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Ruminal protein degradation of feeds and microbial protein synthesis

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Ruminants are a group of animals characterized by the intake of diets that are altered in rumen by anaerobic microorganisms. These microorganisms have ideal conditions in the rumen for their development and growth using dietary protein as feed source. When rumen digesta flows through the gastrointestinal tract, these microorganisms become a source of protein for digestion in the small intestine of ruminants. Thus, to find an appropriate recommendation regarding protein requirements for cattle, we must characterize the changes imposed by these microorganisms and the amount of microbial protein that reaches the small intestine with a specific diet.

INTRODUCTION

The pool of potentially fermentable protein in the rumen includes dietary nitrogenous compounds, endogenous protein from saliva, loss of epithelial cells and lysed rumen microorganisms in the rumen (NRC, 2001). This protein pool that can be significantly changed in this compartment is called rumen degradable protein (RDP). Thus, ruminant´s protein nutrition is dependent on the magnitude and profile of that pool that reaches the small intestine for absorption as amino acids plus dietary protein that has not been degraded in the rumen. This protein fraction is called rumen undegradable protein (RUP). The set of all amino acids that are available for the intestinal digestion and absorption represents the metabolizable protein (MP). The nutritional requirements of MP and crude protein (CP) for beef cattle can be estimated after knowing the changes in dietary nitrogenous compounds by the rumen. Then, it is necessary to know the microbial protein that is produced in the rumen when providing different diets, the factors that affect the production and efficiency of this protein, and understand digestion and absorption of the protein in the gastrointestinal tract.

The literature found different methods to estimate the nitrogen partitioning of the diet into RDP and RUP and its intestinal digestibility. These methods include *in vivo*, *in situ* and a variety of *in vitro* methods (Schwab et al., 2003). The accuracy of *in vivo* methods provide reliable estimates of what happens during nutrient digestion. However, in most situations, the *in vivo* methods can be applied specifically for digestibility trials.

The validation of protocols that allow the use of *in vitro* and *in situ* techniques in an accurately and unbiased manner is required to obtain estimates for ruminal protein degradation, microbial production and indirectly the MP. The total estimated microbial crude protein (MCP) synthesis can also be performed using *in vivo* techniques using microbial markers in the rumen, small intestine or in the urine. Thus, alternative techniques, such as the urinary purine derivatives (PD), can be used to quantify the MCP that leaves the rumen and reaches the small intestine for absorption as amino acids. This technique can reduce handling time of animals during the experiment and dispense the use of fistulated animals. However, the urinary volume estimate must be accurate to minimize experimental errors.

Furthermore, the ability to estimate microbial production and efficiency as a function of the diet offered is an essential mathematical tool to estimate MP requirements. Then, it is necessary to find which are the variables that affect the efficiency of MCP synthesis, and to fit accurate equations to predict it. The objective of this chapter was to present the main techniques involved in the RDP and RUP estimates in feed, including effects of microbial contamination in the ruminal incubation residue. The objective was also to present techniques to quantify MCP and the

factors that affect this synthesis. This chapter also presented validated equations to estimate MCP synthesis.

PROTEIN RUMINAL DEGRADATION

In situ techniques

Among the main differences found in estimating ruminal protein degradation is the choice of technique. The *in situ* technique consists of measuring the ruminal disappearance of the feed by adding ingredients in bags of known porosity, where rumen microorganisms access the feed and degrade it. It allows the quantification of non-degraded residues. The bags are incubated in ruminal digesta of cannulated animals, which characterizes the denomination of *in situ* technique (Orskov et al., 1980). The study of degradability is important to understand feed changes in the rumen, as CP can be degraded and converted into microbial protein.

According to Nocek (1988), using *in situ* technique allows intimate contact between the feed and rumen microorganisms. There is no better way to simulate rumen digestion during certain conditions of temperature, pH, buffer substrate and microbial populations. However, as a limitation, the studied feed is not subjected to all digestive steps such as chewing, rumination, and passage rate. Then, we will be discussed the critical points and some standardizations that made the method as accurate as possible.

a) Non-degraded material losses

The loss of material inside the rumen bag is critical because particles smaller than the size of the bag pores can be lost even without prior degradation. This event can cause overestimation of the soluble fraction or its ruminal degradation rate. However, the reduction of the grinding particle size facilitates microbial access since feed bypasses the processes of chewing and rumination. To minimize this problem, some authors recommend incubations using particle sizes between 1.5- and 3-mm diameter (Huntington and Givens, 1995; Broderick and Cochran, 2000).

Using tropical forage, Casali et al. (2008) recommended particle size of 2 mm for *in situ* incubation for greater accuracy in estimates of degradable fractions. These authors found that the 3 mm size reduced the accuracy of the results probably due to the smaller specific surface for microbial action. NRC (2001) also suggested the standardization of *in situ* incubations using ground particles of 2 mm. Thus, BR-CORTE (2016) recommends milling feed samples with 2 mm sieves to perform *in situ* incubations, although for conducting chemical analyzes the porosity should be 1 mm as suggested by Valente et al. (2011) for more accurate results for neutral detergent fiber (NDF). However, even with the standardization of the particle size, there are losses of undigested material, thus, some authors recommend the correction of *in situ* degradation data by washing the bags in water and determining the immediate loss of particles (Lopez et al., 1994; France et al., 1997).

Hvelplund and Weisbjerg (2000) described a protocol for estimating the extent of particles loss and correction of degradation fractions through the difference between the loss of material from the nylon bags when these were only washed with water and the true solubility measured in filter paper. Water solubility should be measured by adding 0.5 g of sample to 40 mL of water, which should remain at room temperature for 1 h. After this time the material must be transferred to nitrogen-free paper filter to quantify the water-soluble N. The correction for particle loss can be performed using equations proposed by Weisbjerg et al. (1990):

$$DEG_{cor}(ti) = DEG(ti) - P \times \left[1 - \frac{DEG(ti) - P + SOL}{1 - (P + SOL)}\right]$$

$$a_{cor} = a - P$$

$$b_{cor} = b + P \times \left[\frac{b}{1 - (P + SOL)}\right]$$

$$c_{cor} = c$$

where: $DEG_{cor}(ti)$ = degradability corrected in incubation time ti; DEG(ti) = degradability measured in incubation time ti; P = particle loss; SOL = water solubility; a_{cor} = soluble fraction corrected; b_{cor} = potentially degradable

insoluble fraction corrected; c_{cor} = degradation rate corrected; a, b, c = no corrected fractions measured.

b) Microbial contamination of ruminal residue incubation of forage and concentrates

After the end of the *in situ* ruminal incubation, the bags must undergo a cleaning process for the microbial degradation immediate standstill and also for removing ruminal digesta and microbial residue adhered to the feed or in the bags. However, some authors (Nocek and Grant, 1987; Vanzant et al., 1998; Michalet-Doreau and Ould-Bah, 1992) reported that it is difficult to achieve a complete removal of the microbial mass adhered to particles because a specific microbial adhesion is necessary to start particles colonization. Thus, microbial contamination in incubation residues represents an important source of variation, resulting in overestimation of residues and non-degradable fractions. This consequently results in underestimation of the potentially degradable fraction. Especially for the protein fraction of low protein forages, microbial contamination has a greater impact on the estimates of degradable fractions.

However, the procedures to estimate microbial contamination require the use of microbial markers, such as diaminopimelic acid, RNA, ^{35}S and ^{15}N, which have high costs and increase the total time for chemical analysis. A solution to minimize these barriers would be the development of a correction protocol that does not require the use of microbial markers in all procedures, increasing the accuracy of the estimates without raising the experimental cost.

Machado et al. (2013) conducted a study using ^{15}N as a microbial marker to estimate microbial contamination in incubation residues of forage. These authors presented an equation to correct residues after ruminal *in situ* incubation, and to correct degradable fractions, which will be adopted in this edition of BR-CORTE. The authors reported that soluble fraction (A) and potentially degradable (B) in low protein forages can be underestimated if not corrected. The authors recommended the following equations:

(1) $A_{CP}C = 1.99286 + 0.98256 \times A_{CP}NC$

(2) $B_{CP}C = -17.2181 - 0.0344 \times B_{CP}NC + 0.65433 \times CP + 1.03787 \times NDF + 2.66010 \times NDIP - 0.85979 \times iNDF$

(3) $kd_{CP}C = 0.04667 + 0.35139 \times kd_{CP}NC + 0.0020 \times CP - 0.00055839 \times NDF - 0.00336 \times NDIP + 0.00075089 \times iNDF$

where $A_{CP}C$ = soluble fraction of CP corrected for microbial contamination, $A_{CP}NC$ = soluble fraction of CP without correction for microbial contamination, $B_{CP}C$ = potentially degradable fraction of CP corrected for microbial contamination, $B_{CP}NC$ = potentially degradable fraction of CP without microbial correction, $kd_{CP,}C$ = degradation rate of B fraction corrected for microbial contamination, $kd_{CP}NC$ = degradation rate of B fraction without microbial contamination, NDIP = neutral detergent insoluble protein, NDF = neutral detergent fiber, and iNDF = indigestible neutral detergent fiber.

Machado et al. (2013) also suggested that microbial contamination percentage in different incubation times for forages with different CP contents may be obtained by the following equation:

$$\%C = 79.21 \times (1 - e^{-0.0555 \times t}) \times e^{-0.0874 \times CP}$$

where %C = percentage of microbial contamination, t = feed incubation time in hours, CP = crude protein as a percentage of feed in DM basis.

To estimate the impact of microbial contamination on *in situ* incubation residues of concentrate feeds, Menezes et al. (2017) conducted a study using ^{15}N as microbial marker and evaluated 12 concentrate feeds, including six protein and six energetic feeds. Although there was microbial contamination in incubation residues, the authors found no difference among fractions A, B, and kd when values were corrected or not for microbial contamination after 72 hours of ruminal incubation. The authors observed that the greatest contaminations were obtained for corn straw and corncobs, and for sunflower meal and wheat bran, which are feeds with high NDF content. This study suggested that for concentrate feeds the microbial contamination makes an irrelevant contribution to incubation

residues, suggesting that for these feeds it is not necessary to correct for microbial contamination due to the lack of interference in RDP and RUP.

c) Experimental design and incubation times

The experimental protocols adopted by Machado et al. (2013) and Menezes (2016) are proper alternatives to estimate *in situ* ruminal degradation of feeds. These authors conducted repeated incubations of feeds in different animals, using a Latin square design as a tool to collect unbiased samples. According to Machado et al. (2013), the Latin square design can be used to organize data collection, allowing measurement of feed degradation and removing the animal confusion effect. The Latin square design can be used to control sources of variation and avoid animal experimental errors. Machado et al. (2013) reported that the Latin square design does not need to be used to estimate variability or to account for sources of variation in experimental error, but to conduct unbiased data collection.

When the objective of ruminal incubation is to obtain data to estimate intestinal digestibility of RUP, the incubation times proposed by Menezes et al. (2017) should be used. This author conducted a cluster analysis and estimated that, for protein concentrate feeds, the incubation time to estimate RDP should be between 6.8 to 15.4 h, considering kp = 0.05 h^{-1}.

Thus, the literature recommendations of 16 h (Calsamiglia et al., 1995) to obtain RDP feed may not be useful for all types of feed. Menezes et al. (2019) established the times needed to estimate the RDP of concentrated feeds grouped into: high-starch energy concentrates, low-starch energy concentrates, and protein concentrates, obtaining the following intervals, considering kp = 0.05 h^{-1}:

- High-starch energy concentrates – ground corn, ground sorghum and crumbled corn with straw and cob: 16 hours.
- Low-starch energy concentrates – wheat meal, rice meal and soybean hulls: 7 hours.
- Protein concentrates – cottonseed meal, soybean meal, beans, peanut meal, and sunflower meal: 10 hours.

However, it is important to emphasize that these times may not be enough to study CP degradability of tropical forages. Some Brazilian studies (Martins et al., 1999; Cabral et al., 2005; Pires et al., 2006) used 48 hours of incubation to estimate *in situ* degradation for concentrate feeds and 72 hours for forages. Despite several studies evaluating the time needed to estimate the fiber fractions for *in situ* incubation (Casali et al., 2008), few studies have evaluated the time needed to estimate RDP of forages.

d) Conditions inside the incubation bags

According to López (2005), conditions inside the incubation bags should be similar to those in the rumen. Therefore, the choice of suitable nylon for the production of bags is very important. The material must be synthetic and refractory to microbial degradation. Also according to Nocek (1997), the porosity of an adequate bag constitutes the adjustment between the limit to the influx of ruminal content without associating it with the evaluated feeds, thus allowing, the input of microbial populations for degradation; while at the same time, limiting the output of non-degraded feed particles. For many years, nylon bags, ranging from 40 to 60 μm in porosity as recommended by Nocek (1997), have been used as a standard for incubation; however, in the last years, the use of nylon has been questioned in several national and international studies. Hvelplund and Weisbjerg (2000) recommended the use of nylon bags with porosity varying between 30 and 50 μm in studies evaluating the *in situ* degradation of CP. Nevertheless, no studies were found comparing protein degradation in bags with different porosities. Thus, while studies are not conducted to evaluate the ideal porosity of nylon bags to obtain better estimates of RDP of feeds, we recommend the use of nylon bags with porosity of 40-60 μm.

The surface area of the incubated bags in relation to the amount of sample is also an important variable to be considered in the internal conditions of *in situ* degradation. According to Nocek (1988), the optimal amount of sample is that which provides enough quantity for chemical analysis at the end of the degradation process without

excessively filling the bags that delay microbial adhesion, increasing the latency phase and underestimating the digestion rates. After a literature review, the author recommended a sample of 10 to 20 mg/cm^2 of bags for most feeds, highlighting that for concentrate feeds, the higher value can be critical due to the high density and rapid degradation, causing intense gas production per unit of time. Therefore, despite having appeared in the 1980's, the study by Nocek (1988) has not been refuted yet, and is currently used as reference for *in situ* incubation studies.

e) Mathematical models to estimate ruminal degradation of crude protein

Traditional mathematical methods used to describe ruminal degradation generally calculate this variable based on substrate mass retained in the evaluated compartment. Some of these models are first-order (Waldo et al., 1972) that consider only the substrate to be digested, and others are second-order because they also consider the pool of substrates studied and the microbial mass present in the system (France et al., 1990). The Mitscherlich first-order model proposed by Ørskov and McDonald (1979) is most frequently used to evaluate CP residues obtained by *in vitro* and *in situ* methods. This simple negative exponential model is also considered as minimum return model.

The model proposed by Ørskov and McDonald (1979), in first-order kinetics, assumes that the degraded substrate for any time is proportional to the amount of potentially degradable residue in any time at a constant fractional degradation rate. This model is widely used due to its simplicity. On the other hand, this model does not have a wide diversity of changes on fractional rate due to degradation (López, 2008). Thus, López et al. (1999) studied some models that consider that the fractional degradation rate of nutrients is not a constant value, but a variable one; and that some degradation models based on the kinetics of microbial growth have a sigmoidal pattern, indicating an alternative solution for minimum return models or simple exponential models, as it is the case of the model proposed by Van Milgen et al. (1991).

Therefore, the models to adjust the CP degradation curves, both for the exponential and sigmoidal patterns, are presented below considering a constant fractional degradation rate (kd). The incubation CP residues obtained through *in vitro* or *in situ* assays as a function of time can be evaluated using mathematical models proposed by (1) Ørskov and McDonald (1979) and (2) Van Milgen et al. (1991):

(1) $Deg(t) = a + b \times (1 - e^{-kd \times t})$

(2) $Deg(t) = a + b \times [(1 + c \times t) \times (e^{-c \times t})]$

where: Deg (t) represents CP disappearance expressed as a percentage; *a* represents the water soluble fraction at time zero; *b* represents the water insoluble fraction but potentially *c* represents joint fractional lactency and degradation rate (h^{-1}); kd is the degradation rate of the fraction *b*; and *t* is the incubation time (hours).

Mitscherlich's first-order model adapted by Ørskov and McDonald (1979) assumes that degradation occurs at a constant fractional rate after a discrete latency rate; thus, the disappearance rate continuously decreases and there is no tipping point. Then, the authors included the parameter that denotes the immediately soluble fraction.

These authors presented a model with static values for the degradation rate, with a smaller number of parameters to be estimated. Therefore, we recommend the Ørskov and McDonald (1979) model, as it is simple and works relatively well to evaluate protein degradation of feedstuffs. For any model used, from the soluble fraction (a), potentially degradable fraction (b), and degradation rate (kd) measured for CP and using an estimated passage (kp), we can calculate the effective degradability that will correspond to RDP:

$$RDP = a + \left[\frac{(b \times kd)}{(kd + kp)} \right]$$

Therefore, one of the important factors that directly interfere with the RDP values is the passage rate adopted in the calculations of the effective CP degradability. The NRC (2001) previously adopted three different functions to estimate the passage rate of humid forage, dry forage and concentrates. However, Seo et al. (2006) highlighted that the data

compiled to generate these three equations were obtained in experiments that used rare earth elements as main markers, which limit the applicability of equations to current experimental data. Then, Seo et al. (2006) proposed new equations based on a database of 154 studies and 766 observations, which were able to predict the passage rate of several feedstuffs and diets based on external markers. After adjustments, the authors presented the following equations to estimate passage rate (kp) of forage, concentrates, and liquids:

$$kp\ forage = (2.365 + 0.0214 \times FiBW + 0.0734 \times CiBW + 0.069 \times Fi) / 100$$

$$kp\ concentrate = (1.169 + 0.1375 \times FiBW + 0.1721 \times CiBW) / 100$$

$$kp\ liquids = (4.524 + 0.0223 \times FiBW + 0.2046 \times CiBW + 0.344 \times Fi) / 100$$

where: kp = passage rate, h^{-1}; FiBW = forage intake in g DM/kg BW; CiBW = concentrate intake in g DM/kg BW; Fi = forage intake in kg DM.

Chemical analyses to estimate RDP and RUP: protein solubilization and fractioning

The most used method to estimate the fractions of nitrogen compounds in feeds is the subdivision protocol used in the CNCPS (Sniffen et al., 1992; Fox et al., 2000). Originally, the CNCPS divided CP of feedstuffs in 5 fractions, using 3 solvents and a precipitant. The five CP fractions are:

- A, soluble in borate-phosphate buffer (BFB), but without precipitation in trichloroacetic acid (TCA), consisting of non-protein nitrogen compounds (NPN);
- B_1, true protein rapidly degraded in the rumen, soluble in BFB, with precipitation in TCA;
- B_2, true protein and large peptides, moderately degraded in the rumen, calculated as the difference between total CP of feeds and other fractions;
- B_3, true protein slowly degraded in the rumen, calculated by the difference between neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP content) and fraction;
- C, or unavailable protein, equals ADIP.

The NDIP is obtained by estimating the CP in the insoluble residue after treatment with neutral detergent, without the use of sodium sulfite, while the ADIP is estimated after sequential extraction in the residual acid detergent. Fraction A is considered 100% degraded in rumen, while fraction C is considered as 100% undegraded in the rumen.

An interesting aspect of the approach used by CNCPS is that the analyzes (NPN, NDIP, ADIP, and soluble true protein) performed to estimate CP fractions are routine procedures in many laboratories, which facilitates the adoption of this method for use in field conditions (Schwab et al., 2003).

The CNCPS system was recently updated, when Higgs et al. (2015) presented new nomenclature for CP fractions adopted in the current CNCPS. Some changes were made to the analysis methods used by the authors, as follows:

$$P_{A1} = ammonia \times (SP/100) \times (CP/100)$$

$$P_{A2} = [SP \times (CP/100)] - P_{A1}$$

$$CP_1 = CP - (P_{A1} - P_{A2} - CP_2 - IP)$$

$$CP_2 = (NDIP - ADIP) \times (CP/100)$$

$$IP = ADIP \times (CP/100)$$

where: P_{A1} = ammonia; P_{A2} = soluble true protein; CP_1 = insoluble true protein; CP_2 = fiber linked protein; IP = indigestible protein; CP = crude protein; SP = soluble protein in borate-phosphate buffer including sodium azide; NDIP = neutral detergent insoluble protein; ADIP = acid detergent insoluble protein

MICROBIAL PROTEIN SYNTHESIS

Since ruminal microorganisms are the main modifiers of dietary protein, not only the animal's CP requirement must be considered, but also the quantification of N required for the synthesis of ruminal microbial protein. According to Puchala and Kulasek (1992), to obtain the total N required by ruminant, nutritional requirements systems need to provide an estimate of the total amount of protein that is digested and absorbed in the small intestine. This total protein comprises microbial protein synthesized in the rumen and dietary protein that escapes ruminal degradation. The nutritional requirement of

RUP is calculated as the total MP required minus the amount of digestible microbial protein that reaches the duodenum, therefore there is a need to obtain accurate estimates of this variable to quantify the nutritional requirements of MP for ruminants (Firkins, 1996).

According to Broderick and Merchen (1992), microbial markers are necessary to quantify rumen microbial protein. These can be classified as internal and external markers. Internal markers are those inherent to the microorganism, or are already chemical components of the microorganism itself, such as diaminopimelic acid. In addition to the methods mentioned, the most widely used microbial compound as an internal marker is defined as microbial nucleic acids. The high level of RNA in microbial cells makes this compound of great interest in the quantification of the pool of microbial proteins synthesized in the rumen.

External markers are those added to the rumen and able to adhere to microorganisms, as is the case of heavier isotopes such as ^{15}N. An ideal microbial marker should include characteristics such as being easy to quantify, not being present or present in small amounts in feeds, being present in a constant ratio even under experimental conditions and binge biologically stable. The use of each of these markers requires a different technique to estimate the microbial protein, however, most are invasive techniques, as fistulated animals are required to collect ruminal fluid enriched with a marker.

The discovery that urinary purine derivatives (PD) in ruminants are quantitatively important as final products of N metabolism led to further research in the area and to the establishment of relationships between ruminal nucleic acid concentrations and the excretion of urinary PD in ruminants (Topps and Elliott, 1965). This information is the basis of knowledge that led to the use of urinary PD as a non-invasive method to estimate the supply of microbial protein to the intestine in ruminants (Chen and Gomes, 1992).

Comparing ^{15}N and RNA

^{15}N has been widely used as a marker to estimate microbial protein, even though it is a stable isotope, with low environmental risk, lower cost compared to other isotopes for marking all pools of microbial N; moreover, it cannot be found naturally in feedstuffs protein and it does not mark animal protein until marked microbial amino acids are incorporated into their tissues (Broderick and Merchen, 1992). ^{15}N is well distributed in the microbial cell; then, in the case of cell lysis during bacterial isolation, the loss of protoplasm that underestimates nucleic acids is less harmful to the estimate of ^{15}N concentration.

Normally, the marker: microbial N ratio has been obtained in bacteria isolated from the liquid phase of the ruminal digesta, considering that it is similar to mixed ruminal microbial ratio, although there are differences between bacteria from liquid (LAB) and particle (PAB) phases, such as between bacteria and protozoa have been widely reported. The fractions of bacteria associated with the particle phase are greater than those associated to liquid phase, and it can represent more than 90% (Faichney, 1980) of bacteria isolated from animals receiving forage-based diets. Thus, the procedures of bacteria isolation should consider the PAB phase to estimate a more representative marker: total N ratio.

The ^{15}N:^{14}N ratio and the microbial N content, generally, can be obtained from the average in samples of LAB and PAB, since in several cases no differences are found between these two protocols of bacterial isolation (Machado et al., 2013, Rotta et al., 2014a, Prates et al., 2017, Menezes et al., 2017, e Mariz et al., 2018; Table 3.1)

Table 3.1 - Descriptive statistics of the $^{15}N:^{14}N$ ratio and the microbial N content (% OM) obtained in samples of bacteria associated to particles (PAB) and liquid (LAB) phases from different studies

Authors[1]	PAB		LAB	
	$^{15}N:^{14}N$	N (% OM)	$^{15}N:^{14}N$	N (% OM)
Machado et al. (2013)	341[2]	7.07	358[2]	7.20
Rotta et al. (2014a)	0.093[3]	7.80	0.092[3]	8.20
Menezes et al. (2017)	304[2]	5.89	322[2]	5.46
Mariz et al. (2018)	454[2]	7.17	463[2]	7.51
Prates et al. (2017)	0.076[3]	7.27[4]	0.068[3]	7.35[4]

[1]Means of the $^{15}N:^{14}N$ ratio and the microbial N content did not differ by the F test ($P>0.05$), except microbial N in Mariz et al. (2018) that were different between LAB and PAB ($P<0.05$). [2]Δ per thousand. [3]values obtained in omasal samples and considering enrichment of ^{15}N atoms as a percentage; [4]nitrogen on OM basis.

However, unicellular organisms have a high concentration of nucleic acids, especially RNA and purine bases (PB), which makes it interesting to use these organisms as internal microbial markers. About 18% of the total N of ruminal microorganisms is found in nucleic acids and PB contains approximately 11% of total N (Chen and Ørskov, 2003). According to Broderick and Merchen (1992), the use of nucleic acids as markers is well stablished. RNA can be quantified according to the model proposed by Ling and Buttery (1978), while PB according to Ushida et al. (1985).

Most feedstuffs have low concentration of RNA and, according to McAllan and Smith (1973), there is extensive exogenous degradation of RNA in the rumen. Thus, duodenal RNA flow is mainly of microbial origin. However, in proteins from animal byproducts, the RNA concentration is similar to that of microorganisms and, then, the use of RNA as markers is not suitable for animals receiving this type of feed. Although, these feedstuffs are not allowed in Brazil, it does not cause problems with the use of this technique.

According to Rotta et al. (2014b), many of the studies that evaluated different markers to estimate ruminal microbial protein utilized samples from the abomasum and duodenum and the maintenance of cannulated animals in the abomasum and duodenum is difficult and it has high operational costs, causing problems in animal handling. Reynal et al. (2005) and Ipharraguerre et al. (2007) recommended that the calculation of microbial protein flow using ^{15}N as a marker should be performed using omasum samples. However, using ^{15}N and PB as markers, these authors found differences in the values obtained for microbial flow of duodenal samples. Krizsan et al. (2010) suggested that reticulum digesta samples could replace omasal samples. Mariz et al. (2018) studied possible differences between the microbial indicators ^{15}N and PB to estimate ruminal microbial synthesis and efficiency when different levels of protein were applied to the diets of Nellore and crossbred cattle. The authors found no difference in the estimates presented.

Table 3.2 - Effects of different collection sites and microbial markers on microbial nitrogen yield and its efficiency in beef bulls fed corn silage and sugarcane-based diets

	Sampling site				P-value
Markers	Reticulum	Omasum	Abomasum	MSE[1]	L × M[2]
MN[3]	104	114	125	4.59	<0.01
PB[4]	114^{abA}	106^{bA}	130^{aA}	4.78	<0.01
^{15}N	94.1^{bB}	123^{aA}	120^{aA}	4.79	<0.01
MCP[5]/TDN[6]	101	108	118	4.39	<0.01
PB	107^{bA}	93.3^{bB}	117^{aA}	4.44	<0.01
^{15}N	95.0^{bA}	123^{aA}	118^{aA}	4.42	<0.01
MN/fermOM[7]	24.8	31.8	36.2	2.05	<0.05
PB	26.8^{bA}	29.2^{bA}	37.7^{aA}	2.09	<0.05
^{15}N	22.7^{bA}	34.4^{aA}	34.6^{aA}	2.08	<0.05

[1]Mean standard error; [2]Interaction between collection site and microbial marker; [3]Microbial nitrogen; [4]Purine bases; [5]Microbial crude protein; [6]Total digestible nutrients; [7]Fermentable organic matter. Adapted from Rotta et al. (2014b).

The similarity between microbial markers indicated that both ^{15}N and PB are adequate for estimating microbial protein synthesis and microbial efficiency when samples are collected in the omasum. Additionally, Rotta et al. (2014b) conducted a study evaluating these two markers obtained from different sampling sites (Table 3.2). Rotta et al. (2014b) reported that samples obtained from the omasum and abomasum provided similar results for microbial protein yield as well as microbial efficiency when using ^{15}N and PB as markers. Moreover, Rotta et al. (2014b) tested different schemes of sampling, using single, double, and triple markers, isolating different profiles of ruminal digesta such as single phase (single marker system) particle and liquid phase (double marker system), and large and small particles and liquid phases (triple marker system), respectively.

The authors recommended a correction in the estimates of ruminal microbial protein obtained from assays with single and double marker systems for values compatible to the triple markers system, as follows:

$$MNcor\ (g/d) = 49.71 + 0.66 \times MNsingle$$

$$MNcor\ (g/d) = 43.04 + 0.71 \times MNdouble$$

where MNcor is the ruminal microbial nitrogen corrected per day for single or double indicator use. MNsingle is the microbial nitrogen obtained from a single marker scheme, and MNdouble is the microbial nitrogen obtained from a double marker scheme.

Purine derivatives method

Nucleic acids, from bacterial origin that reach the intestine, are mostly digested and absorbed in the small intestine. Absorbed PB are catalyzed to PD (hypoxanthine, xanthine, uric acid, and allantoin) and excreted in the urine (Figure 3.2; Topps and Elliott, 1965). Thus, the microbial N flow in the small intestine can be estimated from the quantification of the excretion of urinary PD (Figure 3.2). Although there are methods to estimate microbial synthesis based on microbial markers (RNA, ^{15}N; Broderick and Merchen, 1992, Tamminga and Chen, 2000), as previously discussed, these methods present difficulties for extensive use because they are extremely invasive and require the use of cannulated animals for the estimation of DM flow through the abomasum or duodenum. These methods based on the estimation of microbial protein flow have been largely used to calibrate some calculation factors using the PD method (Tas and Susenbeth, 2007; Barbosa et al., 2011; Prates et al., 2012).

As it is true for all indirect methods, the method used to estimate microbial production based on urinary PD is susceptible to sources of variation (Chen et al., 1990b, Chen and Gomes, 1992, Tamminga and Chen, 2000, Bowen et al., 2006, Tas and Susenbeth, 2007), and some of the most important factors related to this method have undergone almost constant revisions and updates. The most recent research results related to these factors are discussed in the following items, emphasizing

the use of this method to estimate microbial protein synthesis of cattle raised in tropical conditions, especially in grazing conditions.

a) Urinary collection and experimental sampling

The method to estimate the microbial N flow in cattle is based on the quantification of the daily excretion of urinary PD (allantoin and uric acid). Therefore, daily urinary volume as well as a sample of urine are necessary. The direct quantification of urinary volume can be performed in catheterized animals or any other device that allows the total collection of urine during 24 hours. In females, Foley-type probes are usually used in which urine is passed directly from bladder to a collection recipient. In male animals, funnels are coupled in the foreskin region and are linked directly to a collection recipient. In both cases, the collection is performed for periods of 3 to 5 days with daily quantification and sampling. However, methods of total collection are often laborious which can affect the behavior and welfare of the animals and there are difficulties to apply this technique in grazing animals. In dairy cows, the large amount of urine and the handling of the collection system during milking contribute to making the use of total collection unfeasible and difficulty to conduct. Thus, an alternative technique will be discussed later.

The use of creatinine as a marker for the indirect determination of urinary volume can be an alternative to improve the well-being of animals during experiments. Creatinine is formed in the muscle by the removal of water from creatine phosphate, which is derived from muscle tissue metabolism (Harper et al., 2013). Creatine phosphate is spontaneously degraded at relatively constant rates, forming creatinine. Creatinine is a metabolic product that the body no longer needs, therefore, it is not used for the formation of new compounds, being excreted by the kidneys. The daily production of creatine and, consequently, the excretion of creatinine, depends on the muscle mass and, therefore, is proportional to the body weight of the animal (Koren, 2000). Thus, once the daily creatinine excretion is estimated in relation to the animal's weight and considering this constant concentration throughout the day, it is possible to estimate the urinary volume excreted from the creatinine concentration in a urine sample collected from an animal of known weight.

Creatinine presents constant excretion over 24 hours from constant rates of muscle tissue breakdown. Creatinine excretion is not affected by CP, non-fiber carbohydrates or non-protein nitrogen in the diet (Susmel et al., 1994; Vagnoni et al., 1997; Valadares et al., 1999; Oliveira et al., 2001; Rennó et al., 2000), thus, variations due to diet are not expected.

A meta-analysis was carried out with the results from 25 experiments (Figure 3.1) carried out at the Department of Animal Science of the Federal University of Viçosa (Appendix 3.1), aiming to study the relationship between creatinine excretion and body weight in cattle. The total database contained 882 individual information, and 80% of the data (n = 702) were randomly separated to compose the model-fitting database (Table 3.3), and 20% of the data (n = 180) was selected for the validation process (Table 3.4). Some effects were tested, such as gender and genetic groups (Nellore, *Bos taurus taurus* and crossbred animals), but these variables did not show a significant effect ($P>0.05$). The data were adjusted to an allometric function, making a total number of 702 individual pieces of information.

After the analyses, the following equation was obtained to estimate the daily urinary creatinine excretion (UCE; mg/d) in cattle as a function of body weight (BW; kg):

$$UCE = 66.2158 \times BW^{0.8384}$$

Estimates of model parameters were significant ($P<0.01$) and data fit the model satisfactorily. Therefore, it is recommended that daily excretion of creatinine be estimated using an allometric model, according to the animal's body weight in different genders and genetic groups.

A total of 20% of the data was used for the equation validation procedure, using data observed in 180 experimental units. The predicted data (Table 3.4) were estimated and contrasted with the observed data using the MES software – Model evaluation system (Texas A&M University).

Table 3.3 - Descriptive statistics of data used to fit the allometric model to estimate the relationship between body weight and daily creatinine excretion in urine, containing 80% of the total database collected.

	Creatinine (mg/d)	Body weight (kg)
Mean	8373	369
Median	8205	342
SD	2890	110
Minimum	1266	107
Maxumin	17926	728
n	702	702
Subject	25	25

It was observed that the predicted and observed creatinine data were similar (P>0.05) by the Mayer test (Table 3.5), showed a high correlation and concordance coefficient (CCC = 0.78) and a greater presence of prediction errors of random origin.

These results suggest that the proposed model is accurate for estimating urinary creatinine in cattle (Figure 3.2). The use of creatinine as an accurate tool for estimating urinary volume in different categories of animals makes the process of estimating MCP using PD excreted in urine practical. In Brazil, Pereira (2009) evaluated total creatinine excretion at intervals of 4 to 24 hours and the ratio of purine derivatives, urea, and total nitrogenous compounds to creatinine in Nellore heifers, obtained from spot collections of urine at intervals of 2 hours. The relationship between purine derivatives and creatinine did not vary (P>0.05) during the 24 hours of the day, based on spot collection of urine at 2-hour intervals, suggesting that the calculation of daily PD excretion can be effective in collections obtained at any time of the day.

However, there was an effect of the time of urine collection on the urea:creatinine and Ntotal:creatinine ratios. These relationships were close to the means at two daily points, close to the two daily times of feed supply to the animals (8 AM and 4 PM). Pereira (2009) suggested that the estimation of the excretion of nitrogenous compounds in growing animals can be performed without the need for total collection, using only two spot collections of urine, immediately after supplying the diets.

However, it is emphasized that more research is needed to confirm this suggestion. Silva Junior et al. (2018) studied the relationship between purine derivatives and nitrogenous compounds with creatinine in beef cattle on pasture, to evaluate the possibility of performing collections every 4 hours to measure MCP, nitrogen balance and N excretion.

Table 3.4 - Descriptive statistics of data used to validate the allometric model for estimating the relationship between body weight and daily creatinine excretion in urine, containing 20% of the total database collected.

	Creatinine (mg/d)	Body weight (kg)
Mean	8615	355
Median	8254	318
SD	2887	113
Minimum	18876	743
Maxumin	2887	113
n	180	180
Subject	25	25

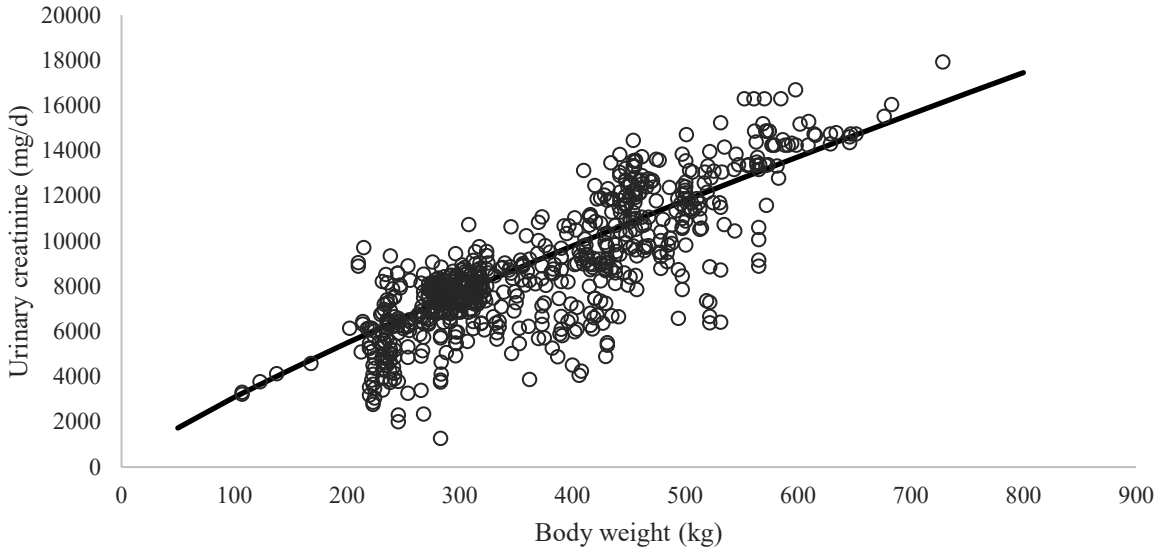

Figure 3.1 - Observed data (o) of the relationship between fasting bovine body weight (kg) and daily urine creatinine excretion (mg/d), and allometric (-) adjustment (UCE = 66.2158× $BW^{0.8384}$), obtained based on a meta-analysis with 25 studies in cattle of different sexual classes and genetic groups.

The authors carried out collections every 4 hours for 5 days and did not detect any difference between the day of collection and the times for the ratio between purine derivatives and creatinine, which allows inferring about the possibility of performing only one urine collection in a given period to estimate the production of MCP in grazing beef cattle, using the technique of urinary purine derivatives. However, due to the variations observed in urea nitrogen and total nitrogen to creatinine ratios over the 24-hour period, Silva Júnior et al. (2018) did not recommend using a sample at just one time to estimate the urinary excretion of nitrogenous compounds.

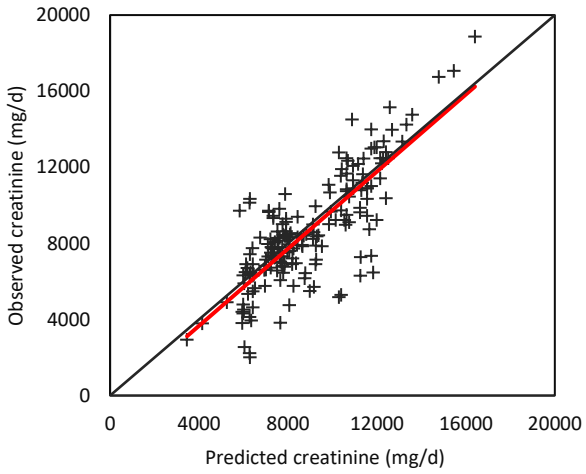

Figure 3.2 - Observed and predicted urinary creatinine values from the proposed equation UCE = 66.2158 × $BW^{0.8384}$, proposed for cattle.

Other results from the same authors (Silva Júnior et al., 2021) demonstrated that urinary creatinine excretion is constant during 24h in grazing cattle. Then, a single spot sample, under grazing conditions, is enough to estimate urinary volume. Once the relationship

between creatinine and purine derivatives is constant, also the purine derivatives can be estimated from only one urinary sample, in pasture conditions. However, two spot samples, 4 hours before and 4 hours after supplying the concentrate, are required to estimate total nitrogen excretion in cattle, since urinary urea nitrogen can vary depending on the diet.

Table 3.5 - Validation of a predicted model for determination of urinary creatinine (UCE mg/d = 66.2158× $BW^{0.8384}$) in cattle, based on body weight, using 20% of the database (n=180)

Item	Predicted creatinine
Mayer test	
Intercept	-390
Slope	1.01
P-value	0.11
RMSE	1725
CCC	0.78
MSEP	
Mean bias %	2.44
Systematic bias %	0.03
Random errors %	97.52
Predicted mean	8614
Mean bias	-269 (3.03%)
P-value	0.04

[1]RMSE: Root of mean square erros; CCC: Concordance correlation coefficient; MSEP: Mean square error of prediction.

b) Urinary recovery of absorbed purines

The relationship between urinary recovery of PD and purine duodenum flow is an important adjustment factor in the method for estimating microbial yield of urinary PD. Several studies have aimed to measure the urinary recovery of post-rumen infused purines from microbial extracts. Urinary excretion of PD was linearly correlated with abomasum infusion of nucleic acids, nucleosides, purines from beer yeast, and with duodenum infusion of nucleic acids, PB, microbial RNA, and yeast RNA (Tas and Susenbeth, 2007). An average equimolar of 0.85 was obtained by Tas and Susenbeth (2007) for urinary recovery of PD infused into the duodenum. In this type of study, the value of PD excretion is linearly related to the value of infused purines (abomasum or duodenum). The slope of the equation gives the recovery value of absorbed purines while the intercept represents the endogenous contribution.

Recent studies conducted in Brazil (Barbosa et al., 2011, Prates et al., 2012) estimated that, for Zebu cattle, the urinary recovery of PD ranged from 0.74 to 0.92 with a mean value 0.80 suggested for practical use, which will be adopted as standard in the BR-CORTE edition for both Zebu and Holstein cattle. Prates et al. (2012) did not observe differences in the recovery rate of absorbed purines between Nellore and Holstein heifers, with no need for different values for each genetic group.

c) Intestinal digestion and absorption of microbial purines

Nucleic acids of bacterial origin, that leave rumen, are extensively degraded in the small intestine and, on average, 85.9% of nucleic acids (Storm et al., 1983), 87-89% RNA, and 80-81% DNA disappeared from small intestine (McAllan, 1980; Storm et al., 1983). Barbosa et al. (2011) evaluated intestinal digestion and absorption of microbial purines in Nellore heifers and estimated the true digestibility coefficient for RNA of 0.93. Although high variability was observed in the true digestibility of ruminal microorganisms purines (Chen and Gomes, 1992, Orellana Boero et al., 2001, Tas and Susenbeth, 2007), the mean value of 0.93 obtained in the study of Barbosa et al. (2011) seems to be adequate for use in Zebu cattle

raised in Brazilian conditions, and is therefore considered as a standard value in this edition of the BR-CORTE.

In the small intestine, purine nucleotides are hydrolyzed into nucleosides (adenosine, guanosine, and inosine) and free bases (adenine and guanine), which are almost completely absorbed by the sodium-potassium dependent pump (McAllan, 1980). In cattle, high activity of the xanthine-oxidase enzyme was observed in the intestinal mucosa and blood plasma (Chen et al., 1990c), making hypoxanthine and xanthine be practically completely degraded to uric acid, unlike sheep. In the liver, uric acid is oxidized to allantoin by the enzyme uricase (Tas and Susenbeth, 2007). Allantoin and uric acid cannot be used by tissues and are excreted mainly in the urine, but also in milk and saliva (Tas and Susenbeth, 2007). In cattle, allantoin is the main PD (more than 80% of the total), while the remainder is composed of uric acid and negligible amounts of xanthine and hypoxanthine (Chen et al., 1990c). Rennó et al. (2000), evaluating the profile of PD excretion in beef heifers, estimated the allantoin and uric acid: total purine ratio of approximately 98%, which indicates that the concentration of xanthine and hypoxanthine in relation to PD would be approximately 2% and that this contribution would irrelevant in the calculation of the microbial protein yield. Thus, the BR-CORTE does not recommend performing xanthine and hypoxanthine analyzes in cattle.

d) Endogenous fraction of urinary purine derivatives

The endogenous fraction of urinary PD includes the portion of PD of nucleic acids from animal tissue degradation (Chen and Gomes, 1992). The direct measurement of endogenous excretion of PD is the use of long-period fasting animals (Chen et al., 1990a; Verbic et al., 1990). Braga et al. (2012) submitted Nellore heifers to feed restriction to evaluate endogenous losses of PD using the following scheme: feeding at 1% BW in the first eight days, 0.5% BW from the ninth to the eleventh day and complete fasting from twelfth to the sixteenth experimental day, totaling 5 days of absolute fasting in which total urine collection was performed. Braga et al. (2012) found an endogenous contribution of 0.332±0.063 mmol/$BW^{0.75}$ and 0.384 g N/$BW^{0.75}$ for growing Nellore heifers.

Alternatively, the endogenous fraction was estimated as the intercept of the linear regression between urinary excretion of PD and post-rumen infused PB. Prates et al. (2012) did not observe differences in endogenous fractions of PD between Nellore and Holstein heifers. Studies conducted in tropical conditions (Barbosa et al., 2011; Prates et al., 2012) with Zebu cattle suggested the use of a mean value of 0.30 mmol/$BW^{0.75}$ as the endogenous fraction of urinary PD, which is the value recommended by the BR-CORTE.

e) The use of urinary allantoin as the only estimator of ruminal microbial protein synthesis

The proportion of allantoin excreted in relation the total PD observed in some studies in the last ten years, we highlight a relationship from 85 to 92 % (Rennó et al., 2000; Magalhães et al., 2005; Pina et al., 2006; Leal et al., 2007; Oliveira et al., 2007; Teixeira et al., 2007; Santos et al., 2010). Then, it becomes interesting for the scientific community to know the estimated relationship between these metabolites and to adjust a mathematical model capable of predicting the uric acid content in the urine.

A meta-analysis involving 38 experiments (Appendix 3.2) performed at the DZO-UFV (Table 3.6), showed that the daily excretion (mmol/d) of uric acid (UA) in the urine can be estimated from the daily excretion of allantoin (ALA) in urine ($P < 0.05$), as follow:

$$UA\ (mmol/d) = 0.1104 \times ALA;\ r^2 = 0.76$$

Also, there was no significant effect ($P = 0.4398$) when the parameters were tested with the intercept, allowing us to estimate a linear model without an intercept.

Table 3.6 - Descriptive statistics of data used to fit linear regression models to estimate the relationship between uric acid and allantoin excreted in cattle urine, and the relationship between allantoin and total purine derivatives (PD)

	Alantoin(mmol/d)	Uric acid (mmol/d)	ALA:PD
Mean	169	20.2	89.0
Median	129	13.5	90.4
DP	123	22.9	4.97
Minimum	18,8	0.30	66.2
Maximum	864	322	99.8
n	1100	1100	1100
Subject	38	38	38

ALA:PD: Percentage of total allantoin in relation to total purine derivatives excreted in the urine.

These results suggest that allantoin can be used as the only predictor of MCP in cattle without the need of uric acid analysis, improving the budget with reagents for analysis and decreasing the time spent on chemical analyzes to estimate MCP synthesis.

Prediction of microbial protein flow of the diet

This version of BR-CORTE maintained the mathematical models proposed to estimate the MCP based on energy and protein intake previously proposed in 2016. A meta-analysis was carried out to evaluate the effect of animal and dietary characteristics on MCP synthesis. In this procedure, 32 studies published in Brazil and abroad were used, as well as theses and dissertations concluded at the Animal Science Department at the Federal University of Viçosa (Appendix 3.3), totaling 2,102 observations (Table 3.7).

The database was used to evaluate three types of energy attributes initially associated with CP intake for each model. The independent variables evaluated were total digestible nutrients intake (TDNI), metabolizable energy intake (MEI) and digested organic matter intake (dMOI). Thus, the complete database comprised all the effects evaluated, with variables classified according to experiment, genetic group (Zebu, beef crossbred, dairy crossbred, and Holstein cattle), gender (bulls, steers, heifers, and cows) and analytical method (PB, PD and ^{15}N). The random effect related to experiments was considered to estimate the parameters of the models.

Table 3.7 - Descriptive statistics of database used to fit the regression models to estimate MCP in beef cattle under tropical conditions.

Item[1]	**n**	**Mean**	**SD[2]**	**Maximum**	**Minimum**
MCP	2102	775	547	3008	66.8
CPI	2102	1.22	0.87	4.39	0.59
DMI	2102	8.52	5.31	23.8	1.76
TDNI	2102	6.22	3.74	16.8	0.83
MEI	2102	22.3	13.3	60.9	3.00
dOMI	1454	5.70	2.98	15.5	0.62
BW	1563	368	125	737	65.3

[1]Microbial crude protein, g/d; Crude protein intake, kg/d; Dry matter intake, kg/d; Total digestible nutrients intake, kg/d; Metabolizable energy intake; Total digestible organic matter intake, kg/d; Crude protein in diet, %; Body weight, kg; [2]Standard deviation.

Random effects such as genetic group, gender, and analytical method were not significant for any proposed equation (P>0.05). Then, the suggested independent variables were evaluated and their parameter estimates were carried out using the Cross Validation procedure (Duchesne and MacGregor, 2001). Linear and quadratic

polynomials were tested because MCP synthesis does not follow a linear behavior and, as in theory, it will reach a plateau. Then, the following equations were obtained (Figure 3.3):

$$MCP = - 53.07 + 304.9 \times CPI + 90.8 \times TDNI - 3.13 \times TDNI^2$$

$$MCP = - 84.87 + 328.7 \times CPI + 28.3 \times MEI - 0.25 \times MEI^2$$

$$MCP = - 93.62 + 381.7 \times CPI + 90.7 \times dOMI - 3.13 \times dOMI^2$$

The database used to estimate the previous equations was developed by data with, on average, 2.83% (± 1.03) of ether extract (EE) in the diet. However, as the NASEM (2016), an equation was developed to estimate microbial protein synthesis for high values of EE, as follows:

$$MCP = - 43.13 + 376.8 \times CPI + 90.9 \times ceeTDNI - 3.22 \times ceeTDNI^2$$

which: MCP is the microbial crude protein synthesis, CPI is the crude protein intake, ceeTDNI is the total digestible nutrients intake corrected for EE.

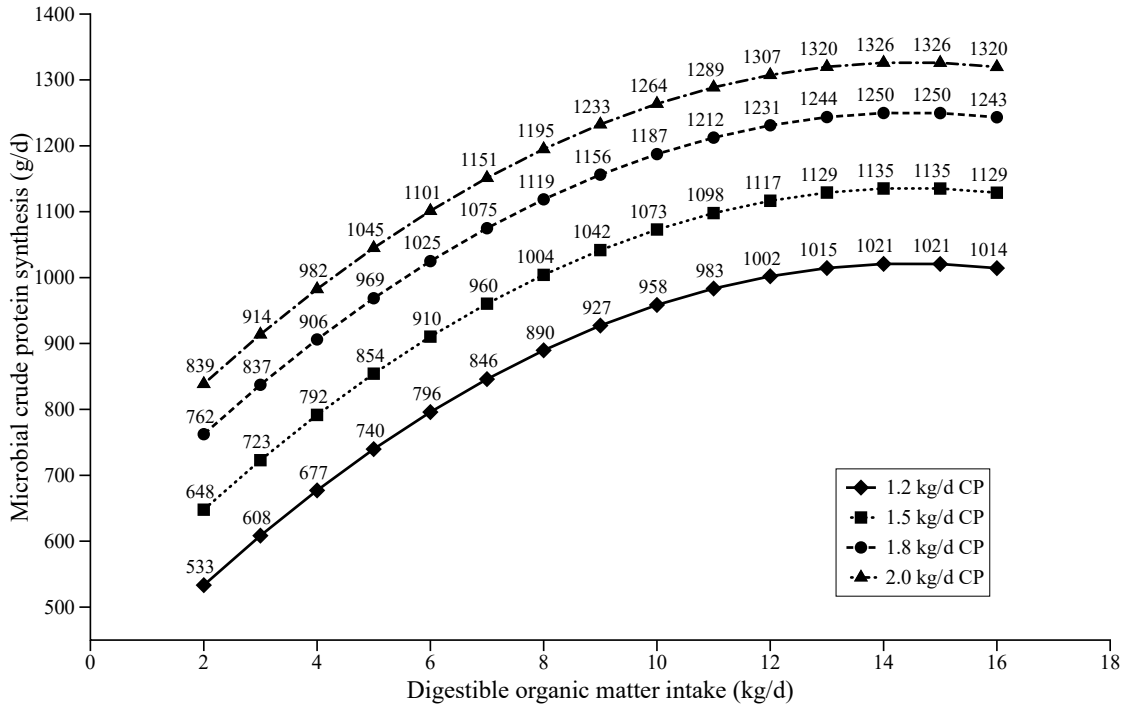

Figure 3.3 - Simulation of MCP estimated from the following equation: $MCP = - 93.62 + 381.7 \times CPI + 90.7 \times dOMI - 3.13 \times dOMI^2$, using four different values for CP intake (1.2; 1.5; 1.8 and 2.0 kg/d) in function of dOMI (kg/d)

The NASEM (2016) suggests an equation for the synthesis of MCP corrected for EE when the EE content in the diet is greater than 3.9%. In Brazil, most beef cattle diets are formulated to contain less than 3.9% EE. However, if the objective is to formulate diets with high EE content, we recommend using the equation proposed by the BR-CORTE which was generated from a database containing 1,437 animals raised under tropical conditions.

A validation process was carried out to verify the prediction accuracy of the MCP equations. The validation was performed using data treatment means (Table 3.8) of 16 published manuscripts (Appendix 3.4). Some criteria were used for the select the manuscripts, as follow: (1) the study was performed in Brazil, (2) the study was performed with Nellore or crossbred cattle, (3) the study was published after 2016 in journals that have the JCR score, (4) the study contained the dependent and independent variables described in the tested equations and, (5) presented standardized residual values calculated for the difference between predicted and observed from -4 to 4.

Table 3.8 - Descriptive statistics of data collected in the literature for validate models to estimate MCP for beef cattle in tropical conditions

Iten[1]	Min	Max	Mean	SD[2]	N
MCP	225.6	1025.0	533.4	182.1	66
TDNI	2.3	7.7	4.3	1.2	66
dOMI	2.1	7.4	4.1	1.1	66
CPI	0.4	1.4	0.8	0.2	66
MEI	9.9	34.0	18.9	5.2	66

[1]Microbial crude protein (MCP) in g/d; intake of total digestible nutrients (ITDN) in kg/d; intake of digestible organic matter (dOMI) in kg/d; crude protein intake (CPI) in kg/d; metabolizable energy intake (MEI) in Mcal/d; Average value; [2]Standard deviation.

There was no effect (P > 0.05) in the Mayer's test for all the variables studied in the validation procedure (Table 3.9), which indicates that the data predicted by the models proposed by the BR-CORTE are the same as those observed in the dataset from the literature (Figure 3.4).

The values of CCC and RMSE are also close to the proposed equations. The equation proposed for the MEI presented the highest QMEP, with the lowest percentage of random errors, which indicates that the accuracy of this model is slightly lower than the others.

The same patterns can be observed when analyzing the mean deviation of -25.7 g (P=0.09), which was greater than the deviation calculated for the other models. Therefore, it is recommended that the model for estimating ruminal MCP should be performed from the MEI only when the values of TDNI and dOMI were not available. Both TDNI and dOMI provided more accurate and precise estimates when compared to those obtained with MEI. The proposed equation based on the dOMI was used to calculate the estimated MCP considering different scenarios (Figure 3.3). The different scenarios have variations in both CP and energy intake, and such equations are used in the BR-CORTE to use the transformation of liquid and metabolizable protein requirements in RDP and RUP requirements, as well as in the estimation of CP requirements in different groups of beef cattle.

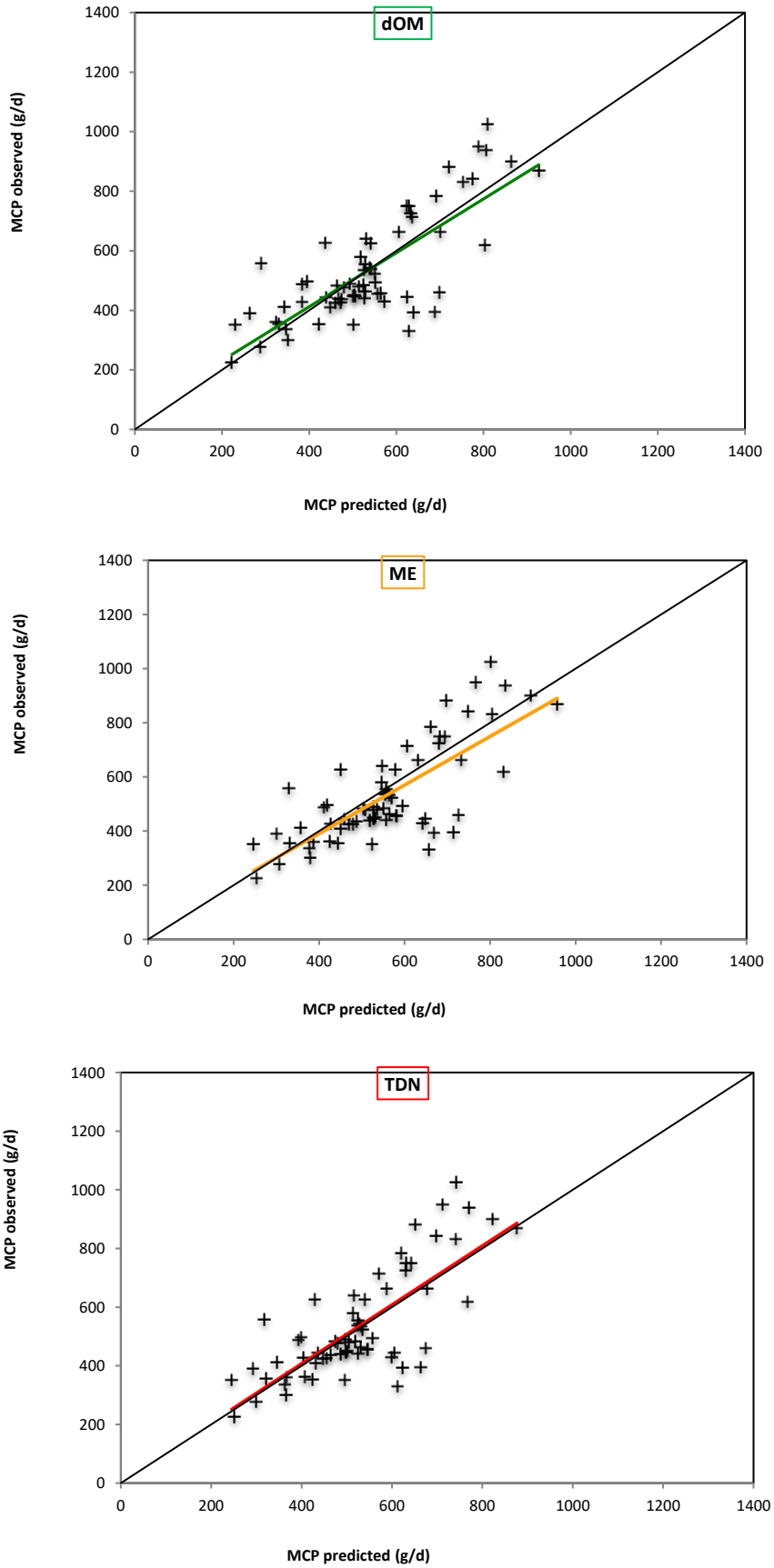

Figure 3.4 - Observed and predicted values of MCP by the equations proposed from the dOMI, MEI and TDNI for beef cattle.

Table 3.9 - Validation of prediction models to estimate MCP (g/d) in beef cattle, based on CP and energy intake in three different presentations (TDN, dOM and ME), using treatment means from 16 published studies (n= 66) collected in scientific literature

Item[1]	TDN	dOM	ME
Mayer test			
Intercept	5.39	46.5	31.89
Slope	1.01	0.91	0.89
P-value	0.85	0.61	0.13
RMSE	117.9	113.6	121.4
CCC	0.73	0.77	0.74
MSEP	13909	12914	22704
Mean bias %	0.50	0.02	4.5
Systematic bias %	0.01	1.52	1.7
Random errors %	99.5	98.5	93.81
Predicted mean			
Mean bias	-8.06	1.79	-25.7
P-value	0.58	0.89	0.09

[1]RMSE: Root of mean square errors; CCC: Concordance correlation coefficient; MSEP: Mean square error of prediction.

GENERAL RECOMMENDATIONS

- **Estimation of microbial contamination of roughage using *in situ* incubation*:***

$A_{CP}C = 1.99286 + 0.98256 \times A_{CP}NC$

$B_{CP}C = -17.2181 - 0.0344 \times B_{CP}NC + 0.65433 \times CP + 1.03787 \times NDF + 2.66010 \times NDIP - 0.85979 \times iNDF$

$kd_{CP}C = 0.04667 + 0.35139 \times kd_{CP}NC + 0.0020 \times CP - 0.00055839 \times NDF - 0.00336 \times NDIP + 0.00075089 \times iNDF$

$\%C = 79.21 \times (1 - e^{-0.0555 \times t}) \times e^{-0.0874 \times CP}$

- **Correction for MCP estimated from assays using single and double indicator system:**

MNcor (g/d) = 49.71 + 0.66 × MNsingle

MNcor (g/d) = 43.04 + 0.71 × MNdouble

- **Endogenous fraction of urinary purine derivatives in Zebu cattle:**

$0.30 \text{ mmol/BW}^{0.75}$

- **Daily excretion of urinary uric acid from daily excretion of urinary allantoin**

UA (mmol/d) = 0.1104 × ALA

- **Estimation of daily urinary creatinine excretion in cattle:**

$UCE \text{ (mg/d)} = 66.2158 \times BW^{0.8384}$

- **Prediction of MCP:**

$MCP = - 53.07 + 304.9 \times CPI + 90.8 \times TDNI - 3.13 \times TDNI^2$

$MCP = - 93.62 + 381.7 \times CPI + 90.7 \times tdOMI - 3.13 \times tdOMI^2$

$MCP = - 84.87 + 328.7 \times CPI + 28.3 \times MEI - 0.25 \times MEI^2$

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APPENDIXES

Appendix 3.1

Azevêdo, J.A.G., Valadares Filho, S.C., Pina, D.S., Detmann, E., Valadares, R.F.D., Pereira, L.G.R., Souza, N.K.P., and Costa e Silva, L.F., 2011. Intake, total digestibility, microbial protein production and the nitrogen balance in diets with fruit by-products for ruminants, *Revista Brasileira de Zootecnia*, 40, 1052-1060.

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Appendix 3.2

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