0.64	0.83	0.78
Total N excretion	Observed	58.49									
	Model A	80.22	30.47	52.10	42.28	50.85	4.45	44.70	0.65	0.79	0.82
	Model G	79.82	28.45	48.65	57.80	56.20	0.31	43.49	0.67	0.83	0.81
	Model I	63.69	20.72	35.43	43.33	6.30	8.79	84.91	0.81	0.82	0.99

[1] Model A, G, and I, base-line models built based on ruminant´s feeding recommendations of the British (AFRC, 1993), German (GfE, 2001), and French (INRA, 2018) systems.
[2] CCC, Concordance Correlation Coefficient and its partitioning into correlation coefficient (ρ) and bias correction factor (Cb); MSE, Mean Square Prediction Error and its partitioning into error due to central tendency (i.e., overall bias; ECT), error due to regression (ER), and error due to disturbance (i.e., random error; ED); RMSE, Root Mean Square Error.
[3] RMSE, Root Mean Square Error corrected for study bias.

Table 4.6.
Relationship between measured and predicted fractions of fecal nitrogen excretion of heifers (n = 12) and steers (n = 83; data set 95).

Equations[1]	Mean g/day	RMSE[2] g/day	RMSE[2] %mean	RMSE[3] %mean	MSE[2] ECT % MSE	MSE[2] ER % MSE	MSE[2] ED % MSE	CCC[2] CCC from -1 to 1	CCC[2] ρ from -1 to 1	CCC[2] Cb from -1 to 1
Observed BEDN	9.72									
$BEDN_A$ = (0.25 x MCP_A + 0.11 x MCP_A) / 6.25	12.76	7.98	82.12	80.74	14.45	67.83	17.72	0.18	0.26	0.67
$BEDN_G$ = 0.15 x (PRCP – RUP x DMI) / 6.25	7.09	4.38	45.02	38.52	36.06	10.50	53.43	0.29	0.40	0.73
$BEDN_I$ = 0.11 x MCP_I / 6.25	5.43	5.53	56.85	42.10	60.27	4.05	35.68	0.14	0.32	0.44
Observed UDN	6.37									
UDN_A = (0.10 x RUP / 6.25 + ADIN) x DMI	8.39	3.91	61.46	56.73	26.55	53.56	19.89	0.45	0.64	0.70
UDN_G = 0.15 x RUP x DMI / 6.25	2.36	4.35	68.36	38.83	84.68	1.23	14.09	0.15	0.69	0.22
UDN_I = 0.19 x RUP x DMI / 6.25	3.04	3.71	58.30	26.62	80.41	0.22	19.37	0.24	0.69	0.35
Observed WSN	5.58									
WSN_A = 0.35 x $BW^{0.75}$ – EUN	12.63	7.66	137.10	59.92	84.63	0.11	15.26	0.15	0.62	0.24
WSN_G = 2.2 x DMI	9.72	4.49	80.33	32.80	84.89	1.45	13.66	0.58	0.90	0.64
WSN_I = 0.02 x NDNDF x DMI / 6.25	3.89	3.13	56.13	61.09	29.06	35.69	35.25	0.50	0.87	0.57

Model A, G, and I, models built based on the British (AFRC, 1993), German (GfE, 2001), and French (INRA, 2018) ruminant´s feeding recommendations.

[1] $BEDN_{A, G, I,}$ microbial and endogenous debris nitrogen excreted via feces as predicted with models A, G, and I, respectively; $UDN_{A, G, I,}$ undigested dietary nitrogen excreted via feces as predicted with models A, G, and I, respectively; $WSN_{A, G, I,}$ fecal water-soluble nitrogen excretion predicted with models A, G, and I, respectively; $MCP_{A\ I}$, microbial crude protein supply of the model A and I (g/day), respectively; PRCP, post-ruminal crude protein intake (g/day) calculated according to Lebzien et al. (1996) equation; RUP, rumen-undegraded crude protein concentration (g/kg DM); DMI, dry matter intake (kg/day); ADIN, acid-detergent-insoluble nitrogen concentration (g/kg DM); BW, body-weight (kg); EUN, endogenous urinary nitrogen (g/day); NDNDF, non-digestible neutral detergent fiber concentration (g/kg DM).

[2] CCC, Concordance Correlation Coefficient and its partitioning into correlation coefficient (ρ) and bias correction factor (Cb); MSE, Mean Square Prediction Error and its partitioning into error due to central tendency (i.e., overall bias; ECT), error due to regression (ER), and error due to disturbance (i.e., random error; ED).

[3] RMSE, Root Mean Square Error corrected for study bias.

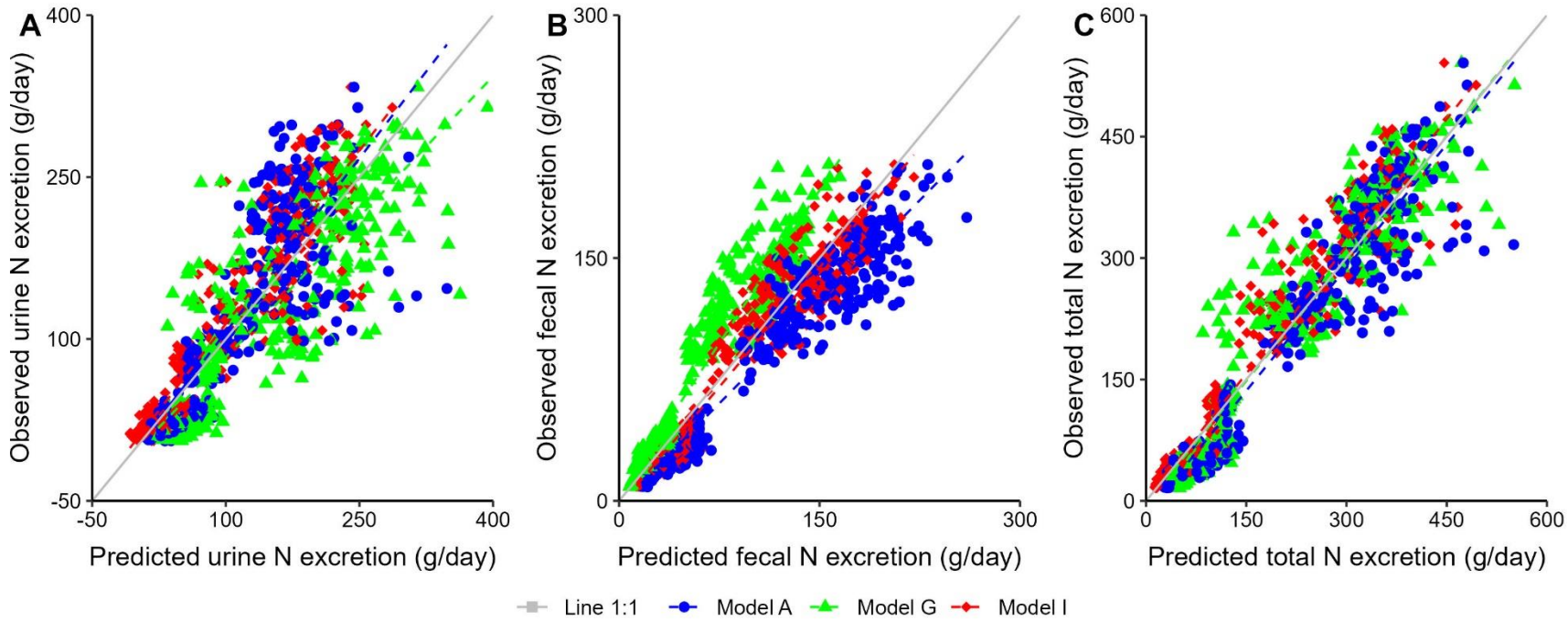


Figure 4.1.
Observed (measured or estimated) urine (Figure 1A), fecal (Figure 1B), and total nitrogen (N) excretion (Figure 1C) and predicted with the models A, G, and I of lactating cows (n = 264), heifers and steers (n = 176) without adjustments (different axis lengths).

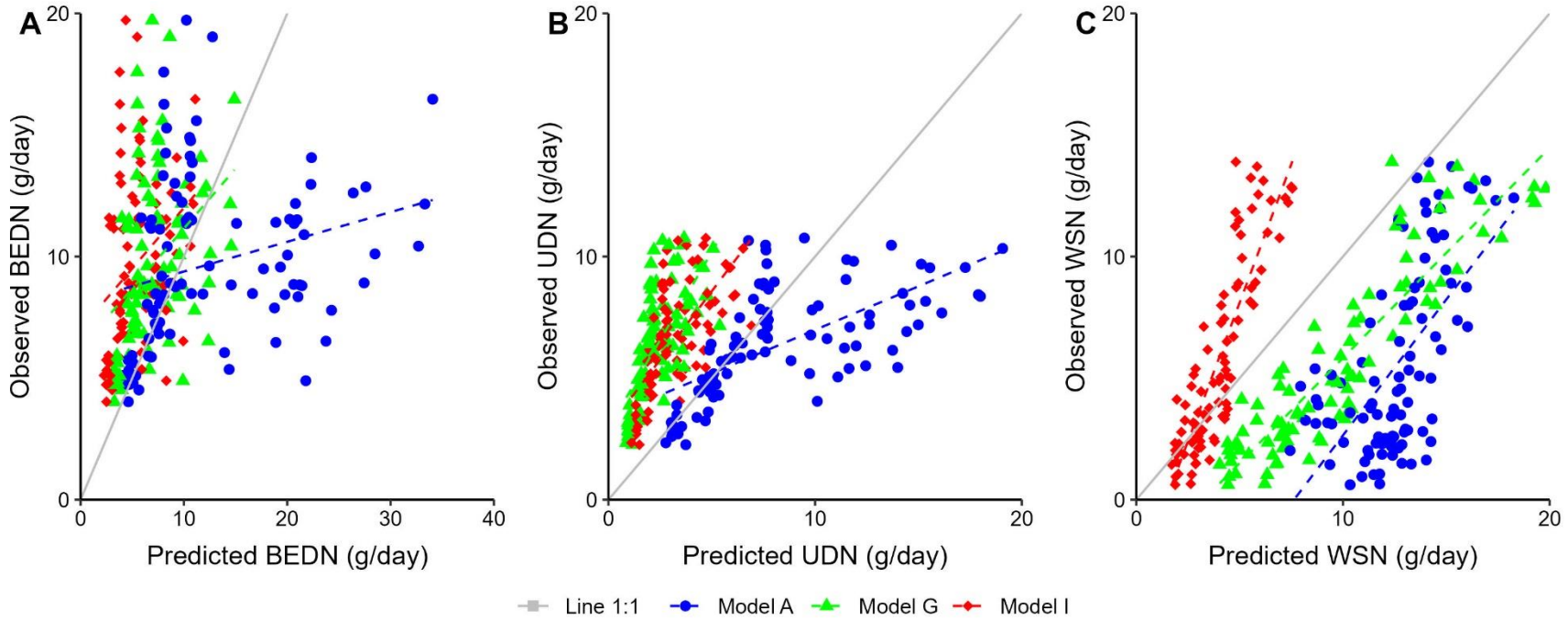


Figure 4.2.

Observed (measured) fecal microbial and endogenous debris nitrogen excretion (BEDN; Figure A), fecal undigested dietary nitrogen excretion (UDN; Figure B), and fecal water-soluble nitrogen excretion (WSN, Figure C) and predicted with models A, G, and I of heifers (n = 12) and steers (n = 83; data set 95) (different x-axis lengths).

4.4 Discussion

4.4.1 Sources of error

The DM intake, N intake, N excretion, and performance levels of cattle in (sub-) tropical husbandry systems included in our database were within the range of published in vivo studies and meta-analyses (Salah et al., 2014; Bateki and Dickhoefer, 2019; Bateki and Dickhoefer, 2020; Castro-Montoya et al., 2019). Therefore, our database is representative and allows for evaluation of the adequacy of semi-mechanistic models for predicting N excretion of cattle in (sub-) tropical husbandry systems under a variety of feeding and management situations that affect N excretion.

Model G adequately predicted TN excretion, but none of the models were able to adequately predict excretion of UN, FN, or of different FN fractions. From an environmental standpoint, there is particular interest to predict UN and FN excretion separately accurately and precisely (Hristov et al., 2019). The low accuracy of models A, G, and I to predict UN and FN excretion could be partially explained by the difficulties in obtaining accurate reference values of UN and FN excretion. For example, total collection of UN excretion is challenging due to technical difficulties with urine collection devices (e.g., harnesses) (Wassie, 2019; Aloba, 2022). Estimating urine volume assuming a constant daily creatinine excretion is also subject to error, due to considerable inter-animal and day to day variability in creatinine excretion (Tas and Susenbeth, 2007). Additionally, the dominant form of N in urine is urea-N (Dijkstra et al., 2013), which volatilizes quickly as ammonia. This may result in underestimations of UN concentration even when urine samples have been acidified.

In the case of reference values of FN excretion, the low concentration of acid insoluble ash in feeds and rations and its high variability (Van Soest, 1994), as well as the variable total fecal recovery of titanium dioxide and the diurnal variation in its fecal excretion (Titgemeyer et al., 2001) can lead to errors in the estimation of FN excretion. Also, FN excretion can be underestimated due to incomplete collection of material and loss of volatile N compounds (Spanghero and Kowalski, 1997). Finally, the N concentration in feces and thus FN excretion may be underestimated by as much as 13% or 16%, due to N losses during freeze-drying or oven-drying, respectively, when compared to fresh fecal samples (Spanghero and Kowalski, 1997; Wassie, 2019).

Due to the uneven distribution of animal class between the measured and estimated reference methods in our database (Table 1), it was not possible to fully isolate the effects of the reference method (measured against estimated) and animal class (lactating cows, heifers,

and steers) on the predictive performance of the evaluated models (Table 4). However, a more detailed analysis of reference method effects was conducted on smaller sub sets. The UN excretion data set of heifers included measured (n = 12) and estimated (n = 32) reference values (not similar data for lactating cows and steers). When estimating the accuracy of all models using both these data sub sets, the RMSE of model A was, however, only slightly greater for the estimated (26% of the observed mean) than the measured reference values (19% of the observed mean). Moreover, for models G (measured = 91% of the observed mean; estimated = 16% of the observed mean) and I (measured = 56% of the observed mean; estimated = 37% of the observed mean) the RMSE was even lower for the estimated than the measured reference values. Similarly, RMSE of estimated FN excretion from heifers and steers (not similar data for lactating cows) was lower when oven-dried fecal samples were used (RMSE from 21 to 40% of the observed mean; n = 80) than for freeze-dried fecal samples (RMSE from 26 to 70% of the observed mean; n = 96). Therefore, inaccuracies in reference values are not the only source of error, and inaccuracies of input variables and lack of parametrization of the models may play a larger role.

4.4.2 Predicting urine, fecal, and total nitrogen excretion by adjusted models

Improving the accuracy of the estimates of individual input variables contributes to improving the adequacy of the model (Tedeschi, 2006). It is, however, not always feasible to improve the adequacy of all input variables. Therefore, efforts should focus on those input variables whose small errors generate larger model prediction errors. In the present study, measured and estimated input variables were used to evaluate the potential of the models and avoid influencing their performance by errors in the input variables. However, in practical conditions, input variables such as DM intake will not be measured but predicted, which may introduce additional errors. Therefore, it is important to identify, which input variables are most prone to generating large errors in the model's predictions in order to develop suitable methodologies to predict them accurately and precisely.

The two models with greater potential for predicting UN and FN excretion (i.e., models A and I) were more sensitive to changes in the efficiency of MP use than other input variables, and efficiency of MP use was more sensitive to changes in rumen-undegraded CP and duodenal microbial CP flow than other variables. While model A used a constant factor, the efficiency of MP use by model I was estimated based on milk protein yield and MP supply (for lactating cows), and metabolic BW (for growing animals). Since efficiency of MP use varies with N and energy supply (Lapierre et al., 2005), the constant factor in model A was replaced with the approach of model I (adjustment two). Since fecal BEDN excretion correlates with

duodenal microbial CP flow (Wassie, 2019) and was more accurately predicted by model G, the approach of models A and I to estimate duodenal microbial CP flow were replaced by the one of model G (adjustment one).

The approaches to predict duodenal microbial CP flow by models A, G, and I differ in their input variables, their structure, and their magnitude. Model A estimated microbial CP flow based on fermentable metabolizable energy concentration and feeding level, whereas model G estimated it based on the rumen-undegraded CP and metabolizable energy intakes. For model I, microbial CP flow was estimated from fermented organic matter intake and the proportion of concentrate in the diet (Table 4). Predicted duodenal microbial CP flow was greater for model G (1155 g/day, SD 783; 9.9 g/MJ ME, SD 0.3; 91 g/kg DM intake, SD 16) and model A (1053 g/day, SD 738; 8.6 g/MJ ME, SD 1.4; 80 g/kg DM intake, SD 21.0) than model I (958 g/day, SD 580; 8.9 g/MJ ME, SD 1.3; 80 g/kg DM intake, SD 4.4) ($P < 0.05$). The differences in magnitude between models suggest that models A and I underestimate duodenal microbial CP flow, while model G may provide a more adequate prediction. Although model G might provide a more adequate prediction of microbial CP flow than the other approaches, it still overestimates *in vivo* measurements of duodenal microbial CP flow of cattle in (sub-) tropical husbandry systems as shown in Wassie et al. (2019). Therefore, to identify the most appropriate approach, the duodenal microbial CP flow predicted by models A, G, and I should be compared with *in vivo* estimates. Rumen-undegraded CP is also an input variable that generated greater prediction errors. Therefore, using tropical diet-specific methodologies and algorithms, as demonstrated in Salazar-Cubillas and Dickhoefer (2021), presents a promising solution for achieving precise and accurate estimates of rumen-undegraded CP.

Adjustments (one and three) to model I were sufficient to adequately predict FN (RMSE of 18% observed mean, systematic error of 1% of the MSE, and CCC of 0.96) and TN excretion (RMSE of 19% observed mean, systematic error of 1% of the MSE, and CCC of 0.96), but not UN excretion (RMSE of 33% observed mean, systematic error of 1% of the MSE, and CCC of 0.91). The prediction of FN excretion by model I was improved, because of an improvement in the prediction of the BEDN fraction (adjustment one), which represents 46% of total excretion of FN (measured BEDN in data set 95). After adjustment one, the linear regression between observed and predicted FN was parallel to line 1:1. Reducing the intercept of the equation to predict FN excretion (adjustment three) improved the accuracy of the FN prediction as the distance between line 1:1 and linear regression of the predictions was reduced. Further evaluations are necessary to determine if the reduction of the slope of model I and the improvement in its accuracy is simply a result of numerical fitting, or if this reduction is necessary for cattle in (sub-) tropical husbandry systems.

The UN excretion by model I was also improved, because adjustments one and three impacted the prediction of its input variables (i.e., MP, efficiency of its use, proportion of N recycled excreted in urine, microbial nucleic acids flow, and N balance; Table 4), which represents the 83% of the total UN excretion predicted by model I (predicted UN by model I; n = 392). Although adjustments to the model I reduced the systematic error to < 5% of the MSE, the RMSE remained high (> 25% of the observed mean) mainly due to a lack of precision rather than accuracy. This suggests that it might not be possible to predict UN excretion of individual animals adequately (expected error of ± 33% of the observed mean) but rather groups of animals. It is likely that the poor adequacy of model I to predict UN excretion is related to the negative UN predictions for animals with low UN excretion.

It appears challenging to predict UN of animals with low N intake and therefore N excretion, and further research is necessary to determine whether the negative values are related to the animal class (heifers and steers) or to animals with a low N intake in general (including lactating cows with a low N intake) and therefore low N excretion. As well as to determine if the proportion of N retention that is overestimated by the default N balance is set at 53% for all animal class and husbandry systems. Further, using empirical equation 22.23 of INRA (2019) to predict UN excretion of cattle in warm areas did not produce adequate predictions than using model I or the adjusted model I. Therefore, it is advisable to use adjusted model I rather than empirical equation 22.23 of INRA (2019) to predict UN excretion of cattle in (sub-) tropical husbandry systems.

In the case of model A, adjustments one and two improved the adequacy of the prediction of UN (RMSE of 37% observed mean, systematic error of 1% of the MSE, and CCC of 0.88) and slightly FN excretion (RMSE of 29% observed mean, systematic error of 71% of the MSE, and CCC of 0.91), but reduced the adequacy of TN excretion (RMSE of 25% observed mean, systematic error of 24% of the MSE, and CCC of 0.94). The reason for this is that in the original model A, the great overestimation of FN excretion (-20.1 g/day) was compensated by the underestimation of UN excretion (+ 7.0 g/day), resulting in a slight underestimation of TN excretion (- 13.1 g/day). However, when the predictions of UN (- 3.2 g/day) and FN (- 20.9 g/day) excretions were adjusted (adjustment one and two), the compensation was no longer present resulting in an overestimation of TN excretion (- 24.1 g/day). Considering that the accuracy of the input variables has been improved, the error of model A is probably related to the parameterization of the FN and UN excretion models (e.g., metabolizable protein supply, proportion of N recycled excreted in urine).

Predicting mean treatments instead of individual observations may lead to improved adequacy estimates; however, in the current study, this was not observed mainly because models were primarily sensitive to feed-related variables, which were constant for all animals under the same treatment. Nevertheless, incorporating animal-dependent characteristics, such as feeding level, into the prediction of diet-dependent input variables shows promise for enhancing model adequacy, as it enables individualized estimates for each animal.

To enhance the adequacy of predictions of N excretion, it is imperative to take digestive interactions into consideration. It should be noted that the present study only accounted for feeding level and did not encompass all digestive interactions present in the original equations. Incorporating these interactions is expected to improve predictions of N excretion, but it requires highly detailed information on the animals' diet and ingredients, which is often limited and subject to significant variation within and between feedstuffs and farming systems in the (Sub-) Tropics. Therefore, there is a clear need for future research to focus on developing novel methodologies for feed quality estimates that account for individual animal characteristics and digestive interactions.

4.5 Conclusions

It was hypothesized that semi-mechanistic models developed based on feeding recommendations for cattle in temperate regions may result in inadequate estimates of TN, UN, and FN, as well as its partitioning into fecal BEDN, UDN, and WSN across different cattle production systems in the (Sub-) Tropics. Our hypothesis was partly accepted due to model developed based on the German feeding recommendations for cattle (GfE, 2001) adequately predicts TN excretion in cattle kept under typical (sub-) tropical husbandry conditions (expected error ±25% observed mean). However, none of the tested models adequately (i.e., RMSE < 25% of the observed mean and a systematic error < 5% of the MSE) predicted the excretion of UN, FN, and of different FN fractions. After adjusting, amongst other model parameters, the estimation of duodenal microbial CP flow, the model developed based on the French feeding recommendations for cattle (INRA, 2019) adequately predicts FN and TN, but not UN excretion and individual fecal N fractions. Errors in the prediction of UN excretion are primarily a result of a lack of precision rather than accuracy.

Models can be improved particularly by providing accurate estimates of duodenal microbial CP and rumen-undegraded CP flow. Prediction of N excretion at individual animal level might be possible if input variables also included animal-related rather than only diet-related variables. Finally, further research is needed to accurately predict N excretion of cattle with low N intakes, as well as to improve the prediction of individual FN fractions.

4.6 Acknowledgments

Our sincere thanks go to A. Sainz-Sánchez, Dr. Termitope, R. Gownipuram, Dr. Wassie, and companion doctoral students for their assistance in providing the data and assisting with the statistical analysis.

4.7 References

AFRC (Agricultural and Food Research Council). 1993. Energy and protein requirements of ruminants: An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. CAB International, Wallingford, UK.

Aiple, K. P., Steingass, H., Menke, K. H. 1992. Suitability of a buffered faecal suspension as the inoculum in the Hohenheim gas test. J. Anim. Physiol. Anim. Nutr. 67(1–5), 57–66. https://doi.org/10.1111/j.1439-0396.1992.tb00583.x

Ali, A. I. M., Wassie, S. E., Korir, D., Goopy, J. P., Merbold, L., Butterbach-Bahl, K., Dickhoefer, U., Schlecht, E. 2019. Digesta passage and nutrient digestibility in Boran steers at low feed intake levels. J. Anim. Physiol. Anim. Nutr. 103(5), 1325–1337. https://doi.org/10.1111/jpn.13158

Ali, A. I. M., Wassie, S. E., Korir, D., Merbold, L., Goopy, J. P., Butterbach-Bahl, K., Dickhoefer, U., Schlecht, E. 2019. Supplementing tropical cattle for improved nutrient utilization and reduced enteric methane emissions. Animals, 9(5), Article 5. https://doi.org/10.3390/ani9050210

Aloba, T., 2022. Effects of ensiling conditions on the nutritional quality of forage legumes and their impacts on rumen fermentation and nutrient utilization by cattle [Dissertation to obtain the doctoral degree of Agricultural Sciences]. University of Hohenheim.

AOAC (Association of Official Analytical Chemists). 2005. Official Methods of Analysis. AOAC International. https://repositorioinstitucional.ceu.es/handle/10637/3158

Arriaga, H., Pinto, M., Calsamiglia, S., Merino, P. 2009. Nutritional and management strategies on nitrogen and phosphorus use efficiency of lactating dairy cattle on commercial farms: An environmental perspective. J. Dairy Sci. 92(1), 204–215. https://doi.org/10.3168/jds.2008-1304

Bateki, C. A., Dickhoefer, U. 2020. Evaluation of the modified LIVestock SIMulator for stall-fed dairy cattle in the Tropics. Animals, 10(5):816. https://doi.org/10.3390/ani10050816

Bateki, C. A., Dickhoefer, U. 2019. Predicting dry matter intake using conceptual models for cattle kept under tropical and subtropical conditions. J. Anim. Sci. 97(9), 3727–3740. https://doi.org/10.1093/jas/skz226

Bibby, J. M., Toutenburg, H. 1977. Prediction and improved estimation in linear models. John Wiley and Sons, London, UK.

Bougouin, A., Hristov, A., Dijkstra, J., Aguerre, M. J., Ahvenjärvi, S., Arndt, C., Bannink, A., Bayat, A. R., Benchaar, C., Boland, T., Brown, W. E., Crompton, L. A., Dehareng, F., Dufrasne, I., Eugène, M., Froidmont, E., van Gastelen, S., Garnsworthy, P. C., Halmemies-Beauchet-Filleau, A., Herremans, S., Huhtanen, P., Johansen, M., Kidane, A., Kreuzer, M., Kuhla, B., Lessire, F., Lund, P., Minnée, E. M. K., Muñoz, C., Niu, M., Nozière, P., Pacheco, D., Prestløkken, E., Reynolds, C. K., Schwarm, A., Spek, J. W., Terranova, M., Vanhatalo, A., Wattiaux, M. A., Weisbjerg, M. R., Yáñez-Ruiz, D. R., Yu, Z., Kebreab, E. 2022. Prediction of nitrogen excretion from data on dairy cows fed a wide range of diets compiled in an intercontinental database: A meta-analysis. J. Dairy Sci. 105(9), 7462–7481. https://doi.org/10.3168/jds.2021-20885

Castro-Montoya, J., Gownipuram, R., Mendoza, M., Solano, N., López, F., Dickhöfer, U., Corea, E. E. 2019. Effects of feeding tropical forage legumes on nutrients digestibility, nitrogen partitioning and performance of crossbred milking cows. Anim. Feed Sci. Technol. 247, 32–40. https://doi.org/10.1016/j.anifeedsci.2018.10.017

Corea, E. E., Aguilar, J. M., Alas, N. P., Alas, E. A., Flores, J. M., Broderick, G. A. 2017. Effects of dietary cowpea (*Vigna sinensis*) hay and protein level on milk yield, milk composition, N efficiency and profitability of dairy cows. Anim. Feed Sci. Technol. 226, 48–55. https://doi.org/10.1016/j.anifeedsci.2017.02.002

Corea, E. E., Castro-Montoya, J., Mendoza, M. V., López, F. M., Martinez, A., Alvarado, M. E., Moreno, C., Broderick, G. A., Dickhoefer, U. 2020. Effect of forage source and dietary rumen-undegradable protein on nutrient use and growth in dairy heifers. Anim. Feed Sci. Technol. 269, 114658. https://doi.org/10.1016/j.anifeedsci.2020.114658

Dijkstra, J., France, J., Assis, A. G., Neal, H. D. S. C., Campos, O. F., Aroeira, L. J. M. 1996. Simulation of digestion in cattle fed sugarcane: Prediction of nutrient supply for milk production with locally available supplements. J. Agric. Sci. 127(2), 247–260. https://doi.org/10.1017/S0021859600078023

Dijkstra, J., Oenema, O., Groenigen, J. W. van, Spek, J. W., Vuuren, A. M. van, Bannink, A. 2013. Diet effects on urine composition of cattle and N2O emissions. Animal, 7(s2), 292–302. https://doi.org/10.1017/S1751731113000578

GfE (Gesellschaft für Ernährungsphysiologie). 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder.DLG-Verlag, Frankfurt, Germany.

Glindemann, T., Tas, B. M., Wang, C., Alvers, S. Susenbeth, A., 2009. Evaluation of titanium dioxide as an inert marker for estimating fecal excretion in grazing sheep. Anim. Feed Sci. Technol. 152, 186–197. https://doi.org/10.1016/j.anifeedsci.2009.04.010.

Herrero, M., Fawcett, R., Jessop, N. 2002. Predicting intake and nutrient supply of tropical and temperate diets for ruminants using a simple dynamic model of digestion. Bioparametrics Ruminant Nutrition Reference Laboratories Monograph. Institute of Ecologyand Resource Management, University of Edinburgh, UK.

Hristov, A. N., Bannink, A., Crompton, L. A., Huhtanen, P., Kreuzer, M., McGee, M., Nozière, P., Reynolds, C. K., Bayat, A. R., Yáñez-Ruiz, D. R., Dijkstra, J., Kebreab, E., Schwarm, A., Shingfield, K. J., Yu, Z. 2019. Invited review: Nitrogen in ruminant nutrition: A review of measurement techniques. J. Dairy Sci. 102(7), 5811–5852. https://doi.org/10.3168/jds.2018-15829

INRA (Institut National de la Recherche Agronomique). 2019. INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, Netherlands.

INRAE, CIRAD, AFZ, and FAO. 2012-2022. Feedipedia: An on-line encyclopedia of animal feeds. https://www.feedipedia.org/

Johnson, A. C. B., Reed, K. F., Kebreab, E. 2016. Short communication: Evaluation of nitrogen excretion equations from cattle. J. Dairy Sci. 99(9), 7669–7678. https://doi.org/10.3168/jds.2015-10730

Jost, D. I., Joergensen, R. G., Sundrum, A. 2013. Effect of cattle faeces with different microbial biomass content on soil properties, gaseous emissions and plant growth. Biol. Fertil. Soils. 49, 61–70. https://doi.org/10.1007/s00374-012-0697-y

Lapierre, H., Berthiaume, R., Raggio, G., Thivierge, M. C., Doepel, L., Pacheco, D., Dubreuil, P., Lobley, G.E. 2005. The route of absorbed nitrogen into milk protein. Anim. Sci. 80, 11–22. https://doi.org/10.1079/ASC41330011

Licitra, G., Hernandez, T. M., Van Soest, P. J. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57(4), 347–358. https://doi.org/10.1016/0377-8401(95)00837-3

Lin, L. I.-K. 1989. A Concordance correlation coefficient to evaluate reproducibility. Biometrics, 45(1), 255-268. PMID: 2720055.

Mason, V. C. 1969. Some observations on the distribution and origin of nitrogen in sheep faeces. J. Agric. Sci. 73(1), 99–111. https://doi.org/10.1017/S0021859600024175

Mason, V. C., Frederiksen, J. H. 1979. Partition of the nitrogen in sheep faeces with detergent solutions, and its application to the estimation of the true digestibility of dietary nitrogen and the excretion of non-dietary faecal nitrogen. Z. Tierphysiol. Tierernahr. Futtermittelkd. 41(3), 121–131. https://doi.org/10.1111/j.1439-0396.1978.tb00573.x

Menke, K. H., Steingass, H. 1987. Estimation of the energetic feed value from chemical analysis, and *in vitro* gas production using rumen fluid. Anim. Res. Develop. 28, 7–55.

Nennich, T. D., Harrison, J. H., VanWieringen, L. M., Meyer, D., Heinrichs, A. J., Weiss, W. P., St-Pierre, N. R., Kincaid, R. L., Davidson, D. L., Block, E. 2005. Prediction of manure and nutrient excretion from dairy cattle. J. Dairy Sci. 88(10), 3721–3733. https://doi.org/10.3168/jds.S0022-0302(05)73058-7

Reed, K. F., Moraes, L. E., Casper, D. P., Kebreab, E. 2015. Predicting nitrogen excretion from cattle. J. Dairy Sci. 98(5), 3025–3035. https://doi.org/10.3168/jds.2014-8397

Rotz, C. A. 2004. Management to reduce nitrogen losses in animal production. J. Anim. Sci. 82(suppl_13), E119– 137. https://doi.org/10.2527/2004.8213_supplE119x

Rufino, M., Herrero, M., Van Wijk, M., Hemerik, L., Ridder, N., Giller, K. 2009. Lifetime productivity of dairy cows in smallholder farming systems of the Central highlands of Kenya. Animal, 3, 1044–1056. https://doi.org/10.1017/S1751731109004248

Sainz-Sanchez, P. A., Korir, D., Ali, A. I. M., Merbold, L., Goopy, J., Schlecht, E., Dickhoefer, U. 2019. Intake, apparent digestibility, and nitrogen balance in steers fed with different grasses grown alone or intercropped with *Lablab purpureus*. Presented at Tropentag, p.191.

Sainz-Sánchez, P., Rojas, G., Castro-Montoya, J., Gómez, C. A., Dickhoefer, U. 2018. Effects of type of concentrate feed and timing of supplementation on performance of lactating dairy cows grazing an alfalfa-rye-grass sward in the Peruvian highlands. Presented at Tropentag, p. 122.

Salah, N., Sauvant, D., Archimède, H. 2014. Meta-analysis of response of growing ruminants to diet in warm conditions. Animal 9(5), 822–830. https://doi.org/10.1017/S175173111400322X

Salazar-Cubillas, K., Dickhoefer, U. 2021. Estimating the proportion of *in situ* rumen-undegraded crude protein from chemical crude protein and fiber fractions in tropical forage grasses and legumes. Anim. Feed Sci. Technol. 282, 115122. https://doi.org/10.1016/j.anifeedsci.2021.115122

Schouten, M., Verwaart, T., Heijman, W. 2014. Comparing two sensitivity analysis approaches for two scenarios with a spatially explicit rural agent-based model. Environ. Model. Softw. 54, 196–210. https://doi.org/10.1016/j.envsoft.2014.01.003

Shannak, S., Südekum, K.-H., Susenbeth, A. 2000. Estimating ruminal crude protein degradation with *in situ* and chemical fractionation procedures. Anim. Feed Sci. Technol. 85(3), 195–214. https://doi.org/10.1016/S0377-8401(00)00146-2

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., Russell, J. B. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 70(11), 3562–3577. https://doi.org/10.2527/1992.70113562x

Spanghero, M., Kowalski, Z. M. 1997. Critical analysis of N balance experiments with lactating cows. Livest. Prod. Sci. 52(2), 113–122. https://doi.org/10.1016/S0301-6226(97)00138-3

Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci. 75, 345–357. https://doi.org/10.3168/jds.S0022-0302(92)77770-4

Tas, B. M., Susenbeth, A. 2007. Urinary purine derivates excretion as an indicator of *in vivo* microbial N flow in cattle: A review. Livest. Sci. 111(3), 181–192. https://doi.org/10.1016/j.livsci.2007.05.010

Tedeschi, L., Chalupa, W., Janczewski, E., Fox, D., Sniffen, C., Munson, R., Kononoff, P., Boston, R. 2008. Evaluation and application of the CPM Dairy Nutrition model. J. Agric. Sci. 146, 171–182. https://doi.org/doi:10.1017/S0021859607007587

Tedeschi, L. O. 2006. Assessment of the adequacy of mathematical models. Agric. Syst 89(2–3), 225–247. https://doi.org/10.1016/j.agsy.2005.11.004

Titgemeyer, E. C., Armendariz, C. K., Bindel, D. J., Greenwood, R. H., Löest, C. A. 2001. Evaluation of titanium dioxide as a digestibility marker for cattle. J. Anim. Sci. 79(4), 1059–1063. https://doi.org/10.2527/2001.7941059x

Valadares, R., Broderick, G. A., Valadares Filho, S. C., Clayton, M. K., 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82, 2686–2696. https://doi.org/10.3168/jds.S0022-0302(99)75525-6

Van Keulen, J. Young, B. A., 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. J. Anim. Sci. 44, 282–287. https://doi.org/10.2527/jas1977.442282x

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Cornell University Press, New York, USA.

VDLUFA (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten). 2012: Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik: (Methodenbuch III). Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

Wassie, S. E. 2019. Nutritional effects on microbial protein synthesis and nitrogen balance in cattle offered low-quality tropical forages [Dissertation to obtain the doctoral degree of Agricultural Sciences]. University of Hohenheim.

Wassie, S. E., Ali, A. I. M., Korir, D., Butterbach-Bahl, K., Goopy, J., Merbold, L., Schlecht, E., Dickhoefer, U. 2019. Effects of feed intake level on efficiency of microbial protein synthesis and nitrogen balance in Boran steers consuming tropical poor-quality forage. Arch. Anim. Nutr. 73(2), 140–157. https://doi.org/10.1080/1745039X.2019.157234

Wilkerson, V. A., Mertens, D. R., Casper, D. P. 1997. Prediction of excretion of manure and nitrogen by Holstein dairy cattle. J. Dairy Sci. 80(12), 3193–3204. https://doi.org/10.3168/jds.S0022-0302(97)76292-1

Chapter 5

5. General discussion

5.1 Nitrogen use efficiency of dairy cattle in tropical husbandry systems

The global demand for milk is projected to increase by 10% within the next decade, particularly in the tropical regions of Africa and Asia (OECD, 2023). Although one third of the global population of dairy cows are found there, their annual milk production is lower than temperate systems (Powell et al., 2013). These low performances result in a high demand for natural resources and high environmental emissions per unit of milk produced (Reid et al., 2004). Hence, improving animal performance and nitrogen (N) use efficiency becomes crucial in tropical dairy systems. However, nutritive value of locally available feeds is poor, and the purchase of protein concentrates capital-intensive (PARI, 2019). Furthermore, there is a lack of information on dietary composition of tropical dairy cattle, coupled with an insufficient integration of variations in digestion process efficiencies between tropical and temperate cattle into available feeding recommendations (Mottet et al., 2017; Pica-Ciamarra et al., 2014). Therefore, to achieve precise alignment of N supply with the N requirements of dairy cattle in tropical husbandry systems, the following challenges need to be addressed (PARI, 2019):

1. There is a need to account for differences in N supply and utilization between cattle in tropical and temperate husbandry systems when establishing protein requirements. An update of these requirements should consider important aspects such as dry matter intake (Bateki et al., 2020; INRA, 2019), efficiency of microbial protein synthesis (Wassie, 2019), body protein turnover (Rufino et al., 2016), efficiency of utilization of metabolizable protein, and animal responses to dietary variations in feed and nutrient supply (INRA, 2019).

2. There is a need for information on the protein value of tropical feedstuffs:

    2.1. A comprehensive feed library is essential, encompassing information on various aspects. These include the degradation of crude protein in the rumen (Salazar-Cubillas and Dickhoefer, 2021), the amino acid composition of undegraded crude protein (Tedeschi et al., 2002) and its intestinal digestibility (Mupeta et al., 1997), along with the concentration of secondary compounds and their impacts on the intake and digestion of tropical feedstuffs.

    2.2. There is a need for adapted laboratory methodology and algorithms for routine evaluation of rumen degradation of crude protein of tropical feedstuffs. Regular evaluation of tropical feedstuffs is required due to the high variability in rumen crude protein degradability between different feedstuffs and within same feedstuffs (Hvelplund and Weisbjerg, 1998).

The findings of chapters 2 and 3 contribute to the challenge of the lack of information on the protein value of tropical feedstuffs by providing adapted laboratory methodology and algorithms for routine evaluation of rumen degradation of crude protein of tropical feedstuffs and by generating information on protein value that can be incorporated into a feed library. Specifically, results of chapter 2 provide with a set of equations to predict the rumen-undegraded (RUP) crude protein proportions of tropical forage grasses and legumes based on chemical crude protein and fiber fractions. Similarly, results of chapter 3 provide a set of equations to predict post-ruminal crude protein (PRCP) supply of tropical forage grasses and legumes based on *in vitro* parameters, chemical composition, and fiber fractions.

The findings of chapter 4 contribute to the challenge of the lack of validation of protein requirements of dairy cattle in tropical regions by providing a semi-mechanistic model that can accurately and precisely predict fecal N (FN) and total N (TN) excretion. Specifically, results of chapter 4 contribute to the identification of the models with the highest potential to predict N excretion of dairy cattle in tropical regions. It also provides the necessary adjustments to improve the accuracy and precision of the models and achieve acceptable predictions of FN and TN. Additionally, the chapter present the main sources of error related to UN excretion and proposes possible solutions to improve the accuracy and precision of UN prediction.

Therefore, the present thesis contributes significantly to overcoming the main challenges associated with the lack of information on cattle husbandry systems in tropical environments. In addition, the results of the present thesis provide valuable information necessary to balance feed N supply and animal requirements in tropical dairy cattle systems. There are, however, still certain challenges that require further research, such as the validation of protein requirements for dairy cattle in tropical regions (e.g., approach to estimate microbial crude protein synthesis), as well as the establishment of a feed library that includes detailed information on feed N partitioning in the rumen of tropical feedstuffs under different phenological conditions and different seasons.

In the following sections of this general discussion, the hypotheses that supported the development of chapters 2, 3, and 4 (sections 5.2 and 5.3) will be presented and discussed, as well as the key challenges and limitations (section 5.4), future recommendations (section 5.5), main conclusions and practical application of the main results obtained in the present thesis (section 5.6).

5.2 Modeling tools for predicting nitrogen partitioning of dairy cattle in tropical husbandry systems

This section outlines the assumptions and hypotheses that guided the development of chapter 4, including the type of model used (i.e., empirical or semi-mechanistic) and the adoption of modeling tools from temperate systems.

1. Semi-mechanistic models are preferred to empirical equations due to their greater robustness (Haddon, 2011). This is because semi-mechanistic models are based on processes and seek to comprehend causality while empirical equations are based on mathematical relationships and not necessarily on any preconceived biological theory (France and Dijkstra, 2006). Therefore, empirical equations have a low level of robustness, because they cannot adequately predict N excretion under conditions other than those used to fit the model (Johnson et al., 2016).

2. The adoption of modeling tools from temperate systems into tropical systems without proper adaptation and validation can lead to imprecise assessments of N partitioning of cattle in tropical husbandry systems. This discrepancy is attributed to variations in N supply and utilization by dairy cattle between tropical and temperate regions (Bateki, 2020; Hernández-Castellano et al., 2019).

Tables 5.1 and 5.2 have been created to facilitate the discussion of section 5.2. Table 5.1 provides a list of semi-mechanistic models and empirical equations developed to predict UN, FN, and TN excretion of lactating cows, heifers, and steers. The semi-mechanistic models include the three models described in chapter 4. These were originally derived from ruminant feeding recommendations of the British (model A), German (model G) and French (model I) systems. Additionally, their adapted versions, as discussed in chapter 4, are included. The empirical equations include those developed to predict UN, FN, and TN excretion of lactating cows, heifers, and steers in tropical (hereinafter referred to as tropical empirical equations) and temperate husbandry systems (hereinafter referred to as temperate empirical equations). Table 5.2 presents the accuracy and precision of semi-mechanistic models and empirical equations in predicting UN, FN, and TN excretion of lactating cows, heifers, and steers in (sub-) tropical husbandry systems. The UN, FN, and TN excretion were evaluated using the same dataset presented in chapter 4. Models with an acceptable accuracy were those with a root mean square error (RMSE) < 25% of the observed mean (Reed et al., 2015) and systematic errors (i.e., the sum of error due to central tendency and error due to regression) < 5% of mean square error (MSE) (Johnson et al., 2016). A higher concordance

correlation coefficient (CCC) was also deemed to be more accurate and precise than predictions with a lower CCC. In Table 5.3, the semi-mechanistic models and empirical equations with the lowest RMSE and highest CCC per N excretion and animal class are listed.

When comparing the number of tropical and temperate empirical equations (n = 46), the number of equations available is greater for temperate (n = 40) than for tropical empirical equations (n = 6). In addition, there are no equations available for predicting UN excretion of dairy cattle in tropical regions or for predicting UN, FN, and TN excretion of heifers and steers separately. Even though there are few tropical empirical equations available, these were more adequate in predicting UN ($RMSE_{tropical}$ = 60% of the observed mean; $RMSE_{temperate}$ from 89 to 135% of the observed mean) and TN excretion of steers ($RMSE_{tropical}$ = 37% of the observed mean; $RMSE_{temperate}$ = 63% of the observed mean), and FN excretion of lactating cows, heifers, and steers ($RMSE_{tropical}$ from 14 - 28% of the observed mean; $RMSE_{temperate}$ from 14 - 84% of the observed mean) than temperate empirical equations. Tropical empirical equations were, however, not adequate enough as none of the equations presented systematic errors < than 5% of the MSE. In addition, the RMSE exceeded 25% of the observed mean for UN, FN, and TN excretion of steers. According to these results, modeling tools developed for temperate systems resulted in inaccurate estimates of N excretion. This was particularly true for UN and TN of steers and for FN of lactating cows, heifers, and steers. Additionally, there is a lack of tropical empirical equations for predicting N excretion, particularly UN for lactating cows. Hence, additional research is needed to develop tropical empirical equations for adequately predicting N excretion of dairy cattle in tropical husbandry systems. This is particularly essential when data limitations prevent the use of semi-mechanistic models and a simplified estimation is needed without the need to understand the mechanism behind.

The UN ($RMSE_{temperate}$ from 24 to 25% observed mean; tropical empirical equation not available) and TN excretion ($RMSE_{temperate}$ from 14 to 15% observed mean; $RMSE_{tropical}$ from 19 to 25% observed mean) of lactating cows and heifers were more adequately predicted by temperate empirical equations than tropical ones. Nevertheless, from these four temperate empirical equations, only equation UN23 (Kebreab, 2010) was able to predict adequately the UN of dairy cattle with a RMSE of 24% of the observed mean and a systematic error of 2% of the MSE. Several factors contribute to explaining why one empirical equation is more adequate than the others (Hanigan and Daley, 2020), but among the most important are the representativeness of the training set and the statistical analyses used to develop the equation.

In terms of representativeness, it is expected that a tropical empirical equation will predict more adequately the N excretion of cattle in tropical husbandry systems than a temperate empirical equation. This expectation relies on the differences between N supply and utilization observed between cattle in tropical and temperate husbandry systems (Bateki, 2020; Hernández-Castellano et al., 2019), which can influence the relationship between N excretion and the independent variables used to predict it. For instance, tropical empirical equation UN13 (Salah et al., 2014) assumed that for an increase in 1 g of N intake the UN excretion of heifers will increase in 0.32 g/day, while for temperate empirical equation UN38 (Reed, 2015) will increase in 0.51 g/day. This lower increment in UN excretion per g of N intake in tropical than in temperate empirical equations can be explained by differences in N incorporated from the rumen hepatic cycle, reduced dry matter intake (INRA, 2019), slower rumen passage rate, and the lower N available concentration in the diet of cattle in tropical compared to temperate husbandry systems (Wassie et al., 2019).

However, having a training set exclusive of N excretion from tropical cattle is not sufficient to be considered representative. Range and distribution, number of observations, use of individual observations or treatment means as well as statistical methodology, should also be taken into consideration (Reed et al., 2015). For instance, the statistical methodology (i.e., genetic algorithm) used by Reed et al. (2015; UN38, TN31, TN41) may explain why their equation is able to predict UN and TN more adequately than tropical empirical equations for heifers (i.e., stepwise regression). However, this does not explain why Reed et al. (2015; UN16) equation did not predict more adequately the UN excretion than Kebreab et al. (2010; UN23) equation, even though Reed et al. (2015; UN16) equation predicted more adequately the UN excretion of temperate lactating cows than Kebreab et al. (2010; UN23) equation in Johnson et al. (2016) study. Therefore, relying only on statistical methods might not be enough to make empirical equations more adequate. Other factors, such as the range and distribution of data, the quantity of observations, and the incorporation of individual observations or treatment means, could play a more substantial role. Therefore, for the development of future empirical equations to predict N excretion of cattle in tropical husbandry systems, it is imperative to select a representative training set that covers a wide range of situations with a representative number of observations and appropriate statistical methodology.

When comparing semi-mechanistic models, empirical equations require fewer input variables (Empirical = 1 to 4 input variables; Semi-mechanistic = from 7 to 10 input variables; Table 5.2). However, they are less robust (Empirical = 46 equations achieved acceptable RMSE for 22 N excretions; Semi-mechanistic = 3 models with 2 adaptations achieved

acceptable RMSE for 17 N excretions). Furthermore, empirical equations require different equations for each animal class and type of N excretion.

Upon prediction of UN, FN, and TN excretion across all animal classes using modified Model I and integrating 8 empirical equations from Table 5.3, it is observed that both models exhibit an RMSE greater than 25% of the observed mean and a systematic error lower than 5% of the MSE. Empirical equations showed a slightly greater level of adequacy ($CCC_{empirical}$ = 0.92; $CCC_{modified\ model\ I}$ = 0.91), primarily attributable to a slightly greater precision (Coefficient of determination (R^2) $_{empirical}$ = 0.93; $R^2_{modified\ model\ I}$ = 0.91). This enhanced precision offset the lower accuracy observed in the empirical equations (Bias correction factor = 0.99; Mean bias = -7.06 g/day) when compared modified model I (Bias correction factor = 1.00; Mean bias = 2.94 g/day).

Regarding FN excretion, both predictions exhibited an RMSE below 25% of the observed mean; however, only the modified model I demonstrated a systematic error below 5% of the MSE. Empirical equations displayed slightly greater adequacy ($CCC_{empirical}$ = 0.97; $CCC_{modified\ model\ I}$ = 0.96) and precision ($R^2_{empirical}$ = 0.97; $R^2_{modified\ model\ I}$ = 0.96), while maintaining same accuracy (Bias correction factor = 1; Mean bias $_{empirical}$ = -3.62 g/day; Mean bias $_{modified\ model\ I}$ = - 0.79 g/day) than modified model I. In the case of TN excretion, both predicitions achieved an RMSE lower than 5% of the observed mean and a systematic error below 5% of the MSE. Furthermore, both predictions exhibit same level of adequacy (CCC = 0.96), precision (R^2 = 0.96), and accuracy (Cb = 1; Mean bias $_{empirical}$ = -7.55 g/day; Mean bias $_{modified\ model\ I}$ = 2.57g/day).

In summary, empirical equations offer simplicity and lower input variables, although with reduced robustness. Despite their slightly better adequacy and precision, they can exhibit lower accuracy compared with semi-mechanistic models. The choice between empirical and semi-mechanistic models for prediction of N excretion hinges on balancing these trade-offs based on specific objectives of the study, expected prediction error, and data availability. Empirical equations are more appropriate when predicting N excreted by individual animals is not required and information on input variables is limited, for example, accounting for the total N excreted by cattle at a national level (e.g., Waldrip et al., 2014). Semi-mechanistic models may be more appropriate if the purpose is, for example, to determine the influence of different feeding strategies on UN and FN excretion or to quantify differences in nutrient use efficiency between farms and manure management systems (e.g., Rufino et al., 2019). From the results presented above it can be concluded that: (1) Semi-mechanistic models are preferred over empirical equations to predict UN, FN, and TN excretion of dairy cattle when high robustness

and prediction at animal level are required. Nevertheless, semi-mechanistic models still require further improvement to adequately predict N excretion, particularly of heifers and steers. (2) The use of temperate empirical equations instead of tropical empirical equations resulted in a lower adequacy of the prediction of N excretion of lactating cows (FN and TN), heifers (FN), and steers (UN, FN, and TN).

Table 5.1
Available semi-mechanistic models and empirical equations for predicting urine nitrogen (UN), fecal nitrogen (FN), and total nitrogen (TN) of lactating cows, heifers, and steers in tropical and temperate husbandry systems.

N°	Equation[1]	Reference
Semi-mechanistic models		
Lactating cows, heifers, and steers		
1a	UN = (1 – Efficiency) x MP / 6.25 + EUN + Nrecycled + MNA	AFRC,1993
1b	UN N° 1a with adjustments 1 and 2	AFRC,1993
2	UN = (PRCP / 6.25 + NBWC) – (FN + Nscurf + PRCPreq / 6.25)	GfE, 2001
3a	UN = MP x (1 – Efficiency) / 6.25 + EUN + Nrecycled + MNA + 0.47 x Nbal	INRA, 2018
3b	UN N° 3a with adjustments 1 and 3	INRA, 2018
4a	FN = BEDN + UDN + WSN	AFRC, 1993
4b	FN N° 4a with adjustments 1 and 2	AFRC,1993
5	FN = BEDN + UDN + WSN	GfE, 2001
6a	FN = BEDN + UDN + WSN + 4.30 x DMI	INRA, 2018
6b	FN N° 6a with adjustments 1 and 3	INRA, 2018
7a	TN = UN1 + FN4	AFRC, 1993
7b	TN N° 7a with adjustments 1 and 2	AFRC,1993
8	TN = UN2 + FN5	GfE, 2001
9a	TN = UN3 + FN6	INRA, 2018
9b	TN N° 9a with adjustments 1 and 3	INRA, 2018

* Equations developed for beef cattle

[1] BEDN, microbial and endogenous debris nitrogen (BEDN = g/day); BW, animal's body-weight (kg); CP, crude protein (% dry matter); DMI, dry matter intake (kg/day); EUN, endogenous urinary nitrogen (g/day); FN, fecal nitrogen excretion (g/day); ME, metabolizable energy (MJ/kg dry matter); MILK, milk production (kg/day); MN, milk nitrogen (g/day); MNA, microbial nucleic acids (g/day); MP, metabolizable protein (g/day); Nbal, nitrogen balance (g/day); NBWC, nitrogen resulting from a negative body-weight change (g/day); NI, nitrogen intake (g/day); Nrecycled, nitrogen recycled excreted in urine (g/day); Nscurf, requirement for scurf nitrogen losses (g/day); PRCP, post-ruminal crude protein intake (g/day); PRCPreq, post-ruminal crude protein net requirements (g/day); TN, total nitrogen excretion (g/day); UN, urine nitrogen excretion (g/day); UDN, undigested dietary nitrogen (g/day); WSN, water-soluble nitrogen (g/day).

(*Table 5.1 Continued)*

N°	Equation[1]	Reference
Empirical equations for cattle in tropical systems		
Lactating cows		
10	FN = 0.08 x NI + 0.60 x (DMI x 1000)/100	Zahra et al., 2020
11	TN = -1.04 + 0.95 x NI – 4.14 x MILK	Garg et al., 2016
12	TN = 0.84 x NI – 23.6	Aarons et al., 2017
Heifers and steers		
13	UN = (0.014 + 0.32 x NI / BW^0.75) x BW^0.75	Salah et al., 2014
14	FN = (0.17 + 0.26 x NI / BW^0.75) x BW^0.75	Salah et al., 2014
15	TN = UN13 + FN14	Salah et al., 2014
Empirical equations for cattle in temperate systems		
Lactating cows		
16	UN = 12.0 + 0.333 × NI	Reed et al., 2015
17	UN = 0.83 × NI − MN-97	Jonker et al., 1998
18	UN = 30.4 × $e^{0.0036 \times NI}$	Castillo et al., 2000
19	UN = −126 + 0.676 × NI	Huhtanen et al., 2008
20	UN = 27 + 0.844 × NI − 13 × DMI	Huhtanen et al., 2008
21	UN = 104 + 0.855 × NI − 13.2 × DMI − 6.8 × ME	Huhtanen et al., 2008
22	UN = 40 + 0.879 × NI – 9 × DMI – 3.9 × MILK	Huhtanen et al., 2008
23	UN = 20 + 0.38 × NI	Kebreab et al., 2010
24	UN = 47.8 + 0.56 × NI − 71.4 × (ME x DMI / BW^0.75)	Kebreab et al., 2010
25	FN = −18.5 + 10.1 × DMI	Reed et al., 2015

* Equations developed for beef cattle

[1] BEDN, microbial and endogenous debris nitrogen (BEDN = g/day); BW, animal's body-weight (kg); CP, crude protein (% dry matter); DMI, dry matter intake (kg/day); EUN, endogenous urinary nitrogen (g/day); FN, fecal nitrogen excretion (g/day); ME, metabolizable energy (MJ/kg dry matter); MILK, milk production (kg/day); MN, milk nitrogen (g/day); MNA, microbial nucleic acids (g/day); MP, metabolizable protein (g/day); Nbal, nitrogen balance (g/day); NBWC, nitrogen resulting from a negative body-weight change (g/day); NI, nitrogen intake (g/day); Nrecycled, nitrogen recycled excreted in urine (g/day); Nscurf, requirement for scurf nitrogen losses (g/day); PRCP, post-ruminal crude protein intake (g/day); PRCPreq, post-ruminal crude protein net requirements (g/day); TN, total nitrogen excretion (g/day); UN, urine nitrogen excretion (g/day); UDN, undigested dietary nitrogen (g/day); WSN, water-soluble nitrogen (g/day).

(*Table 5.1 Continued)*

N°	Equation[1]	Reference
Empirical equations for cattle in tropical systems		
Lactating cows		
26	FN = 52.3 + 0.21 × NI	Castillo et al., 2000
27	FN = −28 + 9.9 × DMI	Huhtanen et al., 2008
28	FN = −21 + 6.73 × DMI + 0.101 × NI	Huhtanen et al., 2008
29	FN = 10 + 0.28 × NI	Kebreab et al., 2010
30	FN = −21 + DMI × 6.25 + NI × 0.17	Higgs et al., 2012
31	TN = 20.3 + 0.654 × NI	Reed et al., 2015
32	TN = 122.61 + 0.778 × NI − 6.93 × DMI	Tomlinson et al., 1992
33	TN = 346 + 2.82 × MILK	Nennich et al., 2005
34	TN = 0.196 × BW + 84.1 × CP × DMI	Nennich et al., 2005
35	TN = 0.691 x NI + 0.094 x BW - 38	Yan et al., 2006
36	TN = 0.77 x NI - 1.687 x MILK + 13	Yan et al., 2006
37	TN =0.749 x NI + 0.065 x BW - 1.515 x MILK - 17	Yan et al., 2006
Heifers		
38	UN = 14.3 + 0.510 × NI	Reed et al., 2015
39	FN = 0.345 + 0.317 × NI	Reed et al., 2015
40	TN = 15.1 + 0.828 × NI	Reed et al., 2015
41	TN = 49.5 + 0.793 × NI − 6.04 × ME + 0.825 × CP + 0.190 × BW^0.75	Reed et al., 2015
42	TN = DMI x (CP/100) x 112.55	Nennich et al., 2005

* Equations developed for beef cattle

[1] BEDN, microbial and endogenous debris nitrogen (BEDN = g/day); BW, animal's body-weight (kg); CP, crude protein (% dry matter); DMI, dry matter intake (kg/day); EUN, endogenous urinary nitrogen (g/day); FN, fecal nitrogen excretion (g/day); ME, metabolizable energy (MJ/kg dry matter); MILK, milk production (kg/day); MN, milk nitrogen (g/day); MNA, microbial nucleic acids (g/day); MP, metabolizable protein (g/day); Nbal, nitrogen balance (g/day); NBWC, nitrogen resulting from a negative body-weight change (g/day); NI, nitrogen intake (g/day); Nrecycled, nitrogen recycled excreted in urine (g/day); Nscurf, requirement for scurf nitrogen losses (g/day); PRCP, post-ruminal crude protein intake (g/day); PRCPreq, post-ruminal crude protein net requirements (g/day); TN, total nitrogen excretion (g/day); UN, urine nitrogen excretion (g/day); UDN, undigested dietary nitrogen (g/day); WSN, water-soluble nitrogen (g/day).

(*Table 5.1 Continued)*

N°	Equation[1]	Reference
Empirical equations for cattle in tropical systems		
Steers		
43	UN = 6.80 + 0.405 × NI	Reed et al., 2015
44	UN = −71.2 + 0.265 × NI + 3.76 × CP + 0.468 × BW^0.75	Reed et al., 2015
45	UN = 0.62 × NI − 3.72 DMI − 3.93	Waldrip et al., 2013*
46	UN = 0.56 × NI − 21.18	Waldrip et al., 2013*
47	UN = 5.91 × CP − 21.52	Waldrip et al., 2013*
48	UN = 6.04 × CP −22.00	Dong et al., 2014*
49	UN = 0.51 × NI −14.12	Dong et al., 2014*
50	FN = 0.506 + 0.352 × NI	Reed et al., 2015
51	FN = 0.154 × NI + 24.28	Waldrip et al., 2013*
52	FN = 1.165 × CP + 30.91	Waldrip et al., 2013*
53	FN = 1.81 × CP + 19.68	Dong et al., 2014*
54	FN = 0.20 × NI + 15.82	Dong et al., 2014*
55	TN = 6.91 + 0.759 × NI	Reed et al., 2015

* Equations developed for beef cattle

[1] BEDN, microbial and endogenous debris nitrogen (BEDN = g/day); BW, animal's body-weight (kg); CP, crude protein (% dry matter); DMI, dry matter intake (kg/day); EUN, endogenous urinary nitrogen (g/day); FN, fecal nitrogen excretion (g/day); ME, metabolizable energy (MJ/kg dry matter); MILK, milk production (kg/day); MN, milk nitrogen (g/day); MNA, microbial nucleic acids (g/day); MP, metabolizable protein (g/day); Nbal, nitrogen balance (g/day); NBWC, nitrogen resulting from a negative body-weight change (g/day); NI, nitrogen intake (g/day); Nrecycled, nitrogen recycled excreted in urine (g/day); Nscurf, requirement for scurf nitrogen losses (g/day); PRCP, post-ruminal crude protein intake (g/day); PRCPreq, post-ruminal crude protein net requirements (g/day); TN, total nitrogen excretion (g/day); UN, urine nitrogen excretion (g/day); UDN, undigested dietary nitrogen (g/day); WSN, water-soluble nitrogen (g/day).

Table 5.2
Evaluation of the accuracy and precision of selected models and equations (Table 5.1) to predict urine nitrogen (UN), fecal nitrogen (FN), and total nitrogen (TN) excretion in tropical and temperate husbandry systems.

N°	Predicting	Root mean square error		Mean square error (%)[1]			CCC[2]
		(g/day)	(% mean)	ECT	ER	ED	-1 to 1
Lactating cows							
Semi-mechanistic models							
1a	UN	63.14	34.26	10.02	9.75	80.23	0.36
1b	UN	54.09	29.35	0.02	9.24	90.74	0.54
2	UN	63.61	34.52	8.80	28.49	62.71	0.55
3a	UN	50.59	27.45	19.76	1.26	78.98	0.62
3b	UN	48.42	26.28	1.27	4.84	93.89	0.64
4a	FN	34.70	25.69	56.16	17.41	26.43	0.58
4b	FN	29.62	21.92	63.45	6.54	30.01	0.63
5	FN	38.28	28.34	77.44	1.52	21.04	0.46
6a	FN	20.26	15.00	0.54	30.13	69.33	0.79
6b	FN	19.31	14.29	4.90	21.21	73.89	0.80
7a	TN	55.24	17.30	1.19	6.79	92.02	0.74
7b	TN	59.79	18.72	14.48	9.75	75.77	0.72
8	TN	62.59	19.60	5.60	30.73	63.67	0.75
9a	TN	49.72	15.57	17.84	7.06	75.10	0.81
9b	TN	47.89	14.99	4.13	9.09	86.78	0.82
Empirical equations for cattle in tropical systems							
10	FN	18.55	13.73	4.41	22.11	73.48	0.82
11	TN	62.01	19.42	4.23	20.63	75.14	0.72
12	TN	63.63	19.92	29.73	22.96	47.31	0.76
Empirical equations for cattle in temperate systems							
16	UN	50.24	27.26	20.17	0.88	78.95	0.56
17	UN	114.63	62.20	64.11	20.72	15.17	0.38
18	UN	57.25	31.07	9.49	22.86	67.65	0.62
19	UN	56.81	30.83	1.25	37.02	61.73	0.67
20	UN	48.93	26.55	0.04	7.09	92.87	0.64
21	UN	52.15	28.30	2.14	6.79	91.07	0.58
22	UN	62.82	34.09	1.57	18.28	80.15	0.41
23	UN	45.12	24.49	2.12	0.02	97.86	0.65
24	UN	50.64	27.48	0.65	1.60	97.75	0.55
25	FN	30.48	22.56	42.31	29.51	28.18	0.67
26	FN	20.74	15.35	31.38	0.33	68.29	0.72
27	FN	23.62	17.48	8.52	44.55	46.93	0.77
28	FN	21.68	16.04	5.05	41.16	53.79	0.79
29	FN	19.47	14.41	0.17	22.35	77.48	0.79
30	FN	37.51	27.76	54.36	27.59	18.05	0.59

[1] ECT, error due to central tendency (i.e., overall bias; ECT); ER, error due to regression; ED, error due to disturbance.
[2] CCC, concordance correlation coefficient.

(Table 5.2 Continued)

N°	Predicting	Root mean square error (g/day)	(% mean)	Mean square error (%)[1] ECT	ER	ED	CCC[2] -1 to 1
Lactating cows							
Empirical equations for cattle in temperate systems							
31	TN	45.00	14.09	1.25	4.17	94.58	0.83
32	TN	57.50	18.00	35.02	0.02	64.96	0.71
33	TN	110.03	34.45	67.81	9.56	22.63	0.20
34	TN	46.65	14.61	10.69	0.77	88.54	0.81
35	TN	47.08	14.74	0.01	14.16	85.83	0.83
36	TN	50.07	15.68	0.09	9.11	90.80	0.80
37	TN	49.67	15.55	0.08	10.46	89.46	0.80
Heifers							
Semi-mechanistic models							
1a	UN	16.97	27.75	57.03	11.66	31.31	0.78
1b	UN	18.47	30.20	58.84	17.52	23.64	0.73
2	UN	13.97	22.85	33.83	5.97	60.20	0.84
3a	UN	24.71	40.41	77.03	5.69	17.28	0.61
3b	UN	21.20	34.67	76.51	4.92	18.57	0.70
4a	FN	12.70	32.73	55.78	32.50	11.72	0.59
4b	FN	13.95	35.95	72.76	17.84	9.40	0.53
5	FN	9.26	23.86	68.91	8.67	22.42	0.66
6a	FN	10.68	27.53	41.76	41.43	16.81	0.67
6b	FN	8.84	22.78	25.53	50.05	24.42	0.73
7a	TN	10.15	10.16	10.76	1.24	88.00	0.96
7b	TN	17.15	17.16	67.40	0.77	31.83	0.89
8	TN	11.23	11.23	0.15	0.78	99.07	0.94
9a	TN	18.20	18.21	66.01	0.16	33.83	0.87
9b	TN	17.15	17.16	67.40	0.77	31.83	0.89
Empirical equations for cattle in tropical systems							
13	UN	26.77	43.78	75.63	13.52	10.85	0.53
14	FN	7.55	19.45	0.38	65.43	34.19	0.79
15	TN	24.95	24.97	83.60	2.20	14.20	0.76
Empirical equations for cattle in temperate systems							
38	UN	15.15	24.77	66.02	0.20	33.78	0.85
39	FN	9.16	23.61	3.37	72.68	23.95	0.74
40	TN	16.27	16.28	47.33	19.44	33.23	0.91
41	TN	15.16	15.17	40.95	20.60	38.45	0.92
42	TN	20.64	20.65	79.05	0.29	20.66	0.84

[1] ECT, error due to central tendency (i.e., overall bias; ECT); ER, error due to regression; ED, error due to disturbance.
[2] CCC, concordance correlation coefficient.

(Table 5.2 Continued)

N°	Predicting	Root mean square error (g/day)	(% mean)	Mean square error (%)[1] ECT	ER	ED	CCC[2] -1 to 1
Steers							
Semi-mechanistic models							
1a	UN	21.58	111.29	56.32	31.19	12.49	0.36
1b	UN	21.57	111.26	51.77	34.74	13.49	0.35
2	UN	34.07	175.73	82.40	11.59	6.01	0.16
3a	UN	16.80	86.64	1.55	76.29	22.16	0.50
3b	UN	18.84	97.16	7.13	75.87	17.00	0.47
4a	FN	15.72	62.19	78.03	10.02	11.95	0.47
4b	FN	21.14	83.61	84.68	7.07	8.25	0.32
5	FN	5.61	22.20	22.00	0.26	77.74	0.81
6a	FN	12.14	48.03	64.77	11.63	23.60	0.56
6b	FN	10.49	41.50	57.43	11.90	30.67	0.62
7a	TN	34.70	77.67	75.17	18.20	6.63	0.44
7b	TN	38.90	87.08	80.84	13.51	5.65	0.38
8	TN	32.21	72.11	77.17	10.84	11.99	0.41
9a	TN	21.50	48.13	30.45	49.23	20.32	0.67
9b	TN	23.35	52.27	30.90	52.09	17.01	0.64
Empirical equations for cattle in tropical systems							
13	UN	11.54	59.54	28.19	30.58	41.23	0.59
14	FN	7.05	27.87	35.71	14.15	50.14	0.79
15	TN	16.45	36.82	39.52	29.84	30.64	0.74
Empirical equations for cattle in temperate systems							
43	UN	22.48	115.95	69.40	19.7	10.90	0.33
44	UN	20.55	106.01	14.28	71.95	13.77	0.44
45	UN	18.45	95.15	13.80	68.84	17.36	0.47
46	UN	18.32	94.50	2.23	81.36	16.41	0.50
47	UN	25.32	130.58	42.20	46.37	11.43	0.29
48	UN	26.17	134.99	43.33	45.97	10.70	0.28
49	UN	17.26	89.03	11.79	69.72	18.49	0.51
50	FN	8.64	34.18	8.00	56.84	35.16	0.77
51	FN	12.19	48.22	80.04	2.3	17.66	0.42
52	FN	18.56	73.41	83.26	1.14	15.60	0.15
53	FN	14.04	55.54	72.60	0.14	27.26	0.32
54	FN	7.89	31.22	57.86	0.01	42.13	0.69
55	TN	28.27	63.29	54.81	34.39	10.80	0.56

[1] ECT, error due to central tendency (i.e., overall bias; ECT); ER, error due to regression; ED, error due to disturbance.
[2] CCC, concordance correlation coefficient.

Table 5.3
Semi-mechanism models and empirical equations (Table 5.1) with the greatest adequacy to predict urine nitrogen (UN), fecal nitrogen (FN), and total nitrogen (TN) excretion of lactating cows, heifers, and steer in tropical husbandry systems.

Predicting	N°	Independent variables	Author	Type	RMSE (% mean)[1]	ED (% MSE)[2]	CCC (-1 to 1)[3]
Semi-mechanistic models							
UN lactating cows	3b	Equations 3b, 6b, 9b, 3a, 9a Rumen-degradable and	Chapter 4	Semi-mechanistic	26.28	93.89	0.64
FN lactating cows	6b	undegraded crude protein, metabolizable	Chapter 4	Semi-mechanistic	19.31	73.89	0.80
TN lactating cows	9b	energy, organic matter, proportion of	Chapter 4	Semi-mechanistic	14.99	86.78	0.82
UN heifers	2	concentrate in the diet, dry matter intake,	GfE, 2001	Semi-mechanistic	22.85	60.20	0.84
FN heifers	6b	body-weight, body weight change, milk	Chapter 4	Semi-mechanistic	22.78	24.42	0.73
TN heifers	8	production, and milk composition	GfE, 2001	Semi-mechanistic	11.23	99.07	0.94
UN steers	3a	Equations 2, 8 Rumen-degradable and rumen-	INRA, 2018	Semi-mechanistic	86.64	22.16	0.50
FN steers	6b	undegraded crude protein, dry matter	Chapter 4	Semi-mechanistic	41.50	30.67	0.62
TN steers	9a	intake, body-weight, body weight change, milk production, and milk protein	INRA, 2018	Semi-mechanistic	48.13	20.32	0.67
Empirical equations							
UN lactating cows	23	Nitrogen intake	Kebreab et al., 2010	Temperate empirical	24.49	97.86	0.65
FN lactating cows	10	Nitrogen intake	Zahra et al., 2020	Tropical empirical	13.73	73.48	0.82
TN lactating cows	31	Nitrogen intake	Reed et al., 2015	Temperate empirical	14.09	94.58	0.83
UN heifers	38	Nitrogen intake	Reed et al., 2015	Temperate empirical	24.77	33.78	0.85
FN heifers	14	Nitrogen intake and body-weight	Salah et al., 2014	Tropical empirical	19.45	34.19	0.79
TN heifers	41	Nitrogen intake, metabolizable energy, crude protein, and body-weight	Reed et al., 2015	Temperate empirical	15.17	38.45	0.92
UN steers	13	Nitrogen intake and body-weight	Salah et al., 2014	Tropical empirical	59.54	41.23	0.59
FN steers	14	Nitrogen intake and body-weight	Salah et al., 2014	Tropical empirical	27.87	50.14	0.79
TN steers	15	Nitrogen intake and body-weight	Salah et al., 2014	Tropical empirical	36.82	30.64	0.76

[1] RMSE, root mean square error.
[2] ED, error due to disturbance.
[2] CCC, concordance correlation coefficient.

5.3 Feed protein partitioning into rumen-degraded and rumen-undegraded crude protein

This section provides the main assumptions and hypotheses that supported the development of chapters 2 and 3. Chapters 2 and 3 were developed on the hypothesis that adopting laboratory methodology from temperate systems without validating and adapting it for tropical systems might result in inaccurate estimates of N partitioning. However, the inaccuracy of estimates of N partitioning models will depend on whether its uncertainty is related to the uncertainty of the model's input variables. To illustrate this point, two examples are provided.

For the first example, the database (n = 392 observations) and model I (INRA, 2018) from chapter 4 were used to determine how uncertainty in the model's input variables affects the accuracy of predicting FN excretion in cattle under tropical conditions. Model I predicts FN excretion based on microbial crude protein synthesis, RUP, and indigestible neutral detergent fiber (chapter 4). Only the model's input variables RUP and indigestible neutral detergent fiber will be used in this example. The levels of uncertainty of the RUP proportion were established to be 15%, 25%, and 78% of the RUP proportion (Table 5.4). The reason for choosing this uncertainty was that the RUP of tropical forages predicted using equations developed for temperate forages presented in chapter 2 (Kirchhof, 2007; Valdés et al., 2011) had a RMSE of 25-78% of the observed mean, whereas when using equations developed for tropical forages in chapter 2 the RMSE was 14-15% of the observed mean. Same uncertainties were selected for indigestible neutral detergent fiber because no other information was available. As shown in Table 5.3, the uncertainty in RUP proportion had a greater impact on the accuracy of predicted FN excretion than those for indigestible neutral detergent fiber; and a high level of uncertainty in RUP proportion (78% in RUP proportion) resulted in inaccurate (RMSE > 25%) predictions of FN excretion by model I (example 1).

The second example is provided by Edmunds et al. (2012), in which metabolizable protein was calculated from *in vitro* PRCP supply (modified Hohenheim gas test) and compared against reference values. The *in vitro* PRCP supply of temperate fresh forages (n = 12) and silages (n = 6) was converted into metabolizable protein by multiplying the supply by 0.73 (i.e., proportion of amino acid-N in duodenal non ammonia-N) and by 0.85 (i.e., proportion of amino acid-N absorbed in the small intestine) (GfE, 2001). The levels of uncertainty associated with the *in vitro* PRCP supply were 11% at a rumen passage rate of 4%/h (Edmunds et al., 2012). Reference values of metabolizable protein of fresh forages (n = 65) and grass silages (n = 500) were derived from averages of intestinally digestible protein (i.e., Dutch equivalent of metabolizable protein) (Duinkerken et al., 2011). For fresh forages

and silages respectively, metabolizable protein calculated from *in vitro* PRCP supply were 94.5 and 82.9 g/kg dry matter and from intestinally digestible protein averages were 98.6 and 82.7 g/kg dry matter. Based on these results, an uncertainty level of 11% of the PRCP by the *in vitro* method supply does not affect the calculation of metabolizable protein with the Dutch system (example 2).

Table 5.4
Effect of the uncertainty of input variables on the prediction of fecal nitrogen excretion of dairy cattle in tropical husbandry systems.

Change in input variables[1]	FN excretion[2] (g/day)	RMSE[3] (g/day)	RMSE (% mean)
+0%RUP, NDNDF	92.20	16.99	19.46
+15%RUP	94.71	18.15	20.79
+25%RUP	96.40	19.18	21.96
+78%RUP	105.33	26.84	30.74
+15%NDNDF	93.42	17.43	19.97
+25%NDNDF	94.23	17.78	20.36
+78%NDNDF	98.54	20.23	23.17

[1] NDNDF, indigestible neutral detergent fiber (g/kg dry matter); RUP, diet's rumen-undegraded crude protein (g/kg crude protein).
[2] FN, fecal nitrogen excretion.
[3] RMSE, root mean square error.

The inaccuracy of estimates of N partitioning models depends on whether its uncertainty is related to the uncertainty of the model's inputs variables (Reeves et al., 1998). An example of this can be seen in example 1, which illustrates that more emphasis should be placed on improving the accuracy of the RUP proportion than indigestible neutral detergent fiber to accurately predict FN excretion by model I. Whereas, improving the accuracy of indigestible neutral detergent fiber is not of high relevance when model I is used to predict FN excretion.

In addition, Example 1 illustrates the possibility of setting a maximum level of uncertainty for the RUP proportion, so that an accurate prediction of the FN is still possible. As discussed in chapters 2 and 3, determining the maximum level of uncertainty or uncertainty threshold for RUP proportion and PRCP supply was one of the major challenges. An uncertainty threshold enables you to determine whether an alternative method is sufficiently accurate to replace a gold standard method. As shown in example 2, the uncertainty associated with the *in vitro* PRCP supply does not significantly affect the accuracy of the calculation of metabolizable protein by the DVE (Norfor, 2011). Therefore, in example 2, the *in vitro* method can replace

the gold standard method for the estimation of the PRCP supply. It is important to note, however, that this will always depend on the purpose for which PRCP supply is used.

Sensibility analysis is a valuable tool for estimating how changes in the model's input variables will affect the accuracy of the model's outcomes. As well as estimating the uncertainty threshold for each model's input variables. Example 1 followed a partial sensibility analysis using a one-at-a-time approach in which each model's input variable varied independently, while all other input variables remain unchanged (Schouten et al., 2014). There are, however, some disadvantages to this approach (Schouten et al., 2014) (e.g., it cannot account for interactions), and therefore a mixed approach is recommended. In a mixed approach, a one-at-a-time approach is combined with a Monte Carlo approach, which includes the selection of random sets of variations in the model's input variables (Schouten et al., 2014). Therefore, inaccuracies in N partitioning models arise from several sources been uncertain on the input variables one of them. This emphasizing the need for improving accuracy of specific input variable to ensure accurate predictions. A mixed approach involving one-at-a-time and Monte Carlo methods is recommended for sensibility analysis, aiding in estimating input variable uncertainty thresholds and interactions to enhance model outcome accuracy.

5.4 Challenge and limitations

Identifying the minimum level of adequacy required to estimate feedstuffs' RUP proportion and PRCP supply was one of the most challenging aspects of the present thesis. This was challenging, because the accuracy of the RUP proportion and PRCP supply depend on the purpose for which these variables are used (section 5.3) and therefore it was not feasible to determine a single threshold. Instead, a list of studies that compare alternative methods with reference values of rumen protein partitioning (i.e., RUP and PRCP) was compiled, and the maximum concordance correlation coefficient achieved by an alternative method was considered to be the minimum level of adequacy. This is, however, not entirely suitable because most studies use *in situ* as reference values rather than *in vivo* methods. It is therefore necessary to validate the methodologies with *in vivo* studies to establish the level of adequacy that an alternative method can achieve.

Obtaining published information on crude protein concentrations and *in situ* RUP proportions of tropical feedstuffs was also challenging. This was required for validation of the developed equations in chapter 2 and 3. Many studies have estimated protein fractions and *in situ* RUP proportions of tropical feedstuffs, however the information reported was mainly presented as means (e.g., Valdés et al., 2011) rather than results per individual feedstuffs.

Consequently, it would be beneficial if authors provided their results not only as means, but also per individual feedstuffs in the appendix section.

5.5 Recommendations

Aside from providing tools to improve the estimation of N use efficiency of cattle in tropical husbandry systems, the present thesis also raised several questions that require further research. These questions are related to (1) estimating the intestinal digestibility of the RUP proportion, (2) estimating the PRCP supply of tropical forages using the *in vitro* method particularly at slow rumen passage rates, (3) evaluating the developed equations to predict RUP proportion and PRCP supply of tropical forages, and (4) improving the prediction of N excretion of dairy cattle in tropical husbandry systems.

Estimating the intestinal digestibility of the RUP will provide complementary information to chapters 2 and 3 as well as relevant information for chapter 4. Most feeding recommendations for ruminants use a fixed factor for intestinal digestibility of RUP. However, a fixed factor is not suitable as intestinal digestibility of RUP varies substantially between and within feedstuffs (Hvelplund and Weisbjerg, 1998). In addition, intestinal digestibility of RUP in many tropical feeds is so low that overlooking this problem would overestimate the supply of absorbed amino acids (Hvelplund and Weisbjerg, 1998). Therefore, further research is necessary to determine the intestinal digestibility of RUP of tropical forages to improve the utilization of protein in the diet of tropical cattle.

It was challenging to estimate PRCP supply using the *in vitro* method, particularly for tropical forage legumes at slow rumen passage rates (chapter 3). The reasons why the *in vitro* method poorly predicts the PRCP supply in tropical forage legumes but moderately predicts the PRCP supply of tropical forage grasses (chapter 3) are yet unclear. One possible explanation might be related to the greater synchrony in the amount and rate of ruminal crude protein and carbohydrate degradation in tropical forage grasses than in forage legumes. It is expected that a greater degree of synchrony will result in optimal *in vitro* fermentation. Therefore, further studies are needed to determine whether the synchrony between protein and carbohydrate degradation (i.e., amount and rate) has an effect on the overall adequacy of the *in vitro* method across various rumen passage rates.

It is also unclear why the accuracy of the *in vitro* method is reduced at slow rumen passage rates (chapter 3). Estimating accurately and precisely the PRCP supply of tropical forages at slow rumen passage rates is crucial. This is because low feed intake levels and thus digesta

passage rates are commonly found in tropical dairy cattle systems. The *in vitro* estimation of PRCP supply at slow rumen passage rates may be improved if a constant amount of protein and carbohydrates is provided in the inoculum. Therefore, further studies are needed to determine whether a constant amount of protein and carbohydrates in the inoculum could prevent an absence of substrate and, consequently, reduce the higher uptake of ammonia-N by the microorganism at slow passage rates.

Evaluation of the developed equations to predict RUP proportions and PRCP supplies of tropical forages is crucial for further calibration and validation. The equations of chapter 2 in the present thesis were validated using internal validation, which is not ideal since the dataset used for development was also used for validation. External validation would be preferable; however, this would require a new dataset. Meta-analysis can be used to build a new dataset, however, there is limited information on fiber concentrations, crude protein fractions, RUP, and PRCP supply of tropical feedstuffs. In addition, this information is usually presented as a mean rather than per individual feedstuff. Therefore, to calibrate and validate our equations as well as to provide information for improving the utilization of protein in tropical cattle's diet, it is imperative to generate a feed library with information on fiber concentrations, crude protein fractions, RUP, and PRCP supply of tropical forages.

Predicting N excretion in tropical husbandry systems for dairy cattle presents specific challenges. These challenges are more pronounced for UN excretion compared to FN and TN excretion and for steers compared to lactating cows and heifers. The limitations of our N excretion dataset (chapter 5) hindered the quantification of the contribution of errors arising from both the inaccuracies of the reference method and the variations among animal classes. Furthermore, a comprehensive dataset encompassing FN fractions of lactating cows, essential for a comprehensive assessment of FN excretion, was also lacking. This absence hinders the evaluation of prediction errors across all animal classes. Furthermore, there is a lack of empirical equations for predicting N excretion of tropical husbandry systems. Therefore, further research is required to improve the prediction of N excretion, particularly UN excretion with greater focus on animals with low N excretion considering the effects associated to reference methods. Furthermore, there is the need for the development of empirical equations aimed at predicting N excretion of dairy cattle within tropical husbandry systems. This need becomes particularly evident when the scarcity of data restricts the utilization of semi-mechanistic models.

5.6 References

Aarons, S. R., Gourley, C. J. P., Powell, J. M., Hannah, M. C. 2017. Estimating nitrogen excretion and deposition by lactating cows in grazed dairy systems. Soil Res. 55, 489–499. https://doi.org/10.1071/SR17033

AFRC (Agricultural and Food Research Council). 1993. Energy and protein requirements of ruminants: An advisory manual prepared by the AFRC Technical Committee on Responses to Nutrients. CAB International, Wallingford, UK.

Bateki, C. A. 2020. Adaptation of herd simulation models to predict the efficiency of the use of resources in tropical ruminant productions systems [Dissertation to obtain the doctoral degree of Agricultural Sciences]. University of Hohenheim.

Castillo, A. R., Kebreab, E., Beever, D. E., France, J. 2000. A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. J. Anim. Feed Sci. 9, 1–32. https://doi.org/10.22358/jafs/68025/2000

Dong, R. L., Zhao, G. Y., Chai, L. L., Beauchemin, K. A., 2014. Prediction of urinary and fecal nitrogen excretion by beef cattle. J. Anim. Sci. 92, 4669–4681. https://doi.org/10.2527/jas.2014-8000

Duinkerken, G. V., Blok, M. C., Bannink, A., Cone, J. W., Dijkstra, J., Vuuren, A. M. V., Tamminga, S., 2011. Update of the Dutch protein evaluation system for ruminants: the DVE/OEB2010 system. J. Agric. Sci. 149, 351–367. https://doi.org/10.1017/S0021859610000912

Edmunds, B., Südekum, K.-H., Spiekers, H., Schuster, M., Schwarz, F. J. 2012. Estimating utilisable crude protein at the duodenum, a precursor to metabolisable protein for ruminants, from forages using a modified gas test. Anim. Feed Sci. Technol. 175, 106–113. https://doi.org/10.1016/j.anifeedsci.2012.05.003

France, J., Dijkstra, J. 2006. Scientific progress and mathematical modelling: different approaches to modelling animal systems, in: Mechanistic Modelling in Pig and Poultry Production. CAB International, Wallingford, UK.

Garg, M. R., Sherasia, P. L., Bhanderi, B. M., Makkar, H. P. S. 2016. Nitrogen use efficiency for milk production on feeding a balanced ration and predicting manure nitrogen excretion in lactating cows and buffaloes under tropical conditions. Anim. Nutr. Feed Tech. 16, 1. https://doi.org/10.5958/0974-181X.2016.00001.9

GfE (Gesellschaft für Ernährungsphysiologie). 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt, Germany.

Haddon, M. 2011. Modelling and quantitative methods in Fisheries. Editorial Chapman and Hall/CRC, Boca Raton, USA.

Hanigan, M. D., Daley, V. L., 2020. Use of mechanistic nutrition models to identify sustainable food animal production. Annu. Rev. Anim. 8, 355–376. https://doi.org/10.1146/annurev-animal-021419-083913

Hernández-Castellano, L. E., Nally, J. E., Lindahl, J., Wanapat, M., Alhidary, I. A., Fangueiro, D., Grace, D., Ratto, M., Bambou, J. C., de Almeida, A. M. 2019. Dairy science and health in the tropics: challenges and opportunities for the next decades. Trop. Anim. Health Prod. 51, 1009–1017. https://doi.org/10.1007/s11250-019-01866-6

Higgs, R. J., Chase, L. E., Van Amburgh, M. E., 2012. Development and evaluation of equations in the Cornell Net Carbohydrate and Protein System to predict nitrogen excretion in lactating dairy cows. J. Dairy Sci. 95, 2004–2014. https://doi.org/10.3168/jds.2011-4810

Huhtanen, P., Nousiainen, J. I., Rinne, M., Kytölä, K., Khalili, H. 2008. Utilization and partition of dietary nitrogen in dairy cows fed grass silage-based diets. J. Dairy Sci. 91, 3589–3599. https://doi.org/10.3168/jds.2008-1181

Hvelplund, T., Weisbjerg, M. R. 1998. *In vitro* techniques to replace *in vivo* methods for estimating amino acid supply. BSAP Occasional Publication 22, 131–144. https://doi.org/10.1017/S0263967X00032419

INRA (Institut National de la Recherche Agronomique). 2019. INRA feeding system for ruminants. Wageningen Academic Publishers, Wageningen, Netherlands.

Johnson, A. C. B., Reed, K. F., Kebreab, E., 2016. Short communication: Evaluation of nitrogen excretion equations from cattle. J. Dairy Sci. 99, 7669–7678. https://doi.org/10.3168/jds.2015-10730

Jonker, J. S., Kohn, R. A., Erdman, R. A. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J. Dairy Sci. 81, 2681–2692. https://doi.org/10.3168/jds.S0022-0302(98)75825-4

Kebreab, E., Strathe, A. B., Dijkstra, J., Mills, J. A. N., Reynolds, C., Crompton, L. A., Yan, T., France, J. 2010. Energy and protein interactions and their effect on nitrogen excretion in dairy cows. Presented at the EAAP Scientific Series, pp. 417–426.

Kirchhof, S., Eisner, I., Gierus, M., Südekum, K.-H. 2010. Variation in the contents of crude protein fractions of different forage legumes during the spring growth. Grass Forage Sci. 65, 376–382. https://doi.org/10.1111/j.1365-2494.2010.00756.x

Mottet, A., de Haan, C., Falcucci, A., Tempio, G., Opio, C., Gerber, P. 2017. Livestock: On our plates or eating at our table? A new analysis of the feed/food debate. Global Food Security, Food Security Governance in Latin America 14, 1–8. https://doi.org/10.1016/j.gfs.2017.01.001

Mupeta, B., Weisbjerg, M. R., Hvelplund, T., Madsen, J., 1997. Digestibility of amino acids in protein rich tropical feeds for ruminants estimated with the mobile bag technique. Anim. Feed Sci. Technol. 69, 271–280. https://doi.org/10.1016/S0377-8401(97)81641-0

Nennich, T. D., Harrison, J. H., VanWieringen, L. M., Meyer, D., Heinrichs, A. J., Weiss, W. P., St-Pierre, N. R., Kincaid, R. L., Davidson, D. L., Block, E. 2005. Prediction of manure and nutrient excretion from dairy cattle. J. Dairy Sci. 88(10), 3721–3733. https://doi.org/10.3168/jds.S0022-0302(05)73058-7

NorFor (Nordic Feed Evaluation System). 2011. The Nordic feed evaluation system. Wageningen Academic Publishers, Wageningen, Netherlands.

OECD (Organisation for Economic Co-operation and Development). 2023. OECD-FAO Agricultural Outlook 2020-2029, OECD Publishing, Paris/FAO, Rome, https://doi.org/10.1787/1112c23b-en

PARI, 2019. PARI Policy Brief No. 17: Improving Protein Nutrition of Dairy Cattle in the Tropics.

Pica-Ciamarra, U., Baker, D., Morgan, N., Zezza, A., Azzarri, C., Ly, C., Nsiima, L., Nouala, S., Okello, P., Sserugga, J. 2014. Investing in the livestock sector: why good numbers matter, a sourcebook for decision makers on how to improve livestock data. World Bank, Washington, DC, USA.

Powell, J. M., MacLeod, M., Vellinga, T. V., Opio, C., Falcucci, A., Tempio, G., Steinfeld, H., Gerber, P. 2013. Feed-milk-manure nitrogen relationships in global dairy production systems. Livest. Sci. 152, 261–272. https://doi.org/10.1016/j.livsci.2013.01.001

Reed, K. F., Moraes, L. E., Casper, D. P., Kebreab, E. 2015. Predicting nitrogen excretion from cattle. J. Dairy Sci. 98, 3025–3035. https://doi.org/10.3168/jds.2014-8397

Reeves, G. K., Cox, D. R., Darby, S. C., Whitley, E. 1998. Some aspects of measurement error in explanatory variables for continuous and binary regression models. Statistics in Medicine 17, 2157–2177. https://doi.org/10.1002/(SICI)1097-0258(19981015)17:19<2157::AID-SIM916>3.0.CO;2-F

Reid, R. S., Thornton, P. K., McCrabb, G. J., Kruska, R. L., Atieno, F., Jones, P. G. 2004. Is it possible to mitigate greenhouse gas emissions in pastoral ecosystems of the tropics? Environ. Dev. Sustain. 6, 91. https://doi.org/10.1023/B:ENVI.0000003631.43271.6b

Rufino, L. M. de A., Detmann, E., Gomes, D. Í., Reis, W. L. S. dos, Batista, E. D., Valadares Filho, S. de C., Paulino, M. F. 2016. Intake, digestibility and nitrogen utilization in cattle fed tropical forage and supplemented with protein in the rumen, abomasum, or both. J. Anim. Sci. Biotechnol. 7, 11. https://doi.org/10.1186/s40104-016-0069-9

Rufino, M., Herrero, M., Van Wijk, M., Hemerik, L., Ridder, N., Giller, K. 2009. Lifetime productivity of dairy cows in smallholder farming systems of the Central highlands of Kenya. Animal 3, 1044–56. https://doi.org/10.1017/S1751731109004248

Salah, N., Sauvant, D., Archimède, H. 2014. Meta-analysis of response of growing ruminants to diet in warm conditions. Animal 9(5), 822–830. https://doi.org/10.1017/S175173111400322X

Salazar-Cubillas, K., Dickhoefer, U. 2021. Estimating the proportion of in situ rumen-undegraded crude protein from chemical crude protein and fiber fractions in tropical forage grasses and legumes. Anim. Feed Sci. Technol. 282, 115122. https://doi.org/10.1016/j.anifeedsci.2021.115122

Schouten, M., Verwaart, T., Heijman, W. 2014. Comparing two sensitivity analysis approaches for two scenarios with a spatially explicit rural agent-based model. Environ. Model. Softw. 54, 196–210. https://doi.org/10.1016/j.envsoft.2014.01.003

Tedeschi, L. O., Fox, D. G., Pell, A. N., Lanna, D. P. D., Boin, C. 2002. Development and evaluation of a tropical feed library for the Cornell Net Carbohydrate and Protein System Model. Sci. Agr. 59, 1–18. https://doi.org/10.1590/S0103-90162002000100001

Tomlinson, A. P., Van Horn, H. H., Nordstedt, R. A., Wilcox, C. J. 1992. Prediction of nitrogen excretion based on diet composition and performance criteria. J. Dairy Sci. 75 (Suppl. 1):176.

Valdés, C., Andrés, S., Giráldez, F. J., García, R., Peláez, R., Calleja, A. 2011. Estimación de la proteína no degradable en el rumen de pastos de la Montaña Leonesa empleando el CNCPS (Cornell). Pastos, paisajes culturales entre tradición y nuevos paradigmas del siglo XXI. Sociedad Española para el Estudio de los Pastos, pp. 401–406.

Wassie, S. E., Ali, A. I. M., Korir, D., Butterbach-Bahl, K., Goopy, J., Merbold, L., Schlecht, E., Dickhoefer, U. 2019. Effects of feed intake level on efficiency of microbial protein synthesis and nitrogen balance in Boran steers consuming tropical poor-quality forage. Arch. Anim. Nutr. 73, 140–157. https://doi.org/10.1080/1745039X.2019.1572343

Waldrip, H. M., Todd, R. W., Cole, N. A., 2013. Prediction of nitrogen excretion by beef cattle: a meta-analysis. J. Anim. Sci. 91, 4290–4302. https://doi.org/10.2527/jas.2012-5818

Yan, T., Frost, J. P., Agnew, R. E., Binnie, R. C., Mayne, C. S. 2006. Relationships among manure nitrogen output and dietary and animal factors in lactating dairy cows. J. Dairy Sci. 89, 3981–3991. https://doi.org/10.3168/jds.S0022-0302(06)72441-9

Zahra, W. A., Middelaar, C. E. van, de Boer, I. J. M., Oosting, S. J. 2020. Predicting nutrient excretion from dairy cows on smallholder farms in Indonesia using readily available farm data. Asian- Australas J. Anim. Sci. 33, 2039– 049. https://doi.org/10.5713/ajas. 20.0089

Chapter 6

6. General conclusions

The findings from the present thesis and literature support the hypothesis that adoption of laboratory methodologies and of modeling tools from temperate systems without validating and adjusting them for tropical systems results in inadequate estimates of protein supply and nitrogen (N) excretion of cattle in tropical husbandry systems, which hampers the assessment of N use efficiency and the adjustment of nutrient supply to the actual requirements of the animal. The adjustment of laboratory methodologies, such as the chemical method used to estimate the protein value of temperate forages, to tropical forages, results in more adequate estimates of the proportion of rumen-undegraded crude protein (RUP) and post-ruminal crude protein (PRCP) supply of tropical forages. The semi-mechanistic model developed by INRA (2018) is, therefore, able to predict the N excretion of cattle more adequately in tropical husbandry systems, because it is sensitive to differences in the RUP proportion and PRCP supply. In addition to increasing the adequacy of these input variables, adjustments made to the microbial protein synthesis and intercept to the fecal N excretion to the semi-mechanistic model developed by INRA (2018) results in a more adequate prediction of N excretion by cattle in tropical husbandry systems.

Nevertheless, not all the adjustments made to the laboratory methodologies and modeling tools from temperate systems were sufficient to achieve an adequate prediction of the protein value of tropical forages and the N excretion of cattle in tropical husbandry systems. Particularly for predicting RUP proportion and PRCP supply of tropical forages legumes at slow rumen passage rates as well as predicting urinary N excretion of cattle in tropical husbandry systems with low N intakes. Therefore, further research is needed to determine the factors that contribute to their poor adequacy.