AP AGRO PRODUCTIVIDAD
Colegio de Postgraduados

Leucaena leucocephala (Lam.) de Wit as protein supply for heifers

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ABSTRACT

Objective: To estimate the percentage of Leucaena in the diet of heifers fed with Taiwan grass, in order to maximize the efficiency of the ruminal supply of microbial Nitrogen (ERSMN).

Design/Methodology/Approach: Five heifers were randomly selected for each of the five treatments (0, 20, 40, 60, and 80% supplementation with Leucaena), according to a 5×5 Latin square experimental design. We determined the ruminal supply of microbial nitrogen (RSMN), ERSMN, and the urea-N by measuring N and purine derivatives in urine. Subsequently, we predicted the duodenal RSMN, the rumen nitrogen balance (RNB), and the urea cost with the Large Ruminant Nutrition System (LRNS v. 1.0.33) model.

Results: The inclusion of Leucaena improved ($P \leq 0.05$) the RSMN. The ERSMN estimated by purine derivatives had a quadratic response ($P \leq 0.05$) at the inclusion level of Leucaena in the diet. The RNB, the cost of urea, and the urea-N increased ($P \leq 0.05$) with a higher inclusion percentage of Leucaena. The maximum ERSMN and N balance were obtained with 20% Leucaena in the ration.

Study Limitations/Implications: The expression of the nitrogen utilization potential of Leucaena for microbial protein synthesis in this study was likely restricted by the limited availability of non-fiber carbohydrates (NFC). Further studies must be conducted to determine the most affordable source of NFC to match Leucaena nitrogen utilization in the rumen.

Findings/Conclusions: Leucaena could be used as an efficient protein source for heifers at a 20% inclusion in their diet.

Key words: *Leucaena leucocephala*, Microbial protein, Purine derivatives, LRNS.

Citation: Castro-González, A., Montero-Lagunes, M., Ríos-Utrera, Á., Ayala-Burgos, A. J., & Juárez- Lagunes, F. I. (2022). *Leucaena leucocephala* (Lam.) de Wit as protein supply for heifers. *Agro Productividad*. https://doi.org/10.32854/ agrop.v15i7.2324

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: January 25, 2022.
Accepted: July 16, 2022.
Published on-line: August 02, 2022.

Agro Productividad, 15(7). July. 2022. pp: 69-77.

INTRODUCTION

Leucaena [*Leucaena leucocephala* (Lam.) de Wit] is known in the tropic as a leguminous quality forage, as a result of its high protein and low fiber contents, as well as its moderate tannin levels (Quero-Carrillo *et al.*, 2014). Several studies have shown that Leucaena improves animal response when used as a protein supplement (Madera *et al.*, 2013; Ku

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Vera *et al.*, 2014; Solorio *et al.*, 2016). However, increasing levels of Leucaena in the diet have been reported to cause a nitrogen (N) loss in urine (Hung *et al.*, 2013; Piñeiro-Vázquez *et al.*, 2017). N loss in animal production systems has important nutritional and environmental effects, including an increase in greenhouse gas emissions. Agriculture is estimated to contribute approximately 8 to 10% to global emissions (O'Mara, 2011). In this regard, ruminants contribute to methane and nitrous oxide emissions (IPCC, 2007; Rotz, 2018). The main source of nitrous oxide in ruminants is urea-N in urine (Hoogendoorn *et al.*, 2010). Reducing urea-N in urine provides the best opportunity to diminish N excretions and this can be achieved through an efficient feeding strategy (Nasiru *et al.*, 2014). Optimizing the use of N in the ruminant diet would be an alternative to reduce N loss in urine, since it improves the synthesis of microbial protein, expressed as microbial N (MN). In forage-based diets, MN is produced in the rumen and requires NH_3 as N and structural carbohydrates (SC) as energy (AFRC, 1993); therefore, the amount of MN that reaches the small intestine depends on the availability of SC and NH_3 in the rumen (Sniffen & Robinson, 1987). There are several methods to estimate the MN supply, including the use of urine purine derivatives (PD) (Chen & Gomes, 1992).

The use of simulation models can be an alternative to predict the amount of N entering the duodenum. Both the Cornell Net Carbohydrate and Protein System (CNCPS) —a system that estimates protein and energy requirements, developed by the University of Cornell— and its 5.0 version —known as Large Ruminant Nutrition System (LRNS v. 1.0.33, Fox *et al.*, 2004)— use a mechanistic model to quantify the supply of bacterial N to the duodenum. In addition, these models predict the rumen N balance, which indicates whether or not the rumen bacterial N requirements are met. When the rumen N balance is positive, excess N is excreted as urea and contributes to the energetic cost of urea synthesis. Non-fiber carbohydrate (NFC) supplements for the rumen are an option to improve the efficiency of microbial synthesis in this organ (Poppi & McLennan, 1995). Products such as citrus pulp, molasses, polished rice, and sorghum can be used as optional NFC sources in ruminant diet in the tropic (Harper *et al.*, 2019). Some studies report that using these supplements in diets with Leucaena in dual-purpose cows improves the efficiency of N use in the diet (Flores-Cocas *et al.*, 2019, Arjona-Alcocer *et al.*, 2020). However, the inclusion levels of Leucaena and the NFC source that would allow capturing the N of this forage is still uncertain. The objective of this work was to estimate the adequate percentage of Leucaena in the diet of heifers fed with *Cenchrus purpureus* cv. Taiwan grass that would both maximize the efficiency of the ruminal supply of microbial N and reduce the excretion of urea-N.

MATERIALS AND METHODS

The two-stage work evaluated the effect on the ruminal supply of microbial N (RSMN) of different Leucaena inclusion levels in the diet of heifers. Stage 1 —conducted on 18-month-old heifers with a live body weight of 295±19 kg— evaluated the effect of the Leucaena inclusion level (determined through purine derivatives) on RSMN. Stage 2 validated the LRNS v. 1.0.33 model (Fox *et al.*, 2004) as a tool to predict the effect of the Leucaena inclusion level on RSMN.

Stage 1. Ruminal supply of microbial N through purine derivatives

The research was conducted at the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Autónoma de Yucatán, in Mérida, México. The region has a tropical climate, an average temperature of 26.8 °C (García, 2004), and an average annual rainfall of 984.4 mm. Five crossbred heifers (*Bos taurus* × *Bos indicus*) with an average live weight of 295 ± 19 kg were used to determine the RSMN through purine derivatives; the heifers were kept in metabolic cages inside a roofed, wall-less building with concrete floor. The basal ration consisted of fresh, chopped Taiwan grass and Leucaena forage, harvested at 60 days of regrowth. Heifers were fed *ad libitum*, allowing a 15% rejection of the amount of food offered the previous day. The estimated DM intake was 7 kg head^{-1} day^{-1}. The grass levels of this intake were increasingly substituted with Leucaena (0, 20, 40, 60, and 80%) for the experimental treatments. Heifers were fed once a day, with the complete feed amount offered at 9:00 h. The N content in Leucaena was 25.6 g kg^{-1} of DM, which equals 160 g of protein per kg^{-1} of DM. During this stage, the total urine volume was collected for 24 h in a 20-L plastic container; 1000 mL of a 10% sulfuric acid solution were added in order to maintain a $\mathrm{pH} < 3$ and avoid the volatilization of N. Urine aliquots (100 mL) were obtained from the total daily volume and then frozen at -4 °C, awaiting the chemical analyses that determined purine derivatives and urea-N (Chen & Gomes, 1992).

Purine derivatives (PD) and microbial N synthesis. Allantoin and uric acid were determined with colorimetry, using a DU-650 spectrophotometer (Beckman Instruments, USA) according to the methodology described by Chen *et al.* (1993). The amount of PD absorption was calculated from the PD excretion (allantoin and uric acid), based on the relation derived from the equation of Chen & Gomes (1992):

$$Y = 0.385 \text{ kg } PV0.75 + 0.85 * X$$

The supply of microbial protein, expressed as microbial nitrogen (SMN), was estimated by PD excretion in urine, based on Chen & Gomes (1992):

$$SMN(g\ N\ d^{-1}) = X(mmol\ d^{-1}) *70/0.116 * 0.83 * 1000 = 0.727 * X$$

where X and Y are PD absorption and excretion in $mmol\ d^{-1}$, respectively.

Efficiency of microbial N in the ruminal supply. The ERSMN was calculated using the following formula:

$$ESRNM = microbial\ N\ (g\ d^{-1})\ kg^{-1}\ DOMR$$

where $DOMR$ is the digestible organic matter in rumen (assuming that ruminal digestion is 650 g kg^{-1} of organic matter digested in the total tract);

$$DOMR = IDOM \times 0.65$$

where $IDOM$ is the ingested digestible organic matter, according to ARC (1980).

Urinary urea-N excretion. The concentration of urea was determined using the urease modified Berthelot reaction, a colorimetric method. The urea-N excreted in urine was determined based on the existent relation between the molecular weight of urea and the molecular weight of its N content (46.65%). The amount of excreted urea (g) was previously obtained. These values were used in the following relation:

$$urea\text{-}N\ (g) = urea\ (g) \times 0.4665.$$

Stage 2. Validation of RSMN with the LRNS model

Database. To validate the LRNS model (Fox *et al*., 2004), we input the observed values for each cow per sampling period (n=25), using dry matter (DM) intake averages per each 5-day period.

Model entries. Table 1 presents a summary of the supplies, animal characteristics, and environment input into the model. The information about the nutritional composition of feed was entered into the model's feed library (Table 2).

Intake and bacterial N predictions with the LRNS model. The LRNS model (Fox *et al*., 2004) was used to predict the rumen N balance (RNB, requirement %), the urea cost (Mcal d^{-1}), and the RSMN (g d^{-1}).

Experimental design and statistical analyses

In Stages 1 and 2, an analysis of variance with a 5×5 Latin square experimental design (Cochran & Cox, 1990) was conducted for the PD, RSMN, ERSMN, urea-N, RNB, and

Table 1. Inputs used to evaluate DM and bacterial N intake predictions with the LRNS model.

Description	Heifers	Description	Units
Animal:		**Environment:**	
Animal Type	Growth/Finishing	Additive	None
Age (mo)	18	Supplemented fat	Not
Sex	Female	Wind Speed (kph)	16
Current Body Weight (kg)	295±19	Previous Temperature (°C)	26
Mature Body Weight (kg)	550	Previous Humidity (%)	80
Body Weight	Live Weight	Current Temperature (°C)	26
Breed Type	Cross Bred (Dual Purpose)	Current Humidity (%)	80
		Sunlight Exposure (h)	4
Grade	Low Marbling (22% body fat)	Storm Exposure	None
		Hair Depth (cm)	0.64
Production:		Floor Mud Depth (cm)	0
Condition score (scale 1 to 9)	5	Hide	Average
Breeding System	*Bos taurus*×*Bos indicus*	Hair Coat	No Mud
Bull's Breed	Holstein	Cattle Panting	None
Dam's Breed	Brahman	Minimum Night Temperature (°C)	18
		Activity	Confinement

Table 2. Inputs of the dietary composition into the model to predict microbial N using the LRNS model.

Composition	Taiwan	Leucaena
DM (% as feed basis)	23.7	32.6
NDF (% DM)	67.0	58.0
Lignin (% NDF)	7.0	16.0
CP (% DM)	6.0	16.0
Ether Extract (% DM)	2.3*	0.7*
Ash (% DM)	5.1*	5.3*
Soluble Protein (% CP)	46.0*	25.0*
Non-protein N (% SP)	2.2*	5.0*
NDIP (% CP)	2.2*	33.5*
ADIP (% CP)	0.9*	12.5*

Cenchrus purpureus cv. Taiwan, Leucaena (*Leucaena leucocephala*); DM: dry matter; NDF: neutral detergent fiber; CP: crude protein; NDIP: neutral detergent insoluble protein; ADIP: acid detergent insoluble protein; *LRNS version 1.0.33 Tropical Feed Library.

urea cost variables, using the SAS generalized linear model procedure (PROC GLM) (2002). The following model was used:

$$Y_{ijk}=\mu+P_i+A_j+T_k+e_{ijk}$$

where Y_{ijk} is the dependent variable, μ the overall mean, P_i the effect of the ith period, A_j the effect of the j^{th} animal, T_k the effect of the k^{th} Leucaena level, and e_{ijk} the experimental error.

The least square means of Leucaena levels were estimated using the LSMEANS option, while the means ($P<0.05$) were compared with Tukey's test. The differences between means ($P<0.05$) were accepted as statistically significant. In addition, we conducted a response surface analysis with orthogonal contrasts (Kaps & Lamberson, 2017) to evaluate the linear, quadratic, and cubic effects of the Leucaena level on the study variables. In Stage 1, the SAS REG procedure (PROC REG) (2002) was used to conduct a regression analysis in order to obtain the equation ($y=a+bx+cx^2$) that can determine the optimal Leucaena level where the ERSMN is maximized. In this equation, the values of "x" (Leucaena level; DM%) was calculated equating to zero the first derivative of the equation —where "y" (ERSMN, g N/kg DOMR) is maximal. To validate the LRNS, we analyzed the mean difference of the variables (DM intake, N intake, and RSMN) —experimentally observed and predicted by LRNS—, by conducting a variance analysis with the SAS GLM procedure (PROC GLM) (2002). We used the following model:

$$Y_{ij}=\mu+T_i+e_{ij}$$

where Y_{ij} is the dependent variable, μ the overall media, T_i the effect of the i^{th} treatment (observed v. predicted), and e_{ij} the experimental error.

RESULTS AND DISCUSSION

Stage 1. Effect of diet on ERSMN

In this study, the Leucaena inclusion level in the diet had no effects ($P>0.05$) on the total PD excretion and the RSMN. However, the ERSMN values were affected with a quadratic response ($P<0.05$) by the Leucaena level in the diet, while the effect of the diet on the urea-N excretion showed a positive linear response ($P<0.05$) (Table 3). The maximum ERSMN was observed with 20% Leucaena (33.4 g N kg^{-1} DOMR), while the ERSMN values were similar with 40 and 60% Leucaena (31 g N kg^{-1} DOMR) (Table 3). These results agree with those found by Hung *et al.* (2013) in buffaloes fed with increasing levels of Leucaena (0 to 45%). The maximum ERSMN (33.4 g N kg^{-1} DOMR) in Hung's study was observed with 20% Leucaena.

Stage 2. Effect of diet on RSMN as predicted by LRNS

The Leucaena level in the diet had a significant impact ($P<0.05$; Table 3) on rumen nitrogen balance (RNB), as a N requirement percentage. The optimal RNB value was reached at 20% Leucaena. These results show that both the ERSMN observed by PD and the ERSMN predicted by LRNS reached their optimal level when heifers were fed with 20% Leucaena. Above this level, the excretion of (observed) urea-N and the (predicted) urea cost increased linearly. These results match the increase of RNB after this level (Table 3).

The effect of the Leucaena level in the diet showed a positive linear response on the observed excretion of urea-N ($P<0.05$) and the predicted urea cost ($P<0.05$) (Table 3). These results could be explained by a lack of energy in rumen for the capture of N, which

Table 3. Efficiency in the ruminal supply of microbial nitrogen and predictions made with the LRNS model for rumen nitrogen balance in heifers supplemented with Leucaena.

Leucaena (% DM)	**Observed (PD)**				**Predicted (LRNS)**		
	TPD (mmol d^{-1})	**RSNM (g N d^{-1})**	**ERSNM (g N kg^{-1} DOMR)**	**Ureic N (g N d^{-1})**	**RNB (% Req)**	**Urea cost (Mcal d^{-1})**	**RSNM (g N d^{-1})**
0	74.1^{a}	47.8^{a}	14.8^{bc}	4.93^{b}	90.2^{e}	0.00^{d}	59.0^{bc}
20	112.6^{a}	72.7^{a}	33.4^{a}	13.5^{b}	100.6^{d}	0.03^{d}	67.2^{ab}
40	98.7^{a}	61.3^{a}	31.3^{ab}	16.6^{b}	107.6^{c}	0.06^{c}	71.8^{ab}
60	102.8^{a}	66.0^{a}	31.7^{ab}	46.6^{a}	112.6^{b}	0.12^{b}	77.2^{a}
80	109.5^{a}	58.1^{a}	24.4^{ab}	56.1^{a}	117.0^{a}	0.15^{a}	79.6^{a}
RMSE	18.5	13.1	7.77	12.1	0.37	0.01	5.9
P-value	0.0911	0.1168	0.0339	0.0005	<.0001	<.0001	0.001
L	*	NS	NS	***	***	***	***
Q	NS	NS	**	NS	***	NS	NS
C	NS	NS	NS	NS	**	*	**

PD: Purine Derivatives; LRNS: Large Ruminants Nutrition System; TPD: Total of purine derivatives; RSMN: Ruminal supply of microbial N; ERSMN: Efficiency in the RSMN; DOMR: Digestible organic matter in rumen; RNB: Rumen N balance (required %); RMSE: Root mean square error. L: Linear contrast; Q: Quadratic contrast; C: Cubic contrast. Means with the same superscript in the same column do not differ significantly ($P>0.05$). *$P<0.05$; ** $P<0.01$; *** $P<0.001$; NS=Not significant.

is the factor that most frequently limits microbial growth (Clark *et al.*, 1992). Karsli & Russell (2001) have pointed out that energy supply is commonly the major limiting factor for microbial growth in rumen. Meanwhile, Orskov (1992) has indicated that microbial protein synthesis can be maximized by synchronizing the availability of fermentable energy and degradable N for rumen microorganisms. Consequently, when the heifers' diet is supplemented with >20% Leucaena as a protein source, a source of energy that allows the capture of the N supplied by Leucaena should be provided to avoid the loss of urea-N in urine. Figure 1 shows that the N intake increases linearly ($P<0.05$), while the ERSMN increases quadratically ($P<0.05$) up to a certain level, after which it starts to drop.

According to the equation ($y=17.49436808+(0.60784137\ x)+(-0.00687749\ x2)$), the inflection point for the optimal level of the RSMN yield (31 g N kg^{-1} MODR) was reached with 44% Leucaena. This result contrasts with the RNB, whose optimal N level in rumen is reached with 20% Leucaena. Therefore, the mathematical equation does not match the dynamics of microorganisms in rumen.

Comparison of data observed with PD *v*. data predicted with the LRNS model

There were no differences ($P>0.05$) between the general averages of DM intake, N intake, and RSMN observed by PD and predicted with the LRNS model (Table 4). This result confirms the usefulness of the LRNS model for the nutritional assessment of tropical forages in ruminant diets.

Figure 2 shows the relation between the intake of N and RSMN in heifers. The N intake increases linearly ($P<0.05$), while the RSMN reaches its maximum value with 20% Leucaena (72.7 g N d^{-1}) ($P>0.05$). A similar response regarding the relation of the intake of N and RSMN was observed with the data predicted with LRNS. The intake of N and the RSMN had a positive linear effect ($P<0.05$) when Leucaena levels in the diet

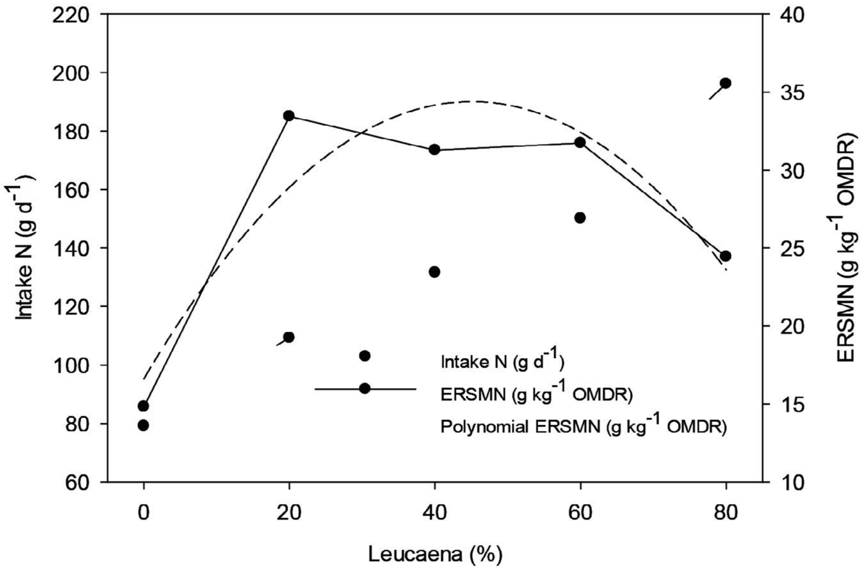

Figure 1. Relation between N intake and the efficiency in the ruminal supply of microbial N (ERSMN, g N kg^{-1} DOMR) in heifers fed with different percentages of Leucaena in their diet.

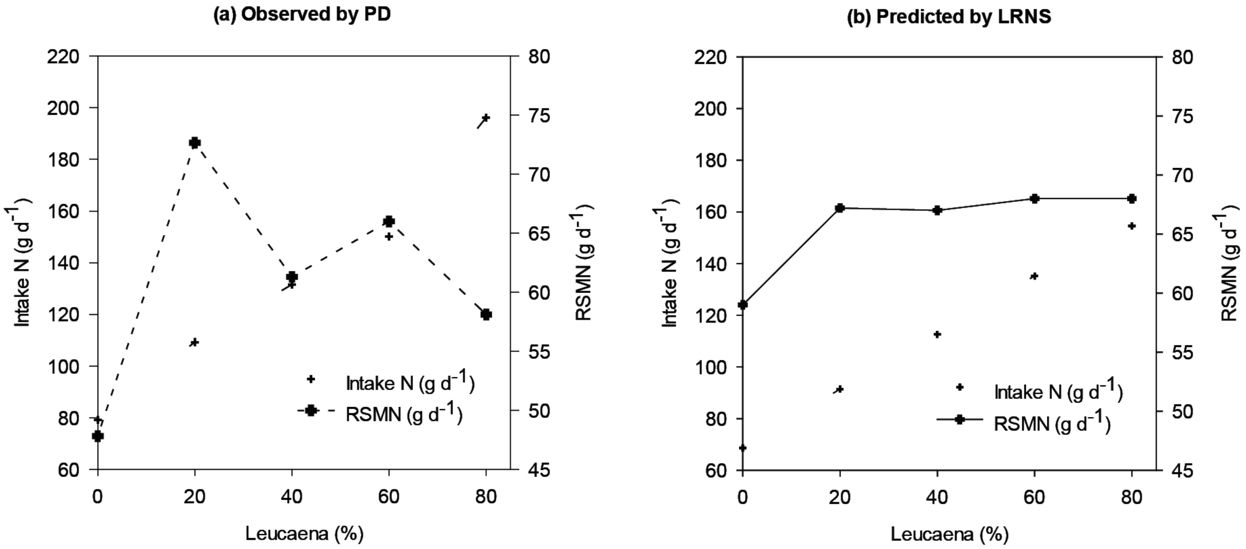

Figure 2. Comparison of the relation between variables: intake of N (g d^{-1}) and ruminal supply of microbial N (RSMN, g d^{-1}) observed by PD (a); and intake of N (g d^{-1}) and RSMN predicted with LRNS (b).

Table 4. Comparison between dry matter intake, N intake, and RSMN observed by PD *v*. LRNS predictions in heifers fed with Leucaena levels.

Variable	Dry Matter Intake (kg d^{-1})	N Intake (g d^{-1})	RSMN (g d^{-1})
Observed by PD	7.02^{a}	133.2^{a}	61.1^{a}
Predicted by LRNS	7.08^{a}	112.5^{a}	65.7^{a}
RMSE	0.88	45.2	14.2
P-value	0.7974	0.1114	0.2612

Means with the same superscript in the same column do not differ significantly ($P>0.05$). PD: Purine Derivatives; LRNS: Large Ruminants Nutrition System; RMSE: Root mean square error. RSMN: Ruminal supply of microbial N.

increased. However, the maximum RSMN value (67 g d^{-1}) remained slightly constant with ≥20% Leucaena. The results for the RSMN match the behavior of urea cost, which increases linearly starting from 20% Leucaena (Table 4).

CONCLUSIONS

Leucaena supplementation significantly increased the RSMN in heifers. In addition, urea-N excretion increased as the Leucaena supplementation level increased. The ERSMN and the RNB reached their optimal level when the heifers' diet was supplemented with 20% Leucaena. For higher levels, we suggest including NFC sources in the diet to capture the N supplied by Leucaena and avoid the loss of N in urine.

ACKNOWLEDGEMENTS AND CONFLICT OF INTERESTS

We certify that no conflict of interests exists with any financial organization regarding the material discussed in this manuscript. We thank Ángel Trinidad Piñeiro Vázquez (ScD) for kindly providing us with the heifer urine samples.

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